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The potential of *Blepharidium guatemalense* for nickel agromining in Mexico and Central America

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Novelty Statement

Our research team is a pioneer in the discovery of metal hyperaccumulator plants in Mesoamerica with at least 13 species discovered in the last 2 years. This study is the first to assess the potential of nickel agromining (phytomining) in Mexico (and in all the American continent), using one of the strongest nickel hyperaccumulators reported so far. The promising results of this study are the basis for optimal agricultural management of *Blepharidium guatemalense*.

The potential of *Blepharidium guatemalense* for nickel agromining in Mexico

Abstract

The aim of this study was to assess the potential of the woody nickel hyperaccumulator species *Blepharidium guatemalense* (Standl.) Standl. for agromining in southeastern Mexico. Pot trials consisting of nickel dosing (0, 20, 50, 100 and 250 mg Ni kg^{-1}), and synthetic and organic fertilization were conducted. Field trials were also undertaken with different harvesting regimes of *B. guatemalense*. Foliar nickel concentrations increased significantly with rising nickel additions, with a 300-fold increase at 250 mg Ni kg^{-1} treatment relative to the control. Synthetic fertilization strongly increased nickel uptake without any change in plant growth or biomass, whereas organic fertilization enhanced plant shoot biomass with a negligible effect on foliar nickel concentrations. A 5-year-old stand which was subsequently harvested twice per year produced the maximum nickel yield $tree^{-1}$ yr^{-1}, with an estimated total nickel yield of 142 kg ha^{-1} yr^{-1}. *Blepharidium guatemalense* is a prime candidate for nickel agromining on account of its high foliar Ni concentrations, high bioconcentration (180) and translocation factors (3.3), fast growth rate and high shoot biomass production. Future studies are needed to test the outcomes of the pot trials in the field. Extensive geochemical studies are needed to identify potential viable agromining locations.

Keywords: *agronomy, fertilization, hyperaccumulation, neotropics, Ni yield, phytomining.*

Introduction

Soil and freshwater ecosystems can be extremely enriched in some trace elements (e.g. Zn, Cu, Ni, Cr, Cd), resulting from lithogenic, pedogenic or anthropogenic inputs (Kabata-Pendias and Pendias, 2001; Sidhu, 2016; Kumar et al., 2019a, 2019b). Metal hyperaccumulation is an unusual phenomenon that occurs in at least 720 plant species. belonging to 130 genera and 52 families distributed worldwide (Reeves et al., 2018). These plant species have evolved physiological mechanisms of metal tolerance, enabling them to take up and accumulate one or more metallic (or metalloid) elements in their leaves to concentration levels two to three orders of magnitude higher than is found in plants growing on non-metalliferous soils (Baker and Whiting, 2002; van der Ent et al., 2013; Merlot et al., 2018). About 75% of hyperaccumulator species are for nickel (Ni) (Reeves 2003; Reeves et al. 2017) and this can be explained by the large extent of Ni-enriched ultramafic outcrops covering approximately 1% of the Earth's land surface (Garnier et al., 2009; Echevarria, 2018). Hyperaccumulator plants are of particular interest for both fundamental research (*e.g.* metal regulation in plants) and practical application. Indeed, metal hyperaccumulator plants can be used as indicators of metals in soils for geochemical prospecting (Baker and Brooks, 1988; Nkoane et al., 2005) and for phytoremediation (Baker and Brooks, 1988; Atma et al., 2017; Sidhu et al., 2018a; Korzeniowska and Stanislawska-Glubiak, 2019; Sidhu et al., 2020). Phytoremediation is a family of plant-based technologies to deal with environmental aspects of sites that requires remediation, including phytostabilization (metals are transformed into non phyto/bioavailable forms), phytoextraction (metals are removed from the soil by plants), and phytomining (metals are removed from soils and recovered from the plant biomass) (Chaney and Baklanov, 2017). Several metal hyperaccumulator plant species are

currently used for phytoextraction and phytomining (Chaney et al., 2007; van der Ent et al., 2015; Sidhu et al., 2018b; Chaney et al., 2018).

Agromining, a type of phytomining, is a complete chain of agro-metallurgical processes that aims to recycle strategic metals from secondary resources (*e.g.* contaminated or naturally metal-enriched soils) by means of the cultivation of selected hyperaccumulator plants ('metal crops') to provide valuable products (Echevarria et al., 2015; Morel et al., 2015; van der Ent et al., 2015, 2018). This 'green' technology is also a cost-effective and environmentally friendly approach for rehabilitating degraded mine sites (Erskine et al., 2018).

Most agromining research to date has focused on nickel because of the relatively high value of this metal on the market (US$ 15 kg^{-1} on the LME, August 28, 2020), and the existence of many Ni-enriched soils and Ni-hyperaccumulator plants around the world. Optimal agronomic practices, based on laboratory and field experiments, have significantly increased the Ni yields of some 'metal crops'. The improvement of the agronomy of the Eurasian species *Odontarrhena muralis sensu lato* (synonym *Alyssum murale s.l.*) resulted in a maximum yield of 112–145 kg Ni ha^{-1} in Albania (Bani et al., 2015b; Bani and Echevarria, 2019), and 400 kg Ni ha^{-1} in the USA (Li et al., 2003; Chaney et al., 2007). Furthermore, pilot-scale agromining trials in western, central and southern Europe have been set-up to improve plant yields whilst simultaneously enhancing soil quality (Kidd et al., 2018). Thus, fertilization regimes, herbicide application, soil tillage, plant density planting, etc. are all currently being tested for high performance Mediterranean 'metal crops' such as *O. muralis* and *Bornmuellera emarginata* (Kidd et al., 2018).

Research on tropical Ni agromining has rapidly progressed in recent years, specifically in Southeast Asia (Sabah, Malaysia). Two promising species, *Phyllanthus rufuschaneyi* and *Rinorea* cf. *bengalensis*, have been tested in pots and in the field to evaluate their response to synthetic fertilization, pH adjustment and organic amendments addition, among other treatments (Nkrumah et al., 2019b, 2019c, 2019d). A maximum Ni yield of 250 kg Ni ha^{-1} has been estimated for a large-scale agromining operation using *P. rufuschaneyi* under field conditions in Sabah (Nkrumah et al., 2019a).

The neotropical region is a major hot-spot for hyperaccumulator plants, with Cuba and Brazil recording ~30% of all currently known Ni hyperaccumulator species (Reeves et al., 2018, 1999). Species from the genera *Buxus* and *Leucocroton* in Cuba have been identified as potential 'metal crops' (> 2% wt Ni in dry leaves) (Reeves et al., 1996; Nkrumah et al., 2016). However, agromining in this region has remained unexplored until recent years. Our field and herbarium surveys have led to the discovery of Ni hyperaccumulation in at least 13 plant species belonging to three families (Oleaceae, Rubiaceae and Violaceae) distributed mainly in the tropical forests of Mexico, Central and South America (McCartha et al., 2019; Nkrumah et al., 2021). Among the discovered species, there is one 'hypernickelophore' (>1 wt% Ni in dry leaves) that seems to be the most promising candidate for Ni agromining in the neotropical regions. *Blepharidium guatemalense* (Standl.) Standl. (Rubiaceae) or 'Popiste', as it is known in the Mayan local language, is a ligneous species mainly distributed in the tropical perennial and sub-perennial rainforests of southern Mexico (Chiapas, Tabasco and Campeche) and Guatemala. This high-biomass tree is able to concentrate up to 4.3 wt% Ni in its dry leaves and has highly Ni-enriched phloem which may have significant role in Ni distribution in this species (Navarrete Gutiérrez et al., 2021). *Blepharidium guatemalense* also grows in fallow fields under 'slash-and-burn' agriculture and pasturelands and has

long been used for building traditional houses (poles for roofing), and as live fences by the local communities (Jimenez-Ferrer et al., 2008). The high density of this wild species observed in some fallow fields is indicative of the current management of this tree, which is referred to by the local people as “tolerated/promoted”. In this plant management approach, local people seek to maintain or even increase the abundance of useful species without protecting (*e.g.* removal of competitors) or cultivating (*e.g.* fertilization practices) it (Casas et al., 2017). Field observations and testimonials from local people suggest that the species grows fast and can be easily propagated via seeds, which are desirable traits of a commercially viable ‘metal crop’ (Chaney et al., 2007; Nkrumah et al., 2018a). Considering the high potential of *B. guatemalense* as a ‘metal crop’ for economic Ni agromining in suitable neotropical regions, it is critical to develop the agronomic systems to optimize Ni yield. This study is the first to assess the response of *B. guatemalense* to different agronomic systems at both laboratory- and field-scale. The specific objectives of this study were to elucidate the effect of Ni (and other elements) uptake and shoot biomass production of *B. guatemalense* in response to: i) soluble Ni additions, ii) synthetic fertilization, iii) organic fertilization and iv) harvesting schedules.

Materials and Methods

Nickel dosing trial

The Ni tolerance and accumulation potential of *B. guatemalense* was assessed through a pot experiment under controlled conditions in the Laboratoire Sols et Environnement (LSE) in Nancy, France (growth chamber at 25°C, 85% relative humidity and a 12 hr daylength). Seeds were collected from one individual in the surroundings of the Universidad Tecnológica de la Selva in Chiapas, Mexico (374 m. a.s.l. 16°49'21.2"N, 91°07'21.5"W) and germinated in pots using a mix of 50% compost and 50% native soil.

A well characterized soil from Champenoux, France was collected from the A horizon (5-15 cm depth), air-dried, sieved to < 2 mm and 1 kg of each placed into 5 plastic bags. The soils were dosed with Ni salt ($NiSO_4$ $6H_2O$): 0 (control), 20 (T1), 50 (T2), 100 (T3) and 250 (T4) mg Ni kg^{-1}, mixed in the plastic bags to avoid Ni losses and then placed in 1kg pots. Plant seedlings of about 2.5 cm height were carefully transplanted into each pot with 3 replicates per treatment, using a fully-randomized design. Plants were watered daily to 80% of the soil water-holding capacity with deionized water and harvested after three months.

Nutrient dosing trial

The response of *B. guatemalense* to synthetic fertilization was evaluated in a pot experiment conducted in a well-ventilated greenhouse at the Universidad Tecnológica de la Selva, Mexico. Soils were collected from the A horizon (5–15 cm depth) at the native habitat, air-dried and sieved to 2 mm. Forty 8-month-old seedlings obtained from seeds mentioned in the Ni dosing trial were carefully transplanted into 1 kg pots. The seedlings were allowed to establish for one month and then assigned to the treatments using a fully-randomized design. Three doses of macronutrients nitrogen, phosphorus and potassium (NPK) and one control with ten replicates each were tested: i) 0:0:0 ii) 50:40:40; iii) 100:80:80 and iv) 120:120:120 kg ha^{-1}. Fertilizers used were urea (46:0:0) and a granulated mixture of NPK (17:17:17) divided into two doses, the first one applied immediately after transplanting, and the second a month later to avoid losses by leaching. An organic treatment of 100 g of vermicompost per pot was also tested. Plants were watered daily with local tap water over a period of 5 months after which they were harvested.

Bulk analysis of harvested plants

Harvested plants were separated into different fractions (roots, stems and leaves), washed with deionized water and placed in a drying oven at 70°C for three days. The dried plant samples were then weighed, ground to 150 µm and digested using 1 mL HNO_3 (65%) and 2 mL H_2O_2 (30%). After 24 h, samples were heated for 2 hrs at 95°C in a block system (*Digi*PREP), left to cool at least for 3 hrs, filtered to < 0.45 µm and adjusted in volume to 10 mL with ultrapure water. The resulting extracts were analyzed by ICP-AES (Thermo Fisher CAP 6300 Duo) for minor elemental quantification. An extra 1:10 dilution was needed for the quantification of major elements. Three blanks and three reference samples of the known hyperaccumulator plant *Noccaea caerulescens*, were used in each analytical series for the quality control of data.

Bulk analysis of soil samples

Soil samples were dried at 70°C for 3 days in a drying oven and sieved to < 2 mm. Subsamples of 0.5 g (± 0.001 g) were finely ground (< 250 µm) and digested by adding 6 mL of 37% HCl and 2 mL of 70% HNO_3 per sample. After allowing to stand for 24 hrs, digests were heated for 3 hrs at 100°C in a block system (*Digi*PREP MS), and then left to cool down to a minimum temperature of 40°C. Solutions were adjusted in volume to 45 mL with ultrapure water, filtered < 0.45 µm, and then readjusted to 50 mL. A final dilution of 1:20 was necessary for major elements. The resulting soil digests/extracts were analyzed by ICP-AES for the quantification of major and minor elements. Trace element availabilities in soil samples were also determined by ICP-AES using diethylenetriaminepentaacetic acid (DTPA-extractant) according to the method of Lindsay and Norvell (1978). Cation exchange capacity (CEC) of soils was determined using cobalt-hexamine trichloride extraction by adding 50 mL of solution (4.458 g of $Co(NH_3)_6Cl_3$ with 700 mL of distilled water). After 1 hr shaking, 10 ml of each sample was filtered at 0.45 µm and measured by spectrophotometrically at a wavelength of 475

nm (BioRad, SmartSpec Plus). Available phosphorus (P) was determined according to the Olsen method (Olsen et al., 1954; ISO 11263, 1994). Soil pH was measured with in a 1:5 soil:water (5 mg soil with 25 mL ultrapure water) suspension after 1 hr shaking at 16 rpm and 1 hr standing.

Harvesting schedule/ nickel yield estimation

The potential biomass and Ni yields were estimated by means of a one-year field experiment. The experimental site was a pastureland, formerly a tropical rainforest located 10 km northeast of Bonampak archaeological site in Chiapas, Mexico (16°44'20.4"N, 90°59'46.2"W). The site was selected due to the high DTPA-available Ni in the soils (Navarrete Gutiérrez et al., 2021), and the presence of many 5-8-year-old trees of *B. guatemalense* distributed in patches of > 8 individuals. Three plots of 15 m x 15 m were delimited, and 6 five-year-old trees (diameter at 1.3 m height > 20 cm) were selected per plot, identified, measured and coppiced at a height of 1.5 m. Prior to harvesting, soil (5–10, 10–25, 25–30 cm depths) and plant samples (three leaves per tree) were collected from each plot for elemental analysis. Total and DTPA-available Ni concentrations in soils as well as foliar Ni concentrations of selected trees are shown in **Table 1**. Two harvesting frequency scenarios were tested with 9 replicates each, one and two times a year, harvesting only leaves and branches. Growth (height) of coppiced trees was measured every 15 days during the first 6 months. In addition, an 8-year-old tree was coppiced to a height of 35 cm to consider a third scenario with a harvesting of the whole biomass (leaves, branches, fruits, flowers and bark) after 8-years cropping. Fresh harvested biomass was separated into the different fractions and weighed. Dried biomass was re-weighed after a two-week period of sun-drying. Leaf samples of each tree were taken for chemical analysis.

Statistical analyses

Descriptive analyses, boxplots and one-way analyses of variance (ANOVA) with a confidence level of 95% were performed using XLSTAT 2019.1.1 version 'Ecology' in Microsoft Excel 365. Normality and homoscedasticity of data were verified using Shapiro and Levene tests. When these two assumptions were fulfilled, t-tests or ANOVA followed by Newman Keuls tests were performed. In specific cases (*e.g.* data ranging over several orders of magnitude), data were log-transformed. Non-parametric tests (Mann-Whitney, Kruskal-Wallis tests) were performed when assumptions of a parametric test were not met.

Results

Nickel dosing trial

Nickel concentrations in the leaves of *B. guatemalense* increased significantly with increasing DTPA-available Ni concentrations (Kruskal-Wallis test, $p < 0.05$), with no significant decrease in shoot (leaves and stem) biomass production (ANOVA, Newman-Keuls test, $p > 0.9$) (**Figure 1**). Nickel concentrations in the leaves of plants from the T4 treatment were almost 300-fold higher than in the control. There were no significant differences in plant growth (height) and leaf count among treatments (ANOVA, Newman-Keuls, $p > 0.8$). Nickel concentrations in stems and roots of the plant increased significantly and steadily with Ni addition (**Figure 2**). Nickel accumulation in primary roots was not significantly different from that found in secondary roots (t-test, $p > 0.9$; data transformed to $\log_{10}$). The bioconcentration factor (BAF = ratio of the metal concentration in leaves to the total Ni concentration in soils, (Mackay and Fraser, 2000)) of the T4 treatment (180), was 70-fold higher than that in the control (**Figure 3)**. The translocation factor (TF = ratio of the Ni concentration in leaves to that in roots, (Mattina et al., 2003)) of the T4 treatment also showed an 8-fold increase (3.3) compared to the control.

Nutrient dosing trial

The effect of the different NPK additions on shoot dry biomass, plant growth and Ni concentrations in the different parts of *B. guatemalense* are shown in **Figure 4**. Although no significant differences were observed in the shoot biomass and in the plant height among treatments (ANOVA. Newman-Keuls, $p > 0.05$) (**Figures 4a, b**), there was a two-fold increase in the number of leaves in the 120:120:120 treatment compared to the control (t-test, $p < 0.05$). There was a slight decrease in Ni concentrations in the leaf

fraction of the plants in the 50:40:40 treatment with respect to the control, but it was not statistically significant (Mann-Whitney test, $p > 0.6$). Nickel concentrations in leaves, stems and roots were significantly higher in the 100:80:80 and 120:120:120 treatments with respect to the control (**Figures 4c, d, e**). There was a three-fold increase in the average foliar Ni concentration in the 120:120:120 treatment compared to that in the control. The shoot Ni yields were significantly different among treatments with an almost five-fold increase for the 120:120:120 treatment with respect to the control (Kruskal-Wallis test – Conover-Iman test, $p < 0.05$).

Concentrations of elements in plant leaves in response to the nutrient dosing are shown in **Table 2**. Most of the elements did not show any significant difference among treatments, except for magnesium (Mg), iron (Fe), manganese (Mn) and zinc (Zn). However, the differences in Mn and Zn foliar concentrations between the 120:120:120 treatment and the control were not significant (Mann-Whitney test, $p > 0.3$). However, in contrast the foliar Mg and Fe concentrations in the 120:120:120 treatment were significantly lower compared to the control (Mann-Whitney test, $p < 0.05$). There were significant differences in the foliar nitrogen (N) concentration within treatments although they did not show an upward trend. No significant differences were found in foliar P and K concentrations in the 120:120:120 treatment with respect to the control (Mann-Whitney test, $p > 0.05$).

The chemical properties of soils (before and after the experiment) are shown in **Table 3**. DTPA-extractable Ni did not significantly change with nutrient addition (ANOVA, Newman-Keuls test, $p > 0.05$). A slight increase in total and extractable K and P concentrations in soils due to the NPK addition was observed. Total N concentrations did not significantly change within treatments (ANOVA, Newman-Keuls test, $p > 0.05$). A slight increase in the total concentrations of Ni, Mg and Fe was observed.

The shoot biomass (leaves and stems) and growth (height) significantly increased in response to vermicompost amendment (t-test, $p < 0.05$). Foliar biomass was three-fold higher than that of the control. However, the Ni concentrations in leaves and roots did not significantly change with respect to the control (t-test, $p > 0.05$), but decreased significantly in the stem fraction (**Figure 5**). The overall shoot Ni yield for the organic treatment was not significantly different to that of the control (t-test, $p > 0.1$), but significantly lower with respect to the NPK 120:120:120 treatment (Mann-Whitney test, $p < 0.05$). Foliar elemental concentrations in both the organic and control treatments are shown in **Table 4**. There was a significant increase in the K concentration in leaves in the organic treatment compared to that in the control (ANOVA, Newman-Keuls test, $p < 0.05$). Iron and Mn concentrations were significantly lower in the organic treatment than in the control (Mann-Whitney test, $p < 0.05$). There was no significant effect on DTPA-extractable Ni concentrations in soils after vermicompost addition (t-test, $p > 0.7$).

Harvesting schedule

The foliar biomass resulting from the harvesting frequency test is shown in **Table 5.** There was a significant difference between the annual average yield per tree produced by the two scenarios (t-test, $p < 0.05$). Harvesting twice per year produced three-fold more dry leaf biomass than only harvesting once per year. For the twice per year harvesting scenario, there was no significant difference between the pre-harvest average leaf biomass (3.2 kg tree^{-1}) and that obtained the year after (3.4 kg tree^{-1}) (t-test, $p > 0.05$). In contrast, the once per year frequency scenario had a significant reduction of about 30% in the average leaf biomass produced in the final harvest (1.0 kg tree^{-1}) with respect to the pre-harvest (3.5 kg tree^{-1}) (t-test, $p < 0.05$). The once per year harvesting scenario had a significantly higher dry biomass of the branches (3.1 kg tree^{-1}) compared to that obtained from the twice per year harvesting regime (1.4 kg tree^{-1}) (t-test, $p < 0.05$). The coppiced

trees had an average height of 1.20 m after 6 months with no significant differences within the three plots (ANOVA, Newman-Keuls test, $p > 0.05$).

The third scenario which was of an 8-year crop followed by the harvesting of the whole biomass including all the parts of the tree (leaves, trunk, branches, flowers and fruit capsules) had a total Ni phytoextraction yield of 154 g per tree. The part that most contributed to the Ni yield was the foliar biomass followed by the trunk and the branches (**Table 6**). Assuming a density of 2500 8-year old trees per hectare, it could be possible to obtain a Ni yield of 240 kg ha^{-1} when harvesting only the foliar biomass and 385 kg ha^{-1} when considering the whole biomass.

Discussion

Agronomy of Blepharidium guatemalense

The ability of *B. guatemalense* to take up Ni from soils is strongly dependent on the DTPA-extractable Ni in soils. The latter is an approximation of the phytoavailable (*i.e.* isotopically-exchangeable or labile) Ni pool in soil (Echevarria et al., 1998; Massoura et al., 2004). These two variables were previously reported to be positively correlated (Pearson, r = 0.5) from foliar and rhizosphere soil of *B. guatemalense* samples collected in the field (Navarrete Gutiérrez et al., 2021). Although Ni uptake and Ni concentrations in the soil solution are generally correlated, many other soil and plant factors can also influence plant Ni uptake such as soil pH (Kabata-Pendias and Pendias, 2001). The maximum Ni accumulation of *B. guatemalense* could not be attained in this study since the maximum concentration in leaves was 3.5 wt% at a DTPA-available Ni of 17 mg kg^{-1} resulting from the highest Ni treatment (addition of 250 mg Ni kg^{-1}). In its natural habitat, *B. guatemalense* is able to accumulate 4.3 wt% Ni and 3.3 wt% Ni from soils with 190 mg kg^{-1} and 460 mg kg^{-1} DTPA-available Ni, respectively (Navarrete et al 2020,

submitted). Thus, the Ni tolerance peak could be 4–5 wt% Ni at 200–300 mg kg^{-1} available Ni in soils. *Blepharidium guatemalense* has a TF >1 when growing in soils with DTPA-available Ni > 2.0 mg kg^{-1}, which is indicative of its effectiveness to translocate Ni from roots to leaves (Baker and Brooks, 1989).

NPK and organic fertilization

Blepharidium guatemalense responded strongly to synthetic fertilization with a three-fold increase in foliar Ni concentrations. This surprising result contrasts with most previous research which reports a negligible effect in shoot Ni concentrations in Ni hyperaccumulators after mineral fertilization (Shallari et al., 2001; Kidd and Monterroso, 2005; Bani et al., 2015a; Nkrumah et al., 2019a). However, this increase on the shoot Ni concentrations has been reported in *B. coddii* with a two-fold increase as a response to N additions (B. H. Robinson et al., 1997). Magnesium and Fe concentrations in plant leaves from the three NPK treatments significantly decreased compared to the non-treated ones but they were within normal ranges for plant growth (Campbell et al., 2012). Shoot biomass of *B. guatemalense* did not significantly change upon NPK additions. There is usually an increase in shoot biomass of Ni hyperaccumulators in response to synthetic fertilization growing in ultramafic substrates (*e.g. P. rufuschaneyi* (Nkrumah et al., 2019a), and *Odontarrhena* spp. (Bani et al., 2015a; Álvarez-López et al., 2016)).

Soils from this region in Chiapas and from other humid-tropical environments in south-eastern Mexico are mostly calcareous (Mendoza-Vega and Messing, 2005). Moreover, these soils are not generally deficient in essential macronutrients as are many ultramafic soils (Proctor and Woodell, 1975), which could partly explain the neutral response in growth of *B. guatemalense* to synthetic fertilization. However, the available P is relatively low in the soils in Chiapas (9.4 mg kg^{-1}) (Navarrete et al., submitted), which is a common

feature in this type of limestone calcareous soils where P is probably adsorbed by calcium carbonate or precipitated as calcium phosphate (von Wandruszka, 2006; Falkowski et al., 2016). Synthetic fertilization is therefore recommended to increase P availability in soils, at least to compensate for plant nutrient uptake over time. Split NPK fertilizer application is recommended to minimize N leaching to the groundwater (Kidd et al., 2015; Nkrumah et al., 2018b). Further experiments should be conducted to evaluate the effect of each nutrient separately, as has been undertaken in Sabah (Nkrumah et al. 2019b, c, d). Considering the large amounts of Ca taken up by *B. guatemalense*, amendments of this element must be made in order to avoid soil depletion over time (Bani et al., 2015a).

Organic fertilization (vermicompost) increased the foliar biomass of *B. guatemalense* three-fold, with no significant reduction in the foliar Ni concentrations. Similarly, higher total Ni yields with composted sewage sludge compared to NPK fertilization have been obtained due to a substantial increase of the biomass in some species of *Odontarrhena* (Álvarez-López et al., 2016). Biochar amendments increased *O. muralis* growth by improving the fertility of ultramafic and constructed soils (Rue et al., 2019). On the other hand, manure compost did not show any positive effect on the biomass of the Ni hyperaccumulator *O. chalcidica* (Broadhurst and Chaney 2016). Fertilization of *B. guatemalense* using vermicompost could be preferred to NPK fertilization when no increase in foliar Ni concentration is needed or when soils are not nutrient deficient. There is evidence that organic matter amendments enhance the growth of 'metal crops' by improving soil drainage and water holding capacity (Angle et al., 2003; Nkrumah et al., 2016, 2018b). In Ni-rich ultramafic soils, organic matter reduces phytoavailable Ni concentrations and may lead to increased or decreased Ni yield depending on shoot Ni concentrations which may depend on soil Ni availability (Bani and Echevarria 2019;

Nkrumah et al. 2019b). Therefore, field trials are needed to assess the effects of organic amendments on the Ni yield of *B. guatemalense* in Ni-rich substrates.

Harvesting scenarios

A natural tree density of 2500 trees per hectare was calculated by measuring the canopy of 15 *B. guatemalense* adult trees (diameter at 1.3 m height > 15 cm) and the distances between them in three different fallow fields with a high tree density in Chiapas (Mexico). The mean Ni concentration in leaves from the trees at the end of our experiment was 16.7 g kg^{-1}. Thus, the Ni phytoextraction yield from the foliar biomass could be 43 kg ha^{-1} yr^{-1} when harvesting once a year and 142 kg ha^{-1} yr^{-1} when harvesting biannually. In the case of combined leaves and branches, it could be possible to obtain 10.3 t and 12 t ha^{-1} yr^{-1} of dry biomass, corresponding to 68 and 154 kg ha^{-1} yr^{-1} Ni yields from the once per year and twice per year harvesting scenarios, respectively. Finally, the third scenario (8-year crop followed by the harvesting of the whole biomass) would generate a Ni yield of 193 kg ha^{-1} when harvesting only the foliar biomass and 385 kg ha^{-1} when considering the whole biomass. Nevertheless, *B. guatemalense* trees can be harvested from the 5th year of culture and still obtain high Ni phytoextraction yields Harvesting twice per year in monoculture is therefore the best regime for profitable Ni agromining in this context. However, the foliar Ni concentrations of *B. guatemalense* in the experimental site (16.7 g kg^{-1}) were above its previously reported average Ni concentration (13.5 g kg^{-1}, n = 78) (Navarrete et al., submitted) and may not be representative. Notwithstanding, most pastures in the region have similar available soil Ni concentrations and *B. guatemalense* foliar Ni concentrations, hence, Ni yields within a range of 23–364 kg Ni ha^{-1} (mean of 115 kg Ni ha^{-1}) could be attained considering foliar Ni concentrations of 0.3–4.3 wt% Ni. Comparable high Ni yields of 250 kg ha^{-1} have been estimated for large-scale tropical agromining using *P. rufuschaneyi* in Sabah, Malaysia (Nkrumah et al., 2019d).

The pastureland used for the harvesting trial had relatively higher total and DTPA-available Ni concentrations in the superficial soil (5–10 cm) compared to that in deeper soil (10–30 cm). Clearly, Ni-rich litter from *B. guatemalense* trees seems to be substantially contributing to the total and available Ni concentrations in the topsoil as it was observed for *O. muralis* in Albania and other Ni hyperaccumulator species (Estrade et al., 2015; Echevarria, 2018). A detailed biogeochemical investigation is required to assess recycling of Ni through Ni-rich leaf litter degradation and the impact of Ni-rich biomass harvesting in an agromining operation on the Ni biogeochemistry.

The phenological cycle of *B. guatemalense* provides valuable information for the better management of the crop. For example, the first harvesting should preferably be done in March, just before the partial leaf fall period (April–May), whereas the second harvest could be in October, four months after the growth of new leaves. Seed collection could be performed during the fruit maturing stage (April–May) based on field observations taken during one year in the native habitat in Ocosingo, Chiapas (April 2018–2019). However, the phenological patterns could be different between populations and may be influenced by local climate (Mantovani et al., 2003). Therefore, specific studies must be performed in different regions to identify possible differences in these patterns and to adjust the management practices accordingly.

Potential sites for agromining

The selection of potential sites is of great importance for the success of Ni agromining (Nkrumah et al., 2018b). A high phytoavailable Ni concentration is always a desirable property of soils for effective Ni agromining (Nkrumah et al. 2016). Considering the strong response of *B. guatemalense* to Ni additions, a high Ni phytoavailability in soils is key to obtaining optimum yields for economic Ni agromining (Massoura et al., 2004).

Soils in the natural habitat of *B. guatemalense* in southeastern Mexico (Chiapas and Tabasco) are not ultramafic but are rather derived from limestone sedimentary rocks (lutite, limonite) (Müllerried, 1957; SGM, 2017). Unpublished data from recent field surveys revealed total Ni concentrations range from 30–1600 mg kg^{-1} in Chiapas and 200–500 mg kg^{-1} in Tabasco, relatively lower than that in ultramafic soils (Kabata-Pendias and Pendias, 2001). The origin of Ni is uncertain but it could be related to volcanic ash deposition from the nearest volcano (*i.e.* El Chichón) (Hernández-Quiroz et al., 2012). Our unpublished data indicates that Ni in Chiapas is found in ferromagnesian clays, mainly associated with Mn oxides. The average phytoavailable Ni in Chiapas is relatively high (50 mg kg^{-1} DPTA-Ni), reaching 200 mg kg^{-1} DPTA-Ni in some cattle farms with a high a density of *B. guatemalense* trees. Thus, a resource 415 kg Ni ha^{-1} is estimated assuming one hectare of soil with an average DTPA-available Ni of 50 mg kg^{-1} to a depth of 1 m and a bulk density of 0.83 g cm^{-3}. It is expected that the subsequent cropping of *B. guatemalense* would progressively decrease Ni toxicity in soils through the reduction of the phytoavailable Ni pool in soils (Morel, 2012). Therefore, the above-mentioned Ni resource in soils per hectare would be completely depleted in 3 years of culture considering an annual yield of 142 kg Ni yr^{-1} after which the site will be suitable for other types of cropping. Research is currently underway to better understand the role of Ni hyperaccumulators in the geochemical cycle of Ni and the replenishment of Ni labile pools in soils over time (Echevarria, 2018; Ratié et al., 2019). Intensive geochemical investigations are needed to determine the total extent of Ni-enriched substrates and to select the most suitable sites for Ni agromining in southeastern Mexico.

Local adaptation of Ni agromining into existing agroforestry systems

The Ni yields mentioned above are expected under a monocropping regime in open pastures or degraded/abandoned land with low food crop productivity. However, *B.*

guatemalense is currently part of some agroforestry systems from which it could currently be harnessed. For example, it is commonly found in backyard gardens of Tabasco, Yucatan and Campeche (Mariaca Méndez, 2012; van der Wal et al., 2011) as well as in silvo-pastoral systems in Chiapas and Tabasco as dispersed trees (Grande et al., 2010; Pignataro et al., 2016, 2017). In eastern Chiapas and in Izabal (Guatemala), the species is used in live fences for cattle pastures (Ramírez-Marcial et al., 2012) (**Figure 6f**). Indeed, *B. guatemalense* is used by local communities due to the hardness of its wood which they use for building houses or for sale in the local market (Standley, 1918; Jimenez-Ferrer et al., 2008; Marinidou and Jimenez-Ferrer, 2010) (**Figure 6g**). Considering a scenario where *B. guatemalense* is used as live fencing in pasturelands (232 trees ha^{-1}, 13.5 g Ni kg^{-1} in leaves, 2 harvest per year), a yield of 11 kg Ni ha^{-1} yr^{-1} could be expected. The economic benefit may seem less than in monoculture, but it will depend on the number of hectares under this system and it would also represent an extra income for local farmers. Future studies must investigate how Ni agromining could be properly integrated into the existing local agroforestry systems.

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Declaration of interest statement

The Authors declare that there is no conflict of interest. The content of the manuscript has not been published or submitted for publication elsewhere in any language. All authors have contributed significantly to the realization of the manuscript in different ways.

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Table 1. Total and DTPA-available Ni in composite soil samples (*n* = 1) of each experimental plot and average Ni concentrations in leaves of 6-years old trees of *B. guatemalense* (*n* = 18) prior to harvesting trial.

Experimental plot	**Horizon (cm)**	**pH**	**Total Ni (mg kg^{-1})**	**DTPA-extractable Ni (mg kg^{-1})**	**Foliar Ni (g kg^{-1})**
C1	5-10	6.3	1030	131	22.5 ± 1.4
	10-25	6.6	544	8.6	
	25-30	6.7	571	5.4	
C2	5-10	6.3	1130	97	15.2 ± 0.5
	10-25	6.5	1000	41	
	25-30	6.6	1010	34	
C3	5-10	6.2	815	114	12.3 ± 0.2
	10-25	6.4	571	30	
	25-30	6.5	626	42	

Table 2. Elemental concentrations in leaves (µg g^{-1}) of *B. guatemalense* under different synthetic fertilization treatments. Values followed by the differen.t letters are significantly different (p value < 0.05) according to Kruskal-Wallis test. Nitrogen values are given as a percentage (%) (ANOVA Newman-Keuls test, $p < 0.05$).

Treatment (NPK)	N (%)	P	K	Ca	S	Mg	Fe	Mn	Zn	Ni
T0, Control (0:0:0)	3.22 ± 0.36 ab	1430 ± 400 a	19 000 ± 5570 a	6700 ± 1670 a	2170 ± 637 a	3240 ± 1409 b	7880 ± 4073 b	238 ± 53 b	107 ± 130 ab	541 ± 204 a
T1 (50:40:40)	3.17 ± 0.34 ab	1500 ± 354 a	24 300 ± 3420 a	4890 ± 720 a	1990 ± 414 a	1810 ± 346 ab	981 ± 930 a	81 ± 28 a	42 ± 20 a	463 ± 140 a
T2 (100:80:80)	3.35 ± 0.37 a	1770 ± 424 a	23 200 ± 2900 a	5770 ± 980 a	2530 ± 1160 a	1840 ± 356 a	714 ± 612 a	104 ± 60 a	130 ± 94 b	1020 ± 431 b
T3 (120:120:120)	2.77 ± 0.37 b	2340 ± 1110 a	23 100 ± 2730 a	5070 ± 623 a	2490 ± 351 a	1590 ± 385 a	2070 ± 1524 ab	206 ± 89 b	93 ± 70 ab	1690 ± 844 b

Table 3. Elemental contents in soil prior to fertilization treatment application (PS), and in soils under the different treatments at the end of the experiment. Available P was obtained through Olsen method.

Treatments	**Ni**	**Zn**	**Ca**	**Mg**	**K**	**P**	**Mn**	**Fe**	**S**
	Total element (mg kg^{-1})								
PS	294	42	7920	5500	2590	250	2910	62 900	658
T0	244 ± 24	42 ± 4.1	7580 ± 1410	4510 ± 605	2440 ± 195	200 ± 46	2440 ± 481	53 350 ± 3244	695 ± 116
T1	273 ± 31	54 ± 12	8670 ± 981	5360 ± 775	2710 ± 293	406 ± 106	2250 ± 358	57 700 ± 4277	745 ± 50
T2	284 ± 9	57 ± 11	9210 ± 1410	5620 ± 408	2860 ± 143	451 ± 114	2220 ± 238	58 200 ± 3182	773 ± 100
T3	285 ± 15	47 ± 3.2	8170 ± 639	5950 ± 771	3050 ± 361	402 ± 76	2230 ± 391	60 700 ± 4232	708 ± 45
T Org	276 ± 10	56 ± 4.9	10 400 ± 644	5700 ± 367	2920 ± 228	457 ± 74	2470 ± 265	58 500 ± 1724	865 ± 51
	DTPA-extractable element (mg kg^{-1})								
PS	11	3.0	-	245	22	1.4	39	44	-
T0	10 ± 1.7	3.3 ± 1.2	-	255 ± 18	21 ± 5.6	2.1 ± 1.6	36 ± 5.0	43 ± 4.5	-
T1	13 ± 2.5	9.0 ± 5.3	-	269 ± 28	41 ± 22	13 ± 5.6	38 ± 4.9	51 ± 4.6	-
T2	13 ± 1.4	8.3 ± 3.8	-	258 ± 26	50 ± 13	13 ± 7.3	35 ± 7.1	49 ± 6.1	-
T3	13 ± 2.4	4.8 ± 1.2	-	242 ± 19	58 ± 14	17 ± 8.1	40 ± 4.6	50 ± 8.4	-
T Org	11 ± 2.0	8.6 ± 2.7	-	252 ± 27	49 ± 9.4	27 ± 11	27 ± 5.3	37 ± 5.3	-
	CEC (cmol+ kg^{-1})						*pH*		
PS	-		39	5.7	0.5		6.8		
T0	-		37 ± 4.7	5.6 ± 0.1	0.5 ± 0.4		6.7		
T1	-		40 ± 2.2	6.3 ± 0.3	0.9 ± 0.9		6.7		
T2	-		40 ± 3.9	6.4 ± 0.2	1.0 ± 0.7		6.7		
T3	-		37 ± 5.6	5.8 ± 0.3	1.1 ± 0.5		6.9		
T Org	-		42 ± 0.9	6.4 ± 0.2	1.0 ± 0.5		6.8		

Table 4. Elemental concentrations in leaves (µg g^{-1}) of *B. guatemalense* under organic fertilization. Values followed by the different letters are significantly different (*p* value < 0.05) according to ANOVA Newman-Keuls test. Values followed by different symbols mean significant differences between the position according to Mann-Whitney test (*p* value < 0.05).

Treatment (NPK)	**Ni**	**Zn**	**N (%)**	**P**	**K**	**Ca**	**S**	**Mg**	**Fe**	**Mn**
T0, Control (0:0:0)	541 ± 204 a	107 ± 130 ¤	3.22 ± 0.36 a	1430 ± 400 a	19 000 ± 5570 b	6700 ± 1670 a	2170 ± 637 a	3240 ± 1409 ¤	7880 ± 4070 a	238 ± 53 a
T4, Organic	414 ± 106 a	39 ± 7.0 ǂ	3.09 ± 0.35 a	1480 ± 206 a	25 900 ± 4520 a	5800 ± 721 a	1960 ± 212 a	2070 ± 303 ǂ	1397 ± 1790 b	79 ± 61 b

Table 5. Biomass values refer to dry weight (* average value, n = 9). Different letters express significant differences (ANOVA, Newman-Keuls test after data transformation to log, $p < 0.001$). Mean Ni concentration in leaves from trees in the experimental site was of 16.7 g kg^{-1}. Leaf biomass yield was calculated for a density of 2500 trees per hectare.

Harvesting frequency	**Leaf biomass yield (kg $tree^{-1}$)**	**Leaf biomass yield (t ha^{-1})**	**Ni phytoextraction yield (kg ha^{-1})**
Once per year	1.02 ± 0.8 b*	2.55	43
Twice per year	3.40 ± 1.3 a*	8.49	142
Single coppicing after 8th year	5.68	14.2	237

Table 6. Biomass obtained from an eight-year-old tree of *B. guatemalense* in a cattle pasture. (*) Mean Ni concentration values obtained from field samples (number of samples $n > 9$) (Navarrete et al. 2020, submitted), except for the trunk ($n = 1$).

Plant part	**Proportion of the tree (%)**	**Dry biomass weight (kg)**	**Ni concentration in biomass* (g kg^{-1})**	**Ni phytoextracted (g)**
Leaves	18	5.68	13.5	77
Trunk	51	16.8	2.55	43
Bark	0.3	0.60	4.36	2.6
Branches	25	8.43	3.29	28
Flowers	2.5	0.06	5.93	0.4
Fruits	3.0	1.06	2.65	2.8
Total	*100*	*32.6*		*154*

Figure captions

Figure 1. Nickel concentrations in leaves and biomass production of *B. guatemalense* under different doses of Ni in soils. Nickel concentrations in leaves with different letters indicate significant differences according to Kruskal-Wallis test (alpha = 0.05). For shoot biomass, same letters indicate no significant differences according to ANOVA, Newman-Keuls test, (alpha = 0.05).

Figure 2. Nickel concentrations in the different parts of *B. guatemalense* under different Ni additions to soils. Values with different letters indicate significant differences according to Kruskal-Wallis test (alpha = 0.05, Conover-Iman as post-hoc test).

Figure 3. Bioconcentration and translocation factors of *B. guatemalense* under different Ni doses in soils.

Figure 4. Response of *B. guatemalense* to synthetic fertilization after 5-months pot experiment. For a) biomass and b) height, same letters represent no significant differences (ANOVA, Newman-Keuls test, alpha = 0.05). For Ni concentrations in leaves, stems and roots (c, d, e), significant differences are indicated by different letters (Kruskal-Wallis test, alpha = 0.05).

Figure 5. Shoot biomass (a), height (b) and Ni concentrations in leaves, stems and roots (c, d, e) of *B. guatemalense* in response to organic fertilization after 5-months pot experiment, p values < 0.05 are significantly different (t-test, alpha = 0.05).

Figure 6. Details of a), b), c) and e) different harvesting frequencies of *B. guatemalense* tested in the field in Chiapas, Mexico, d) green Ni-rich phloem in the bark, f) trees used as living fences in Izabal, Guatemala, and g) trunks used as house roofs in Tabasco, Mexico.

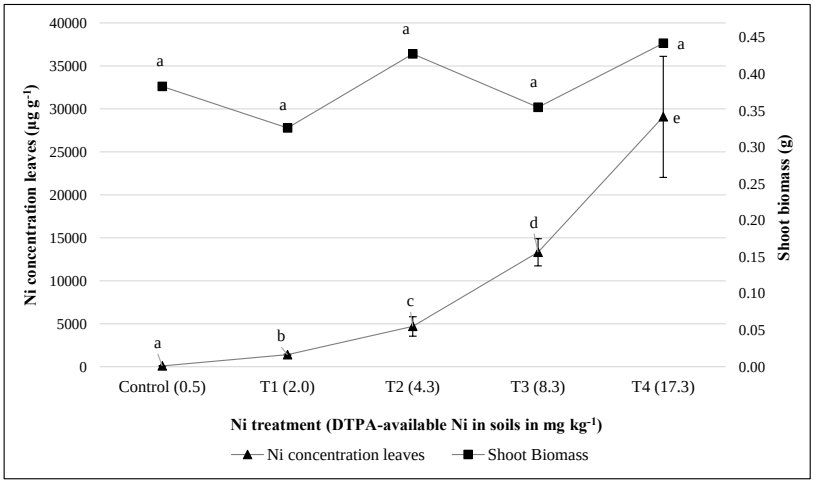

Figure 1

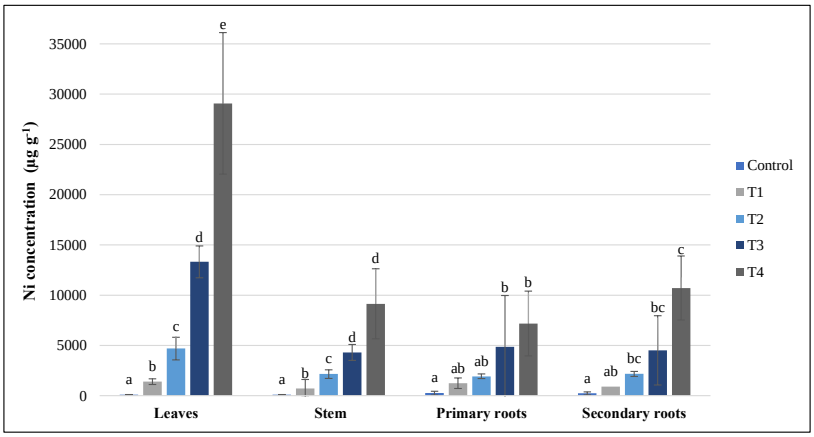

Figure 2

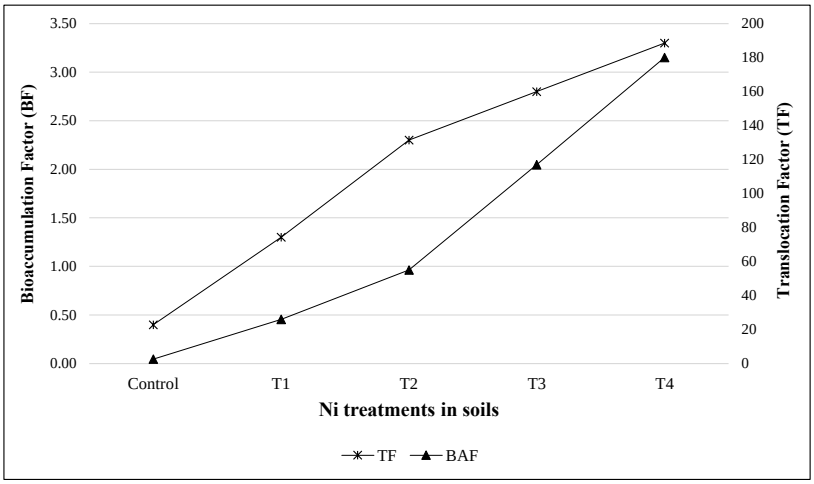

Figure 3

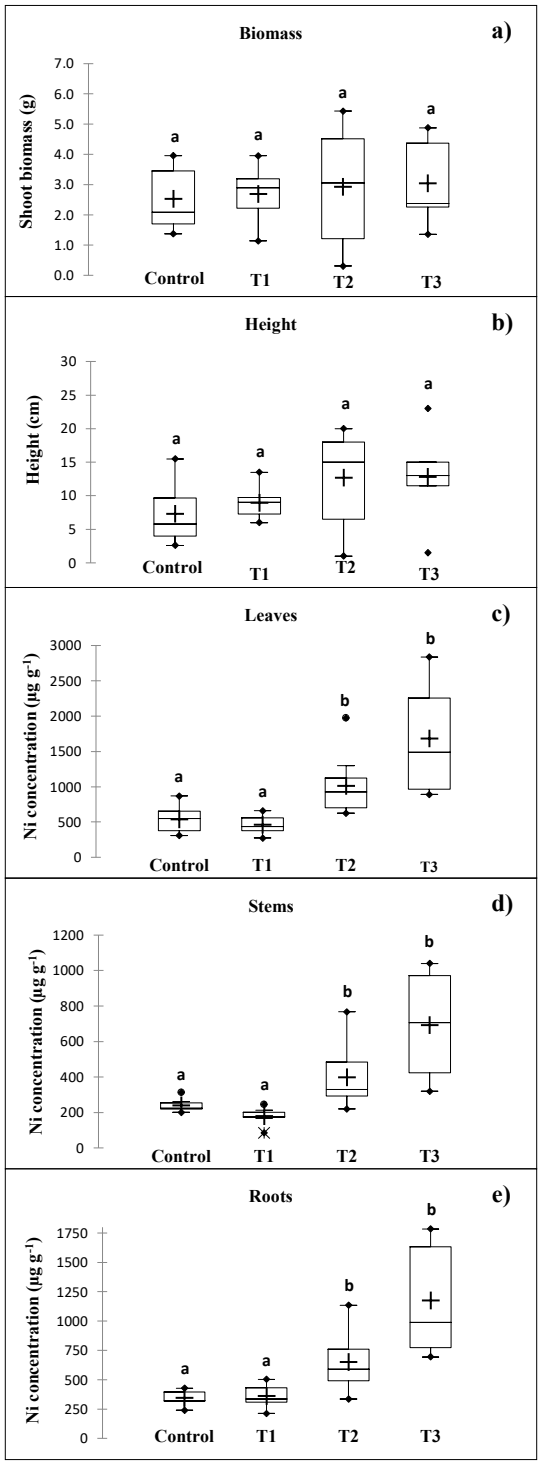
Biomass
a)
Shoot biomass (g)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
a
a
a
a
Control
T1
T2
T3
Height
b)
Height (cm)
30
25
20
15
10
5
0
a
a
a
a
Control
T1
T2
T3
Leaves
c)
Ni concentration (µg g-1)
3000
2500
2000
1500
1000
500
0
a
a
b
b
Control
T1
T2
T3
Stems
d)
Ni concentration (µg g-1)
1200
1000
800
600
400
200
0
a
a
b
b
Control
T1
T2
T3
Roots
e)
Ni concentration (µg g-1)
1750
1500
1250
1000
750
500
250
0
a
a
b
b
Control
T1
T2
T3

Figure 4

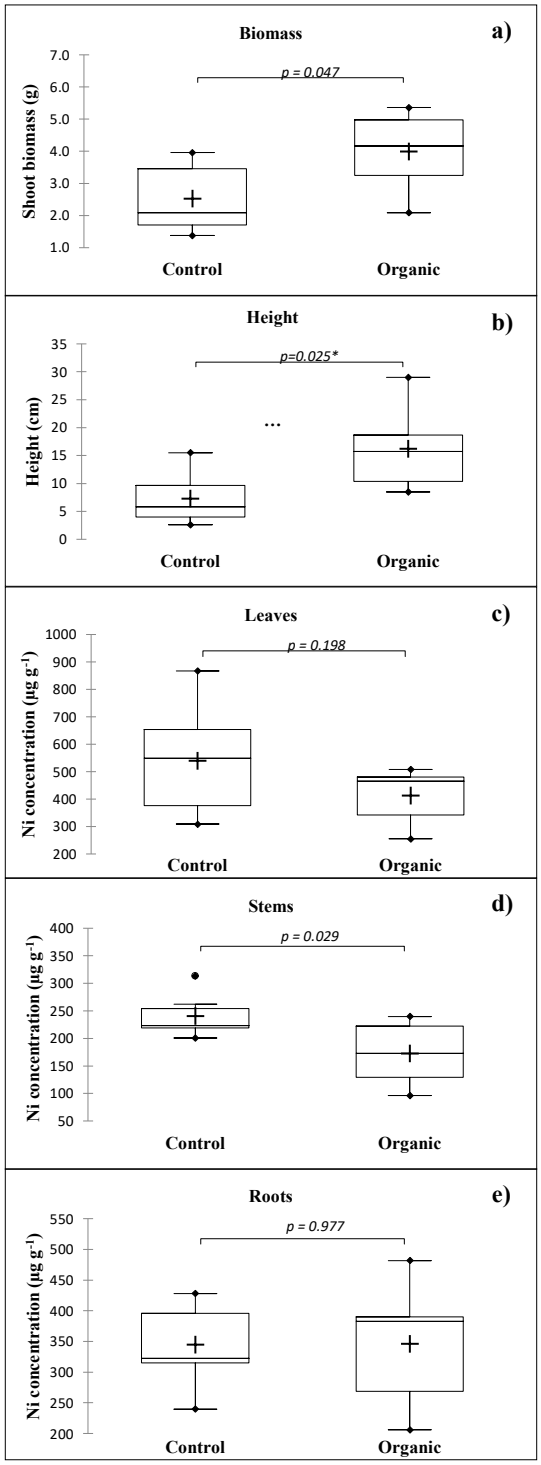
Biomass
a)
p = 0.047
Shoot biomass (g)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
Control
Organic
Height
b)
p=0.025*
Height (cm)
35
30
25
20
15
10
5
0
Control
Organic
Leaves
c)
p = 0.198
Ni concentration (µg g-1)
1000
900
800
700
600
500
400
300
200
Control
Organic
Stems
d)
p = 0.029
Ni concentration (µg g-1)
400
350
300
250
200
150
100
50
Control
Organic
Roots
e)
p = 0.977
Ni concentration (µg g-1)
550
500
450
400
350
300
250
200
Control
Organic

Figure 5

Figure 6