Stress responses and nickel and zinc accumulation in different accessions of Stellaria media (L.) Vill. in response to solution pH variation in hydroponic culture

Title: Stress responses and nickel and zinc accumulation in different accessions of *Stellaria media* (L.) Vill. in response to solution pH variation in hydroponic culture

Running title: Effect of pH on Ni and Zn uptake in *Stellaria media*

Authors: Mirko Salinitro[a], Antony van der Ent[b], Alice Tognacchini[c], Annalisa Tassoni[a*]

[a] Department of Biological, Geological and Environmental Science, University of Bologna, Via Irnerio 42, 40126, Italy

[b] Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, Sir James Foots Building (47A), The University of Queensland, Brisbane QLD 4072, Australia

[c] University of Natural Resources and Life Sciences, Department of Forest and Soil Sciences, Institute of Soil Research, Konrad-Lorenz-Straße 24, 3430 Tulln, Vienna, Austria

E-mails:

mirko.salinitro2@unibo.it

a.vanderent@uq.edu.au

alice.tognacchini@boku.ac.at

annalisa.tassoni2@unibo.it

*** Correspondence:** Annalisa Tassoni, e-mail: annalisa.tassoni2@unibo.it, Telephone: +39 051 2091278

Abstract

In most non-hyperaccumulating plants, Ni and Zn uptake is negatively correlated with soil pH, however, few studies so far have investigated how pH influences the activity and uptake of Ni and Zn in plants grown in a hydroponic system, which generally allows culture variables to be singularly manipulated.

In this study, the non-accumulator *Stellaria media* (L.) Vill. (Caryophyllaceae) displayed opposite trends of Ni and Zn uptake along a pH gradient (between 5 to 8 for Zn and between 5 to 6.5 for Ni), when grown in hydroponics. In all treatments, the solution metal concentration was fixed at 0.1 mM Ni or 0.55 mM Zn. Nickel accumulation increased with increasing pH with an average concentration in shoots of 167 μg/gDW at pH 5 and of 250 μg/gDW at pH 6.5. In contrast, Zn accumulation decreased with increasing pH, with an average concentration in shoots varying from 1640 μg/gDW at pH 5 to 435 μg/gDW at pH 8. Assessment of total polyphenol and flavonoid contents and of antioxidant activity showed that these parameters were positively correlated with Ni or Zn accumulation in *S. media* shoots, while photosynthetic pigments content and root and shoot biomass were negatively correlated with Ni and Zn accumulation. The study was carried out on five different *S. media* populations, which did not show differences in relation to the accumulation of metals and synthesis of antioxidant compounds, nonetheless showing a different biomass production under control conditions.

Keywords: Antioxidant compounds; flavonoids; heavy metals; metal uptake; oxidative stress; plant stress; polyphenols.

Abbreviations:

AA, ascorbic acid; ABTS, 2,2'-azino-bis(3-etilbenzotiazolin-6-sulfonic) acid; CAT, catechin; DOC, dissolved organic carbon; DW, dry weight; FW, fresh weight, GA, gallic acid; HMs, heavy metals; ROS, reactive oxygen species.

1. Introduction

The relationship between metal availability and soil parameters has been intensively studied over the last decades, to better understand which parameters have the highest impact on metal activity in the soil, hence on uptake and toxicity for plants (Kukier et al., 2004; Pérez-Esteban et al., 2014; Zia et al., 2018). A significant number of studies have shown that soil pH and dissolved organic carbon (DOC) strongly influence metal concentrations and speciation in soil solutions, although the specific effects vary between different metals (Kunhikrishnan et al., 2017). Other studies have identified total organic matter content, Fe-Mn oxides and pH as major factors determining metal availability in soil solution (Pérez-Esteban et al., 2014). Ni and Zn uptake is typically strongly correlated with soil solution pH while weakly influenced by other factors (Kukier et al., 2004; Zia et al., 2018). These elements are also common pollutants in urban environments since they are widely used in metal alloys and tires (McKenzie et al., 2009). It has been demonstrated that soluble organic carbon does not affect Zn concentrations in soil solution because of the low affinity of Zn for organic compounds (Wong et al., 2007), while for Ni, soil characteristics like clay content and cationic exchange capacity (CEC) cannot be totally excluded, along with pH, to explain metal availability (Zia et al., 2018). A correlation between low pH and increased Zn solubility was observed in several studies. Above pH 7.5, most of the Zn present in soils is in fact bound to clays and organic matter rather than in solution, while below pH 7, it is mostly available as hydrated Zn^{2+} ions (Kukier et al., 2004; Park et al., 2011). Similarly, at pH values below 6, the Ni pool is almost entirely available as the hydrated Ni^{2+} ion is mostly present in water solution. On the contrary, above pH 6, Ni availability decreases as it is mostly absorbed by a multitude of soil components capable of complexing this metal (*e.g.* OH^-, CO^{3-}) (Uren, 1992). However, studies carried out on Ni uptake reported contrasting results. A study on the effect of different soil pH on Ni concentration in the hyperaccumulators *Alyssum murale* Waldst. & Kit. and *Alyssum corsicum* Duby concluded that an increase in soil pH was associated with an increase in shoot Ni concentration (Li et al., 2003). These results were in contrast with other data demonstrating that the increase of soil pH led to a decrease of Ni concentration in various non-accumulator species (Kukier and Chaney, 2004; L'Huillier and Edighoffer, 1996) and in the hyperaccumulators *Berkheya coddii* Roessler and *Alyssum bertolonii* Desv. (Robinson et al., 1999). Contrasting results were also found by Kukier et al. (2004) studying *A. corsicum* and *A. murale* in different Ni-contaminated soils. Additionally, it was recently pointed out that the bioavailability of metals varies with plant species as a result of rhizosphere

mechanisms like root acidification or alkalinisation (Chaignon et al., 2002; Hinsinger and Courchesne, 2008; Wenzel et al., 2003). For instance, the availability of Zn was found to be higher than expected in tobacco plants due to a roots-induced pH decrease (Loosemore et al., 2004). Despite the many studies carried out on the synergic influence of many factors on metal availability in the soil (Kukier et al., 2004), limited research was performed under hydroponic conditions (Kumar et al., 2012; Viehweger and Geipel, 2010). In contrast to complex soil system in which the concomitant effect of several variables cannot be distinguished, hydroponics allows to investigate how a single variable (*i.e.* pH or metal ionic activity) influences the availability and the subsequent uptake of a specific metal in plants. Moreover, acidic root exudates can be effectively counterbalanced in hydroponics as a result of their dilution and neutralization in the nutrient solution (Viehweger and Geipel, 2010).

The presence of heavy metals (HMs) in plant cells can disrupt physiological and biochemical functions causing the formation of reactive oxygen species (ROS), especially in non-accumulator plants (Kisa, 2018; Ovecka and Takac, 2014). Under normal conditions, ROS are tightly controlled by the plant's complex antioxidant system (Bhaduri and Fulekar, 2012). In fact, plants try to protect cells against oxidative damage by producing a wide range of antioxidant enzymes, like superoxide dismutase, peroxidase and catalase (Kisa, 2018), as well as many molecules, among which polyphenols and flavonoids, with HM chelating ability and antioxidant action in plants (Michalak, 2006).

In the present experimental design, five different accessions of the non-accumulator plant *Stellaria media* (Caryophyllaceae) were used. *S. media* is a common annual herbaceous plant native to Europe and widely naturalized in all continents. It commonly grows in disturbed habitats, such as road margins, crop fields and bare soil deposits, and, due its widespread presence, it is also a suitable species for metal bioindication (Salinitro et al., 2019). Moreover, its fast growth and adaptability make this species suitable for laboratory experiments under hydroponic conditions.

The main objectives of this study were to evaluate the effect of pH on the uptake of Ni and Zn in different accessions of the non-accumulator plant *Stellaria media* grown hydroponically. In addition, to better highlight the possible toxic effects caused by Zn and Ni accumulation in plant tissues, the levels of some polyphenols and flavonoids with antioxidant activity and of photosynthetic pigments were measured.

2. Material and methods

2.1. Materials

Five different accessions of *Stellaria media* were collected in the year 2017 from five different locations, to test whether plant Ni and Zn uptake and possible consequent stress reactions are related to plant genetic variability present in the original habitat. In each location, seeds were sampled at several stations to cover the whole genetic variability. Two accessions were from the urban areas of Milan and Bologna (Italy), two accessions were from woodland locations located on the outskirts of Milan and Bologna and one accession was from the ultramafic outcrop at Mount Prinzera (Parma, Italy). The urban locations were characterized by high and polymetallic trace element contamination of the soils, whereas the woodland locations were characterized by low concentrations of trace elements. The ultramafic station was characterized by high concentrations of Cr and Ni only. Detailed information on the seed accession locations are listed in Supplementary Table S1 and in Salinitro et al. (2019). Seed collections were carried out at the end of the vegetative season of the plants (late April). Plants were cut and air-dried for one week, then shaken to allow the release of seeds. Seeds were sieved to remove plant particles, air-dried for 1 week, then stored in plastic tubes at room temperature until sowing. For germination, seeds were soaked overnight in tap water and subsequently placed on a substrate composed of perlite, vermiculite and quartz sand in a ratio of 1:1:1. Seeds were watered with tap water and kept in the dark at 20°C for 2–3 days until germination. Seedlings were then transferred to a growth cabinet with a 12-12 hours light-dark photoperiod at 25°C degrees for 1 week, before transplanting them into the hydroponic system.

2.2. Hydroponic culture system

The hydroponic system was composed of four separate rectangular tanks (21 cm height x 30 cm width x 40 cm length; 25 L each) filled with Vega Classic nutrient solution (Canna, Brisbane QLD, Australia) diluted 1:250 with deionized water. The diluted solution contained: 16.3 mM N, 1.2 mM P, 5.7 mM K, 4.4 mM Ca, 1.3 mM Mg, 1.1 mM S, 14 μM Fe-DTPA (diethylenetriaminepentaacetic acid ferric complex), 26 μM B, 0.6 μM Cu, 10.2 μM Mn, 0.8 μM Mo, 0.2 μM Ni, 4.3 μM Zn. The solution was kept constantly aerated by placing an aquarium air stone at the bottom of each tank.

The nutrient solution was spiked with $Zn(NO_3)_2 \cdot 6H_20$ or $Ni(NO_3)_2 \cdot 6H_2O$ to obtain a nominal Zn and Ni concentration of 0.55 mM and 0.10 mM, respectively (Table 1), which corresponded to the average total metal concentration measured in the four most polluted soil

samples collected in the cities of Bologna and Milan (from where the *S. media* urban accessions were collected) (Salinitro et al., 2019).

Zinc was tested at pH values ranging from 5 to 8, while Ni was tested only at pH levels ranging from 5 to 6.5, as higher pH values in combination with Ni stress induced Fe deficiency which led to plant death (Table 1). Control plants were cultivated at pH 5 with normal Vega Classic nutrient (Canna, Brisbane QLD, Australia) solution. The control sample was performed without the addition of any further salt, so the amount of nitrate was slightly lower compared to the treatments (-1% for Ni treatment and -6% for Zn treatment) to which $Zn(NO_3)_2$ or $Ni(NO_3)_2$ salts were added. The pH was automatically maintained at the set value ± 0.1 with 0.1 M KOH solution (Table 1). A 5% (v/v) replacement of nutrient solution was performed daily. For each accession, five plants were grown in 5 cm round plastic baskets filled with a foam disk to allow the roots to be immersed in the nutrient solution. Before transfer to hydroponic systems, plants were thoroughly washed to remove any substrate residues. Plants were grown for 20 days with a 12-12 hours light-dark photoperiod, under high intensity LED lights RAY22 physioSpecGreenhouse (Fluence Science, Austin, TX, USA), with photosynthetically active radiation (PAR) 550 µmol m^2/s), at 22/20°C day/night (Fig. 1).

At the end of the cultivation period, plants were harvested and divided into shoots and roots. Plants were rinsed with de-ionised water and subsequently ground in liquid nitrogen. The bulk samples were divided into fresh aliquots (stored at -80° C) used for spectrophotometric analyses and dry aliquots used for metal content analyses. To obtain dry samples, the aliquots were dried at 80°C for 24 hours. Dried shoot weight was on average 13.5 % of the fresh weight, whereas dried root weight was on average 7.3 % of the fresh weight.

2.3. Spectrophotometric quantifications.

For spectrophotometric analyses, 0.1 g of freshly ground plant samples were extracted with 1 mL of 95% (v/v) methanol and shaken overnight at room temperature (Ferri et al., 2013). After centrifugation at 15300 × *g* for 5 minutes at room temperature, the supernatant was recovered and used in the total polyphenols, total flavonoids and antioxidant activity quantification reactions.

Total polyphenol colorimetric quantification was performed through the Folin-Ciocalteu assay (Ferri et al., 2013). The results were expressed as mg of gallic acid equivalents per g of fresh weight (mg GA eq/gFW) by means of a dose-response calibration curve (between 0 and 15 µg of gallic acid).

Total flavonoid colorimetric quantification was performed as described in Zhishen et al. (1999). The results were expressed as mg of catechin equivalents per g of fresh weight (mg CAT eq/gFW) by means of a dose-response calibration curve (between 2 and 14 µg of catechin).

Antioxidant activity quantification was performed using the ABTS (2,2'-azino-bis(3-etilbenzotiazolin-6-sulfonic)) acid reagent colorimetric assay (Ferri et al., 2013) and the results were expressed as mg of ascorbic acid (AA) equivalents per g of fresh weight (mg AA eq/gFW) by means of a dose-response calibration curve (between 0 and 2 µg of AA).

For the determination of photosynthetic pigments, a modified method from Radwan et al. (2007) was used. Freshly ground plants (0.1 g) were extracted with 1.5 mL of 85% (v/v) acetone and mixed twice for 30 seconds; the samples were then centrifuged at 4°C, 665 x *g* for 5 minutes and the supernatant recovered.

The supernatant was analysed at three different wavelengths (663, 644 and 452.5 nm) and the obtained absorbance values were processed to give the pigment concentrations in mg/gFW with the following equations:

chlorophyll *a* = 10.3 x Abs_{663} – 0.098 x Abs_{644},

chlorophyll *b* = 19.7 x Abs_{644} – 3.87 x Abs_{663},

carotenoids = 4.2 x $Abs_{452.5}$ – [(0.0264 x chl-*a*) + (0.426 x chl-*b*)]

All spectrophotometric analyses were performed with a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

2.4. Nickel and zinc quantification

For the quantification of metals, ground roots and shoots were oven dried at 80°C until constant weight (24 h). Samples were pre-digested at room temperature with 2 mL 70% (v/v) HNO_3 for 1 day, and then digested on a heat block, 1 h at 70°C and 1 h at 125°C, following a modified method from Huang et al. (1985). After digestion, sample volumes were brought up to 10 mL with de-ionised water. Experimental blanks were included in the plant digestion. For quality control, five replicates of certified reference material (Apple leaves NIST® SRM® 1515) were digested together with the samples. Recovery rates of Ni and Zn were within ± 5% of the target concentrations of the certified reference (Ni 98.0%; Zn 103.5%). In the same way, quality control solutions were included in the measurement with recovery rates of 95.8% for Ni and 96.6% for Zn. Limits of quantification of the target elements were 0.007 mg/kg for Ni and 0.003 mg/kg for Zn. The analyses were performed with an iCAP 7400 series ICP-OES

simultaneous spectrometer (ThermoFisher Scientific, Cambridge, UK). Data were expressed as μg/g of sample dry weight (DW).

2.5. Data analysis

All the statistical analyses were performed using R software version 1.3.5 (R Core Team, Vienna, Austria). The differences in metal uptake and metabolite production were evaluated among the different accessions and pH treatments. For each treatment 5 biological replicates for each accession were analysed in 2 technical replicates (n=5). Data were tested for normality using the Shapiro-Wilk normality test and for homogeneity using Levene's test for homogeneity of variance with default parameters from the package "car" (https://CRAN.R-project.org/package=car). The non-parametric Kruskal-Wallis test, followed by Dunn's multiple pairwise comparison post-hoc test from the dunn.test package (https://CRAN.R-project.org/package=dunn.test), was used to evaluate the differences among compared groups (*p-values* are reported in brackets in the section Results). Spearman correlation coefficients were calculated to determine the relationship between metal uptake and metabolite production. Linear regression models were used to describe the relations between metal uptake and flavonoids, polyphenols and antioxidants. Non-linear regression was used to describe the relation between Ni uptake-fresh biomass and Zn uptake-chlorophylls production (R^2 and *p-values* are reported in brackets in the section Results). Graphical elaborations were performed using the R package ggpubr (https://CRAN.R-project.org/package=ggpubr). To calculate free ionic activity of Ni and Zn at every pH tested a simulation was performed using the software GEOCHEM-EZ 1.0 (free software available at http://www.PlantMineralNutrition.net). The ionic activity of a metal expresses the ease with which the specific metal undergoes chemical reactions and interactions with biological membranes and other chemical species. The salts contained in the nutrient solution and solution pH were used as input data, and the parameter *precipitation allowed* was set as default.

3. Results

3.1. Population biomass variability

Differences in shoot biomass among populations were only observed in control samples (Figs. 2 and 3). With respect to the plants grown under control conditions, those coming from ultramafic and Bologna urban accessions showed the lowest biomass with an average shoot

weight of 7.7 gFW and 6.4 gFW, respectively. The Milan urban accession showed an intermediate shoot weight with an average of 15.1 gFW, while Milan and Bologna woodland accessions displayed the highest average shoot weight of 22.8 gFW. The shoot biomasses were no longer significantly different in the plant samples with higher Zn and Ni contents which exerted toxic effects that buffered population variability (Figs. 3A, B).

3.2. Effect of pH on Ni and Zn uptake

An overall different shoot biomass production was noticed between plants grown with Ni and plants grown with Zn (Fig. 3). For Ni and Zn treatments, the average shoot weight was 0.11 gFW and of 2.18 gFW, respectively. Root biomass was not significantly different among different samples with an average weight of 0.04 and of 0.52 gFW for Ni and Zn treatments, respectively (the complete raw dataset is reported in Supplementary Table S2).

Data showed that different pH treatments clearly affected the uptake of Ni and Zn ($p=< 0.01$ both for shoots and roots) but in opposite ways (Fig. 4). Ni accumulation increased with the increase of solution pH, both in roots and shoots (Figs. 4A, B), with roots accumulating on average six-times more Ni than shoots. Ni concentration in plant roots varied from an average 1036 μg/gDW at pH 5 to 1522 μg/gDW at pH 6.5 (Fig. 4A). A similar trend was observed in shoots with values ranging between an average of 167 μg/gDW, at pH 5, to 250 μg/gDW at pH 6.5. In contrast, Zn accumulation decreased with increasing pH values (Figs. 4C, D) with concentrations in roots being 5- to 20-fold higher than in shoots, respectively, in pH 8 and pH 5 treatments. Zn concentration in roots varied between an average of 32700 μg/gDW in plants grown at pH 5 and 3390 μg/gDW in plants grown at pH 8 (Fig. 4C). Shoot Zn uptake ranged between 1647 μg/gDW and 435 μg/gDW, respectively, in plants grown at pH 5 and pH 8 (Fig. 4D). Ion activity of Ni did not show significant changes among pH treatments, except for a slight decrease at increasing pH, whereas Zn ion activity decreased at higher pH treatment (Table 2).

The translocation of both Ni and Zn from roots to shoots was affected by their availability. The translocation of Ni from roots to shoots was around 25 % in control plants, where Ni levels were very low, whereas in all the other treatments translocation was around 12% to increase up to 16% at pH 6.5 ($R^2 = 0.604$, $p<0.01$) (Fig. 5A). When Zn levels were low (*i.e.* in the control) or its availability was low (*i.e.* in the pH 8 treatment), plant transferred up to the 15% of Zn from roots to shoots. Conversely, with the increase of Zn availability, the translocation decreased to around 3% and went slightly up to 5% at pH 5 ($R^2 = 0.913$, $p<0.01$) (Fig. 5B).

3.3. Assessment of Ni and Zn toxicity

One of the parameters which best reflected plant stress connected to Ni and Zn uptake was the reduction in shoot biomass. Ni toxicity was similar among all plants grown at different pH values with a biomass reduction of around 95% compared to the control for all treatments; a strong negative correlation between the two variables was found (R^2 = 0.890, $p<0.01$) (Fig. 6A). The complete Zn uptake curve, from toxicity to deficiency caused by pH, was highlighted in this study and the correlation between Zn uptake and shoot biomass reduction was high (R^2 = 0.652, $p<0.01$, control was not included in the regression) (Fig. 6B). Plants showed strong Zn toxicity at pH 5, with a reduction in shoot biomass of 97% compared to the control. At pH 6, 6.5 and 7 plants had similar biomass values as the control, while at pH 8 plants suffered a marked biomass reduction (90% compared to the control) due to Zn and Fe deficiency caused by alkaline pH (Table 2).

In addition to shoot biomass reduction, increasing Ni concentrations caused a reduction in total flavonoid amount and total chlorophyll-*a*, chlorophyll-*b* and carotenoids content in plant aerial parts (R^2 = 0.793, $p<0.01$ and R^2 = 0.680, $p<0.01$, respectively) (Figs. 7B, D). On the other hand, total polyphenol content was weakly positively correlated with Ni concentration (R^2 = 0.309, $p<0.05$) (Fig. 7A). Finally, antioxidant activity, to which both polyphenols and their subclass flavonoids contribute, seemed not to be correlated with Ni uptake (R^2 = 0.037, $p=0.17$) (Fig. 7C).

In the case of Zn uptake, general trends were substantially clearer. Values for the pH 8 treatment were reported in graphs (in grey) but not included in regression models because, as previously explained, the observed stress symptoms were not caused by Zn and Ni toxicity but related to metal deficiency due to the low activity of Zn and Fe (Table 2). Regarding flavonoid content, the trend was reversed compared to Ni, in fact, in shoots flavonoids increased with increasing Zn concentration (R^2 = 0.852, $p<0.01$) (Fig. 8B). Moreover, as reported for Ni, total polyphenols concentration was positively correlated with Zn concentration (R^2 = 0.728, $p<0.01$) (Fig. 8A). Because both polyphenols and flavonoids may contribute to antioxidant activity, this parameter also showed a clear positive correlation with Zn concentration (R^2 = 0.751, $p<0.01$) (Fig. 8C). Photosynthetic pigments content slightly increased in treatments at pH 6 and 6.5 compared to the control, and then sharply decreased at pH 5 (R^2 = 0.476, $p=<0.05$).

4. Discussion

The aim of our research was to study the effect of pH on Zn and Ni uptake in the non-accumulator plant *Stellaria media*. The lack of *buffer factors* (*i.e.* clay presence, organic matter) when using hydroponics, on one side made the effects caused by pH more evident, but on the other side, made plants more vulnerable to Ni and Zn toxicity. All plants undergoing Ni treatments suffered chlorosis due to Ni toxicity, while in Zn treatments at pH 8 plants suffered Zn and Fe deficiency (Table 2). In general, Ni caused stronger deleterious effects than Zn, partly because of the intrinsic higher toxicity of Ni (Kabata-Pendias, 2010) and partly because higher translocation occurred from roots to shoots (Fig. 5A, B).

The present experimental design, moreover, aimed at the assessment of possible variations among the five different *S. media* plant populations tested. The hypothesis that populations coming from different habitats could have developed different levels of metal tolerance has already been confirmed by other studies (Collier et al., 2010) that took into consideration populations growing on standard and mine soils. Our data did not confirm such a hypothesis as all populations appeared homogeneous in Zn and Ni uptake ($p=0.92$ for Ni, and $p=0.91$ for Zn) and in flavonoid, polyphenol and photosynthetic pigment production. Therefore, it appears that differences in Ni and Zn concentrations and availability between urban, woodland and ultramafic soils were not marked enough to allow diversification among populations. Despite that, under control conditions, the populations differed in plant size. In fact, plants coming from contaminated environments, such as urban and ultramafic accessions, showed a lower shoot biomass than woodland accessions (Figs. 2, 3A, 3B). At increasing pH, a corresponding increase in Ni uptake was observed both in roots and shoots of *S. media* (Fig. 4). The conceptual model of such a response is known as the *biotic ligand model* (Di Toro et al., 2001). When metal ionic activity is kept constant, an increase in pH may cause higher binding of metal cations to biotic ligands (biological membrane and transporter proteins) because of the deprotonation of transporters (Lòpez et al., 2000). Our results showed that Ni activity can be considered almost constant at all pH values tested (Table 2), an ideal situation that could happen only in total absence of other factors able to bind or release Ni ions from the solution. In fact, soil pot experiments carried out on non-accumulator plants like crop plants (Kukier and Chaney, 2004; L'Huillier and Edighoffer, 1996) reported opposite trends in Ni uptake in relation to pH increase.

The uptake of Ni may vary according to the type of media (*i.e.* soil, perlite, hydroponic, etc.) in which the plant is grown. In fact, studies conducted on oat with inert substrates (Weng et al., 2003) found that Ni concentration in shoots increased with an increase of pH, analogously to our results (Fig. 4A).

As stated in several previous studies (Pérez-Esteban et al., 2014; Zia et al., 2018), soil pH is the main variable affecting bioavailability and uptake of Zn. This study is consistent with the literature since a strong negative correlation between Zn uptake and pH was observed (Fig. 4). This behaviour can be explained by the chemical forms that Zn assumes at different pH values (Kukier et al., 2004). Solution pH influences the speciation of Zn: at pH values below 7.7, Zn^{2+} predominates and plants can easily absorb it, but above pH 7.7, the main species is $ZnOH^{+}$ which is no longer bioavailable (Kiekens, 1995; Reddy et al., 1995). Moreover, as confirmed by the GEOCHEM-EZ simulation (Table 2), free Zn activity decreased with increasing pH values, coupled with Zn precipitation at the highest pH.

Unlike Ni, the behaviour of Zn in hydroponics closely simulates the one in soil, as soluble Zn is mainly present as inorganic forms, mostly free cations (94–98%), in soil solution. Moreover, few interactions occur with soil and organic matter (Meers et al., 2006; Pérez-Esteban et al., 2014).

Zn and Ni toxicity, induced by differential uptake among pH treatments, influenced several plant parameters, most importantly the biomass produced. As expected, Zn and Ni toxicity caused a reduction in biomass and chlorosis, as widely reported in the literature (Jayakumar et al., 2007; Weng et al., 2003) (Fig. 6, 7D, 8D). Beside these effects, the production of secondary metabolites (like flavonoids and polyphenols) connected with oxidative stress was investigated. A positive correlation between the production of these compounds and metal uptake was observed both for Ni (R^2 = 0.793, for polyphenols) and Zn (R^2 = 0.852, R^2 = 0.728, for flavonoids and polyphenols, respectively) (Fig. 7A, 8A, 8B). For Ni treatments, data were grouped in two main clusters: control and pH treatments. All treated plants exhibited acute Ni toxicity; therefore, the production of polyphenols and flavonoids was quite similar for all pH treatments and strongly different from the control (Fig. 7). Flavonoids (a subfamily of polyphenols) were negatively correlated with Ni uptake (Fig. 7B), despite most studies (Winkel-Shirley, 2002) reported opposite trends. This negative trend was probably caused by the high toxicity of Ni which suppressed flavonoid production as found by Jayakumar et al. (2007). A similar trend was detected for polyphenols, which showed overall growing similar levels in samples from pH 5 to 6, followed by a sharp decrease at pH 6.5, probably as a result of acute Ni toxicity. In fact, higher Ni concentrations were detected in *S. media* shoots at pH 6.5 (250 μg/gDW). Analogous results were reported for maize plants by Kisa et al. (2016), who observed a general increase of phenolic compounds in all treatments with Cu, Pb and Cd. Similarly L., Lavid et al. (2001) showed that increased Cd accumulation in *Nymphaea alba* leaves triggered the synthesis of polyphenols and peroxidase enzyme

involved in metal detoxification. The opposite trends of flavonoid and polyphenol production were probably the cause that no correlation between antioxidant capacity and Ni content was found (Fig. 7C).

The lower toxicity of Zn, instead, allowed us to observe a gradual production of antioxidant activity which steadily increased from control treatment to high Zn stress (pH 5 treatment), with no signs of inhibition by excessive toxicity. Polyphenols, flavonoids and total antioxidant capacity all increased with increasing metal concentration, acting clearly against the Zn-induced oxidative stress (Jayakumar et al., 2007; Kisa et al., 2016; Kumar et al., 2012; Winkel-Shirley, 2002). In addition, polyphenols can act as HM chelating agents thanks to their capacity to form insoluble complexes with divalent and trivalent cations thereby reducing their intracellular concentrations (Lavid et al., 2001).

Photosynthetic pigments content was negatively affected by both Ni and Zn uptake causing chlorosis (Fig. 8D). The presence of metals and metalloids in plant organs exerts a wide array of effects, which include low chlorophyll synthesis, changes in the chlorophyll *a*/chlorophyll *b* ratio and poor photosynthetic activity (Küpper et al., 2007; Viehweger and Geipel, 2010). Consequently, one of the most visible effects of metal toxicity is diffuse leaf chlorosis (Chandra and Kang, 2016).

5. Conclusions

This study showed that pH is a major variable affecting the uptake of Zn and Ni in *Stellaria media* grown under hydroponic conditions. pH is positively correlated with Ni uptake, but negatively correlated with Zn uptake. The results obtained in hydroponics confirmed previous data on Zn uptake obtained in soil. Conversely, the relation between pH and Ni uptake showed an opposite trend to that detected in soil. Therefore, our findings confirm that many factors play an important role in controlling Ni availability and uptake in soil, while Zn absorption is mainly controlled by pH.

The study showed a higher accumulation of flavonoids and polyphenols in shoots having the highest Zn and Ni concentrations, where these molecules may act as reactive oxygen species scavengers. On the contrary, in the same samples, shoot biomass production and photosynthetic pigments content decreased. Finally, our results demonstrated the suitability of using hydroponic systems to study single culture variables that may affect metal uptake as well as the possibility to use polyphenols and flavonoids as indicators of metal-related stress.

Contributions

MS designed the study and acquired and interpreted the data; AVDE designed and financed the study, interpreted data and drafted the article; AT acquired data; AT critically revised the manuscript and financed the study.

Acknowledgments

MS was the recipient of a Marco Polo PhD travel grant from the Department of Biological Geological and Environment al Sciences of the University of Bologna (Italy). We wish to thank Dr. Marianne Louise van Buuren for editing English language.

Supplementary Data

Supplementary Table S1. *Stellaria media* seed collection detailed stations.

Supplementary Table S2. Complete dataset.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated or analysed during this study are included in the published article and in Supplementary Table S2.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Bhaduri, A., Fulekar, M., 2012. Antioxidant enzyme responses of plants to heavy metal stress. Reviews in Environmental Science and Bio/Technology 11, 55-69.

Chaignon, V., Bedin, F., Hinsinger, P., 2002. Copper bioavailability and rhizosphere pH changes as affected by nitrogen supply for tomato and oilseed rape cropped on an acidic and a calcareous soil. Plant Soil 243, 219-228.

Chandra, R., Kang, H., 2016. Mixed heavy metal stress on photosynthesis, transpiration rate, and chlorophyll content in poplar hybrids. For. Sci. Technol. 12, 55-61.

Collier, M.H., Keane, B., Rogstad, S.H., 2010. Productivity differences between dandelion (*Taraxacum officinale*; Asteraceae) clones from pollution impacted versus non-impacted soils. Plant Soil 329, 173-183.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. 20, 2383-2396.

Ferri, M., Gianotti, A., Tassoni, A., 2013. Optimisation of assay conditions for the determination of antioxidant capacity and polyphenols in cereal food components. J. Food Comp. Anal. 30, 94-101.

Hinsinger, P., Courchesne, F., 2008. Biogeochemistry of metals and metalloids at the soil–root interface, in: Violante, A., Huang, P.M., Gadd, G.M. (Eds.) Biophysic- Chemical Processes of Heavy Metals and Metalloids in Soil Environments, John Wiley & Sons, Hoboken, USA, pp. 267-311.

Huang, C.Y., Schulte, E.E., 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Commun. Soil Sci. Plant Anal. 16, 943-958.

Jayakumar, K., Jaleel, C., Vijayarengan, P., 2007. Changes in growth, biochemical constituents, and antioxidant potentials in radish (*Raphanus sativus* L.) under cobalt stress. Turk. J. Biol. 31, 127-136.

Kabata-Pendias, A., 2010. Trace Elements in Soils and Plants, 4th ed., Taylor & Francis Group, Boca Raton, FL, USA.

Kiekens, L., 1995. Zinc, in: Alloway, B.J. (Ed.) Heavy Metals in Soils, Blackie Academic and Professional, London, UK, pp. 284-305.

Kisa, D., 2018. The responses of antioxidant system against the heavy metal-induced stress in tomato. J. Nat. Appl. Sci. 22, 1-6.

Kisa, D., Elmastas, M., Ozturk, L., Kayir, O., 2016. Responses of the phenolic compounds of *Zea mays* under heavy metal stress. Appl. Biol. Chem. 59, 813-820.

Kukier, U., Chaney, R.L., 2004. *In situ* remediation of nickel phytotoxicity for different plant species. J. Plant Nutr. 27, 465-495.

Kukier, U., Peters, C.A., Chaney, R.L., Angle, J.S., Roseberg, R.J., 2004. The effect of pH on metal accumulation in two *Alyssum* species. J. Environ. Qual. 33, 2090-2102.

Kumar, A., Prasad, M.N.V., Sytar, O., 2012. Lead toxicity, defense strategies and associated indicative biomarkers in *Talinum triangulare* grown hydroponically. Chemosphere 89, 1056-1065.

Kunhikrishnan, A., Choppala, G., Seshadri, B., Wijesekara, H., Bolan, N.S., Mbene, K., Kim, W.I., 2017. Impact of wastewater derived dissolved organic carbon on reduction, mobility, and bioavailability of As(V) and Cr(VI) in contaminated soils. J. Environ. Manage. 186, 183-191.

Küpper, H., Parameswaran, A., Leitenmaier, B., Trtilek, M., Setlik, I., 2007. Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. New Phytol. 175, 655-674.

L'Huillier, L., Edighoffer, S., 1996. Extractability of nickel and its concentration in cultivated plants in Ni rich ultramafic soils of New Caledonia. Plant Soil 186, 255-264.

Lavid, N., Schwartz, A., Yarden, O., Tel-Or, A., 2001. The involvement of polyphenols and peroxidase activities in heavy metal accumulation by epidermal glands of waterlily (Nympheaceae). Planta 212, 323-331.

Li, Y.M., Chaney, R.L., Brewer, E.P., Angle, J.S., Nelkin, J., 2003. Phytoextraction of nickel and cobalt by hyperaccumulator *Alyssum* species grown on nickel-contaminated soils. Environ. Sci. Technol. 37, 1463-1468.

Loosemore, N., Straczek, A., Hinsinger, P., Jaillard, B., 2004. Zinc mobilisation from a contaminated soil by three genotypes of tobacco as affected by soil and rhizosphere pH. Plant Soil 260, 19-32.

Lòpez, A., Làzaro, N., Priego, J.M., Marques, A.M., 2000. Effect of pH on the biosorption of nickel and other heavy metals by *Pseudomonas fluorescens* 4F39. J. Ind. Microbiol. Biotech. 24, 146-151.

McKenzie, E.R., Money, J.E., Green, P.G., Young, T.M., 2009. Metals associated with stormwater-relevant brake and tire samples. Sci. Total Environ. 407, 5855-5860.

Meers, E., Unamuno, V.R., Du Laing, G., Vangronsveld, J., Vanbroekhoven, K., Samson, R., Diels, L., Geebelen, W., Ruttens, A., Vandegehuchte, M., Tack, F.M.G., 2006. Zn in the soil solution of unpolluted and polluted soils as affected by soil characteristics. Geoderma 136, 107-119.

Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol. J. Environ. Stud. 15, 523-530.

Ovecka, M., Takac, T., 2014. Managing heavy metal toxicity stress in plants: biological and biotechnological tools. Biotechnol. Adv. 32, 73-86.

Park, J.H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N., Chung, J.W., 2011. Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. J. Hazard. Mater. 185, 549-574.

Pérez-Esteban, J., Escolastico, C., Masaguer, A., Vargas, C., Moliner, A., 2014. Soluble organic carbon and pH of organic amendments affect metal mobility and chemical speciation in mine soils. Chemosphere 103, 164-171.

Radwan, D.E.M., Fayez, K.A., Mahmoud, S.Y., Hamad, A., Lu, G., 2007. Physiological and metabolic changes of *Cucurbita pepo* leaves in response to zucchini yellow mosaic virus (ZYMV) infection and salicylic acid treatments. Plant Physiol. Biochem. 45, 480-489.

Reddy, K.J., Wang, L., Gloss, S.P., 1995. Solubility and mobility of copper, zinc and lead in acidic environments. Plant Soil 171, 53-58.

Robinson, B.H., Brooks, R.R., Clothier, B.E., 1999. Soil amendments affecting nickel and cobalt uptake by *Berkheya coddii*: potential use for phytomining and phytoremediation. Ann. Bot. 84, 689-694.

Salinitro, M., Tassoni, A., Casolari, S., de Laurentiis, F., Zappi, A., Melucci, D., 2019. Heavy metals bioindication potential of the common weeds *Senecio vulgaris* L., *Polygonum aviculare* L. and *Poa annua* L. Molecules 24, 2813.

Uren, N.C., 1992. Forms, reactions, and availability of Nickel in soils. Adv. Agron. 48, 141-203.

Viehweger, K., Geipel, G., 2010. Uranium accumulation and tolerance in *Arabidopsis halleri* under native versus hydroponic conditions. Environ. Exp. Bot. 69, 39-46.

Weng, L.P., Lexmond, T.M., Wolthoorn, A., Temminghoff, E.J.M., Van Riemsdijk, W.H., 2003. Phytotoxicity and bioavailability of nickel: chemical speciation and bioaccumulation. Environ. Toxicol. Chem. 22, 2180-2187.

Wenzel, W.W., Bunkowski, M., Puschenreiter, M., Horak, O., 2003. Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. Environ. Pollut. 123, 131-138.

Winkel-Shirley, B., 2002. Biosynthesis of flavonoids and effects of stress. Curr. Opin. Plant Biol. 5, 218-223.

Wong, J.W.C., Li, K.L., Zhou, L.X., Selvam, A., 2007. The sorption of Cd and Zn by different soils in the presence of dissolved organic matter from sludge. Geoderma 137, 310-317.

Zhishen, J., Mengcheng, T., Janming, W., 1999. The determination of flavonoid in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555-559.

569 Zia, A., van den Berg, L., Ahmad, M.N., Riaz, M., Zia, D., Ashmore, M., 2018. Controls on
570 accumulation and soil solution partitioning of heavy metals across upland sites in United
571 Kingdom (UK). J. Environ. Manage. 222, 260-267.

572
573

Figure legends

Figure 1. *S. media* plants after 20 days of growth in hydroponics in the presence of 0.55 mM Zn. Each tank corresponded to a pH treatment (ranging from pH 5 to 8). The control tank had no Zn added to it and was kept at pH 5.

Figure 2. Different plant populations of *S. media* L. Vill. after 1 week of hydroponic growth in control conditions (no metal, pH 5.0). A) Bologna woodland; B) Milan woodland; C) Milan urban; D) Bologna urban; E) Ultramafic.

Figure 3. Shoot biomass variability in *S. media* plants from five different populations, after hydroponic culture at different pHs. Plants treated with Ni (A) and with Zn (B) at different pHs. B, Bologna urban; M, Milan urban; N, Bologna woodland; P, ultramafic; T, Milan woodland. Highlighted with grey circles are the ultramafic and Bologna urban accessions, which always showed the smallest plant size under low stress conditions. Each population was analysed in five biological replicates (n=5). The complete dataset is available in Supplementary Table S2.

Figure 4. Accumulated Ni and Zn concentrations in *S. media* roots and shoots after hydroponic culture at different pHs. Ni uptake in roots (A) and shoots (B). Zn uptake in roots (C) and shoots (D). All treatments resulted significantly different ($p<0.05$) after Kruskal-Wallis and Dunn's post-hoc tests, except for Ni treatments at pH 5, 5.5, 6 ($p>0.05$). Each bar is represented by data coming from five accessions analysed in five biological replicates (n=25). The complete dataset is available in Supplementary Table S2.

Figure 5. Ni and Zn root-shoot translocation gradients in relation to metal concentrations in *S. media* roots after hydroponic culture at different pHs. Ni (A) and Zn (B) translocation. A) Linear model (R^2 = 0.604, $p<0.01$); B) Non-linear model (R^2 = 0.913, $p<0.01$). Each treatment is represented by data coming from five accessions analysed in five biological replicates (n=25). The complete dataset is available in Supplementary Table S2.

Figure 6. *S. media* shoot biomass variation in relation to Ni or Zn accumulation after hydroponic culture at different pHs. Biomass variation in Ni (A) and Zn (B) treatments. A) Non-linear model (R^2 = 0.890, $p<0.01$); B) Non-linear model (R^2 = 0.652, $p<0.01$). Each treatment is represented by data coming from five accessions analysed in five biological replicates (n=25). The complete dataset is available in Supplementary Table S2.

Figure 7. Polyphenol, flavonoid, photosynthetic pigment and antioxidant activity levels in *S. media* shoots in relation to accumulated Ni concentration after hydroponic culture at different pHs. A) Total polyphenols, expressed as mg of gallic acid (GA) equivalents/gFW; B) total flavonoids, expressed as mg of catechin (CAT) equivalents/gFW; C) antioxidant activity, expressed as mg of ascorbic acid (AA) equivalents/gFW; D) total content of chlorophyll-*a*, chlorophyll-*b* and carotenoids, expressed in mg/gFW. A) Linear model (R^2 = 0.309, $p<0.05$); B) Linear model (R^2 = 0.793, $p<0.01$); C) Linear model (R^2 = 0.037, p=0.17); D) Linear model (R^2 = 0.680, $p<0.01$). Each treatment is represented by data coming from five accessions analysed in five biological replicates (n=25). The complete dataset is available in Supplementary Table S2.

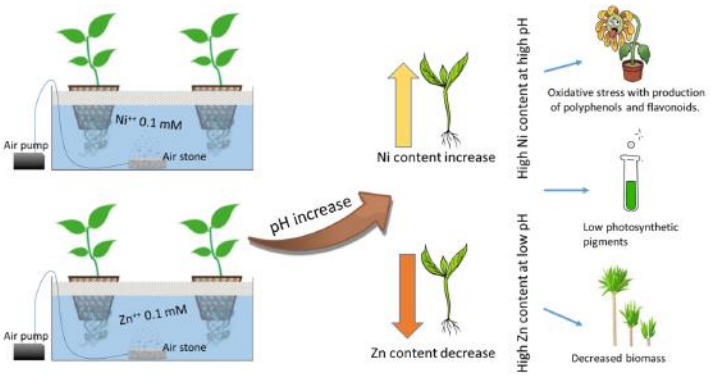
Figure 8. Polyphenol, flavonoid, photosynthetic pigment and antioxidant activity levels in *S. media* shoots in relation to accumulated Zn concentration after hydroponic culture at different pHs. A) Total polyphenols expressed as mg of gallic acid (GA) equivalents/gFW; B) total flavonoids expressed as mg of catechin (CAT) equivalents/gFW; C) antioxidant activity expressed as mg of ascorbic acid (AA) equivalents/gFW; D) total content of chlorophyll-*a*, chlorophyll-*b* and carotenoids expressed in mg/gFW. A) Linear model (R^2 = 0.728, $p<0.01$); B) Linear model (R^2 = 0.852, $p<0.01$); C) Linear model (R^2 = 0.751, $p<0.01$); D) Non-linear model (R^2 = 0.476, $p=<0.05$). Each treatment is represented by data coming from five accessions analysed in five biological replicates (n=25). The complete dataset is available in Supplementary Table S2.

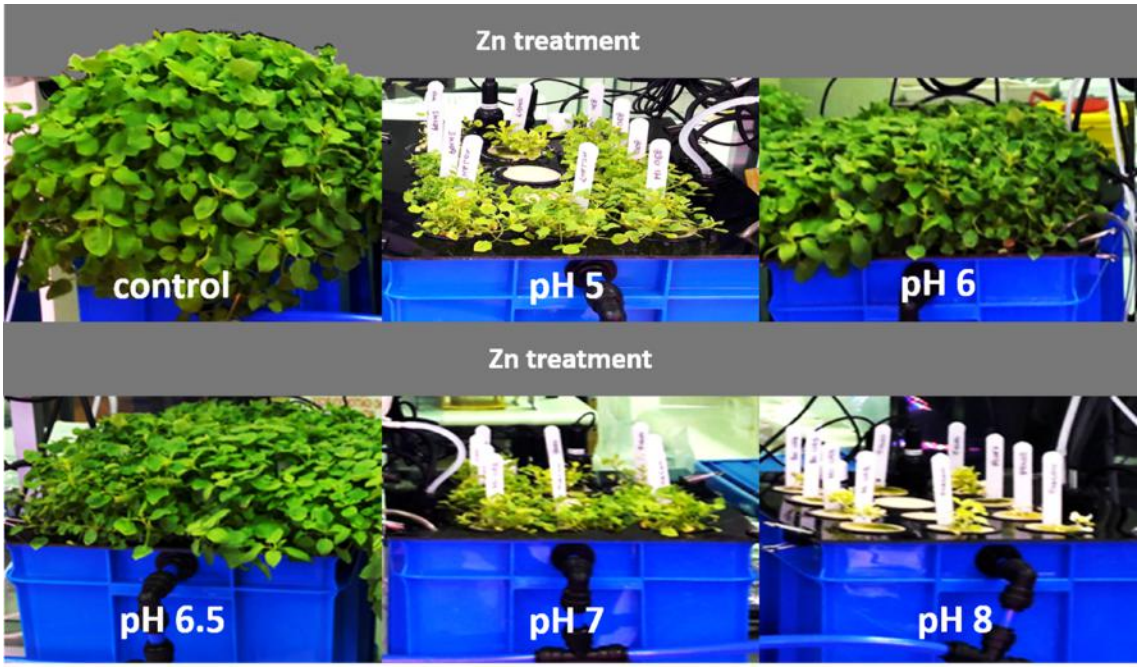
Table 1. Different pH treatments and initially supplied Zn and Ni concentrations during hydroponic culture of *Stellaria media*.

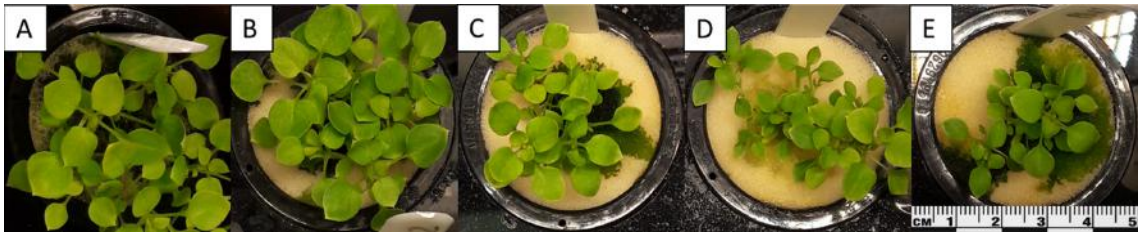
pH	**Zn (mM)**	**Ni (mM)**
5.0	0 (Control)	0 (Control)
5.0	0.55	0.10
5.5	n.d.	0.10
6.0	0.55	0.10
6.5	0.55	0.10
7.0	0.55	no growth
8.0	0.55	no growth

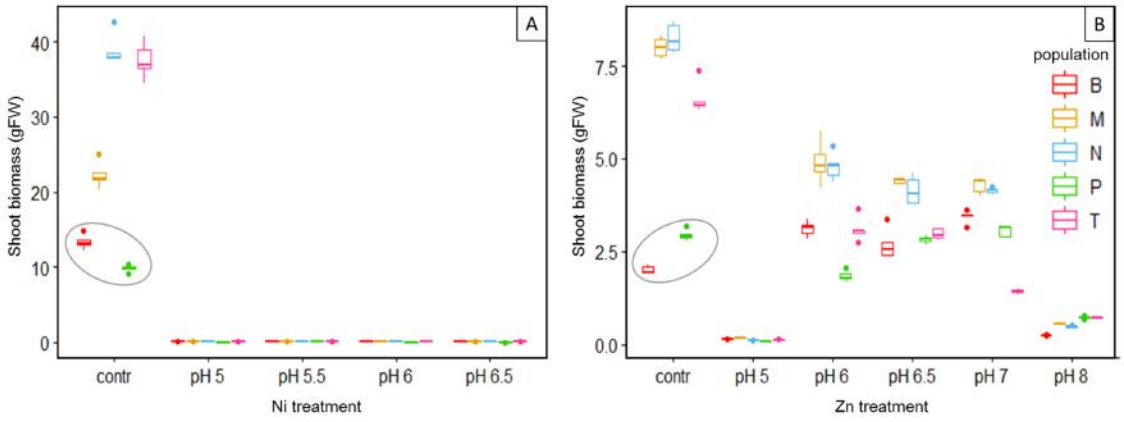
Table 2. Ni and Zn ionic activity at different pH treatments calculated with the software GEOCHEM-EZ.

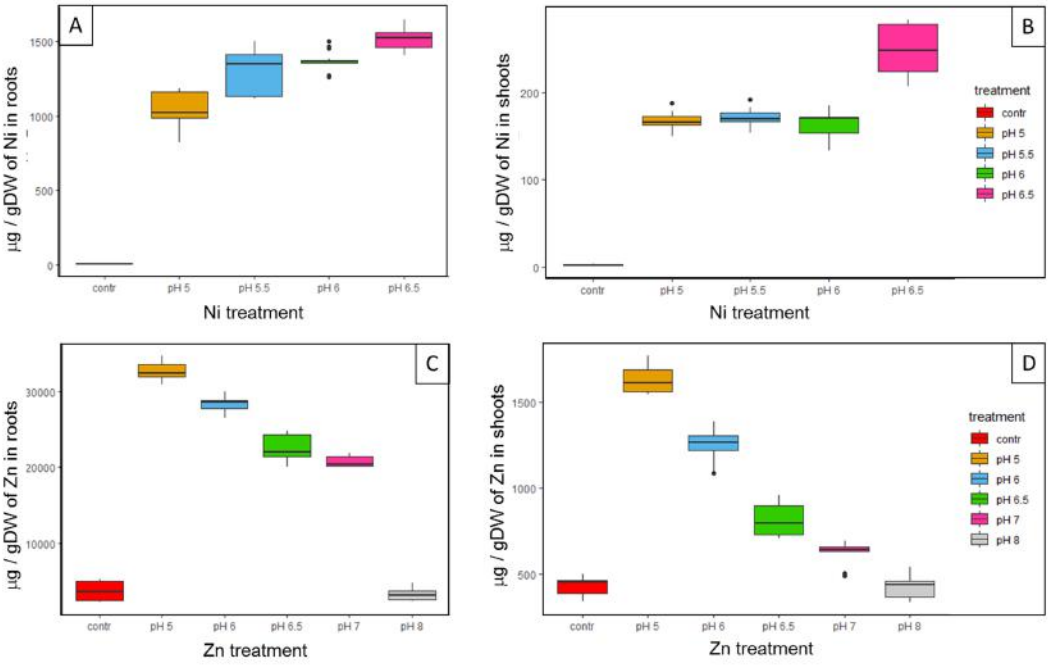
	Ionic activity in Zn treatment			**Precipitates µg/L**		
pH	Zn	Fe	PO_4^-	$ZnPO_4$	$FeOH^-$	$FePO_4$
8	3.904×10^{-7}	3.198×10^{-21}	9.213×10^{-9}	4.672	0.192	-
7	2.221×10^{-6}	3.190×10^{-18}	6.752×10^{-9}	1.104	0.187	-
6.5	7.006 v 10^{-6}	3.311×10^{-17}	1.202×10^{-10}	1.079	0.183	-
6	2.642 v 10^{-5}	2.395×10^{-16}	1.653×10^{-11}	0.990	0.185	-
5	2.584×10^{-4}	1.533×10^{-14}	2.590×10^{-13}	-	-	0.090
	Ionic activity in Ni treatment			**Precipitates µg/L**		
pH	Ni	Fe	PO_4^-	$ZnPO_4$	$FeOH^-$	$FePO_4$
6.5	3.337×10^{-5}	2.339×10^{-17}	1.668×10^{-10}	-	-	0.093
6	3.517×10^{-5}	1.788×10^{-16}	2.252×10^{-11}	-	-	0.093
5.5	3.590×10^{-5}	1.595×10^{-15}	2.520×10^{-12}	-	-	0.093
5	3.654×10^{-5}	1.535×10^{-14}	2.616×10^{-13}	-	-	0.093

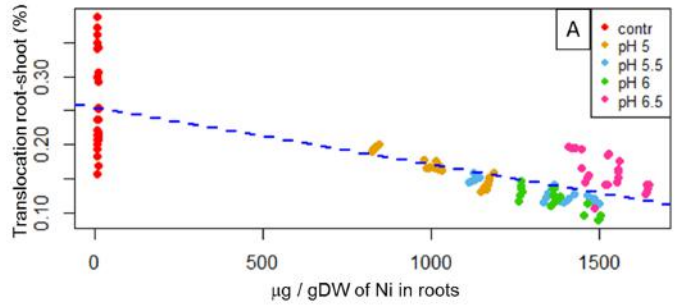

Air pump
Ni++ 0.1 mM
Air stone
Air pump
Zn++ 0.1 mM
Air stone
pH increase
Ni content increase
Zn content decrease
High Ni content at high pH
Oxidative stress with production of polyphenols and flavonoids.
Low photosynthetic pigments
High Zn content at low pH
Decreased biomass

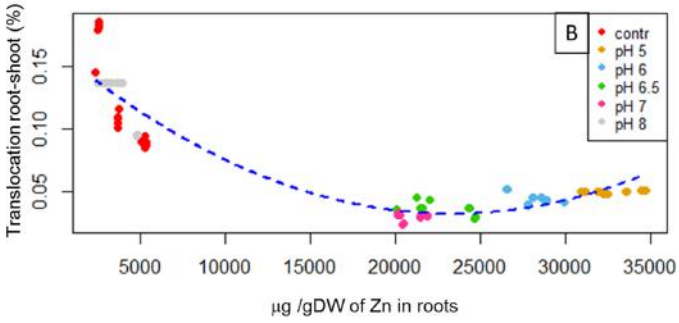

Zn treatment
control
pH 5
pH 6
Zn treatment
pH 6.5
pH 7
pH 8

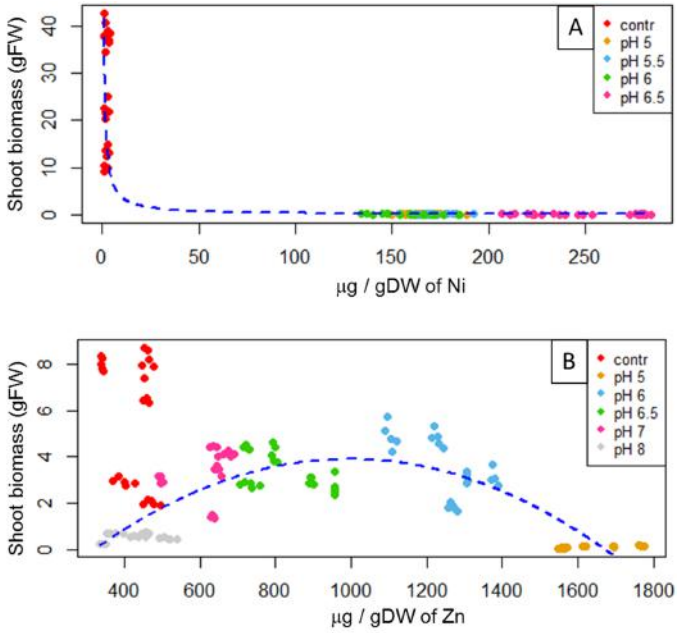

A
B
C
D
E
см 1 2 3 4 5


A
Shoot biomass (gFW)
40
30
20
10
0
contr
pH 5
pH 5.5
pH 6
pH 6.5
Ni treatment
B
population
B
M
N
P
T
Shoot biomass (gFW)
7.5
5.0
2.5
0.0
contr
pH 5
pH 6
pH 6.5
pH 7
pH 8
Zn treatment

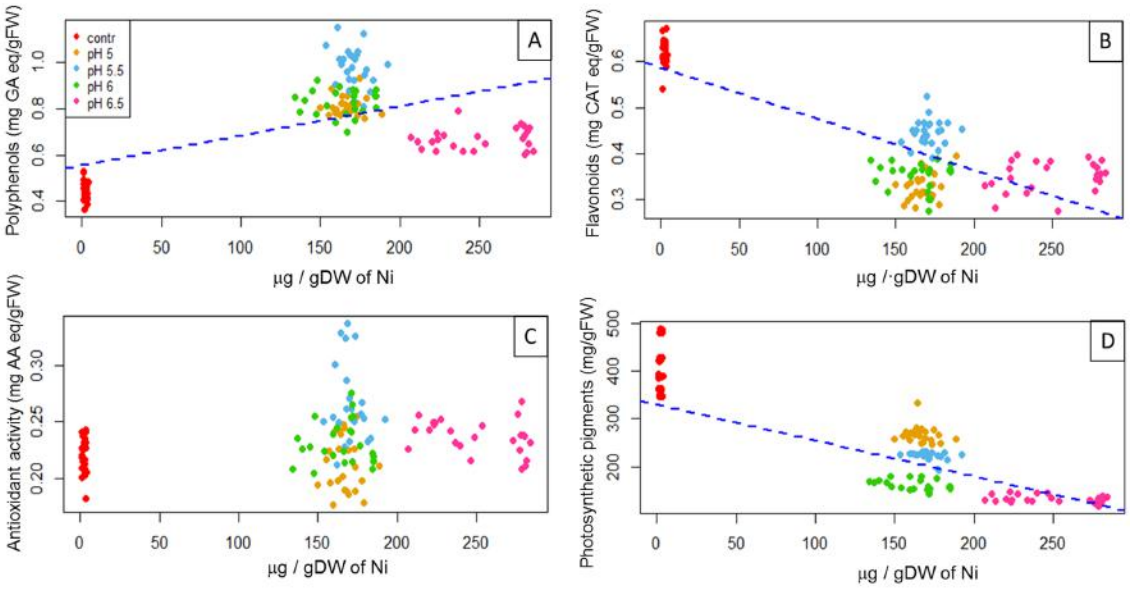

A
µg / gDW of Ni in roots
1500
1000
500
0
contr
pH 5
pH 5.5
pH 6
pH 6.5
Ni treatment
B
µg / gDW of Ni in shoots
200
100
0
treatment
contr
pH 5
pH 5.5
pH 6
pH 6.5
Ni treatment
C
µg / gDW of Zn in roots
30000
20000
10000
contr
pH 5
pH 6
pH 6.5
pH 7
pH 8
Zn treatment
D
µg / gDW of Zn in shoots
1500
1000
500
treatment
contr
pH 5
pH 6
pH 6.5
pH 7
pH 8
Zn treatment

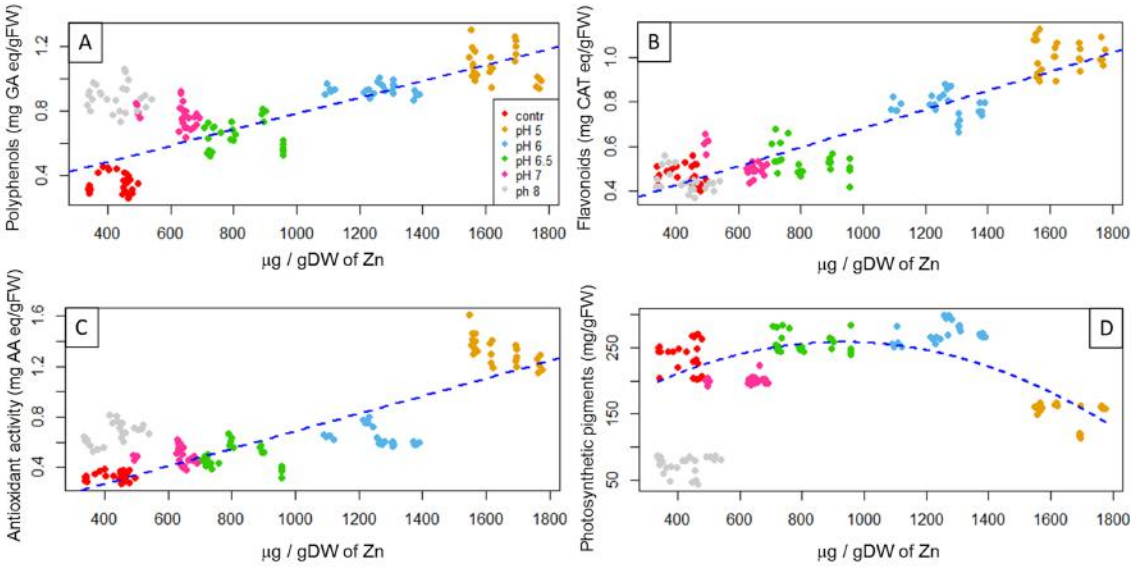

A
contr
pH 5
pH 5.5
pH 6
pH 6.5
Translocation root-shoot (%)
0.10
0.20
0.30
0
500
1000
1500
µg / gDW of Ni in roots


B
contr
pH 5
pH 6
pH 6.5
pH 7
pH 8
Translocation root-shoot (%)
0.05
0.10
0.15
5000
10000
15000
20000
25000
30000
35000
µg /gDW of Zn in roots


A
contr
pH 5
pH 5.5
pH 6
pH 6.5
Shoot biomass (gFW)
0
10
20
30
40
0
50
100
150
200
250
µg / gDW of Ni

B
contr
pH 5
pH 6
pH 6.5
pH 7
pH 8
Shoot biomass (gFW)
0
2
4
6
8
400
600
800
1000
1200
1400
1600
1800
µg / gDW of Zn


A
contr
pH 5
pH 5.5
pH 6
pH 6.5
Polyphenols (mg GA eq/gFW)
0.4
0.6
0.8
1.0
0
50
100
150
200
250
µg / gDW of Ni
B
Flavonoids (mg CAT eq/gFW)
0.3
0.4
0.5
0.6
µg /·gDW of Ni
C
Antioxidant activity (mg AA eq/gFW)
0.20
0.25
0.30
µg / gDW of Ni
D
Photosynthetic pigments (mg/gFW)
200
300
400
500
µg / gDW of Ni


A
Polyphenols (mg GA eq/gFW)
0.4
0.8
1.2
400
600
800
1000
1200
1400
1600
1800
µg / gDW of Zn
contr
pH 5
pH 6
pH 6.5
pH 7
ph 8
B
Flavonoids (mg CAT eq/gFW)
0.4
0.6
0.8
1.0
400
600
800
1000
1200
1400
1600
1800
µg / gDW of Zn
C
Antioxidant activity (mg AA eq/gFW)
0.4
0.8
1.2
1.6
400
600
800
1000
1200
1400
1600
1800
µg / gDW of Zn
D
Photosynthetic pigments (mg/gFW)
50
150
250
400
600
800
1000
1200
1400
1600
1800
µg / gDW of Zn

Title: Stress responses and nickel and zinc accumulation in different accessions of *Stellaria media* in response to solution pH variation in hydroponic culture

Authors: Mirko Salinitro[a], Antony van der Ent[b], Alice Tognacchini[c], Annalisa Tassoni[a*]

Highlights

- *Stellaria media* shows a positive correlation between Ni uptake and pH in hydroponics
- *Stellaria media* shows a negative correlation between Zn uptake and pH in hydroponics
- Metal uptake was positively correlated with polyphenols and flavonoids synthesis
- Hydroponic systems are suitable to study single variables affecting metal uptake

Contributions

MS designed the study, acquired and interpreted the data; AVDE designed and financed the study, interpreted data and drafted the article; AT acquired data; AT critically revised the manuscript and financed the study.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.