Nickel uptake in hydroponics and elemental profile in relation to cultivation reveal variability in three *Hypericum* species

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Abstract

The *Hypericum* species (*H. perforatum*, *H. olympicum,* and *H. orientale*) were cultured in hydroponics with excess nickel (Ni, 1 or 100 µM Ni) to compare the metallic and metabolite content. Identical species were collected outdoor to assess the same parameters (including uranium and lanthanides) with total of 53 elements. The results showed that Ni was less accumulated in shoots in hydroponics (translocation factor of 0.01 – 0.25) and the highest absolute amount was detected in *H. olympicum*. Essential elements were typically depleted by Ni excess, but Co and Na increased. Soluble phenols, sum of flavonols and catechin rather increased in response to Ni but quercetin glycosides and free amino acids decreased in the shoots of *H. olympicum* mainly. Comparison of laboratory and outdoor growing plants showed more phenols in outdoor samples but not in *H. olympicum* and individual metabolites differed too. Plants cultured in hydroponics contained lower amount of non-essential, toxic and rare earth elements (30 to 100-fold) and shoot bioaccumulation factor in outdoor samples was low for most elements (<0.01) but not for Cd and Pt. Data reveal that *H. olympicum* is a potent source of phenolic metabolites whereas *H. orientale* accumulates many elements (38 out of 53 elements).

Keywords: bioremediation; heavy metals; medicinal plants; phenolic metabolites; rare earth elements.

1. Introduction

Nickel (Ni) is an essential "ultramicronutrient" for some plant species and is frequently found in the environment, including urban and agricultural soils, with levels above 200 mg Ni/kg of soil considered to be contaminated soils (Kováčik et al. 2011; Kováčik et al. 2012; Pavlova and Karadjova 2013). If tested under laboratory conditions and/or in hydroponics, Ni toxicity is typically lower compared to other metals such as Cd but it is not a general rule for all species (Kováčik et al. 2019). Ni uptake differs in relation to plant species and/or experimental setups though various species in the same study were rather rarely compared (Soudek et al. 2009; Antonkiewicz et al. 2016).

Plants contain small amounts of about 90 elements, only some of which are essential for them: if we consider Hoagland's solution (with some modifications), it contains 12 nutrients (Supplementary Table S1). Non-essential, toxic and rare elements are commonly present in the soil (Ramos et al. 2016; Modabberi et al. 2018; Cicchella et al. 2020) and taken up in plants but their accumulation in plants has only rarely been complexly studied (Bonanno 2011; Kováčik et al. 2014; Dołęgowska et al. 2022). These elements are often taken up depending on their availability in the soil, although this is not a general phenomenon (Kováčik et al. 2016), and they can pose a health risk if they accumulate in excessive amounts in crops or medicinal plants (Kováčik et al. 2012; Kováčik et al. 2014; Valivand and Amooaghaie 2021a). However, mainly the quantification of trace elements including so-called the rare earth elements (REE) is not frequent because it needs sensitive techniques (Dołęgowska et al. 2022).

Unlike animals, plants produce a variety of metabolites, among which phenolic compounds are quantitatively abundant. They can contribute to the antioxidant protection and other physiological processes of plants by several mechanisms and, in terms of human

nutrition, they are potent antioxidants with a wide range of health benefits (Franklin and Dias 2011; Kováčik et al. 2012; Kováčik et al. 2019; Singh et al. 2021).

The *Hypericum* genus includes ca. 500 species of herbs or shrubs with numerous health positive effects (Franklin et al. 2017). Despite the numerous species of this genus, *Hypericum perforatum* in particular has been studied in terms of metal excess and subsequent effect on metabolites under laboratory conditions (Babula et al. 2015; Kováčik et al. 2022). Several reports from the real field conditions also reported the accumulation of metals in *H. perforatum* originated from Czech Republic (Sládková et al. 2015), Italy (Bonari et al. 2019), Bulgaria (Pavlova and Karadjova 2013) or Turkey (Kadioglu et al. 2005).

Therefore, we selected three *Hypericum* species for this research to assess the effect of excess Ni on its uptake and accumulation of essential elements in hydroponics along with quantification of selected phenolic metabolites by LC-MS. At the same time, these species can survive the winter period in real soil conditions in Slovakia (*H. perforatum* is a native species), so we can compare the accumulation of non-essential, toxic and rare elements in plants growing outdoors and in hydroponics. Although the accumulation of toxic or rare elements in hydroponics may arise from pre-cultivation in sand or from distilled water used for cultivation, differences between species can still be expected. Analyses of plants and respective soil samples were precisely done with ICP-MS device, and to our knowledge, no such data are available for the genus *Hypericum*. Phenolic metabolites quantified as a function of cultivation method is another original aspect of this work, and correlations between elements or between metals and metabolites were also evaluated.

2. Materials and methods

2.1. Cultivation of plants and experimental design

Fourteen-day old seedlings of *Hypericum perforatum*, *Hypericum olympicum* and *Hypericum orientale* (seeds originated from the Centre of Medicinal Plants, Masaryk University in Brno) pre-cultured in sand were placed to 1/4 strength of Hoagland solution (i.e. macronutrients reduced to 1/4) containing 1.01 mM $Ca(NO_3)_2.4H_2O$, 0.13 mM $(NH_4)H_2PO_4$, 1.51 mM KNO_3, 0.4975 mM $MgSO_4.7H_2O$ and standard dose of micronutrients (μM): 125 NaOH, 288 KOH, 89.2 EDTA, 89.6 $FeSO_4.7H_2O$, 9.68 H_3BO_3, 2.03 $MnCl_2.4H_2O$, 0.314 $ZnSO_4.7H_2O$, 0.210 $CuSO_4.5H_2O$, 0.139 Na_2MoO_4 and 0.0859 $CoCl_2.6H_2O$ (Kováčik 2013, for final dose of essential elements per L, see Supplementary Table S1). Uniform plants were cultivated in dark plastic boxes with 5 L of continually aerated solutions (10 plants per box). The whole experiment was carried out in a growth chamber under controlled conditions: 12-h day (6.00 am to 6.00 pm), the photon flux density was ~300 μmol m^{-2} s^{-1} PAR at the leaf level supplied by cool white fluorescent tubes L36W/840 (Lumilux, Osram, Germany) with a 25/20°C day/night temperature and relative humidity of ~60 %. Solutions were renewed weekly to prevent nutrient depletion and plants that had been cultivated hydroponically over 4 weeks were used in the experiment and further cultured for 7 days in the same Hoagland solution with no nickel (Ni) addition (control) or with Ni added in the form of chloride in a final concentration of 1 or 100 μM and pH was checked to be 6.0 in all treatments. After 7 days of exposure, individual plants were separated to shoots and roots (roots double washed with deionized water), dried at powdered using IKA® A11 basic analytical mill.

In order to compare minerals and metabolites of laboratory-cultured plants with those growing outdoor, shoots (without flowers) of respective species growing naturally near the faculty (planted two years ago) were collected, washed with deionized water, dried and powdered as above. Processing of some samples involved cold mortar and pestle with the

addition of inert so-called sea sand (to achieve complete tissue disruption; Penta Ltd., Prague, Czech Republic) followed by centrifugation (14 000 g for 15 min at 5°C, Hettich Mikro 200R). Soil from the given area was also collected to allow quantification of elements and calculation of bioaccumulation factor.

2.2. Quantification of elements

The deionized water of 0.055 μS cm^{-1} conductivity produced using the Milli-Q® water purification system (Millipore Corp., Bedford, USA) was used to prepare all solutions. Sub-boiled nitric acid was prepared from 65%, w/w HNO_3 of Selectipur quality (Lach-Ner, Neratovice, Czech Republic) using the distillation equipment BSB-939-IR (Berghof, Eningen, Germany). Hydrogen peroxide (≥ 30%) and 37% HCl, both of TraceSelect quality, were purchased from Fluka Chemie AG (Buchs, Switzerland).

Microwave digestions and extractions of plant and soil samples were performed in a closed microwave oven system speedwave XPERT (Berghof, Eningen, Germany) with the power output of dual magnetrons 2 x 1000 W and the optical sensors for contactless real-time recording of the sample temperature and pressure in each vessel. The high-pressure resistant (up to 100 bar) TFM ™ -PTFE vessels DAK100 were used for sample digestion.

Plant samples (100 mg) were mineralized in microwave digestion vessels with 5 mL of 16% HNO_3 and 2 mL of 30% H_2O_2 with controlled temperature program up to 220°C and digested solutions were quantitatively transferred to polypropylene flasks and diluted with water up to 25 mL. Soil samples (500 mg) were mineralized in *aqua regia* in the mixture of 7 mL of 37% HCl and 2.5 mL of 65% HNO_3 with controlled temperature program up to 200°C, filtered through 0.45 μm Nylon syringe filters (Whatman Autovial) and diluted with deionized water into a 50 mL volumetric flask. All samples were prepared in three replicates. Blanks, consisting of reagents, were subjected to a similar preparation procedure.

The Agilent 7900 ICP-MS fitted with standard nickel cones, glass concentric nebulizer MicroMist (400 µL min^{-1}), the Peltier-cooled (2 °C) quartz spray chamber, and 2.5-mm internal diameter quartz torch was used for the analysis (Varrà et al. 2021). For precise delivery of samples and ISTD, a low-pulsation, 10-roller peristaltic pump with three separate channels was involved. The instrument was equipped with an octopole-based collision cell for effective and reliable removal of multiple polyatomic interferences using kinetic energy discrimination (KED) in a standard helium ("He") or high energy helium ("HE He") mode. The instrument was automatically tuned in the ICP-MS MassHunter software during each start-up sequence to obtain the highest possible sensitivity for elements of low, middle and high m/z. The working parameters of the collision cell for helium ("He") and high energy He ("HE He") modes were adjusted manually. All plasma and ion lens tuning parameters were consistent for all cell modes (see Supplementary Table S2 for technical details).

Concentrations of individual elements were determined with external calibration using the following analytical solutions prepared daily by appropriate dilution of multi-element solutions "A" (500 µg L^{-1}), "B" (50 + 10 µg L^{-1}) and "C" (50 mg L^{-1}) in 25 mL volumetric flasks: blank, 1, 5, 10, 50, 100 µg L^{-1} of Li, B, Al, V, Cr, Fe, Ni, Co, As, Se, Rb, Sr, Zr, Mo, Ru, Pd, Cd, Sn, Sb, Cs, Ba, Hf, Re, Pt, Tl, Pb, Bi, Th; 0.1, 0.5, 1, 5, 10 µg L^{-1} La, Ce, Pr, Nd, U; 0.02, 0.1, 0.2, 1, 2 µg L^{-1} of Y, Tb, Ho, Yb, Sm, Eu, Gd, Er, Lu, and Dy; 0.5, 1, 5, 10 mg L^{-1} of Na, Mg, P, K, Ca, Mn, Cu, and Zn. Linear calibrations were obtained, with coefficients of determination >0.998 for all elements. To compensate possible instrumental drift and matrix effects, a 200 µg L^{-1} Rh ISTD was simultaneously aspired and mixed with samples (see Supplementary Table S3 for details).

The trueness, intra‑ day and inter-day assay precisions were examined by analyzing three replicates of the four commercially supplied certified reference materials (CRMs), three times during the same day or on three different days over a period of one month. The CRMs

and the results of certified and measured concentrations of the target analytes are mentioned in Supplementary Table S4.

2.3. Assay of metabolites

Total soluble phenols and flavonols were measured in extracts prepared with 80% aqueous methanol (extraction 50 mg DW/5 mL) using Folin-Ciocalteu phenol reagent or $AlCl_3$ reagent with detection at 750 nm and gallic acid as standard or detection at 420 nm and quercetin as standard, respectively. The assay mixture for phenols contained 0.03 mL of extract, 0.47 mL of redistilled water, 0.975 mL of 2% Na_2CO_3 and 0.025 mL of 2 N Folin-Ciocalteu reagent while the mixture for flavonols contained 0.5 mL of extract and 1 mL of 2% $AlCl_3$ in methanol (Kováčik et al. 2011). Due to yellow-green color of shoot samples, parallel controls with no $AlCl_3$ addition were used as a blank for the assay of flavonols. Free amino acids were assayed in extracts prepared in 60% aqueous ethanol (50 mg DW/5 mL) and quantified using the ninhydrin method according to Jiang et al. (2013): 0.1 mL of extract with 0.2 mL of 1.15% ninhydrin ethanol solution was heated over 25 min at 60 °C in closed Eppendorf tubes. After cooling, volume was made up to 1 mL and absorbance was monitored at 570 nm with glycine as standard. Spectrophotometry for all measurements was done with T60 UV/VIS (PG Instruments, UK).

For the quantification of individual phenolic metabolites in the shoots of laboratory or outdoor grown plants, 50 mg of dry tissue was extracted twice with 70% methanol by ultrasound, centrifuged and filtered through 0.22 µm Nylon filters. Quali-quantitative analyses of selected metabolites (see specification in Supplementary Table S5) was done by an UHPLC system (DionexUltiMate 3000, Thermo Fisher Scientific, Waltham, MA, USA) coupled to a Q-Exactive Orbitrap mass spectrometer (UHPLC, Thermo Fisher Scientific, Waltham, MA, USA). A Luna Omega PS 1.6 µm column (50×2.1 mm, Phenomenex,

Torrance, CA, USA) with a temperature set at 25°C was used under the mobile phase consisted of 0.1% formic acid in water (eluent A) and 0.1% formic acid in acetonitrile (eluent B) with gradient program 0-1 min 0% B, 1-2 min 0-95% B, 2-2.5 min 95-95% B, 2.5-5 min 95–75% B, 5-6 min 75-60% B. The flow rate was 0.4 mL/min with the injection volume of 5 µL and the autosampler temperature of 10°C. The mass spectrometer operated in negative ion mode (ESI-) setting two scan events (full scan and all ion fragmentation, AIF). Full scan data were acquired setting a resolving power of 35,000 FWHM at m/z 200 whereas AIF scan events were acquired by setting a resolving power of 17,500 FWHM and collision energy values of 10, 20, and 45 eV. In both cases, the instrument was set to spray voltage -3.5 kV, sheath gas flow rate 45 arbitrary units, capillary temperature 275°C, auxiliary gas heater temperature 350°C, S-lens RF level 50, and the scan range was m/z 80-1200. External calibration mode was performed for the acquisition of the chromatograms, and Quan/Qual Browser Xcalibur software, v. 3.1.66.10 (Xcalibur, Thermo Fisher Scientific, Waltham, MA, USA) was used for data acquisition and processing.

2.4. Statistical analyses

Data were evaluated using ANOVA followed by a Tukey's test at $P<0.05$, with bivariate Pearson's correlation analysis (MINITAB Release 11, Minitab Inc., State College, Pennsylvania) or Student's *t*-test (comparison of laboratory and outdoor species). The normality and homogeneity of variance of data was checked by applying the Shapiro-Wilk's and Levene's tests, respectively ($p \leq 0.05$). If the normal distribution and/or homogeneity of variance assumption was violated, the Box-Cox normalizing and variance-stabilizing transformation was employed. The principal component analysis (PCA) was conducted using MATLAB® R2021a software (The MathWorks, Inc., USA). As predictors have widely

different scales, data were standardized using the z-score by subtracting the mean and dividing by the standard deviation each column of input data matrix before fitting.

3. Results and discussion

3.1. General responses of plants

No growth retardation was observed in response to Ni excess (probably due to the use of older seedlings and subsequent longer cultivation of plants in hydroponics). In addition, no chlorotic symptoms were visible as evidence of mineral nutrient depletion.

3.2. Accumulation of Ni and essential elements in hydroponically-grown plants

Ni accumulation increased with increasing external dose from 1 to 100 µM Ni in both shoots and roots, but the intensity varied: an approximately 10 – 30-fold increase was observed in shoots and an approximately 100 – 240-fold increase in roots (Table 1). In other words, amount of Ni in the roots roughly reflected an increase in the external Ni availability but the shoot Ni amount did not, indicating preferential retention of Ni in the roots. This is a common metal movement behavior in so-called excluder species, which include the vast majority of vascular species, including medicinal plants (chamomile, Kováčik et al. 2009b) or crops (Antonkiewicz et al. 2016). In line with our data, maize, field bean or lettuce cultured in hydroponics with the addition of up to 10 mg Ni/L (~170 µM) contained much more Ni in the roots, leading to translocation factor (TF, shoot/root ratio) of 0.04 – 0.08 in individual species (Antonkiewicz et al. 2016). The same was observed in barley cultivated with Ni excess, where TF reached only 0.014 (Thomas 2021). Our values gave TF of 0.01 – 0.03 for 100 µM Ni and 0.19 – 0.24 for 1 µM Ni dose (Table 1) while medicinal plant chamomile cultured with 3 or 120 µM Ni in hydroponics had TF of 0.16 and 0.11 (Kováčik et al. 2009b) and dandelion cultured with 30 µM Ni had a TF value of 0.12 – 0.19 (Kováčik et al. 2019), indicating higher

mobility and higher Ni accumulation in the shoots compared to *Hypericum* species analyzed in the present work. In contrast, (hyper)accumulator species such as *Alyssum* sp. accumulate Ni preferentially in shoots independently of the external Ni dose, as observed in experiment with up to 1 mM Ni, where the TF value was higher than 1 (Asemaneh et al. 2006).

Regarding species-specific differences, we found several original findings. First, *Hypericum orientale* contained the highest amount of Ni in control shoots or shoots and roots exposed to 1 µM Ni, and this was reflected in the corresponding TF (Table 1). Second, *H. olympicum* contained the highest Ni amount in 100 µM Ni treatment, even 4-fold higher in the shoots in comparison with common medicinal species *H. perforatum* (Table 1). These data indicate the lowest eventual health risk arising from low Ni accumulation in *H. perforatum*. Consistent with our results, in several *Allium* species exposed to 50 or 250 µM Ni in hydroponics, the amount of Ni in shoots varied by about 5-fold in onion or garlic cultivars and retention of Ni in bulbs and roots was observed (Soudek et al. 2009). Also, comparison of 72 rice cultivars cultured with 10 µM Ni revealed that Ni accumulation and translocation are significantly influenced by the genotypes (Wang et al. 2019). Traces of Ni detected in control plants of individual species may arise from the pre-cultivation of seedlings in the sand as previously detected in chamomile (3 and 6 µg/g DW in control shoots and roots) under the same culture conditions (Kováčik et al. 2009b). We may also compare shoot bioaccumulation factor (BAF) of Ni-exposed species: the highest amount of Ni in the shoots of 1 µM Ni-exposed *H. orientale* was reflected in high shoot BAF value (56 vs. 30 and 36 in two other species) and the highest amount of Ni in the shoots of 100 µM Ni-exposed *H. olympicum* was reflected in high shoot BAF (11.5 vs. 9.9 and 2.8 in two other species). For comparison, *H. perforatum* exposed to 10 µM Cd or La showed shoot BAF of 21.2 and 2.9 (i.e. ~7-times lower for La, Babula et al. 2015) and *H. perforatum* exposed to 100 µM Sr had shoot BAF of 46 (Kováčik et al. 2022), indicating higher root-to-hoot mobility of Sr and probably of Cd in

comparison with Ni in *Hypericum*. In agreement, Ni translocation to the shoot was much lower than for Cd in barley (Thomas 2021).

For the purpose of this work, essential elements are all nutrients which were added to hydroponics in the form of Hoagland solution (i.e. 12 elements quantified in Table 1 and mentioned in Supplementary Table S1). Generally, 1 µM Ni dose evoked less negative impact (if any) on the accumulation of nutrients in individual species (Table 1). Negative relations between heavy metals and essential macronutrients such as Ca/K are the most commonly studied: it was also found that Ni uptake in hyperaccumulator is mainly mediated by Ca channels while K channel blocker had no effect (Mohseni et al. 2019). We observed variation in 100 µM Ni-induced depletion of Ca in individual species: 100 µM Ni significantly depleted shoot or root Ca content in two out of three species (Table 1) and the correlation between Ca and Ni amount in all species showed significant negative trend ($r = -0.4985$ and $r = -0.4936$ in shoots and roots, respectively). In line with our data, Ca had negative impact on Ni accumulation in *Cucurbita pepo*, confirming negative relation in various species (Valivand and Amooaghaie 2021a). Despite depletion of Mg (a component of the chlorophyll molecule) in *H. perforatum* and *H. olympicum* shoots (significant $r = -0.6309$), no visible chlorotic symptoms were observed. Ni-induced decrease in K and P accumulation was observed in shoots of two out of three species and the correlations with Ni content were not significant ($p = 0.254$ and 0.336) while it was slightly significant for root K versus Ni ($r = -0.3904$, $p = 0.044$). On the contrary, 10 µM Ni excess had almost negligible impact on P amount in rice (Wang et al. 2019) and 120 µM Ni excess showed no impact on Mg amount in chamomile tissue, where accumulation of K dropped by ca. 25% (Kováčik et al. 2009b).

Among micronutrients, shoot accumulation of Fe, B, Zn and Cu decreased at least in one out of three species under 100 µM Ni treatment (correlation between shoot Fe and B versus Ni was significantly negative, $r = -0.5158$ and -0.7037) but the content of Mn and Mo

remained unaffected. Similar observations were done in the root tissue (significant negative correlation between B and Ni, r = -0.4745). Notwithstanding this, Zn amount increased in *H. olympicum* roots under high Ni dose, which has no immediate explanation. We note much higher content of Fe in the roots of all species compared to shoots, as previously observed in other plants (Kováčik et al. 2009b; Wang et al. 2019). Depletion of Fe in some individual species is in line with report from some Ni-exposed rice cultivars (Wang et al. 2019). Unlike our data where Cu remained unaffected by Ni excess in the roots, chamomile exposed to 60 or 120 µM Ni even showed higher accumulation (Kováčik et al. 2009b). All these observations seem to be species-specific and probably affected by other factors such as the amount of secondary or chelating metabolites. It was therefore interesting to find that the accumulation of Co (considered as beneficial for some plants and it is a component of the modified Hoagland solution we used, see Supplementary Table S1) increased in response to 100 µM Ni in almost all organs/treatments (for all species, r = 0.4106 and 0.9292 in shoots and roots, respectively) and the significance of this observation needs further study. Sodium is another beneficial element for some plants which is used (in the form of NaOH) to balance pH of the solution: its amount even increased in *H. orientale* shoots and roots under 100 µM Ni and the same was observed in *H. olympicum* roots, suggesting that Na may replace, at least partially, potassium in osmotic processes. Calculation of the translocation factor (shoot/root ratio) revealed the highest value of some nutrients (K, P and Mn) in control or 100 µM Ni-exposed (K, Ca, P, B, Mn and Zn) *H. orientale* plants, indicating effective root-to-shoot translocation and thus better resistance to Ni excess. In comparison with the previous study in hydroponics (modified nitrogen content), control *H. perforatum* plants in the present work had similar TF values of K, Ca, Mg and Fe (Kováčik et al. 2022). The quantitative comparison of non-essential, toxic or rare elements is provided in the section 3.5.

3.3. Accumulation of Ni and essential elements in outdoor growing plants

To compare Ni uptake in hydroponics with natural soil conditions, shoots of all three *Hypericum* species growing naturally near the faculty (planted two years ago) were collected and analyzed for the same 12 essential elements (added to hydroponics) plus Ni (cf. Tables 1 and 2). In agreement with control plants in hydroponics, the amount of Ni was the highest in *H. orientale* (Table 2) with values in two other species around 1 ppm (1 µg/g) typical for common plant tissue. In line with these data, dandelion collected from urban localities contained ca. 3 µg Ni/g in the leaves and even less in the inflorescence (Kováčik et al. 2016). In the extensive study from Italy involving several sites with various edaphic conditions, aerial parts of *H. perforatum* contained 1.3 – 2.4 µg Ni/g and the highest value (7.7 µg Ni/g) was reported at the locality with ultramafic magmatic rocks (Bonari et al. 2019). Similarly, data from serpentine sites in Bulgaria revealed 1.2 – 11.7 µg Ni/g in flowering shoots of *H. perforatum* (Pavlova and Karadjova 2013) so we may conclude that Ni content in outdoor growing shoots (no flowers during harvest) is within common range in plants. It was interesting to find that the correlation of shoot Ni in all three species was highly significant between laboratory and outdoor samples ($r = 0.8084$) and the accumulation of Ni was significantly higher in *H. orientale* under both modes of cultivation.

Outdoor plants revealed abundance of elements in descending order K < Ca < P < Mg and the lowest amount of Cu, Co and Mo, which was also observed in almost all hydroponically grown counterparts of the respective species (cf. Tables 1 and 2). Among essential nutrients (the same as added to hydroponics mentioned above), it was visible that *H. orientale* shoots contained the highest amount of many elements, mainly ca. doubled amount of Ca and Fe (Table 2). In *H. perforatum* shoots, we found similar content of Cu (6.6 – 8.6 µg/g), Zn (22 – 30.7 µg/g) and Co (0.09 – 0.97 µg/g) as reported in natural populations from

Italy (Bonari et al. 2019) and similar values (5.6 – 9.1 µg Cu/g, 23 – 46 µg Zn/g, 12 – 32 µg Mn/g, but only 40 – 99 µg Fe/g) were reported in plants from Bulgaria (Pavlova and Karadjova 2013) despite serpentine nature of some localities in the cited papers. On the contrary, polluted locality from Turkey revealed almost 500 µg Fe/g (Kadioglu et al. 2005) and *H. perforatum* samples from a former military area in the Czech Republic contained much higher amount of Cu (188 µg/g) and Zn (95.4 µg/g) if compared to our data (Sládková et al. 2015 and Table 2).

3.4. BAF of essential elements in hydroponically versus outdoor-grown plants

The Faculty of Education of the University of Trnava (western Slovakia), where the *Hypericum* plants grew outdoor, is located in an industrial area with a car service or metalworking companies nearby. Despite this fact, e.g. K and Fe amounts in this soil (Supplementary Table S6) were comparable with 29.5 mg Fe or 8.8 mg K/g in garden soil originated from western Slovakia (Kováčik et al. 2014) while soil from eastern Slovakia contained only ca. 3 mg Fe or 2.3 mg K/g (Kováčik et al. 2012). Other essential elements such as Mg, Mn, Zn, Cu or Na were present at the level similar to papers cited above, while Ca was much more accumulated in the soil in the present study and the explanation is unclear. Additional toxic or rare elements are commented in the next section.

The composition of the nutrient solution used for hydroponics is mentioned in the method section and quantity of essential elements (i.e. 12 elements which were added to hydroponics, including Na and Co, which are considered beneficial to some plants) is presented in Supplementary Table S1. If we theoretically consider 1 mL of solution as 1 g, we may compare the amount of nutrients in the soil and the solution (Supplementary Tables S1 and S6): all 12 elements are much more abundant in the soil compared to hydroponics by a factor of 100 – 5000 (e.g. 7.43 mg K/g soil vs. 0.07 mg K/mL or 0.1139 mg Zn/g soil vs.

0.00002053 mg Zn/mL). We note that the full amount of elements (quantified as pseudo-total content) is not available in the soil (typically about 1% of the total content is water soluble, Kováčik et al. 2014), so the final comparison with hydroponics would be "less dramatic". Notwithstanding this, the accumulation of K, Zn or Mo was higher in hydroponically-grown control shoots (cf. Tables 1 and 2). Subsequent analyses showed a highly positive correlation in the accumulation of the 12 essential elements between plants growing in the laboratory and outdoors, mainly in the case of *H. perforatum* (r = 0.9510), followed by *H. olympicum* (r = 0.7924) and *H. orientale* (r = 0.5446).

Since the metal content in tissue is also a function of their amount in the environment (content in soil or solution, as above), we calculated the bioaccumulation factor (BAF, content in shoot/soil or solution) of the three species (controls only) to compare the efficiency of uptake of essential elements. Because of lower amount of essential elements in hydroponics (in comparison with soil), BAF values of all species were much higher in hydroponics (Supplementary Table S7), often by a factor over 100 (in the case of Zn, there was almost 10,000-fold difference). A comparison with the previous studies using plants growing in the soil showed that e.g. four crops had higher BAF values of K, Ca or Zn while BAF values of Mg, Mn, Cu or Na were rather similar (Kováčik et al. 2014). Also, flowers of medicinal plant chamomile had higher BAF values of K, Ca, Na or Fe while BAF values of Mg, Zn or Cu were similar to present data (Kováčik et al. 2012). In the *H. perforatum* aerial parts originated from various localities in Italy, BAF (aerial part/total soil metal content) of Cu was 0.12 – 0.67 and of Zn 0.15 – 0.39 (Bonari et al. 2019), which is similar to our range (Supplementary Table S7). Lower shoot BAF values for Cu (0.19 – 0.26), Mn (0.009 – 0.026) or Co (0.002 – 0.014) were reported in *H. perforatum* from Bulgaria (Pavlova and Karadjova 2013). However, BAF of essential micronutrients such Cu, Zn or Mn is rather similar in *Hypericum* species from various countries and edaphic conditions but BAF of essential macronutrients

seems to be lower in comparison with crops mentioned above. At the same time, plants growing outdoor had BAF values over 1 for K, P, B and Mo, suggesting that given elements are actively accumulated in their shoots. Among control plants growing in hydroponics, the highest BAF values were also observed for P and B and BAF of Zn was exceptionally high (Supplementary Table S7). Previous study with *H. perforatum* in hydroponics also showed (in control plants) that BAF of K was higher than that of Ca or Fe and absolute values differed only up to 2-fold (Kováčik et al. 2022). On the contrary, dandelion in hydroponics showed higher BAF values of K, Ca or Mg (750, 272 and 167, respectively), indicating higher accumulation of given elements in comparison with *Hypericum* (Kováčik et al. 2019). In the subsequent correlation analyses, BAF values for Zn were excluded (because they show great numerical difference between laboratory and outdoor plants) and the remaining 11 essential elements revealed the same trend as observed above for the absolute content of elements, i.e. the strongest positive correlation in *H. perforatum* (r = 0.7434), followed by *H. olympicum* (r = 0.6920) and *H. orientale* (r = 0.6321). It therefore seems that the accumulation of elements as well as their bioaccumulation is in not extensively affected by the mode of cultivation though some elements showed variability.

3.5. Non-essential, toxic and rare elements in outdoor growing versus hydroponic plants

Non-essential elements such as Al, Sr, Ti, Ba, Li, Sn, Sb, Be, V and else are commonly present in soil. The amount of mentioned elements (Supplementary Table S6) is lower or within the range observed in the soils from urban areas e.g. in Italy and Iran, i.e. ~22 – 32 mg Al/g, 1 mg Ti/g, 300 µg Ba/g, 40 – 300 µg Sr/g, 60 µg V/g, 6 µg Sn/g or 2 µg Sb/g (Bonanno 2011; Modabberi et al. 2018; Bonari et al. 2019; Cicchella et al. 2020). It seems that the amount of given elements is not higher than the usual soil content. Accumulation of Sr in shoots we observed (Table 3) is similar to 7 – 19 µg Sr/g in shoots of *H. perforatum* from in

Italy (Bonari et al. 2019) but we observed higher shoot BAF values (0.18 – 0.39, Supplementary Table S8) compared to mentioned study owing to lower soil Sr amount (BAF of 0.05 – 0.17, Bonari et al. 2019). Interestingly, accumulation of some elements found in *Hypericum* species (Table 3) is similar to data from *H. perforatum* in the Czech Republic, e.g. 8 µg Ba/g, 2.6 µg Ti/g or 287 ng Sb/g (Sládková et al. 2015) and respective BAF values (Supplementary Table S8) of Ti and V were similar. However, the BAF values of many non-essential elements were low (<0.01, Supplementary Table S8) indicating their low accumulation in plants despite availability in the soil. On the other hand, higher BAF values of Sb and Sr reflect higher accumulation in plants and Sr was 2nd the most abundant element in *Hypericum* species growing outdoor (Table 3).

Specific non-essential elements are those which are commonly considered as toxic metals, mainly Cd, Cr, Pb or As. Cadmium is a contaminant of global concern and its amount in the soil (Supplementary Table S6) was within the range observed in other Slovak soils though the absolute values were rather low in the industrial city Košice (6 – 114 ng Cd/g, Kováčik et al. 2016). Also, the amount of Cr in soil of the present study was similar to earlier data from Košice (35 – 61 µg/g) but Pb content (126 – 243 µg/g in Košice) was much lower here (24.8 µg/g, Supplementary Table S6). Amount of As we detected in the soil is similar to other cities (Modabberi et al. 2018; Cicchella et al. 2020). It was surprising to find that the amount of Cd in outdoor plants (Table 3) was much higher compared to dandelion leaves collected in another Slovak city (2 – 103 ng/g, Kováčik et al. 2016) and various crops cultured in the soil with higher Cd amount also contained less Cd in the shoots (55 – 279 ng/g, Kováčik et al. 2014). In agreement, BAF value over 2 was recorded in all *Hypericum* species (Supplementary Table S8) while it was less than 1 in crops (Kováčik et al. 2014) or in *Hypericum* from serpentine localities (Pavlova and Karadjova 2013). On the contrary, several species of *Hypericum* from Austria contained higher Cd amount in aerial parts and had higher

BAF values as we found (close to or over 2.5), indicating that at least some populations are prone to higher accumulation of Cd (Chizzola and Lukas 2005). In the mentioned dandelion, Cr content and its BAF values were similar to present data while Pb amount in *Hypericum* was considerably lower (Table 3 and Kováčik et al. 2016). Compared to other species, *Hypericum* species appear to accumulate more Cd but less Pb. Similar trend of BAF values for Cd and Pb was detected in *Juncus effusus* (Dołęgowska et al. 2022).

Rare earth elements (REE) are identified by the IUPAC as a group of 17 elements with similar physicochemical characteristics: 15 out of 17 these elements belong to the group of lanthanides, including La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu plus Sc and Y (Ramos et al. 2016). Tens of µg/g (15.4 – 86) were detected for Ce > La > Nd > Y while other elements (Eu, Yb, Er, Dy, Gd, Sm and Pr) accumulated in the range of 1.2 – 9.1 µg/g and values below 1 µg/g were detected for Tb, Ho and Lu (Supplementary Table S6). These values are in line with general trend and quantity in the earth's crust (Ramos et al. 2016) and similar quantitative values (tens of µg/g for Ce, La and Nd but values below 1 µg/g for Tb and Ho) were detected in rhizosphere soil in Poland (Dołęgowska et al. 2022). It was interesting to find that the accumulation of given REE in *Hypericum* plants reflected an order detected in the soil, i.e. the highest amount of Ce, La, Nd and Y but the lowest amount of Tb, Ho and Lu was observed (Table 3). For this reason, BAF of all these elements was at the level below 0.01 and reflect very low accumulation in plants (Supplementary Table S8). The same quantitative trend of REE was found in *Juncus effusus* plants in Poland and respective BAF values were also at the level we recorded (Dołęgowska et al. 2022), indicating generally low bioaccumulation of given elements in plants.

Rhenium was the only element detected in the soil (Supplementary Table S6), which was not present in the plants (Table 3). Its quantity was slightly higher that the data in agricultural soils (0.23 – 0.34 ng/g, Tagami and Uchida 2008) so further monitoring of this

element in soils is needed. Radioactive metallic elements U and Th were detected in the soil differing by ca. 10-fold in favor of Th (Supplementary Table S6) and the quantitative levels are similar to those from soils in Italy (Cicchella et al. 2020). Their accumulation in plants was ca. 1000-fold lower than in the soil (i.e. ng versus µg), yielding BAF values below 0.01 as observed for REE. Amount of valuable elements Pt and Pd differed by a factor of ca. 70 (5 versus 340 ng/g, Supplementary Table S6) and similar maximal values for Pd were observed in the soil from Italy (432 ng/g, Cicchella et al. 2020). Relatively high amount of Pd may originate from automobile catalytic converters in urban areas as previously suggested by Zuzolo et al. (2018). It was therefore interesting to find that Pd and Pt content in plant shoots was similar (12 – 27 ng/g, Table 3), yielding low BAF value for Pd but BAF value over 3 for Pt (Supplementary Table S8). High content of Pd (in comparison with Pt) has been recorded in the leaves and roots of grass *Phragmites* in Italy (Bonanno 2011). Monitoring of these rare elements is therefore a challenge for future in the urban areas.

Owing to numerous non-essential elements detected in the soil (41 elements including Ni), we monitored their accumulation not only in the outdoor-growing plants as mentioned above but also in respective control plants of individual species cultured in hydroponics (Table 3). Data revealed that the order of the accumulation of non-essential elements differed in outdoor and laboratory plants, with Al and Sr being the major ones in both culture regimes. The 11 elements were undetectable or present at the level below 0.1 ng/g in hydroponic shoots (Table 3). However, the accumulation of all elements was significantly lower (at least 10-fold) in hydroponically grown plants, and some elements, such as Pr, were even 80-fold less abundant (Table 3). For this reason, sum of REE was ca. 60 – 100-times lower in hydroponics (in comparison with outdoor-growing plants) and sum of all non-essential elements was ca. 30 – 40-times lower. Another calculation in individual species revealed that the amount of REE from all non-essential elements was similar, i.e. 0.29 – 0.35% in species

growing outdoor and 0.12 – 0.15% in species growing in hydroponics (calculable from Table 3). It remains an open question what the source of non-essential elements in hydroponically grown plants is: pre-cultivation in sand (prior to transfer to hydroponics) and eventual traces of elements in distilled water used for hydroponic cultivation are the most probable reasons. However, values we detected in hydroponics are much lower than those recorded in soybean pre-cultured in quartz sand and then cultured in nutrient solution where control shoots contained up to 21 µg Cd/g and 187 µg Al/g (Shamsi et al. 2007), i.e. 400- and 20-times more than the highest values we observed in *H. orientale*. However, it was clearly observed that the accumulation of non-essential elements was typically the highest just in *H. orientale* under both modes of cultivation (Table 3). It was also found that bioaccumulation of given elements is very low but BAF values of Cd and Pt reached values over 1 and further field studies are needed (Supplementary Table S8).

3.6. Metabolites in hydroponically and outdoor growing plants

The accumulation of soluble phenols (the test is often referred to as "total phenolic content", which is not true) was highest in the control *H. perforatum* shoots and significant difference was observed between *H. olympicum* and *H. orientale* control shoots and roots (Fig. 1). The impact of Ni excess on phenols was rather negligible and only 100 µM Ni elevated them in *H. perforatum* shoots and roots by ca. 20% (Fig. 1). In agreement, chamomile cultured in hydroponics with 3 – 120 µM Ni showed enhanced accumulation of phenols in the roots at the highest Ni dose only (Kováčik et al. 2009a). Another medicinal plant dandelion responded to 30 µM Ni by an increase in soluble phenols mainly in the leaves and responses in roots were less intense compared to excess Cd (Kováčik et al. 2019). It seems that the reactions of phenols to Ni excess are less intense and, in agreement, even dose of 50 mg Ni/L (~850 µM) were needed to increase them in *Cucurbita pepo* (Valivand and Amooaghaie 2021b).

Assay of the sum of flavonols ($AlCl_3$ reagent) revealed a trend different from that of soluble phenols and *H. olympicum* control shoots and roots contained the highest amount (Fig. 1). Unlike our data, natural population of *H. perforatum* and *H. olympicum* from Bulgaria generally did not reveal significant differences of sum of flavonols (Krasteva et al. 2013) probably due to the use of hyperoside for calibration (we used aglycone quercetin) but reported quantities are similar to our data (11 – 16 mg/g DW). Responses to excess Ni also varied, with no effect observed in the shoot of either species, but a significant effect of at least 100 µM Ni was observed in the roots of *H. olympicum* and *H. orientale* (Fig. 1). Roots that are in direct contact with a solution containing Ni ions can be expected to accumulate more flavonols as potent antioxidant substances. A more pronounced effect of Ni on the amount of flavonols was also found in chamomile roots (in comparison with shoots) exposed to 60 or 120 µM Ni (Kováčik et al. 2009a).

Subsequent detailed profiling of 16 individual phenolic metabolites carried out by LC-MS was done in the shoot due to its use for medicinal purposes. As expected, great variability between species was observed, e.g. *H. olympicum* contained ca. 10 – 20-times more chlorogenic acid and ca. 2 – 3-times more of the major flavonoids (quercetin-3-O-glucoside and quercetin-7-O-glucoside) than the other two species (Table 4). However, the greatest difference was observed for rutin, which was almost 2500-times more abundant in *H. perforatum* than in *H. orientale*. Similar considerable variability of phenolic metabolites has also been observed in other species of the genus *Hypericum* (Camas et al. 2014). In terms of the impact of Ni excess, we found rather negligible effect as mentioned above for sum of flavonols. Interestingly, catechin was the only metabolite that increased approximately 30-100% in response to 100 µM Ni in all species and partially in response to 1 µM Ni in two of the three species (Table 4). The increase in an isomer of catechin (epicatechin) was previously observed in *H. perforatum* shoots exposed to Cd and La excess in hydroponics (Babula et al.

2015) so it seems that this flavan-3-ol may have a role in response to metals. Among phenolic acids, chlorogenic acid and related metabolite 3-O-feruloylquinic acid decrease under 100 µM Ni at least in one species while Ni had no impact in the shoots of medicinal plant chamomile (Kováčik et al. 2009a). Flavone apigenin-7-O-glucoside was less abundant metabolite and its quantity decreased in two out of three species under 100 µM Ni treatment (*H. perforatum* and *H. olympicum*) and the same was observed for two flavonol glycosides (quercetin 3-rhamnoside and quercetin 7-rhamnoside, Table 4): correlation analyses confirmed that Ni accumulation had significantly negative impact ($p < 0.01$) on the amount of quercetin 3-rhamnoside and quercetin 7-rhamnoside in *H. olympicum* ($r = -0.8189$ and $r = -0.8928$, respectively) and negative correlation between sum of metabolites (detected by LC-MS, Supplementary Fig. S2) and Ni content has also been observed in this species ($r = -0.7048$) but the correlation was positive in *H. orientale* ($r = 0.6978$). When comparing individual species, phenolic metabolites decreased especially in *H. olympicum* shoots exposed to 100 µM (10 out of 16 compounds, Table 4). Such reactions are rather surprising because the accumulation of phenols usually increases with metal excess even in *Hypericum*, although the final reactions of the individual metabolites depend on the applied metal (see variable impact of Cd and La, Babula et al. 2015). However, detailed quantification of metabolites allows us to see subtle differences between treatments that are not visible in the spectrophotometric assay (µg/g versus mg/g DW), and therefore we noted the specificity of the Ni effect. In agreement, Ni excess had negative impact on the accumulation of specific metabolites in *H. perforatum* (Murch et al. 2003). Correlation analyses showed a strong positive correlation of the sum of individual metabolites (Supplementary Fig. S2) with the sum of flavonols ($r = 0.8981$) but not with soluble phenols ($r = 0.0848$) in the shoot tissue, which is in line with the fact that mainly individual flavonoids were quantified by LC-MS. Correlation between shoot Ni amount and sum of flavonols or sum of metabolites was low in *H. perforatum* ($p = 0.15$

and 0.54) and similar result is calculable between methanol extract of *H. perforatum* flavonoids and Ni content in samples originated from various localities in Bulgaria (r = 0.036, Pavlova et al. 2015). In contrast, we found that Ni was positively correlated with shoot soluble phenols in *H. perforatum* (r = 0.7873) so it probably contains metabolites that were not included in our assay. In agreement, common weed *Stellaria media* showed a positive correlation of Ni content with phenols but negative with flavonoids when cultured under 100 µM Ni (Salinitro et al. 2020). Overall, data indicate that Ni had more pronounced impact on phenols in the roots (where also strongly positive correlation between root Ni and soluble phenols or sum of flavonols in *H. perforatum* or *H. orientale* was visible, r = 0.8838 and 0.7820) while detailed analyses in shoots revealed even negative impact on individual metabolites mainly in *H. olympicum*.

Owing to availability of outdoor growing plants, comparison of metabolite production with laboratory-cultured plants is an interesting aspect. We observed, as expected, that the amount of soluble phenols and flavonols in two out of three species was higher in outdoor plants (Supplementary Fig. S1), which may most likely be induced by meteorological factors such as sunlight or temperature change. We have, however, no exact explanation why soluble phenols in *H. olympicum* did not differ and flavonols were even less accumulated in outdoor plants (Supplementary Fig. S1). Also sum of individual metabolites where flavonoid dominated (calculated from Table 2) confirmed that they were less accumulated in outdoor plants of *H. olympicum* (Supplementary Fig. S2). Detailed quantification of individual metabolites revealed another interesting finding: phenolic acids (chlorogenic acid and 3-O-feruloylquinic acid) were less accumulated in all outdoor plants but flavonoids including major compounds catechin, rutin (in *H. perforatum* mainly) or quercetin-7-O-glucoside were more accumulated in outdoor plants (but not in *H. olympicum* as mentioned above, cf. Tables 2 and 4 combined in Supplementary Fig. S2). These data indicate that environmental factors

have great impact on the biosynthesis of phenolic metabolites, but individual species also differed as observed in the case of *H. olympicum*.

Amino acids, as key components of proteins, are often affected by the excess of metals. We found the highest amount of free amino acids (FAA) in control shoots of *H. olympicum* and 100 µM Ni dose depleted them (by 23%, Fig. 1), leading to a strongly negative correlation between shoot Ni and FAA (r = -0.9022). On the contrary, *H. orientale* cultured with 1 µM Ni revealed slightly but significantly enhanced amount of FAA, which is in line with various reaction of phenols in the given species as mentioned above. In the roots, 100 µM Cd dose depleted FAA in two out of three species and no significant stimulation was observed (Fig. 1). Unlike present data, chamomile exposed to 120 µM Ni had elevated amount of FAA by ca. 26% where Ni was also much accumulated in the shoot tissue (Kováčik et al. 2009b). On the contrary, *Trigonella corniculata* cultured with 25 – 100 mg Ni/kg soil (ca. 0.43 – 1.7 mM) had reduced amount of FAA by ca. 80% at the highest dose (Younis et al. 2020). These data indicate that biochemical responses to Ni excess differ even between species of the same genus. Subsequent comparison of laboratory and outdoor-growing plants revealed considerably lower amount of FAA in the shoot tissue of all species growing outdoor and non-significant differences between species (Supplementary Fig. S1). For this reason, correlation of FAA content between laboratory and outdoor-growing plants was insignificant (r = 0.078). This may be related either to the higher availability of soluble nitrogen in hydroponics (leading to faster metabolism and production of amino acids) or to the less stressful conditions in hydroponics. However, all the metabolic data clearly showed not only different effects of nickel in hydroponics, but also species-specific differences. *H. olympicum* was found to contain the highest amount of valuable phenols (chlorogenic acid and quercetin glucosides).

3.7. The principal component analyses

Using the parameters selected in the shoots and roots, a graphical representation of the sample similarities and dissimilarities of the samples was obtained by a Principal Component Analysis (PCA). 2-D biplots visualize both the orthonormal principal component coefficients for each variable and the principal component scores for each observation in a single plot. The direction and length of the vector indicate how each variable contributes to the two main components of the plot. The points are scaled with respect to the maximum score value and maximum coefficient length, so only their relative locations can be determined from the plot. Figure 2A illustrates that the amount of individual phenols contributed in particular to the separation of individual species. Within the first two principal components (PC), 77.2 % of the total variance was explained, with 45.39 % in the first dimension and an additional 31.79 % in the second dimension. Separation in the roots (Fig. 2B) did not follow the same pattern as in the shoots, because the individual phenolic metabolites were not quantified in the root tissue. The samples are separated by species type along PC1, with *H. perforatum* samples located on the left-hand side of the PCA biplot (Figure 2B) with respect to the FAA and Na content. The *H. orientale* and *H. olympicum* samples are positioned in the middle and right-hand sides of the biplot, respectively. *H. olympicum* was separated with respect to micronutrients mainly in 1 µM treatment (Fig. 2B). Along PC2, mainly Ni, Ca, K, and B contents separate the *H. orientale* samples, depending on the treatment.

Comparison of controls of individual species cultured in hydroponics (Fig. 3A) versus outdoor growing plants (Fig. 3B) gave very similar conclusions for the separation of the three groups of objects, i.e. the three groupings of the plant objects were reasonably separated on the PC1 and PC2. The PC1 versus PC2 plot accounted for 89.47 and 90.84 % of the data variance for hydroponic controls (Fig. 3A) or for outdoor controls (Fig. 3B). Mainly individual flavonoids (rutin and quercetin derivatives) contributed to this separation.

4. Conclusions

This study revealed that Ni accumulated most in *H. olympicum* growing in hydroponics (67.9 and 2433.9 µg/g DW in shoots and roots, respectively, when treated with 100 µM Ni) and that essential elements were rather suppressed by excess Ni in all species (except Co and Na). High Ni accumulation in *H. olympicum* could be a reason for depletion of free amino acids and some individual phenolic metabolites (quercetin glycosides mainly) in the shoots (and significant negative correlations were confirmed) while soluble phenols and sum of flavonols rather increased in shoot or root tissue of individual species. Plants grown outdoor contained lower amounts of some essential elements (K, Zn or Mo) than plants grown in hydroponics, but the amounts of other elements were approximately similar, suggesting that uptake efficiency, especially in *H. perforatum*, is not influenced by the mode of cultivation. On the contrary, plants cultured in hydroponics contained lower amount of non-essential, toxic and rare earth elements (30 – 100-fold). Higher amount of soluble phenols and sum of flavonols in outdoor species was expected but it was not observed in *H. olympicum*: detailed quantification of individual phenols confirmed this trend and it nicely supports various regulation of metabolism even in the same genus. However, we identified *H. olympicum* as a promising source of valuable metabolites (chlorogenic acid and quercetin derivatives) and control *H. orientale* accumulated elements the most efficiently (38 out of 53).

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Author contribution

Experimental design, plant cultivation and spectrophotometry (JK and MV), assay of elements (LH and JP), LC-MS analyses (GG and YR), statistics (JK and LH), manuscript preparation (JK) and manuscript revision (LH and YR).

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Table 1. Accumulation of essential macronutrients and micronutrients (present in the cultivation solution) including nickel in the shoots and roots of three *Hypericum* species cultured in hydroponics and exposed to nickel (1 or 100 μM) over 7 days. Data are means (n = 3) and for the lucidity of table, SD values are not shown. Values within rows followed by the same letter(s) are not significantly different according to Tukey's test (at P<0.05 level). TF indicates translocation factor (ratio of shoot/root content of the given element in the given treatment).

	Hypericum perforatum			*Hypericum olympicum*			*Hypericum orientale*		
shoot	control	1 Ni	100 Ni	control	1 Ni	100 Ni	control	1 Ni	100 Ni
Ni (μg/g DW)	0.44 g	1.75 ef	16.6 c	0.58 g	2.09 e	67.9 a	1.43 f	3.30 d	58.2 b
K (mg/g DW)	21.5 c	23.1 c	16.3 d	25.0 bc	24.2 bc	23.9 bc	29.8 ab	33.2 a	22.4 c
Ca (mg/g DW)	7.19 a	7.40 a	5.65 b	7.29 a	5.88 b	5.80 b	5.54 b	5.99 b	5.23 b
P (mg/g DW)	2.49 c	2.61 c	1.79 d	3.23 b	3.25 b	3.02 bc	4.79 a	5.07 a	3.08 bc
Mg (mg/g DW)	2.43 a	2.46 a	1.83 bc	2.47 a	2.46 a	1.96 bc	1.94 bc	2.12 ab	1.56 c
Fe (μg/g DW)	131.3 ab	125.5 ab	100.7 bc	134.4 a	140.1 a	97.5 c	92.0 c	103.2 bc	93.8 c
B (μg/g DW)	68.1 ab	63.3 abc	41.4 d	72.1 a	55.9 bc	46.3 cd	75.2 a	68.6 abc	45.4 d
Mn (μg/g DW)	60.3 cd	65.3 c	49.9 d	94.7 ab	93.6 ab	90.2 ab	100.4 ab	103.5 a	84.3 b
Zn (μg/g DW)	51.5 c	43.4 c	28.9 d	84.0 a	86.3 a	67.0 b	88.6 a	88.8 a	66.7 b
Na (μg/g DW)	14.0 cd	12.9 cd	16.6 bc	40.9 a	39.8 a	40.0 a	11.3 cd	8.87 d	22.9 b
Cu (μg/g DW)	4.57 b	4.56 b	4.01 b	4.50 b	4.26 b	4.84 b	6.81 a	7.15 a	4.55 b
Mo (μg/g DW)	2.45 b	2.63 b	2.28 b	8.40 a	10.8 a	10.5 a	2.63 b	2.77 b	2.42 b
Co (μg/g DW)	0.45 cd	0.45 cd	0.43 d	0.52 bc	0.54 b	0.66 a	0.27 e	0.28 e	0.39 d
root									
Ni (μg/g DW)	1.28 e	9.32 d	1499.6 b	1.62 e	10.9 cd	2433.9 a	1.51 e	14.6 c	1524.7 b
K (mg/g DW)	51.1 bc	49.3 bc	48.4 c	56.9 ab	45.7 c	48.2 c	60.1 a	45.2 c	36.5 d
Ca (mg/g DW)	5.63 ab	5.29 bc	5.97 ab	6.26 a	5.92 ab	4.78 c	6.32 a	4.97 c	3.74 d
P (mg/g DW)	2.88 cd	2.79 cd	2.76 cd	5.48 a	5.04 a	5.27 a	4.20 b	3.04 c	2.24 d
Mg (mg/g DW)	6.72 b	6.91 b	6.95 b	4.97 c	3.19 d	3.54 cd	10.5 a	7.83 b	4.56 c
Fe (μg/g DW)	1619 d	1650 d	1641 d	4863 a	4640 a	4745 a	3604 b	3380 b	2475 c
B (μg/g DW)	23.7 b	22.4 b	20.0 b	28.6 a	20.3 b	21.2 b	32.1 a	20.5 b	14.4 c
Mn (μg/g DW)	50.8 c	38.5 d	40.6 cd	145.3 a	104.7 b	111.3 ab	36.5 de	26.0 e	14.8 f
Zn (μg/g DW)	291.0 d	306.3 d	340.5 d	524.6 bc	508.3 bc	811.2 a	587.1 b	472.6 c	445.7 c
Na (μg/g DW)	210.7 a	128.0 bc	147.3 b	79.7 d	73.2 d	104.9 c	67.2 d	69.2 d	96.4 c

Cu (µg/g DW)	9.10 c	9.20 c	9.96 bc	28.1 a	27.8 a	29.6 a	14.6 b	13.1 bc	14.2 bc
Mo (µg/g DW)	4.01 d	3.85 d	3.99 d	6.81 b	6.83 b	6.43 b	8.75 a	6.34 bc	5.51 c
Co (µg/g DW)	3.06 e	3.27 de	7.68 b	3.37 de	3.16 de	8.89 a	3.93 d	3.11 de	4.97 c
TF values									
Ni	0.36 b	0.19 c	0.01 e	0.35 b	0.19 c	0.03 d	1.02 a	0.24 bc	0.03 d
K	0.42 de	0.47 cd	0.33 e	0.44 cd	0.52 bc	0.49 bc	0.50 bc	0.73 a	0.61 ab
Ca	1.27 ab	1.40 a	0.94 c	1.16 ab	0.99 bc	1.21 ab	0.87 c	1.20 ab	1.41 a
P	0.86 c	0.93 bc	0.64 d	0.58 d	0.63 d	0.57 d	1.14 b	1.66 a	1.37 ab
Mg	0.36 c	0.35 c	0.26 cd	0.49 b	0.78 a	0.55 b	0.18 d	0.27 cd	0.34 c
Fe	0.081 a	0.076 a	0.061 b	0.027 cd	0.030 cd	0.021 d	0.025 cd	0.031 cd	0.037 c
B	2.87 a	2.82 ab	2.08 b	2.52 b	2.75 ab	2.20 b	2.34 b	3.21 a	3.15 a
Mn	1.18 e	1.70 d	1.23 de	0.65 f	0.89 e	0.81 ef	2.83 c	3.97 b	5.79 a
Zn	0.17 ab	0.14 bc	0.09 c	0.16 ab	0.17 ab	0.08 c	0.15 ab	0.19 a	0.15 ab
Na	0.07 e	0.10 e	0.11 e	0.51 a	0.54 a	0.38 b	0.16 d	0.12 de	0.23 c
Cu	0.50 ab	0.49 ab	0.40 bc	0.16 d	0.15 d	0.16 d	0.47 ab	0.54 a	0.32 c
Mo	0.61 cd	0.68 c	0.57 d	1.23 b	1.58 a	1.63 a	0.30 f	0.43 e	0.44 e
Co	0.15 a	0.14 ab	0.05 c	0.15 a	0.17 a	0.07 c	0.06 c	0.09 bc	0.08 bc

Table 2. Accumulation of essential mineral elements (including nickel) and selected metabolites (µg/g DW for individual phenols, mg/g DW for soluble phenols and flavonols and µmol/g DW for amino acids) in the control shoots of three *Hypericum* species growing under natural soil conditions (see Supplementary Table S6 for elements in the soil). Data are means ± SDs (n = 3). Values within rows followed by the same letter(s) are not significantly different according to Tukey's test (at P<0.05 level). Note high content of chlorogenic acid and quercetin glucosides in *H. olympicum*. nd – not detected.

minerals in shoot	*H. perforatum*	*H. olympicum*	*H. orientale*
Ni (µg/g DW)	1.51 ± 0.18 b	1.49 ± 0.32 b	2.48 ± 0.29 a
K (mg/g DW)	10.1 ± 1.17 a	11.6 ± 0.59 a	11.6 ± 0.74 a
Ca (mg/g DW)	5.48 ± 0.22 b	4.70 ± 0.34 b	9.96 ± 0.86 a
P (mg/g DW)	2.35 ± 0.21 a	1.62 ± 0.14 b	2.10 ± 0.07 a
Mg (mg/g DW)	2.09 ± 0.13 ab	1.83 ± 0.14 b	2.40 ± 0.12 a
Fe (µg/g DW)	132.5 ± 15.8 b	147.3 ± 16.5 b	382.0 ± 19.3 a
B (µg/g DW)	75.3 ± 5.09 a	69.7 ± 4.30 a	76.6 ± 4.34 a
Mn (µg/g DW)	46.3 ± 3.90 a	22.6 ± 1.72 b	38.0 ± 2.19 a
Zn (µg/g DW)	29.9 ± 1.65 a	17.2 ± 1.60 b	27.8 ± 1.43 a
Na (µg/g DW)	38.7 ± 3.43 b	45.4 ± 5.20 b	65.5 ± 3.42 a
Cu (µg/g DW)	11.0 ± 1.21 a	6.06 ± 0.29 b	9.98 ± 0.66 a
Mo (µg/g DW)	0.67 ± 0.023 b	1.28 ± 0.13 a	1.25 ± 0.11 a
Co (µg/g DW)	0.34 ± 0.016 a	0.18 ± 0.022 b	0.20 ± 0.013 b
phenols in shoot			
chlorogenic acid	38.7 ± 4.65 c	3426.2 ± 316.9 a	166.9 ± 11.7 b
3-O-feruloylquinic acid	88.1 ± 4.13 b	303.0 ± 33.7 a	36.3 ± 3.76 c
apigenin-7-O-glucoside	23.9 ± 1.61 a	17.5 ± 1.77 b	9.74 ± 0.72 c
catechin	848.3 ± 16.0 a	301.4 ± 29.7 b	852.7 ± 37.9 a
myricetin-3-O-hexoside	101.1 ± 10.0 b	505.0 ± 12.6 a	89.3 ± 5.1 b
rutin	1438.7 ± 52.6 a	32.1 ± 2.74 b	1.15 ± 0.23 c
quercetin-3-O-glucoside	582.5 ± 45.0 b	1146.0 ± 27.7 a	566.8 ± 26.2 b
quercetin-7-O-glucoside	1017.3 ± 28.5 b	1416.1 ± 100.6 a	933.4 ± 22.3 b
taxifolin 3-O-rhamnoside	92.8 ± 5.31 a	15.2 ± 1.86 b	13.1 ± 1.79 b
quercetin 3-rhamnoside	139.5 ± 9.18 a	124.7 ± 9.83 a	43.2 ± 1.46 b
quercetin 7-rhamnoside	185.7 ± 17.4 a	168.5 ± 14.9 a	53.4 ± 3.78 b
quercetin-3-arabinoside	58.1 ± 4.45 b	23.0 ± 1.86 c	172.8 ± 16.0 a
quercetin	80.8 ± 5.11 b	6.04 ± 1.28 c	346.6 ± 21.9 a
skyrin-2-O-glucopyranoside	61.7 ± 2.78 a	30.9 ± 1.51 b	nd
biapigenin	0.93 ± 0.052	nd	nd
amentoflavone	1.23 ± 0.10 a	0.25 ± 0.04 b	nd
soluble phenols	49.2 ± 2.48 a	31.7 ± 1.88 b	49.6 ± 4.91 a
flavonols	19.6 ± 1.42 a	13.8 ± 1.01 b	17.5 ± 0.83 a
free amino acids	42.5 ± 5.96 a	39.4 ± 4.31 a	38.2 ± 4.24 a

Table 3. Accumulation of non-essential, toxic or rare elements in the control shoots of three *Hypericum* species growing under natural soil conditions or cultured in hydroponics (control plants growing in Hoagland solution only). Data are means (n = 3) and for the lucidity of table, SD values are not shown. Values within rows followed by the same small or capital letter(s) are not significantly different according to Tukey's test (at P<0.05 level). [x] indicates the sum of REE (rare earth elements) and the sum of all elements in this table including respective Ni content in control plants of individual species mentioned in Tables 1 and 2 (both sums are in µg/g DW). nd – not detected.

	growing under natural soil conditions			cultured in hydroponics		
	H. perforatum	*H. olympicum*	*H. orientale*	*H. perforatum*	*H. olympicum*	*H. orientale*
Al (µg/g DW)	106.7 c	142.1 b	513.9 a	2.13 C	4.01 B	9.75 A
Sr (µg/g DW)	12.9 b	11.7 b	25.4 a	0.85 A	0.73 B	0.71 B
Ba (µg/g DW)	8.42 b	4.55 c	11.9 a	0.11 B	0.11 B	0.16 A
Ti (µg/g DW)	3.80 b	4.23 b	15.4 a	0.14 B	0.19 B	0.40 A
Cr (µg/g DW)	1.75 b	1.87 b	3.27 a	0.35 C	0.48 B	1.46 A
Rb (µg/g DW)	1.01 c	3.06 a	1.61 b	0.54 B	0.56 B	0.69 A
Cd (ng/g DW)	463.5 c	1029.0 a	644.3 b	31.3 B	24.5 C	52.2 A
Pb (ng/g DW)	383.2 b	419.1 b	892.6 a	13.8 A	14.9 A	16.4 A
Sb (ng/g DW)	330.8 a	299.4 a	218.9 b	24.1 C	51.7 B	65.5 A
Ce (ng/g DW)	176.8 c	250.2 b	841.0 a	3.43 B	3.65 B	9.50 A
Li (ng/g DW)	172.9 c	337.5 b	691.2 a	nd	nd	nd
V (ng/g DW)	170.6 b	211.0 b	776.4 a	3.72 C	5.65 B	14.8 A
Sn (ng/g DW)	156.0 ab	120.8 b	183.1 a	22.8 A	11.7 C	16.4 B
Zr (ng/g DW)	116.2 b	117.1 b	284.5 a	12.0 AB	9.36 B	16.4 A
La (ng/g DW)	88.6 c	116.7 b	439.3 a	1.70 B	1.64 B	4.13 A
As (ng/g DW)	56.7 b	46.0 b	129.1 a	nd	nd	nd
Y (ng/g DW)	34.4 b	44.1 b	210.6 a	0.85 C	1.39 B	2.47 A
Se (ng/g DW)	47.2 b	48.3 b	82.5 a	nd	nd	nd
Nd (ng/g DW)	38.5 b	49.2 b	227.9 a	0.31 C	0.66 B	2.14 A
W (ng/g DW)	29.4 b	26.1 b	43.7 a	2.71 B	2.78 B	5.05 A
Th (ng/g DW)	17.2 b	19.9 b	111.4 a	0.41 B	0.46 B	0.82 A
Cs (ng/g DW)	12.7 c	29.2 b	54.3 a	0.66 B	0.68 B	1.17 A
Pr (ng/g DW)	14.6 b	22.5 b	79.9 a	0.18 C	0.26 B	0.54 A
Pd (ng/g DW)	14.4 b	12.3 b	27.6 a	1.38 A	1.35 A	1.18 A

Pt (ng/g DW)	18.3 a	16.1 a	15.5 a	15.2 A	16.7 A	17.3 A
Sm (ng/g DW)	11.7 b	13.4 b	57.6 a	0.15 B	0.18 B	0.59 A
Gd (ng/g DW)	8.36 b	10.0 b	47.3 a	< 0.1	< 0.1	0.41
Dy (ng/g DW)	7.03 b	9.09 b	38.6 a	< 0.1	< 0.1	0.36
Bi (ng/g DW)	7.81 b	7.69 b	13.4 a	0.60 A	0.46 B	0.41 B
Eu (ng/g DW)	4.40 b	4.18 b	15.3 a	< 0.1	< 0.1	0.16
Hf (ng/g DW)	4.42 b	4.05 b	8.70 a	1.03 B	0.94 B	1.56 A
U (ng/g DW)	3.56 b	3.91 b	15.7 a	0.17 B	0.16 B	0.24 A
Be (ng/g DW)	3.61 b	4.32 b	17.5 a	0.16 B	0.15 B	0.44 A
Er (ng/g DW)	3.60 b	4.79 b	18.8 a	< 0.1	< 0.1	< 0.1
Yb (ng/g DW)	2.84 b	3.93 b	14.5 a	< 0.1	< 0.1	< 0.1
Tl (ng/g DW)	1.56 b	1.79 b	5.62 a	0.26 AB	0.19 B	0.33 A
Tb (ng/g DW)	1.52 b	1.97 b	7.96 a	< 0.1	< 0.1	< 0.1
Ho (ng/g DW)	1.25 b	1.70 b	6.58 a	< 0.1	< 0.1	< 0.1
Lu (ng/g DW)	0.47 b	0.57 b	2.04 a	< 0.1	< 0.1	< 0.1
Re (ng/g DW)	nd	nd	nd	nd	nd	nd
sum of REE [x]	0.3935 c	0.5323 b	2.0078 a	0.00662 C ***	0.00774 B ***	0.0201 A ***
sum of all [x]	137.1 b	170.9 b	577.8 a	4.28 C ***	6.26 B ***	13.4 A ***

Table 4. Accumulation of selected individual phenolic metabolites (µg/g DW) in the shoots of three *Hypericum* species cultured in hydroponics and exposed to nickel (1 or 100 µM) over 7 days. Data are means (n = 3) and for the lucidity of table, SD values are not shown. Values within rows followed by the same letter(s) are not significantly different according to Tukey's test (at P<0.05 level). nd – not detected.

	Hypericum perforatum			*Hypericum olympicum*			*Hypericum orientale*		
shoot	control	1 Ni	100 Ni	control	1 Ni	100 Ni	control	1 Ni	100 Ni
chlorogenic acid	144.3 d	137.7 d	171.2 d	3073.1 a	3289.9 a	2791.4 a	354.7 b	296.9 bc	234.3 c
3-O-feruloylquinic acid	166.0 c	160.1 c	143.3 cd	487.7 a	343.8 b	368.9 b	117.4 d	114.6 d	120.4 cd
apigenin-7-O-glucoside	33.0 a	33.6 a	24.3 bc	35.3 a	28.8 ab	17.4 c	10.2 d	14.1 cd	9.26 d
catechin	499.4 b	643.1 a	647.5 a	170.3 f	168.2 ef	224.2 e	297.8 de	400.5 c	593.6 a
myricetin-3-O-hexoside	14.5 c	14.6 c	14.1 c	240.7 a	212.5 a	212.6 a	31.4 b	30.3 b	34.9 b
rutin	1042.8 a	981.3 a	1055.0 a	42.7 b	38.3 b	44.1 b	0.42 c	0.41 c	0.45 c
quercetin-3-O-glucoside	528.3 c	490.4 c	503.6 c	1819.2 a	1694.4 ab	1601.5 b	549.3 c	524.5 c	521.9 c
quercetin-7-O-glucoside	615.2 c	537.5 d	582.4 cd	2392.7 a	2027.3 b	2071.8 b	648.9 c	646.0 c	653.7 c
taxifolin 3-O-rhamnoside	282.5 a	258.2 a	246.8 a	26.4 b	16.7 cd	17.1 cd	12.3 d	11.1 d	19.1 c
quercetin 3-rhamnoside	226.8 b	218.7 bc	166.8 c	294.3 a	285.1 a	225.4 b	30.2 d	32.6 d	31.9 d
quercetin 7-rhamnoside	274.8 b	255.5 b	196.1 c	323.2 a	328.4 a	262.6 b	35.0 d	38.5 d	35.7 d
quercetin-3-arabinoside	28.4 cd	24.2 d	26.3 d	39.7 b	36.4 bc	28.3 cd	158.7 a	168.8 a	163.0 a
quercetin	128.0 b	125.2 b	123.8 b	10.4 c	8.86 c	10.5 c	263.7 a	278.8 a	289.3 a
skyrin-2-O-glucopyranoside	47.8 a	50.9 a	48.4 a	36.7 b	25.8 c	15.3 d	nd	nd	nd
biapigenin	2.64 a	2.98 a	2.70 a	nd	nd	nd	nd	nd	nd
amentoflavone	0.87 d	2.54 c	2.41 c	6.92 b	9.65 a	2.93 c	nd	nd	nd

Figure heads

Figure 1. Accumulation of soluble phenols, sum of flavonols ($AlCl_3$ reaction) and free amino acids (FAA) in the shoots and roots of three *Hypericum* species cultured in hydroponics and exposed to nickel (1 or 100 µM) over 7 days. Data are means ± SDs shown as bars (n = 3). Columns followed by the same letter(s) are not significantly different according to Tukey's test (at P<0.05 level).

Figure 2. Biplot illustrating PCA analyses of selected parameters in the shoots (A) and roots (B) of three *Hypericum* species cultured in hydroponics and exposed to nickel (1 or 100 µM) over 7 days. TPC – total phenolic content (meaning soluble phenols), SF – sum of flavonols ($AlCl_3$ reaction), FAA – free amino acids. Color identifies species and shape treatments.

Figure 3. The PCA analyses of selected parameters in the control shoots of three *Hypericum* species cultured in hydroponics (laboratory, A) or growing outdoor (B, near the faculty as mentioned in Method section). TPC – total phenolic content (meaning soluble phenols), SF – sum of flavonols ($AlCl_3$ reaction), FAA – free amino acids.

Figure 1

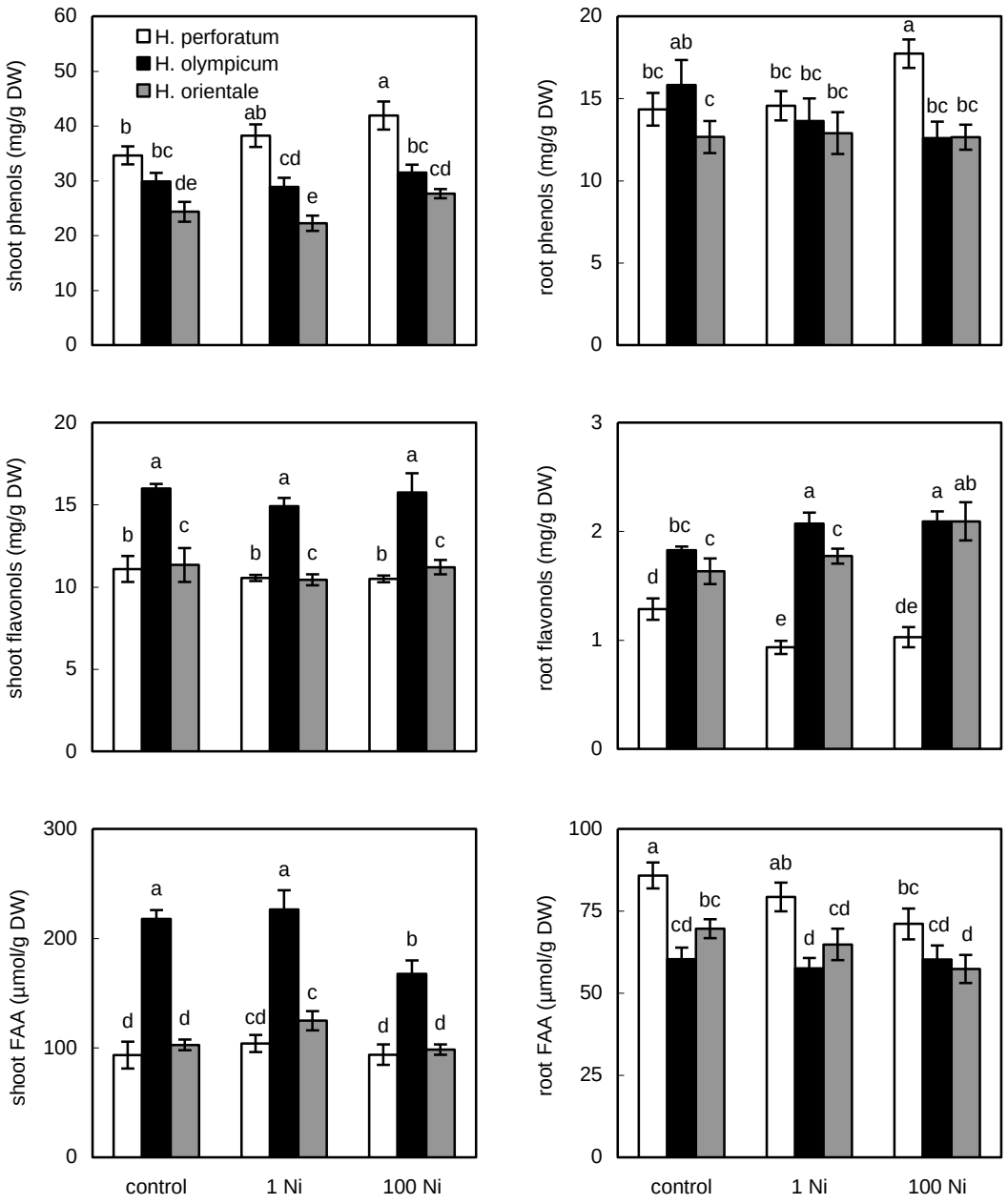

H. perforatum
H. olympicum
H. orientale
shoot phenols (mg/g DW)
root phenols (mg/g DW)
shoot flavonols (mg/g DW)
root flavonols (mg/g DW)
shoot FAA (µmol/g DW)
root FAA (µmol/g DW)
control
1 Ni
100 Ni

Figure 2

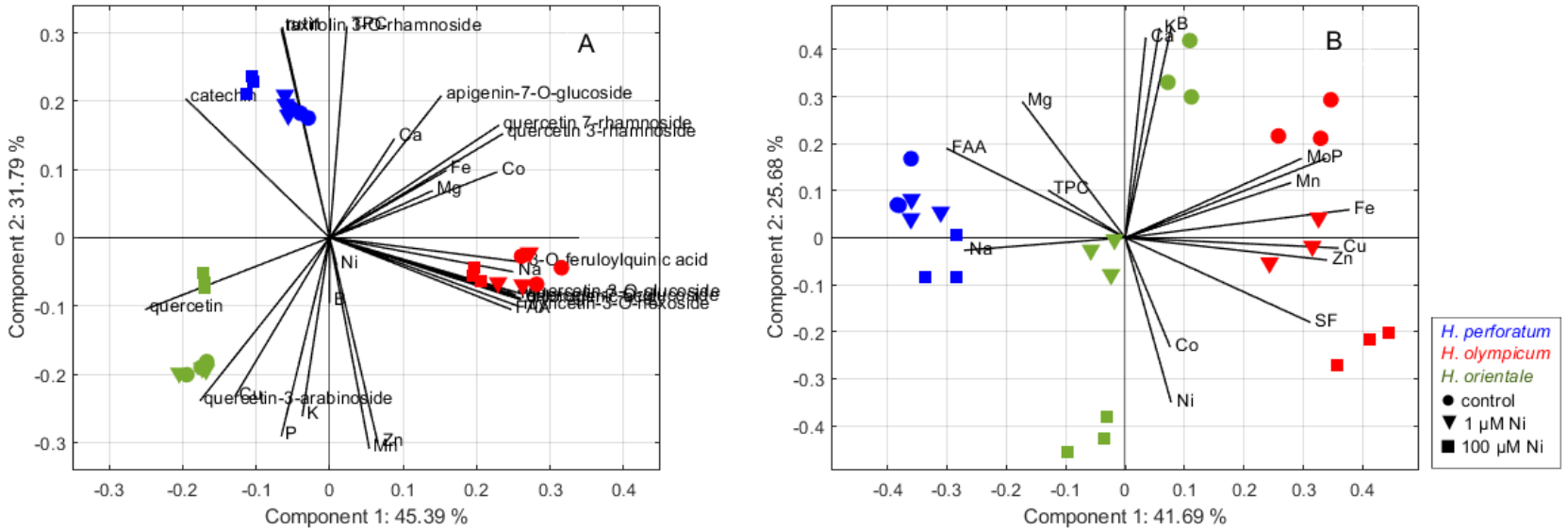
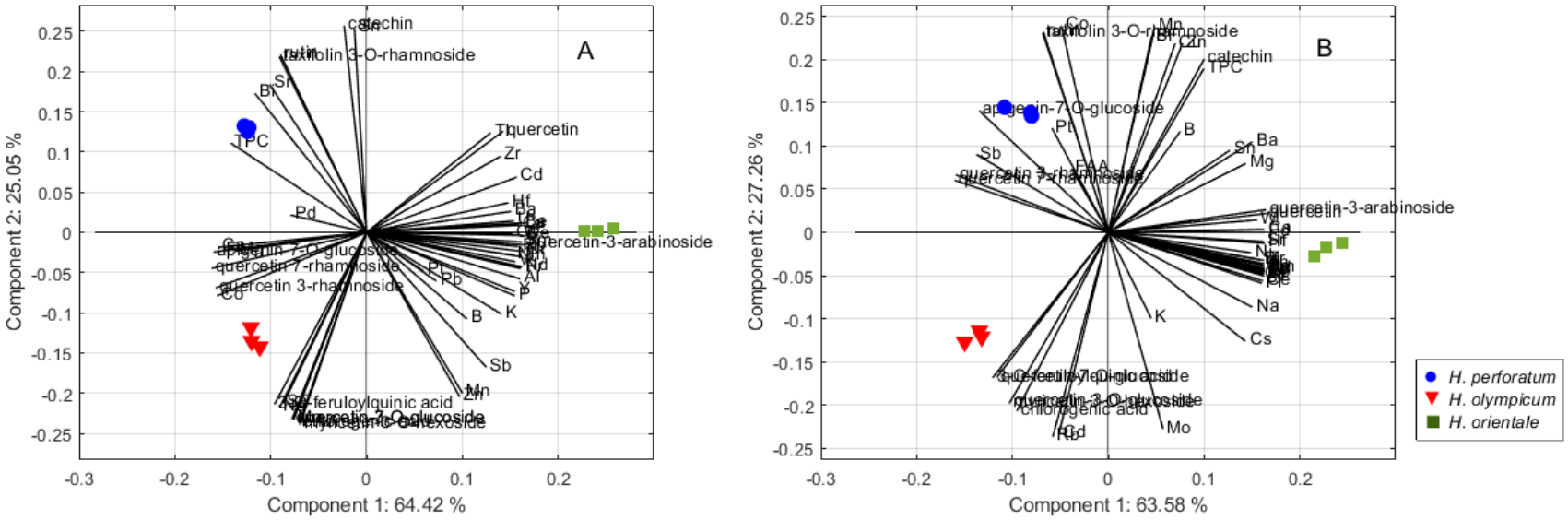

A
B
Component 2: 31.79 %
Component 1: 45.39 %
Component 2: 25.68 %
Component 1: 41.69 %
catechin
apigenin-7-O-glucoside
quercetin 7-rhamnoside
quercetin 3-rhamnoside
Ca
Fe
Co
Mg
Ni
Na
quercetin
quercetin-3-arabinoside
K
P
Mn
Zn
FAA
TPC
Mo
Cu
SF
H. perforatum
H. olympicum
H. orientale
control
1 µM Ni
100 µM Ni

Figure 3


A
B
Component 2: 25.05 %
Component 1: 64.42 %
Component 2: 27.26 %
Component 1: 63.58 %
catechin
TPC
Pd
Zr
Cd
Sb
B
K
Pb
Mn
Na
Cs
Mo
Mg
Ba
quercetin-3-arabinoside
chlorogenic acid
H. perforatum
H. olympicum
H. orientale