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

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1	Title: Stress responses and nickel and zinc accumulation in different accessions of Stellaria
2	media (L.) Vill. in response to solution pH variation in hydroponic culture
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4	Running title: Effect of pH on Ni and Zn uptake in Stellaria media
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31 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 36 opposite trends of Ni and Zn uptake along a pH gradient (between 5 to 8 for Zn and between 37 5 to 6.5 for Ni), when grown in hydroponics. In all treatments, the solution metal 38 concentration was fixed at 0.1 mM Ni or 0.55 mM Zn. Nickel accumulation increased with 39 increasing pH with an average concentration in shoots of 167 µg/gDW at pH 5 and of 250 40 µg/gDW at pH 6.5. In contrast, Zn accumulation decreased with increasing pH, with an 41 average concentration in shoots varying from 1640 µg/gDW at pH 5 to 435 µg/gDW at pH 8. 42 43 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, 44 45 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 46 populations, which did not show differences in relation to the accumulation of metals and 47 synthesis of antioxidant compounds, nonetheless showing a different biomass production 48 under control conditions. 49

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

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56 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.

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63 **1. Introduction**

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The relationship between metal availability and soil parameters has been intensively studied 65 over the last decades, to better understand which parameters have the highest impact on metal 66 activity in the soil, hence on uptake and toxicity for plants (Kukier et al., 2004; Pérez-Esteban 67 et al., 2014; Zia et al., 2018). A significant number of studies have shown that soil pH and 68 dissolved organic carbon (DOC) strongly influence metal concentrations and speciation in 69 soil solutions, although the specific effects vary between different metals (Kunhikrishnan et 70 71 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 72 73 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 74 75 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 76 77 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 78 79 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 80 studies. Above pH 7.5, most of the Zn present in soils is in fact bound to clays and organic 81 matter rather than in solution, while below pH 7, it is mostly available as hydrated Zn^{2+} ions 82 (Kukier et al., 2004; Park et al., 2011). Similarly, at pH values below 6, the Ni pool is almost 83 entirely available as the hydrated Ni²⁺ ion is mostly present in water solution. On the contrary, 84 above pH 6. Ni availability decreases as it is mostly absorbed by a multitude of soil 85 components capable of complexing this metal (e.g. OH⁻, CO³⁻) (Uren, 1992). However, 86 studies carried out on Ni uptake reported contrasting results. A study on the effect of different 87 soil pH on Ni concentration in the hyperaccumulators Alyssum murale Waldst. & Kit. and 88 Alyssum corsicum Duby concluded that an increase in soil pH was associated with an increase 89 90 in shoot Ni concentration (Li et al., 2003). These results were in contrast with other data 91 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 92 the hyperaccumulators Berkheya coddii Roessler and Alyssum bertolonii Desv. (Robinson et 93 al., 1999). Contrasting results were also found by Kukier et al. (2004) studying A. corsicum 94 and A. murale in different Ni-contaminated soils. Additionally, it was recently pointed out 95 that the bioavailability of metals varies with plant species as a result of rhizosphere 96

mechanisms like root acidification or alkalinisation (Chaignon et al., 2002; Hinsinger and 97 98 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., 99 2004). Despite the many studies carried out on the synergic influence of many factors on 100 metal availability in the soil (Kukier et al., 2004), limited research was performed under 101 hydroponic conditions (Kumar et al., 2012; Viehweger and Geipel, 2010). In contrast to 102 complex soil system in which the concomitant effect of several variables cannot be 103 distinguished, hydroponics allows to investigate how a single variable (*i.e.* pH or metal ionic 104 105 activity) influences the availability and the subsequent uptake of a specific metal in plants. 106 Moreover, acidic root exudates can be effectively counterbalanced in hydroponics as a result 107 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 108 109 functions causing the formation of reactive oxygen species (ROS), especially in nonaccumulator plants (Kisa, 2018; Ovecka and Takac, 2014). Under normal conditions, ROS are 110 111 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 112 113 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 114 antioxidant action in plants (Michalak, 2006). 115

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.

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129 2. Material and methods

131 *2.1. Materials*

Five different accessions of Stellaria media were collected in the year 2017 from five 132 different locations, to test whether plant Ni and Zn uptake and possible consequent stress 133 reactions are related to plant genetic variability present in the original habitat. In each 134 location, seeds were sampled at several stations to cover the whole genetic variability. Two 135 accessions were from the urban areas of Milan and Bologna (Italy), two accessions were from 136 woodland locations located on the outskirts of Milan and Bologna and one accession was 137 from the ultramafic outcrop at Mount Prinzera (Parma, Italy). The urban locations were 138 characterized by high and polymetallic trace element contamination of the soils, whereas the 139 woodland locations were characterized by low concentrations of trace elements. The 140 141 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 142 143 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 144 145 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 146 overnight in tap water and subsequently placed on a substrate composed of perlite, 147 vermiculite and quartz sand in a ratio of 1:1:1. Seeds were watered with tap water and kept in 148 the dark at 20°C for 2–3 days until germination. Seedlings were then transferred to a growth 149 cabinet with a 12-12 hours light-dark photoperiod at 25°C degrees for 1 week, before 150 transplanting them into the hydroponic system. 151

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153 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 μ M Fe-DTPA (diethylenetriaminepentaacetic acid ferric complex), 26 μ M B, 0.6 μ M Cu, 10.2 μ M Mn, 0.8 μ M Mo, 0.2 μ M Ni, 4.3 μ M Zn. The solution was kept constantly aerated by placing an aquarium air stone at the bottom of each tank.

161 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 162 Zn and Ni concentration of 0.55 mM and 0.10 mM, respectively (Table 1), which 163 corresponded to the average total metal concentration measured in the four most polluted soil 164 samples collected in the cities of Bologna and Milan (from where the *S. media* urban165 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 166 ranging from 5 to 6.5, as higher pH values in combination with Ni stress induced Fe 167 deficiency which led to plant death (Table 1). Control plants were cultivated at pH 5 with 168 normal Vega Classic nutrient (Canna, Brisbane QLD, Australia) solution. The control sample 169 was performed without the addition of any further salt, so the amount of nitrate was slightly 170 lower compared to the treatments (-1% for Ni treatment and -6% for Zn treatment) to which 171 172 $Zn(NO_3)_2$ or $Ni(NO_3)_2$ salts were added. The pH was automatically maintained at the set value \pm 0.1 with 0.1 M KOH solution (Table 1). A 5% (v/v) replacement of nutrient solution 173 174 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. 175 176 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, 177 178 under high intensity LED lights RAY22 physioSpecGreenhouse (Fluence Science, Austin, TX, USA), with photosynthetically active radiation (PAR) 550 μ mol m²/s), at 22/20°C 179 180 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.

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188 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). <u>A</u>fter centrifugation at $15300 \times g$ 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). 198 Total flavonoid colorimetric quantification was performed as described in Zhishen et al. 199 (1999). The results were expressed as mg of catechin equivalents per g of fresh weight (mg 200 CAT eq/gFW) by means of a dose-response calibration curve (between 2 and 14 μ g of 201 catechin).

- Antioxidant activity quantification was performed using the ABTS (2,2'-azino-bis(3etilbenzotiazolin-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 μ g 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
- 209 for 5 minutes and the supernatant recovered.

210 The supernatant was analysed at three different wavelengths (663, 644 and 452.5 nm) and the

- 211 obtained absorbance values were processed to give the pigment concentrations in mg/gFW
- 212 with the following equations:
- 213 chlorophyll $a = 10.3 \text{ x Abs}_{663} 0.098 \text{ x Abs}_{644}$,
- 214 chlorophyll $b = 19.7 \text{ x Abs}_{644} 3.87 \text{ x Abs}_{663}$,
- 215 carotenoids = $4.2 \times Abs_{452.5} [(0.0264 \times chl-a) + (0.426 \times chl-b)]$
- All spectrophotometric analyses were performed with a Cary 60 UV-Vis spectrophotometer
- 217 (Agilent Technologies, Santa Clara, CA, USA).
- 218
- 219 2.4. Nickel and zinc quantification

For the quantification of metals, ground roots and shoots were oven dried at 80°C until 220 constant weight (24 h). Samples were pre-digested at room temperature with 2 mL 70% (v/v) 221 HNO₃ for 1 day, and then digested on a heat block, 1 h at 70°C and 1 h at 125°C, following a 222 modified method from Huang et al. (1985). After digestion, sample volumes were brought up 223 to 10 mL with de-ionised water. Experimental blanks were included in the plant digestion. For 224 225 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 \pm 226 5% of the target concentrations of the certified reference (Ni 98.0%; Zn 103.5%). In the same 227 way, quality control solutions were included in the measurement with recovery rates of 95.8% 228 for Ni and 96.6% for Zn. Limits of quantification of the target elements were 0.007 mg/kg for 229 Ni and 0.003 mg/kg for Zn. The analyses were performed with an iCAP 7400 series ICP-OES 230

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

233

234 2.5. Data analysis

All the statistical analyses were performed using R software version 1.3.5 (R Core Team, 235 Vienna, Austria). The differences in metal uptake and metabolite production were evaluated 236 among the different accessions and pH treatments. For each treatment 5 biological replicates 237 for each accession were analysed in 2 technical replicates (n=5). Data were tested for 238 239 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-240 241 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-242 243 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 244 245 were calculated to determine the relationship between metal uptake and metabolite production. Linear regression models were used to describe the relations between metal 246 247 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 248 249 $(\mathbf{R}^2 \text{ and } p\text{-values are reported in brackets in the section Results})$. Graphical elaborations were performed using the R package ggpubr (https://CRAN.R-project.org/package=ggpubr). To 250 calculate free ionic activity of Ni and Zn at every pH tested a simulation was performed using 251 **GEOCHEM-EZ** 1.0 252 the software (free software available at http://www.PlantMineralNutrition.net). The ionic activity of a metal expresses the ease with 253 which the specific metal undergoes chemical reactions and interactions with biological 254 255 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 256 default. 257

258

259 **3. Results**

260

261 *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).

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271 *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).

277 Data showed that different pH treatments clearly affected the uptake of Ni and Zn (p = < 0.01both for shoots and roots) but in opposite ways (Fig. 4). Ni accumulation increased with the 278 279 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 280 281 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 282 pH 6.5. In contrast, Zn accumulation decreased with increasing pH values (Figs. 4C, D) with 283 concentrations in roots being 5- to 20-fold higher than in shoots, respectively, in pH 8 and pH 284 5 treatments. Zn concentration in roots varied between an average of 32700 µg/gDW in plants 285 grown at pH 5 and 3390 µg/gDW in plants grown at pH 8 (Fig. 4C). Shoot Zn uptake ranged 286 between 1647 µg/gDW and 435 µg/gDW, respectively, in plants grown at pH 5 and pH 8 287 (Fig. 4D). Ion activity of Ni did not show significant changes among pH treatments, except 288 for a slight decrease at increasing pH, whereas Zn ion activity decreased at higher pH 289 290 treatment (Table 2).

The translocation of both Ni and Zn from roots to shoots was affected by their availability. 291 292 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 293 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 294 the control) or its availability was low (i.e. in the pH 8 treatment), plant transferred up to the 295 15% of Zn from roots to shoots. Conversely, with the increase of Zn availability, the 296 translocation decreased to around 3% and went slightly up to 5% at pH 5 ($R^2 = 0.913$, p < 0.01) 297 298 (Fig. 5B).

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300 *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 301 reduction in shoot biomass. Ni toxicity was similar among all plants grown at different pH 302 values with a biomass reduction of around 95% compared to the control for all treatments; a 303 strong negative correlation between the two variables was found ($R^2 = 0.890$, p < 0.01) (Fig. 304 6A). The complete Zn uptake curve, from toxicity to deficiency caused by pH, was 305 highlighted in this study and the correlation between Zn uptake and shoot biomass reduction 306 was high ($R^2 = 0.652$, p < 0.01, control was not included in the regression) (Fig. 6B). Plants 307 showed strong Zn toxicity at pH 5, with a reduction in shoot biomass of 97% compared to the 308 309 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 310 311 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 8 319 treatment were reported in graphs (in grey) but not included in regression models because, as 320 previously explained, the observed stress symptoms were not caused by Zn and Ni toxicity 321 but related to metal deficiency due to the low activity of Zn and Fe (Table 2). Regarding 322 flavonoid content, the trend was reversed compared to Ni, in fact, in shoots flavonoids 323 increased with increasing Zn concentration ($R^2 = 0.852$, p < 0.01) (Fig. 8B). Moreover, as 324 reported for Ni, total polyphenols concentration was positively correlated with Zn 325 concentration ($R^2 = 0.728$, p < 0.01) (Fig. 8A). Because both polyphenols and flavonoids may 326 327 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 328 increased in treatments at pH 6 and 6.5 compared to the control, and then sharply decreased at 329 pH 5 ($\mathbb{R}^2 = 0.476, p = < 0.05$). 330

331

332 **4. Discussion**

The aim of our research was to study the effect of pH on Zn and Ni uptake in the non-333 334 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 335 on the other side, made plants more vulnerable to Ni and Zn toxicity. All plants undergoing 336 Ni treatments suffered chlorosis due to Ni toxicity, while in Zn treatments at pH 8 plants 337 suffered Zn and Fe deficiency (Table 2). In general, Ni caused stronger deleterious effects 338 than Zn, partly because of the intrinsic higher toxicity of Ni (Kabata-Pendias, 2010) and 339 340 partly because higher translocation occurred from roots to shoots (Fig. 5A, B).

341 The present experimental design, moreover, aimed at the assessment of possible variations 342 among the five different S. media plant populations tested. The hypothesis that populations 343 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 344 345 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 346 347 Zn) and in flavonoid, polyphenol and photosynthetic pigment production. Therefore, it appears that differences in Ni and Zn concentrations and availability between urban, 348 woodland and ultramafic soils were not marked enough to allow diversification among 349 populations. Despite that, under control conditions, the populations differed in plant size. In 350 fact, plants coming from contaminated environments, such as urban and ultramafic 351 accessions, showed a lower shoot biomass than woodland accessions (Figs. 2, 3A, 3B). At 352 increasing pH, a corresponding increase in Ni uptake was observed both in roots and shoots of 353 S. media (Fig. 4). The conceptual model of such a response is known as the biotic ligand 354 model (Di Toro et al., 2001). When metal ionic activity is kept constant, an increase in pH 355 may cause higher binding of metal cations to biotic ligands (biological membrane and 356 transporter proteins) because of the deprotonation of transporters (Lòpez et al., 2000). Our 357 results showed that Ni activity can be considered almost constant at all pH values tested 358 (Table 2), an ideal situation that could happen only in total absence of other factors able to 359 360 bind or release Ni ions from the solution. In fact, soil pot experiments carried out on nonaccumulator plants like crop plants (Kukier and Chaney, 2004; L'Huillier and Edighoffer, 361 362 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 367 the main variable affecting bioavailability and uptake of Zn. This study is consistent with the 368 literature since a strong negative correlation between Zn uptake and pH was observed (Fig. 4). 369 370 This behaviour can be explained by the chemical forms that Zn assumes at different pH 371 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 372 ZnOH⁺ which is no longer bioavailable (Kiekens, 1995; Reddy et al., 1995). Moreover, as 373 confirmed by the GEOCHEM-EZ simulation (Table 2), free Zn activity decreased with 374 375 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; PérezEsteban et al., 2014).

Zn and Ni toxicity, induced by differential uptake among pH treatments, influenced several 380 381 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 382 383 al., 2007; Weng et al., 2003) (Fig. 6, 7D, 8D). Beside these effects, the production of 384 secondary metabolites (like flavonoids and polyphenols) connected with oxidative stress was investigated. A positive correlation between the production of these compounds and metal 385 uptake was observed both for Ni ($R^2 = 0.793$, for polyphenols) and Zn ($R^2 = 0.852$, $R^2 =$ 386 0.728, for flavonoids and polyphenols, respectively) (Fig. 7A, 8A, 8B). For Ni treatments, 387 data were grouped in two main clusters: control and pH treatments. All treated plants 388 exhibited acute Ni toxicity; therefore, the production of polyphenols and flavonoids was quite 389 similar for all pH treatments and strongly different from the control (Fig. 7). Flavonoids (a 390 391 subfamily of polyphenols) were negatively correlated with Ni uptake (Fig. 7B), despite most 392 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 393 394 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, 395 probably as a result of acute Ni toxicity. In fact, higher Ni concentrations were detected in S. 396 media shoots at pH 6.5 (250 µg/gDW). Analogous results were reported for maize plants by 397 Kisa et al. (2016), who observed a general increase of phenolic compounds in all treatments 398 with Cu, Pb and Cd. Similarly L., Lavid et al. (2001) showed that increased Cd accumulation 399 in Nymphaea alba leaves triggered the synthesis of polyphenols and peroxidase enzyme 400

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).

404 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), 405 with no signs of inhibition by excessive toxicity. Polyphenols, flavonoids and total 406 antioxidant capacity all increased with increasing metal concentration, acting clearly against 407 the Zn-induced oxidative stress (Jayakumar et al., 2007; Kisa et al., 2016; Kumar et al., 2012; 408 409 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 410 411 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).

418

419 **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.

433

434 Contributions

435	MS designed the study and acquired and interpreted the data; AVDE designed and financed				
436	the study, interpreted data and drafted the article; AT acquired data; AT critically revised the				
437	manuscript and financed the study.				
438					
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443					
444	Supplementary Data				
445					
446	Supplementary Table S1. Stellaria media seed collection detailed stations.				
447	Supplementary Table S2. Complete dataset.				
448					
449	Competing interests				
450	The authors declare that they have no known competing financial interests or personal				
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453	Data availability				
454	All data generated or analysed during this study are included in the published article and in				
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460 461					
462	References				
463 464	Bhaduri A Fulekar M 2012 Antioxidant enzyme responses of plants to heavy metal stress				
465	Reviews in Environmental Science and Bio/Technology 11, 55-69				
465	Chaignon V Bedin F Hinsinger P 2002 Copper bioavailability and rhizosphere pH				
467	changes as affected by nitrogen supply for tomato and oilseed rape cropped on an acidic and a				
468	calcareous soil Plant Soil 243 219-228				
-100	Curcurous 5011. 1 min 5011 275, 217 220.				

- 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.
- 471 Collier, M.H., Keane, B., Rogstad, S.H., 2010. Productivity differences between dandelion
- 472 (Taraxacum officinale; Asteraceae) clones from pollution impacted versus non-impacted
- 473 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.
- 480 Hinsinger, P., Courchesne, F., 2008. Biogeochemistry of metals and metalloids at the soil-
- 481 root interface, in: Violante, A., Huang, P.M., Gadd, G.M. (Eds.) Biophysic- Chemical
- 482 Processes of Heavy Metals and Metalloids in Soil Environments, John Wiley & Sons,
- 483 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.
- 486 Jayakumar, K., Jaleel, C., Vijayarengan, P., 2007. Changes in growth, biochemical
- 487 constituents, and antioxidant potentials in radish (*Raphanus sativus* L.) under cobalt stress.
- 488 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.
- 491 Kiekens, L., 1995. Zinc, in: Alloway, B.J. (Ed.) Heavy Metals in Soils, Blackie Academic and
- 492 Professional, London, UK, pp. 284-305.
- 493 Kisa, D., 2018. The responses of antioxidant system against the heavy metal-induced stress in
- 494 tomato. J. Nat. Appl. Sci. 22, 1-6.
- 495 Kisa, D., Elmastas, M., Ozturk, L., Kayir, O., 2016. Responses of the phenolic compounds of
- 496 Zea mays under heavy metal stress. Appl. Biol. Chem. 59, 813-820.
- 497 Kukier, U., Chaney, R.L., 2004. *In situ* remediation of nickel phytotoxicity for different plant
- 498 species. J. Plant Nutr. 27, 465-495.
- 499 Kukier, U., Peters, C.A., Chaney, R.L., Angle, J.S., Roseberg, R.J., 2004. The effect of pH on
- 500 metal accumulation in two *Alyssum* species. J. Environ. Qual. 33, 2090-2102.

- 501 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.
- 504 Kunhikrishnan, A., Choppala, G., Seshadri, B., Wijesekara, H., Bolan, N.S., Mbene, K., Kim,
- 505 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, 183191.
- Küpper, H., Parameswaran, A., Leitenmaier, B., Trtilek, M., Setlik, I., 2007. Cadmiuminduced inhibition of photosynthesis and long-term acclimation to cadmium stress in the
 hyperaccumulator *Thlaspi caerulescens*. New Phytol. 175, 655-674.
- 511 L'Huillier, L., Edighoffer, S., 1996. Extractability of nickel and its concentration in cultivated
- 512 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.
- 516 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.
- 524 24, 146-151.
- 525 McKenzie, E.R., Money, J.E., Green, P.G., Young, T.M., 2009. Metals associated with 526 stormwater-relevant brake and tire samples. Sci. Total Environ. 407, 5855-5860.
- 527 Meers, E., Unamuno, V.R., Du Laing, G., Vangronsveld, J., Vanbroekhoven, K., Samson, R.,
- 528 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.
- 531 Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing
- under heavy metal stress. Pol. J. Environ. Stud. 15, 523-530.
- 533 Ovecka, M., Takac, T., 2014. Managing heavy metal toxicity stress in plants: biological and
- 534 biotechnological tools. Biotechnol. Adv. 32, 73-86.

- 535 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.
- 537 Hazard. Mater. 185, 549-574.
- 538 Pérez-Esteban, J., Escolastico, C., Masaguer, A., Vargas, C., Moliner, A., 2014. Soluble
- 539 organic carbon and pH of organic amendments affect metal mobility and chemical speciation
- 540 in mine soils. Chemosphere 103, 164-171.
- 541 Radwan, D.E.M., Fayez, K.A., Mahmoud, S.Y., Hamad, A., Lu, G., 2007. Physiological and
- 542 metabolic changes of *Cucurbita pepo* leaves in response to zucchini yellow mosaic virus
- 543 (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.
- 546 Robinson, B.H., Brooks, R.R., Clothier, B.E., 1999. Soil amendments affecting nickel and
- 547 cobalt uptake by *Berkheya coddii*: potential use for phytomining and phytoremediation. Ann.
- 548 Bot. 84, 689-694.
- 549 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.
- 552 Uren, N.C., 1992. Forms, reactions, and availability of Nickel in soils. Adv. Agron. 48, 141-553 203.
- Viehweger, K., Geipel, G., 2010. Uranium accumulation and tolerance in *Arabidopsis halleri*under native versus hydroponic conditions. Environ. Exp. Bot. 69, 39-46.
- 556 Weng, L.P., Lexmond, T.M., Wolthoorn, A., Temminghoff, E.J.M., Van Riemsdijk, W.H.,
- 557 2003. Phytotoxicity and bioavailability of nickel: chemical speciation and bioaccumulation.
- 558 Environ. Toxicol. Chem. 22, 2180-2187.
- 559 Wenzel, W.W., Bunkowski, M., Puschenreiter, M., Horak, O., 2003. Rhizosphere
- 560 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 mulberryand their scavenging effects on superoxide radicals. Food Chem. 64, 555-559.

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

574 **Figure legends**

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576

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.

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- 581

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.

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586

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.

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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.

601

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.

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Figure 7. Polyphenol, flavonoid, photosynthetic pigment and antioxidant activity levels in S. 615 media shoots in relation to accumulated Ni concentration after hydroponic culture at different 616 pHs. A) Total polyphenols, expressed as mg of gallic acid (GA) equivalents/gFW; B) total 617 flavonoids, expressed as mg of catechin (CAT) equivalents/gFW; C) antioxidant activity, 618 expressed as mg of ascorbic acid (AA) equivalents/gFW; D) total content of chlorophyll-a, 619 chlorophyll-b and carotenoids, expressed in mg/gFW. A) Linear model ($R^2 = 0.309$, p < 0.05); 620 B) Linear model ($R^2 = 0.793$, p < 0.01); C) Linear model ($R^2 = 0.037$, p = 0.17); D) Linear 621 model ($R^2 = 0.680$, p < 0.01). Each treatment is represented by data coming from five 622 accessions analysed in five biological replicates (n=25). The complete dataset is available in 623 624 Supplementary Table S2.

625 626

Figure 8. Polyphenol, flavonoid, photosynthetic pigment and antioxidant activity levels in S. 627 media shoots in relation to accumulated Zn concentration after hydroponic culture at different 628 pHs. A) Total polyphenols expressed as mg of gallic acid (GA) equivalents/gFW; B) total 629 flavonoids expressed as mg of catechin (CAT) equivalents/gFW; C) antioxidant activity 630 expressed as mg of ascorbic acid (AA) equivalents/gFW; D) total content of chlorophyll-a, 631 chlorophyll-b and carotenoids expressed in mg/gFW. A) Linear model ($R^2 = 0.728$, p < 0.01); 632 B) Linear model ($R^2 = 0.852$, p < 0.01); C) Linear model ($R^2 = 0.751$, p < 0.01); D) Non-linear 633 model ($R^2 = 0.476$, p = < 0.05). Each treatment is represented by data coming from five 634 accessions analysed in five biological replicates (n=25). The complete dataset is available in 635 Supplementary Table S2. 636

637

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

	Ionic ac	ctivity in Zn t	reatment	Precipitates µg/L		
pН	Zn	Fe	PO_4^-	ZnPO ₄	FeOH	FePO ₄
8	3.904×10^{-7}	$3.198\times10^{\text{-}21}$	9.213 x 10 ⁻⁹	4.672	0.192	-
7	2.221×10^{-6}	3.190×10^{18}	6.752 x 10 ⁻⁹	1.104	0.187	-
6.5	7.006 v 10 ⁻⁶	3.311×10^{-17}	1.202 x 10 ⁻¹⁰	1.079	0.183	-
6	2.642 v 10 ⁻⁵	$2.395 imes 10^{-16}$	1.653 x 10 ⁻¹¹	0.990	0.185	-
5	$2.584 imes 10^{-4}$	$1.533\times10^{\text{-}14}$	2.590 x 10 ⁻¹³	-	-	0.090
	Ionic activity in Ni treatment			Precipitates µg/L		
pН	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}	$1.788\times10^{\text{-16}}$	$2.252\times10^{\text{-}11}$	-	-	0.093
5.5	3.590×10^{-5}	$1.595 imes 10^{-15}$	$2.520 imes 10^{-12}$	-	-	0.093
5	3.654×10^{-5}	$1.535 imes 10^{-14}$	2.616×10^{-13}	-	-	0.093

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



















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.