Organic amendments for improving biomass production and metal yield of Ni-hyperaccumulating plants

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Abstract

Ni phytomining is a promising technology for Ni recovery from low-grade ores such as ultramafic soils. Metal-hyperaccumulators are good candidates for phytomining due to their extraordinary capacity for Ni accumulation. However, many of these plants produce a low biomass, which makes the use of agronomic techniques for improving their growth necessary.

In this study, the Ni hyperaccumulators *Alyssum serpyllifolium* subsp. *lusitanicum*, *A. serpyllifolium* subsp. *malacitanum*, *A. bertolonii* and *Noccaea goesingense* were evaluated for their Ni phytoextraction efficiency from a Ni-rich serpentine soil. Effects of soil inorganic fertilisation (100:100:125 kg NPK ha⁻¹) and soil organic amendment addition (2.5, 5 or 10% compost) on plant growth and Ni accumulation were determined. All soil treatments greatly improved plant growth, but the highest biomass production was generally found after addition of 2.5 or 5% compost (w/w). The most pronounced beneficial effects were observed for *N. goesingense*. Total Ni phytoextracted from soils was significantly improved using both soil treatments (inorganic and organic), despite the decrease observed in soil Ni availability and shoot Ni concentrations in compost-amended soils. The most promising results were found using intermediate amount of compost, indicating that these types of organic wastes can be incorporated into phytomining systems.

Keywords

Phytomining, serpentine soils, soil fertility, inorganic fertilisation, compost, agromining

1. Introduction

Metal hyperaccumulator plants present an extreme ability to accumulate trace elements in their harvestable tissues. To date, approximately 500 taxa of metal hyperaccumulators have been identified, of which over 90% accumulate nickel (Ni) (Pollard et al., 2014). The Brassicaceae family, and specifically the genus *Alyssum*, present the greatest number of Ni hyperaccumulators (Pollard et al., 2014). The vast majority of Ni-hyperaccumulating species are endemic to soils developed over ultramafic rocks (naturally rich in metals such as Co, Cr and Ni), and can present up to 1-3% dry weight Ni content in their shoots (Reeves 2006). This characteristic makes these plants particularly useful for application in phytoextraction or phytomining (Chaney et al., 2010). Phytoextraction techniques are focused on the remediation of degraded or contaminated soils using plants which are able to accumulate high amount of metals in their harvestable plants, while phytomining is the phytoextraction and recovery of metals from the harvested plant biomass for commercial gain (Chaney et al., 2007; Tang et al., 2012). Ni phytomining was proven to be economically feasible in the USA and Canada (Chaney et al., 2007; Li et al., 2003), and more recently, in Europe (Bani et al., 2015; Bani et al., 2007). To date the Ni-hyperaccumulating *Alyssum* species, *A. murale* and *A. corsicum* (native to Mediterranean serpentine soils), have shown the highest potential application in phytomining processes (Chaney et al., 2007; Li et al., 2003). Selected plants for Ni phytomining purposes should be able to hyperaccumulate this metal and also to tolerate and thrive under the adverse growth conditions which are frequently present in metal-rich environments (Harris et al., 2009). The use of native plant species has several advantages, both for practical reasons and also as a means of supporting the conservation of serpentine biodiversity. For example, *A. murale* occurs widely as a native weed of agricultural land on ultramafic Vertisols in

Albania, and was successfully implemented in phytomining cropping systems in the Albanian regions of Pojskë and Prrenjas (Bani et al., 2015; Bani et al., 2007). However, a low biomass production can be an important bottleneck limiting the practical application of hyperaccumulators in phytomining (Kidd et al., 2015; McGrath and Zhao, 2003; Mench et al., 2010; Robinson et al., 1997).

Over the last decade several authors have highlighted the need to optimise the phytomining process using appropriate agronomic and crop management practices, such as pH adjustments, fertilisation regimes, design of cropping patterns (co-cropping or rotation), bioaugmentation using plant-associated microbial inoculants, or the use of plant growth regulators (PGRs) (Bani et al., 2015; Cabello-Conejo et al., 2014a; Cabello-Conejo et al., 2014b; Chaney et al., 2007; Kidd et al., 2015; Tang et al., 2012; Durand et al., 2015). In fact, the concept of agromining was introduced by van der Ent et al. (2015) to emphasise that phytomining forms part of an integrated agricultural chain. Several studies have shown the importance of soil fertilisation to increase plant yields and phytoextracted Ni. In field-scale experiments in serpentine soils, Bani *et al.* (2015) found a high positive response in biomass production of *A. murale* using NPK and Ca fertilisers. Robinson et al. (1997) enhanced the biomass of *A. bertolonii* with NPK fertilisers by 3-fold. Inorganic fertilisation also increased the biomass of *A. serpyllifolium* ssp. *lusitanicum* growing on a mine-soil by a factor of 10; as a consequence the total Ni phytoextracted experimented a 6-fold increase (Kidd and Monterroso, 2005).

Municipal wastes such as sewage sludges (biosolids) have been used as an amendment to agricultural soils for many years now, and are an economically attractive alternative

to disposal through landfill and incineration. However, few studies have considered the use of organic soil amendments to increase Ni phytomining efficiency. Ultramafic soils are characterised by a low fertility, low water holding capacity and poor structure. Compost addition could be a useful means of increasing soil fertility and soil structure (Bernal et al., 2007; Fagnano et al., 2011; Martínez-Fernández et al., 2014). Moreover, the use of composted solid organic residues, such as municipal solid wastes (MSW) or sewage sludge, also constitutes a means of recycling waste products, thus contributing towards the objectives of the EU's Zero Waste Policy (2013).

The objective of this study was to evaluate the use of composted MSW as a means of increasing the phytoextraction efficiency of different Ni hyperaccumulating plants grown in ultramafic soils. The effects of different fertilisation regimes (compost addition or mineral fertilisers) on biomass production and Ni removal of the hyperaccumulators, *Alyssum serpyllifolium* subsp. *lusitanicum* (two populations), *A. serpyllifolium* subsp. *malacitanum* (two populations), *A. bertolonii* (one population) and *Noccaea goesingense* (one population), were compared.

2. Material and methods

2.1 Plant species, study soil and experimental set-up

Soil was collected from the ultramafic area of Barazón (Galicia) in NW Spain (where *A. serpyllifolium* subsp. *lusitanicum* is a native species). Soils were air-dried, sieved through an 8-mm stainless steel sieve and homogenised. Soil treatments included untreated control (no amendment), inorganic fertilisation (NPK), and different rates of an organic amendment addition (commercial compost based on municipal solid wastes

(MSW)). Inorganic fertilisers were added as pure chemical reagents: N was added at 100 kg ha⁻¹ as NH₄NO₃, and P and K were added as KH₂PO₄ at 100 and 125 kg ha⁻¹, respectively (based on a soil depth of 20 cm and bulk density of 1.1 g cm⁻³). The compost amendment was applied at three different rates of addition: 2.5%, 5 % and 10% w/w. The commercial compost used in this study was provided by a local SME company dedicated to the recycling of waste products (Tratamientos Ecológicos del Noroeste (TEN) s.l.) and is primarily based on municipal sewage sludges and wood chippings. The main properties of the compost are a slightly acidic pH (6.2), high amount of nutrients such as available P (P_{olsen}: 425 mg kg⁻¹), organic C (22.2 %) or total N (1.6 %), and high cation exchange capacity (CEC: 57.6 cmol_c kg⁻¹). The CEC was dominated by Ca (40.4 cmol_c kg⁻¹), and exchangeable K was 4.4 cmol_c kg⁻¹. After incorporation of amendments the soils were adjusted to 70% of their water holding capacity (which was measured according the method of Guitián and Carballas (1976)) and left for 2 weeks to attain equilibrium, after which time aliquots were taken of each treatment for soil analyses.

Seeds of *Alyssum serpyllifolium* subsp. *lusitanicum* Dudley and P. Silva (hereafter referred to as *A. pintodasilvae*) from Barazón (NW Spain) and Samil (Trás-Os-Montes, NE Portugal), *A. serpyllifolium* subsp. *malacitanum* Rivas Goday (hereafter referred to as *A. malacitanum*) from Sierra Bermeja and Sierra de Aguas (S Spain), *Alyssum bertolonii* subsp. *bertolonii* Desv from Imprunetta (Italy), and *Noccaea goesingense* Hálácsy from Redlschlag (E Austria) were collected and germinated on a 2:1 perlite:quartz mixture (2:1 v/v) in a growth chamber under controlled conditions (temperature 22-25 °C, PPFD (Photosynthetic photon flux density) of 190 μmol m⁻² s⁻¹, under a 16/8 h light/dark cycle). Seeds were watered daily with deionised water until

germination and thereafter with a Ni-rich serpentine-like macro-nutrient solution which consisted of 2 mM MgSO_4 , 0.8 mM $\text{Ca}(\text{NO}_3)_2$, 2.5 mM KNO_3 , 0.1 mM K_2HPO_4 , 20 μM FeEDDHA, 10 μM H_3BO_3 , 2 μM MnCl_2 , 1 μM ZnSO_4 , 0.5 μM CuSO_4 , 0.2 μM Na_2MoO_4 and 300 μM NiSO_4 (based on Chaney et al. (2008)). Plastic pots were filled with 1 kg of soil and one four-week-old seedling of each of the six tested taxa was transplanted to each pot. Five replicate pots were established for each soil treatment (untreated, NPK, 2.5%, 5% and 10%) and plants were grown in an environmentally controlled growth chamber for five months (temperature 22-25 °C, PPFD of 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$, under a 16/8 h light/dark cycle).

2.2 Plant and soil analysis

General physicochemical properties and the total and available metal concentrations of all soils were determined before planting. Soil pH was measured in H_2O using a 1:2.5 soil:solution ratio. Total C and N were analysed by combustion with a CHN analyser (Model CHN-1000, LECO Corp., St Joseph, MI). Available P was determined by Olsen's NaHCO_3 method (Olsen et al., 1954). Exchangeable cations were extracted with 1M NH_4Cl (1:20 soil:solution ratio) after 16 hours of equilibration, and Co and Ni availability was evaluated by extraction with 10 mM $\text{Sr}(\text{NO}_3)_2$ in a 1:4 soil:solution ratio after 2h shaking (Everhart et al., 2006). Soils (0.25 g) were digested in a 2:1 mixture of concentrated HNO_3 : HCl (65% and 37% respectively and trace analysis grade). Blanks and soil reference materials (ultramafic rock SO-3, Canadian Certified Reference Materials Project) were also included in each digestion batch to ensure quality control. After plant growth, soil Ni availability was evaluated by extraction with $\text{Sr}(\text{NO}_3)_2$. All these soil extracts and the corresponding blanks were then filtered through cellulose paper (Filter-Laboratory 1242, 90-mm diameter, 7-9 μm pore) and

analysed by simultaneous inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista Pro, Australia).

At harvest, plants were thoroughly washed with pressurised tap water to remove any adhering soil particles, and rinsed in deionised water. Shoots and roots were separated, dried for 48 h at 40 °C and weighed to determine dry weight (DW) biomass. Plant tissues (0.5 g) were digested in a 2:1 HNO₃:HCl mixture and Ca, Fe, K, Mg, Ni, and P were measured also by ICP-OES. Data were expressed in mg kg⁻¹ DW plant material. Blanks and reference materials for vegetation (hay powder No. 129, Community Bureau of References, EU) were also included in each digestion batch.

The ability of the plants to bioconcentrate Ni in their aboveground biomass (Bioconcentration Factor, BCF) was calculated as the ratio of the shoot Ni concentration and the Sr(NO₃)₂-extractable concentration in the soil. Total Ni phytoextracted (Ni yield) was calculated as the product of the shoot DW yield and the shoot Ni concentration.

2.3 Statistical analyses

Significant effects of soil treatments on soil properties, plant biomass production, ionome, metal accumulation, and metal removal were determined by one-way ANOVA followed by the “post-hoc” Tukey test. All statistical analyses were performed with *SPSS 21.0* software (SPSS Inc., Chicago, IL).

3. Results

3.1 Effect of fertilisation and compost amendments on soil physicochemical properties

Table 1 shows the physicochemical properties of all the soil treatments before planting. Untreated soils presented a slightly acidic pH (5.8), low amount of available P (7.3 mg kg⁻¹) and low total C and N content (3.03% and 0.16% respectively). The cation exchange capacity was low (6.6 cmol_c kg⁻¹) and the Ca/Mg ratio lower than 1. Total metal content was high for Co (73 mg kg⁻¹), Ni (927 mg kg⁻¹) and Cr (1297 mg kg⁻¹) and metal availability. Exchangeable Ni and Co represented approximately 1-2% of the total concentration and Sr(NO₃)₂-extractable Ni and Co <1% (Table 1).

The main change induced by the addition of inorganic fertilisers was a significant increase in available P (from 7.3 mg kg⁻¹ to 22.1 mg kg⁻¹; $p < 0.05$). In addition, a decrease in the total Ni concentration was observed (from 927 mg kg⁻¹ to 819 mg kg⁻¹; $p < 0.05$). Compost addition (at all three rates) significantly increased total C and N content, available P, cation exchange capacity (CEC) only significant for 10% ($p < 0.05$), and also improved the Ca/Mg ratio (reaching close to 1 in the 10% compost treatment) ($p < 0.05$). These effects of compost on soil properties were generally more pronounced with an increase in the rate of addition; for example, available P and CEC increased from 18.1 mg kg⁻¹ and 2.7 cmol_c kg⁻¹ in 2.5% treatment to 97.5 mg kg⁻¹ and 11.9 cmol_c kg⁻¹ in the 10% treatment, respectively. Exchangeable K (cmol_c kg⁻¹) also increased from 0.13 in the untreated soil to 0.47 after inorganic fertilisation and up to 1.54 after 10% of compost addition. In contrast, amending with compost tended to decrease the total soil metal concentrations, and this decrease was significant in the 2.5% and 10% compost treatments (Table 1). In the case of Ni, the total concentration was reduced from 937 mg kg⁻¹ in untreated soil to 707.3 mg kg⁻¹ in the 10% compost

treatment. There was also a strong reduction in Ni availability: NH_4Cl -extractable Ni concentrations decreased by 3-, 3- and 11-fold after addition of 2.5%, 5% and 10% compost, respectively, while in the same treatments $\text{Sr}(\text{NO}_3)_2$ -extractable Ni concentrations decreased by 4-, 3- and 6-fold. A similar decrease was observed in Co (1.2 to 2.6-fold for NH_4Cl -extractable concentrations and 2 to 7-fold for $\text{Sr}(\text{NO}_3)_2$ -extractable concentrations).

3.2 Plant biomass production, shoot Ni concentration, Ni yield and bioconcentration factor (BCF) in untreated soils

Table 2 shows the shoot DW, Ni concentrations, Ni yield and Ni BCF of the six hyperaccumulator plants growing in the untreated soil. No significant differences in biomass production were found between species. Plant growth was poor and shoot DW ranged from $11.6 \text{ mg plant}^{-1}$ in *A. pintodasilvae* (Samil population) to $33.8 \text{ mg plant}^{-1}$ in *N. goesingense*, following the decreasing order: *N. goesingense* > *A. bertolonii* > *A. malacitanum* (S. Bermeja) = *A. pintodasilvae* (Barazón) > *A. malacitanum* (S. Aguas) > *A. pintodasilvae* (Samil). Regarding shoot Ni concentrations, the S. Bermeja population of *A. malacitanum* presented the highest concentrations (with a mean concentration of $9523 \text{ mg Ni kg}^{-1}$) and the Barazón population of *A. pintodasilvae* the lowest ($5714 \text{ mg Ni kg}^{-1}$). Some differences were observed (although not always significant) in Ni removal (Ni yield) between populations and/or species: *A. bertolonii* and *A. malacitanum* (S. Bermeja) showed the highest Ni yields (mean values of 299 and $279 \text{ mg plant}^{-1}$, respectively), while the other plants extracted between $117 \text{ mg plant}^{-1}$ (*A. pintodasilvae* from Samil) to $152 \text{ mg plant}^{-1}$ (*A. malacitanum* from S. Aguas).

All the plant species presented extremely high BCF (based on the $\text{Sr}(\text{NO}_3)_2$ -extractable Ni fraction) and values ranged from 15.3×10^2 in *A. malacitanum* (S. Aguas) to up to 27.5×10^2 in *A. malacitanum* (S. Bermeja).

3.3 Effect of fertilisation and compost amendments on plant biomass production

All the soil treatments increased (although not always significantly) plant growth and biomass production, and this effect was most extreme in *N. goesingense* where both inorganic fertilisation and compost addition had pronounced beneficial effects (Figure 1). In general, and for all three *Alyssum* species, the lowest improvement in shoot biomass production was observed after NPK fertilisation. In this case, the most pronounced effect was observed in the S. Bermeja population of *A. malacitanum* (DW yield increased by 9-fold; from $0.03 \text{ g plant}^{-1}$ to $0.28 \text{ g plant}^{-1}$; $p > 0.05$) and in both populations of *A. pintodasilvae* (DW yield increased from $0.01 \text{ g plant}^{-1}$ to $0.24 \text{ g plant}^{-1}$ (24-fold) and from $0.03 \text{ g plant}^{-1}$ to $0.30 \text{ g plant}^{-1}$ (10-fold) in Samil and Barazón respectively; $p > 0.05$). In contrast, in the case of *N. goesingense* the NPK treatment increased shoot DW from $0.03 \text{ g plant}^{-1}$ to $2.86 \text{ g plant}^{-1}$ (representing an increase of up to 95-fold; $p < 0.05$).

After compost addition, *N. goesingense* presented again the highest response for all rates of addition, and DW yield was increased by up to 162-fold compared to yields obtained in untreated soil after addition of 5% compost ($p < 0.05$). In general, all rates of addition had a beneficial effect on biomass production, but the most pronounced increase in DW yields was obtained with 2.5% or 5% compost. The mean biomass of all plants grown in untreated soil was $0.03 \text{ g plant}^{-1}$, and this increased in the 5% compost treatment to up to $0.68 \text{ g plant}^{-1}$ and $0.73 \text{ g plant}^{-1}$ in *A. pintodasilvae* (Barazón and

Samil populations, respectively; $p < 0.05$), 1.56 g plant⁻¹ and 1.49 g plant⁻¹ in *A. malacitanum* (S. Bermeja and S. Aguas populations, respectively; $p < 0.05$), 1.87 g plant⁻¹ in *A. bertolonii* ($p < 0.05$), and finally, 4.86 g plant⁻¹ in *N. goesingense* ($p < 0.05$). Increasing the compost rate from 2.5% to 5% tended to increase plant biomass production (although not significantly); but an increase in the compost dose from 5% to 10% did not lead to any further improvements in shoot DW. In fact, in the case of *A. pintodasilvae* (Barazón population) and *A. malacitanum* (both populations) there was a substantial decrease in shoot DW compared to the lower doses (Figure 1).

3.4 Effect of fertilisation and compost amendments on plant nutritive status

Table 3 shows the shoot macro- and micro-nutrient concentrations for each of the six hyperaccumulators. The shoot ionome was dependent on the plant (sub)species and the soil treatment. All the soil treatments increased shoot P concentrations, and this effect was generally statistically significant ($p < 0.05$). Compost addition led to a more marked increase in P concentrations than NPK fertilisation, and the highest shoot P concentrations were found in either the 5% or 10% compost rates (of depending on the plant (sub)species and/or population). Shoot P concentration in plants growing in untreated soils ranged from 0.36 g kg⁻¹ in *N. goesingense* to 0.99 g kg⁻¹ in *A. pintodasilvae* (Samil) and increased up to 7.74 g kg⁻¹ in *A. malacitanum* (S. Bermeja) in 5% compost (which corresponds with a 10-fold increase; $p < 0.05$). On the other hand, after inorganic fertilisation the maximum shoot P concentration reached was 2.89 g kg⁻¹ in *A. pintodasilvae* (Barazón), representing an increase of up to 3-fold ($p < 0.05$). Shoot Ca concentrations varied markedly according to the treatment: NPK fertilisation decreased the shoot Ca concentrations of all six hyperaccumulators; while compost amendments increased Ca accumulation. This effect was stronger in those plants grown

in the two higher addition rates (5% and 10%). Shoot Ca concentrations of untreated plants ranged from 10.9 g kg⁻¹ in *N. goesingense* to 37.4 g kg⁻¹ in *A. pintodasilvae* (Samil), while in the NPK fertilised soil they ranged from 2.8 g kg⁻¹ in *N. goesingense* to 12.4 g kg⁻¹ in *A. pintodasilvae* (Samil) (representing a 3- to 4-fold decrease). On the other hand, the highest shoot Ca concentration were found in *A. pintodasilvae* (Samil) grown in the compost-amended soils: 49.4 g Ca kg⁻¹ in the 10% compost and 45.6 g Ca kg⁻¹ in the 5% compost treatment, respectively. Shoot K concentration in untreated plants ranged from 11.7 g kg⁻¹ in *A. pintodasilvae* (Barazón) to 22.0 g kg⁻¹ in *A. malacitanum* (S. Aguas). All soil treatments; both NPK fertilisation and compost amendment, increased shoot K concentrations. In general, the highest improvements were observed after NPK fertilisation (up to 37.4 g kg⁻¹ in *A. pintodasilvae* (Samil)) and the 2.5% compost amendment rate (up to 35.3 g kg⁻¹ in *A. pintodasilvae* (Samil)); while the highest compost rate had the least effect on shoot K accumulation. In contrast, all soil treatments led to a significant decrease in shoot Fe concentration and no differences were observed amongst treatments. Shoot Fe concentrations were considerably higher in *N. goesingense* growing in untreated soils (2.25 g kg⁻¹) compared to the *Alyssum* hyperaccumulators (ranging from 0.37 g kg⁻¹ to 0.86 g kg⁻¹). Variable effects on shoot Mg concentrations were observed, although some tendencies could be seen. The NPK treatment tended to increase Mg concentrations in plants (except *N. goesingense*), although this was only significant for *A. bertolonii* ($p < 0.05$). On the contrary, compost treatments reduced Mg concentrations, and this effect was less pronounced in plants grown at the highest compost rate of 10% (except in *A. bertolonii*).

3.5 Effect of fertilisation and compost amendments on soil Ni availability, shoot Ni concentration, Ni yield and bioconcentration factor (BCF)

Soil Ni availability was assessed at the end of the experiment through $\text{Sr}(\text{NO}_3)_2$ extraction (Figure 2). After plant growth, in untreated soils available Ni ranged from 4.23 mg kg⁻¹ in soils planted with *A. pintodasilvae* (Barazón) to 3.25 mg kg⁻¹ in soils planted with *A. bertolonii*. Compared to untreated soil, a reduction in soil Ni availability was detected after plant growth in all the soil treatments, except in two cases with NPK fertilisation (Figure 2). Soil Ni availability was lowest in the 10% compost treatment, and especially after growth of *A. pintodasilvae* (Barazón); in this treatment $\text{Sr}(\text{NO}_3)_2$ -extractable Ni concentrations were 0.08 mg kg⁻¹ (53-fold lower than in untreated soils and 15-fold lower than in the 2.5% compost treatment; $p < 0.05$). In the NPK treatment, $\text{Sr}(\text{NO}_3)_2$ -extractable Ni concentrations in pots planted with *A. pintodasilvae* (Samil) increased from 3.44 mg kg⁻¹ (untreated soil) to 3.62 mg kg⁻¹ ($p > 0.05$) and in *A. bertolonii* available Ni was significantly increased (from 3.45 mg kg⁻¹ to 3.93 mg kg⁻¹; $p < 0.05$).

In contrast to what was observed for plant biomass production, but in agreement with the soil Ni availability, compost treatments significantly reduced shoot Ni concentrations compared to plants growing in untreated soils (Figure 3a). On the other hand, the NPK treatment had no pronounced effect on shoot Ni accumulation although led to a significant reduction in the shoot Ni concentration in *A. malacitanum* (S. Bermeja) and *N. goesingense* ($p < 0.05$), while a slight increase (but not significant) was observed in *A. pintodasilvae* (Samil). In contrast, the reduction in shoot Ni concentrations of plants growing in compost-amended soils was consistently observed in all six hyperaccumulators and at all three rates of addition ($p < 0.05$). Moreover, an increase in the compost rate (from 5% to 10%) was accompanied by further declines in shoot Ni concentration (Figure 2). The maximum decrease in shoot Ni concentration

after addition of 2.5% compost was 4-fold (this was recorded for the S. Bermeja population of *A. malacitanum*, decreasing from 9528 mg Ni kg⁻¹ in untreated soil to 2584 mg Ni kg⁻¹ in 2.5% compost). While a 29-fold decrease in shoot Ni concentration was observed in *A. pintodasilvae* (Barazón) after 10% compost addition (decreasing from 5714 mg Ni kg⁻¹ in untreated soil to 197 mg Ni kg⁻¹ in 10% compost). Contrary to what was observed with Ni availability, compost treatment did not generally lead to a decrease in the Ni BCF and, in some cases, even increased it (for example in the 2.5% compost treatment; Figure 3b). This increase in the Ni BCF in the low compost treatment was significant for *A. pintodasilvae* (Samil) (36.7×10^2 ; $p < 0.05$). On the other hand, a significant reduction in Ni BCF was generally only observed at the highest compost treatment (10%): the lowest BCF values were found in *A. pintodasilvae* (Barazón) (3.2×10^2) and in *N. goesingense* (5.9×10^2). The 5% compost treatment and NPK fertilisations significantly decreased Ni BCF in *A. malacitanum* (S. Bermeja) and *N. goesingense* (only for NPK) ($p < 0.05$).

Compared to untreated soils, all the soil treatments (with the exception of 10% compost in *A. pintodasilvae* (Barazón)) increased the amount of phytoextracted Ni (Ni yield) (Figure 3c). The highest beneficial effects of soil treatments on plant Ni yield were generally obtained in the 2.5% and 5% compost treatments. Moreover, these positive effects were more marked in *N. goesingense* (in this plant species both the NPK treatment and compost additions caused a strong increase in Ni yield). Ni removal by *N. goesingense* was 8.8 mg Ni plant⁻¹ in NPK fertilised soil, 7.9 mg plant⁻¹ in 2.5% compost, and 8.8 mg plant⁻¹ in 5% compost. However, its efficiency of Ni phytoextraction was significantly reduced at the higher compost (10%) addition (2.4 mg Ni plant⁻¹; $p < 0.05$). For *Alyssum*, plant Ni yields in compost-amended soils were

similar amongst the two *A. serpyllifolium* subspecies and their populations (ranging from 1.6 to 2.8 mg Ni plant⁻¹ in 2.5%, from 1.3 to 2.9 mg Ni plant⁻¹ in 5% and from 0.05 to 0.8 mg Ni plant⁻¹ in 10%), while *A. bertolonii* achieved a higher Ni yield in the compost-amended soils (3.1 mg Ni plant⁻¹, 4.4 mg Ni plant⁻¹ and 1.5 mg Ni plant⁻¹ in 2.5%, 5% and 10% compost respectively). For *Alyssum*, the highest benefits of the NPK treatment on Ni yield were observed in both populations of *A. pintodasilvae* (1.8 mg Ni plant⁻¹ for the Barazón population and 1.9 mg Ni plant⁻¹ for Samil) and the S. Bermeja population of *A. malacitanum* (1.9 mg Ni plant⁻¹).

4. DISCUSSION

The six hyperaccumulator plants showed a similar growth in the untreated soil. The Barazón population of *A. pintodasilvae* did not show better growth despite the fact that this subspecies is native to the site where the soil was collected. Plant growth was extremely poor in the untreated soil, and much lower (approximately 10-fold) than what was expected in comparison to previous studies where *A. pintodasilvae* was grown in the same soil (Cabello-Conejo et al., 2014a). Nonetheless, all six hyperaccumulators presented shoot Ni concentrations which were well above the threshold value defined for Ni hyperaccumulation (1000 mg kg⁻¹). In the case of the S. Bermeja population of *A. malacitanum* plants obtained close to 1% DW of Ni in their aboveground tissues. However, limited growth reduced the corresponding amount of phytoextracted Ni (in untreated soils less than 0.1% of the total Ni in soil was removed by plants (data not shown)). In the case of *Alyssum*, differences were observed in shoot Ni accumulation between species, subspecies and populations of subspecies. *Alyssum malacitanum* (only the S. Bermeja population) and *A. bertolonii* were able to obtain higher shoot concentrations of Ni compared to *A. pintodasilvae*.

Serpentine soils typically present unfavourable conditions for plant growth, such as a deficiency in essential nutrients (such as N, P and K) and organic matter. Maximising the performance and yield of hyperaccumulator crops is therefore essential in the development of phytomining systems. This study showed that inorganic fertilisation could improve soil fertility and hence plant growth, and confirmed the existence of nutritional deficiency in serpentine soils. A significant increase in plant DW yield, and shoot K and P concentrations, was observed in all six hyperaccumulators in NPK-treated soils. These essential nutrients are known to be low in ultramafic soils and yet fairly accumulated by most hyperaccumulator plants (Zhang et al., 2014). These results support previous studies which have also shown stimulation in the biomass production of *Alyssum* species after the addition of inorganic fertilisers (Cabello-Conejo et al., 2014b; Chaney et al., 2008; Robinson et al., 1997). Cabello-Conejo et al. (2014b) obtained a similar DW yield in the SB population of *A. malacitanum* as that obtained in this study: this subspecies was grown in the same serpentine soil and with the same NPK fertilisation as used here (growth time was 4 months compared to 5 months here). Inorganic fertilisation consisting in 100:150:200 kg ha⁻¹ NPK was also shown to effectively increase DW yields (10-fold) of *A. pintodasilvae* in contaminated mine-soils (Kidd and Monterroso, 2005). Moreover, mineral fertilisation (120 kg NPK ha⁻¹ and 77 kg Ca ha⁻¹) has been successfully used on a field basis to improve the biomass production of *Alyssum murale*, obtaining yields of up to 9.0 t ha⁻¹ (Bani et al., 2015).

In general, compost amendment had an even higher growth-promoting effect than inorganic fertilisation. Composts can not only provide a source of essential nutrients (which are limiting in serpentine soils) but also improve soil structure and porosity, and

water holding capacity, which could explain the more pronounced effects of compost additions that were observed on plant yields. The addition of composted MSW improved soil physicochemical properties, such as nutrient availability, CEC, or organic matter content. The compost used in this study also significantly improved the soil Ca/Mg ratio. Shoot Ca concentrations were often reduced in plants grown in NPK-treated compared to untreated soils, whereas compost additions led to a significant improvement in the shoot Ca concentration. This was particularly the case at the 5 and 10% addition rates. Serpentine soils characteristically present a low Ca concentration (and unfavourable Ca/Mg ratio for plant growth) and Ni hyperaccumulators take up large amounts of Ca from them, hence the repeated harvest and removal of biomass during phytomining processes requires the inclusion of Ca fertilisation to maintain fertility at appropriate levels (Chaney et al., 2008; Chaney et al., 2007; Kukier et al., 2004). Other authors have added gypsum to serpentine soils to overcome this limitation (Cabello-Conejo et al. 2013), but it seems that organic amendments (such as the composts used here) could also fulfil this plant requirement. Plants grown in compost-amended soils also presented a higher shoot P and K concentration than plants grown in untreated soil. On the other hand, shoot Fe and Mg concentrations were reduced (in both compost- and NPK-treated soils) but neither element reached deficiency levels (50-150 mg kg⁻¹ for Fe, 1.5-3.5 g kg⁻¹ for Mg, according to Marschner, 1995).

The Ca/Mg ratio has been shown to be a relatively important factor for growth and Ni uptake of hyperaccumulators. Ghasemi and Ghaderian (2009) studied the effect of the Ca/Mg quotient in two populations (serpentine and non-serpentine) of the hyperaccumulator *A. inflatum* and found that the serpentine population showed a higher tolerance to a low Ca/Mg ratio. The same authors also observed an increase in plant Ni

accumulation with increasing Ca/Mg ratio in hydroponic solutions. However, in the present study, a decrease in shoot Ni concentration was found alongside the increase in the soil Ca/Mg ratio produced in compost-amended soils. Similarly, Brooks et al. (1981) showed that *A. serpyllifolium* was able to tolerate higher Ni concentrations in the presence of high concentrations of Ca in the soil by lowering its Ni uptake. Thus when using compost amendments it will be important to manipulate the soil Ca/Mg ratio in order to obtain an optimum balance between plant growth and Ni accumulation in phytomining systems.

The use of organic amendments based on waste products has rarely been considered for stimulating the growth of hyperaccumulating plants in serpentine soils. Here, the stimulation in plant growth was observed in hyperaccumulating plants belonging to two distinct genera (*Alyssum* and *Noccaea*). In fact, *N. goesingense* responded most to the different soil treatments in terms of biomass production (and consequently Ni removal). The serpentine site of Redlschlag where *N. goesingense* is endemic presents soil physicochemical characteristics which are likely to be more favourable for plant growth than the serpentine site of Barazón where the soil in this study was collected (unpublished data). The adaptation of this species to the soil conditions in its natural habitat could explain the stronger positive response observed in its growth after improvements in soil fertility. Ni-hyperaccumulating subspecies of *Alyssum serpyllifolium* have often been suggested to present a limited application potential in phytomining systems compared to other *Alyssum* sp., such as *A. murale* or *A. corsicum*, due to their low productivity (Cabello-Conejo et al., 2013). However, the shoot biomass of *A. malacitanum* (both populations) obtained in the soils with 5% w/w compost was close to that of *A. bertolonii*. Moreover, the stimulation in biomass produced through

compost additions was 4-fold higher than that obtained using phytohormone applications by Cabello-Conejo et al. (2014b). These authors obtained a mean shoot DW yield in *A. murale* (grown in the same serpentine soil as used here) which was of a similar magnitude to that obtained in *A. malacitanum* with 5% w/w compost addition.

The beneficial effects of compost addition were no longer observed at the highest rate of addition (10% w/w). Other authors have found a similar decrease in plant yields of agricultural crops with an increase in the rate of addition of compost to metal-contaminated soils. Alvarenga et al. (2009) found that increasing compost (based on municipal residues) addition from 50 Mg ha⁻¹ to 100 Mg ha⁻¹ to a mine-soil did not induce a higher biomass production of perennial ryegrass. These authors attributed this to an increase in soil salinity due to the addition of soluble salts with the compost. In this study, a possible explanation for the decrease in the yields of *Alyssum* could be the addition of potentially toxic metals to the soil (other than Co, Cr or Ni) together with the compost. The pseudo-total soil Cu concentration was 7-fold higher in the 10% compost-amended soil (77.8 mg kg⁻¹) compared to the untreated soil (11.0 mg kg⁻¹). Copper has previously been shown to be phytotoxic to *A. pintodasilvae* at high concentrations (Kidd and Monterroso, 2005), and minimal Cu tolerance was observed in a range of Ni-hyperaccumulators within the *Alyssum* genus (Ghasemi et al., 2015). Other studies suggest that the decrease in soil Ni availability could also negatively affect the growth of hyperaccumulators. For example, Sellami et al., (2012)) found the growth of *A. murale* was enhanced in soils amended with industrial sewage sludge with increasing Ni concentration compared to the non-amended control, which could indicate the high Ni requirement of this plant. In the present study, the reduced growth observed at 10% compost addition could reflect a similar Ni requirement (also reflected in the significant

decrease in Ni BCF at the highest dose of compost in four of the Ni hyperaccumulators). Our results therefore show that compost addition can increase soil organic matter content and fertility of serpentine soils, however, it will be important to optimise the rates of addition to achieve optimal plant growth and metal extraction.

Soil Ni availability differed between soil treatments, and was dependent on the type of treatment (inorganic vs. organic fertilisation) and also on the rate of compost addition (in the case of organic amendment). Before plant growth, Ni availability was not modified by NPK fertilisation compared to untreated soil. Similarly, Robinson et al., (1997) found no differences in soil Ni extractability after incorporating inorganic fertilisers (NH_4NO_3 , K_2SO_4 , $\text{Ca}(\text{H}_2\text{PO}_4)_2$) in a serpentine soil where *A. bertolonii* was grown. In contrast to NPK fertilisation, compost addition led to a strong reduction in soil Ni availability. This could partly, but not entirely, be explained by a soil dilution effect; after compost addition a decrease of up to 1.6-fold was recorded in the pseudo-total Ni concentration after 10% w/w compost addition. However, the corresponding decrease in the NH_4Cl -extractable Ni concentration (11-fold) indicates that processes other than simple soil dilution were influencing metal mobility. Organic soil amendments have frequently been shown to decrease soil metal bioavailability through the formation of organo-metallic insoluble complexes (Mench et al., 2000; Vangronsveld et al., 2009). The composting of organic residues also reduces the dissolved organic carbon thus stabilising the wastes and decreasing metal mobility (Kiikkilä et al., 2002). The effect of compost addition on soil Ni availability reinforces the need for optimising the rate of addition to reach maximal biomass productivity but minimise reductions in plant-available metal fractions and thus metal uptake and accumulation.

After plant growth, soil Ni availability was consistently lower than in untreated soil in all compost-amended treatments and for all plant species. In the 10% compost treatment, the $\text{Sr}(\text{NO}_3)_2$ -extractable concentration was reduced by up to 7-fold compared to before plant growth, which could again suggest Ni was limiting plant growth in this treatment. On the other hand, plant growth modified soil Ni availability in the NPK treatment, and this was dependent on the plant species. In general, a slight decrease in $\text{Sr}(\text{NO}_3)_2$ -extractable Ni concentrations compared to untreated soil was found in those pots cultivated with *A. pintodasilvae* and *A. malacitnanum*. In the case of *N. goesingense* a similar decrease was observed but much more pronounced (more than 2-fold), which could not be attributed to a more prolific plant growth and Ni uptake. Finally, after growth of *A. bertolonii* a significant increase in soil Ni availability was observed. In addition, to this plant species effect, the type and composition of inorganic fertilisers could be selected in order to enhance metal bioavailability and therefore the efficiency of phytoextraction (Kidd et al., 2015). For example, acidification induced by ammonium-fed plants can promote mobilisation of metals in soils (Kidd et al., 2015). However, Schwartz et al. (2003) showed that at higher N additions, NO_3^- significantly increased Cd uptake by *Noccaea caerulescens* compared to NH_4^+ . This effect would be consistent with other studies showing an increase in Ni uptake of hyperaccumulators with increasing soil pH, thus indicating that the hyperaccumulation process does not seem to be related to soil acidification (Li et al., 2003). It would be interesting to test different fertilisation regimes for achieving an optimal balance between plant biomass stimulation, enhanced soil metal availability and hence plant uptake and accumulation. Additionally, in long-term phytomining strategies if biomass production is to be

maintained over several years, it will be necessary to adjust the fertiliser application so as to replace exported nutrients with each harvest.

No positive effects on shoot Ni concentrations were found after either NPK or compost treatment. In fact, after NPK fertilisation shoot Ni concentrations were significantly reduced in *N. goesingense* (but not *Alyssum* (sub)species) compared to plants grown in untreated soil. In agreement, Bani et al. (2015) found that soil fertilisation did not influence Ni uptake by *A. murale*. Some authors have observed a decrease in shoot Ni concentrations alongside the stimulation in biomass production; for example, both Robinson et al. (1997) and Kidd and Monterroso (2005) observed a slight, but significant, decrease in Ni uptake of *A. bertolonii* and *A. pintodasilvae* after fertilisation. However, these authors observed that Ni yield was still increased due to the promotion in plant biomass production. The same was true in this study, where phytoextracted Ni was increased after NPK addition due to the fertiliser-induced promotion in growth. A significant and negative effect on shoot Ni concentrations was also observed after compost amendment, and this was found for all rates of addition (in line with the observed reductions in soil Ni availability). However, the pronounced positive effect on plant biomass production significantly increased phytoextracted Ni. Moreover, since compost had a more beneficial effect on plant yield than NPK fertilisation, the overall phytoextracted Ni was also higher in these treatments. Most literature related to the use of organic amendments in soil phytoremediation focuses on (aided) phytostabilisation, while few studies have evaluated their use in metal phytoextraction using metal-accumulating plant species, and rarely with hyperaccumulators. Fiorentino et al. (2013) improved the growth of *Arundo donax* growing in a Cd-contaminated soil after compost application at the same time than an increase in Cd, Cu and Cr uptake. Pachura et al.

(2015), observed an increase in Cd uptake after addition of compost based on sewage sludge and forest waste by *Virginsia fanpetals* growing in a Cd- and Zn- contaminated soil. Schwartz et al. (2003) reported increases in Cd uptake, biomass production, and Zn/Cd yields of *N. caerulea* after addition of non-contaminated sludge to a co-contaminated soil with inorganic and organic pollutants. To the best of our knowledge this is the first study to demonstrate the potential use of organic waste products as a means of improving Ni phytoextraction by Ni-hyperaccumulators. Field-based studies will be required to evaluate the long-term effects and potential benefits of compost amendments in phytomining systems. The need for additional compost dressings over time will also have to be considered.

CONCLUSIONS

In conclusion, both types of soil treatment (inorganic fertilisation and organic amendments) improved the total amount of Ni phytoextracted, and this was mainly through stimulation in biomass production and not through an improved Ni uptake by the hyperaccumulating plants. All the plant species accumulated Ni concentrations characteristic of hyperaccumulators, however the most efficient plants in terms of Ni removal were *N. goesingense*, *A. bertolonii* and the SB population of *A. malacitanum*. In these plants, the best soil treatments were 2.5 or 5.0% w/w compost addition (and also the NPK treatment in the case of *N. goesingense*). The strong reductions in soil Ni availability (and corresponding plant Ni accumulation) observed after compost addition highlight the need for further optimisation regarding the final rates of addition. Full deployment of phytomining systems at field scale will require agronomic practices which aim to maximise biomass production and Ni yield of hyperaccumulators.

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Table 1 Physicochemical properties of the untreated and treated serpentine soil before planting of *Alyssum* (sub)species and *Noccaea goesingense*. Different letters indicate significant differences ($p < 0.05$) between treatments.

	Untreated	Soil treatments			
		NPK	Compost		
			2.5%	5%	10%
pH _{H2O}	5.8 ± 0.01 b	5.7 ± 0.02 c	6.2 ± 0.03 a	5.5 ± 0.01 d	5.7 ± 0.01 c
pH _{KCl}	4.9 ± 0.04 bc	4.8 ± 0.01 c	5.1 ± 0.15 bc	5.2 ± 0.02 ab	5.5 ± 0.04 a
P _{olsen} (mg kg ⁻¹)	7.3 ± 0.86 d	22.1 ± 1.24 c	18.1 ± 0.75 c	28.8 ± 0.44 b	97.5 ± 2.39 a
CEC (cmol _c kg ⁻¹)	6.6 ± 1.95 b	8.1 ± 0.19 b	8.6 ± 0.65 b	10.9 ± 2.88 b	23.8 ± 1.94 a
Exchangeable K (cmol _c kg ⁻¹)	0.13 ± 0.03 c	0.47 ± 0.02 bc	0.54 ± 0.03 bc	0.61 ± 0.14 b	1.54 ± 0.15 a
Exchangeable Ca (cmol _c kg ⁻¹)	0.50 ± 0.12 c	0.54 ± 0.03 c	2.69 ± 0.17 bc	4.47 ± 1.24 b	11.90 ± 1.01 a
Ca / Mg	0.09 ± 0.01 b	0.08 ± 0.00 b	0.51 ± 0.02 a	0.78 ± 0.04 a	1.20 ± 0.04 a
% C	3.03 ± 0.01 d	2.74 ± 0.02 e	3.68 ± 0.02 c	4.11 ± 0.06 b	6.17 ± 0.05 a
% N	0.16 ± 0.01 c	0.14 ± 0.01 c	0.23 ± 0.00 b	0.24 ± 0.01 b	0.41 ± 0.01 a
<i>Total metal concentration (mg kg⁻¹)</i>					
Co	73.1 ± 0.5 a	69.6 ± 1.4 ab	62.2 ± 1.3 b	71.8 ± 1.4 a	62.0 ± 3.8 b
Ni	937.0 ± 7.3 a	818.8 ± 14.0 bc	753.5 ± 4.5 cd	878.6 ± 2.4 ab	707.3 ± 42.7 d
Cr	1297.6 ± 18.9 a	1200.9 ± 26.8 a	1203.1 ± 17.6 a	1218.7 ± 12.9 a	965.2 ± 43.7 b
<i>NH₄Cl-extractable metal concentration (mg kg⁻¹)</i>					
Co	0.75 ± 0.20 ab	0.91 ± 0.07 a	0.63 ± 0.04 ab	0.56 ± 0.07ab	0.29 ± 0.03 b
Ni	20.8 ± 4.2 a	21.9 ± 0.5 a	6.7 ± 0.6 b	6.8 ± 0.8 b	1.9 ± 0.1 b
<i>Sr(NO₃)₂-extractable metal concentration (mg kg⁻¹)</i>					

Co	0.22 ± 0.01 a	0.25 ± 0.01 a	0.09 ± 0.01 b	0.14 ± 0.01 b	0.03 ± 0.01 c
Ni	3.43 ± 0.04 a	3.23 ± 0.04 b	0.95 ± 0.01 d	1.22 ± 0.04 c	0.57 ± 0.02 e

Table 2 Mean shoot biomass, shoot Ni concentration, Ni BCF and Ni yield of the different Ni-hyperaccumulating plants in the untreated soil. Different letters indicate significant differences ($p < 0.05$; $n = 5$) between plant (sub)species.

		Shoot DW (mg plant ⁻¹)	Ni (mg kg ⁻¹)	Ni yield (mg plant ⁻¹)	BCF x 10 ²
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
<i>A. pintodasilvae</i>	Barazón	29.2 ± 11.2 a	5713 ± 647 b	148 ± 39 a	16.4 ± 1.6 c
	Samil	11.6 ± 3.1 a	6665 ± 1304 ab	117 ± 48 a	19.2 ± 3.6 bc
<i>A. malacitanum</i>	S. Bermeja	29.6 ± 3.5 a	9527 ± 815 a	279 ± 33 a	27.6 ± 1.7 a
	S. Aguas	27.8 ± 3.5 a	5178 ± 682 b	152 ± 34 a	15.3 ± 13.2 c
<i>A. bertolonii</i>		33.3 ± 5.2 a	8727 ± 688 ab	299 ± 61 a	25.6 ± 1.5 ab
<i>Noccaea goesingense</i>		33.8 ± 4.0 a	6241 ± 759 ab	211 ± 36 a	18.9 ± 1.2 bc

Table 3 Shoot macro- and micro-nutrients concentrations in the six hyperaccumulators grown in untreated, NPK fertilised and compost-amended soils (mean concentration $\pm$ SE). Different letters indicate significant differences between treatments for each plant ($p < 0.05$; $n = 5$).

		P (g kg ⁻¹)	K (g kg ⁻¹)	Ca (g kg ⁻¹)	Mg (g kg ⁻¹)	Fe (g kg ⁻¹)	
<i>A. pintodasilvae</i>	Barazón	Untreated	0.94 ± 0.08 c	11.7 ± 2.3 c	24.1 ± 2.0 b	19.1 ± 1.6 a	0.60 ± 0.07 a
		NPK	2.89 ± 0.15 b	37.4 ± 1.0 a	12.1 ± 0.3 c	21.3 ± 1.1 a	0.18 ± 0.02 b
		2.50%	4.83 ± 0.52 a	33.3 ± 3.8 ab	31.3 ± 2.3 b	12.4 ± 1.1 b	0.21 ± 0.02 b
		5%	6.40 ± 0.65 a	26.9 ± 1.5 b	43.9 ± 2.6 a	9.1 ± 0.8 b	0.24 ± 0.05 b
		10%	2.90 ± 0.39 b	13.6 ± 1.3 c	29.6 ± 3.7 b	22.9 ± 2.4 a	0.27 ± 0.06 b
	Samil	Untreated	0.99 ± 0.03 c	17.1 ± 3.9 c	37.4 ± 6.9 bc	18.9 ± 1.9 a	0.86 ± 0.20 a
		NPK	2.35 ± 0.10 b	33.2 ± 1.1 a	12.4 ± 1.3 d	20.5 ± 0.4 a	0.36 ± 0.08 b
		2.50%	3.22 ± 0.11 b	35.3 ± 4.0 a	34.4 ± 0.9 c	12.4 ± 1.2 b	0.16 ± 0.02 b
		5%	4.31 ± 0.11 a	28.8 ± 1.9 bc	45.6 ± 1.2 ab	8.6 ± 0.6 b	0.27 ± 0.02 b
		10%	4.80 ± 0.33 a	27.2 ± 2.1 bc	49.4 ± 2.2 a	8.9 ± 0.7 b	0.25 ± 0.01 b
<i>A. malacitanum</i>	S. Bermeja	Untreated	0.74 ± 0.09 c	19.7 ± 1.4 a	27.0 ± 2.6 b	16.7 ± 0.8 ab	0.46 ± 0.07 a
		NPK	1.80 ± 0.29 c	27.8 ± 3.9 a	11.3 ± 0.9 c	20.9 ± 2.3 a	0.24 ± 0.05 b
		2.50%	3.62 ± 0.99 bc	27.7 ± 6.4 a	26.2 ± 6.0 b	8.7 ± 2.0 c	0.10 ± 0.02 b
		5%	7.74 ± 0.79 a	25.6 ± 1.8 a	40.6 ± 1.1 a	6.9 ± 0.4 c	0.11 ± 0.01 b
		10%	6.74 ± 1.34 ab	21.1 ± 2.2 a	43.9 ± 2.3 a	11.1 ± 2.0 bc	0.18 ± 0.01 b
	S. Aguas	Untreated	0.52 ± 0.10 d	22.0 ± 2.6 ab	16.6 ± 1.4 c	15.2 ± 0.6 ab	0.37 ± 0.05 a
		NPK	1.93 ± 0.09 cd	31.1 ± 4.0 a	9.3 ± 1.1 c	18.4 ± 0.8 a	0.29 ± 0.04 a
		2.50%	3.41 ± 0.13 bc	32.3 ± 2.1 a	28.2 ± 1.4 b	10.9 ± 0.6 bc	0.10 ± 0.01 b
		5%	5.07 ± 0.63 ab	28.1 ± 1.9 ab	43.0 ± 2.5 a	7.8 ± 0.7 c	0.10 ± 0.01 b
		10%	6.63 ± 1.22 a	19.0 ± 2.2 b	45.2 ± 3.8 a	13.3 ± 3.4 abc	0.11 ± 0.01 b
<i>A. bertolonii</i>	Untreated	0.67 ± 0.11 c	17.0 ± 1.7 a	14.3 ± 1.4 c	10.6 ± 1.5 b	0.68 ± 0.12 a	
	NPK	2.44 ± 0.23 bc	22.3 ± 1.4 a	6.1 ± 0.2 c	16.1 ± 1.0 a	0.21 ± 0.02 b	
	2.50%	4.42 ± 0.97 ab	26.5 ± 4.1 a	26.0 ± 3.9 b	10.7 ± 0.8 b	0.12 ± 0.01 b	
	5%	5.40 ± 0.44 a	26.0 ± 2.1 a	33.6 ± 2.0 a	6.4 ± 0.5 c	0.12 ± 0.01 b	
	10%	4.13 ± 0.19 ab	22.2 ± 1.3 a	37.0 ± 1.8 a	5.1 ± 0.3 c	0.12 ± 0.02 b	
<i>Noccaea goesingense</i>	Untreated	0.36 ± 0.02 c	10.2 ± 2.8 b	10.9 ± 1.8 c	13.0 ± 1.3 a	2.25 ± 0.58 a	
	NPK	1.30 ± 0.13 c	17.5 ± 2.5 ab	2.8 ± 0.3 c	5.9 ± 0.7 b	0.22 ± 0.05 b	
	2.50%	2.50 ± 0.29 b	17.3 ± 2.2 ab	12.8 ± 1.5 b	7.8 ± 0.9 b	0.17 ± 0.03 b	
	5%	3.70 ± 0.29 a	26.1 ± 0.4 a	22.3 ± 2.2 ab	7.3 ± 0.9 ab	0.27 ± 0.03 b	
	10%	3.06 ± 0.42 ab	23.7 ± 6.7 ab	22.5 ± 2.9 a	10.8 ± 2.4 ab	0.36 ± 0.08 b	

Figure 1 Effects of soil treatments (inorganic fertilisation (NPK) and organic amendment (addition rates of 2.5, 5 and 10%) on biomass production of the different Ni-hyperaccumulators. Different letters above bars indicate significant differences between treatments for each plant ($p < 0.05$; $n = 5$). Note that graphs use a different y-axis scale.

Figure 2 Effects of soil treatments (inorganic fertilisation (NPK) and organic amendment (addition rates of 2.5, 5 and 10%) on soil Ni availability assessed through $\text{Sr}(\text{NO})_3$ extraction. Different letters above bars indicate significant differences between treatments ($p < 0.05$; $n = 5$).

Figure 3 Effects of soil treatments (inorganic fertilisation (NPK) and organic amendment (addition rates of 2.5, 5 and 10%) on (a) shoot Ni concentration, (b) Ni BCF and (c) Ni yield. Different letters above bars indicate significant differences between treatments ($p < 0.05$; $n = 5$).

Figure1

Figure2

Figure3

Ni-hyperaccumulators

Alyssum pintodasilvae

Barazón (NW Spain)

Samil (Portugal)

Alyssum malacitanum

S. Bermeja
(S Spain)

S. Aguas
(S Spain)

Alyssum bertolonii

Imprunetta (Italy)

Noccaea goesingense

Redlschlag (Austria)

NPK fertilisation and compost amendment improve Ni phytoextraction in Ni-rich serpentine soils