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Mathieu Scattolin[1], Steve Peuble[1], Fernando Pereira[1], Frédéric Paran[1], Jacques Moutte[1], Noureddine Menad[2] and Olivier Faure[1*]

[1] *Mines Saint-Etienne, Univ Lyon, Univ Jean Moulin, Univ Lumière, Univ Jean Monnet, ENTPE, INSA Lyon, ENS Lyon, CNRS, UMR 5600 EVS, Centre SPIN, Département PEG, F - 42023 Saint-Etienne, France*

[2] *BRGM, Water, Environment Process and Analysis, Waste and Raw Materials & Recycling Unit, 3 Avenue Claude Guillemin, BP 36009, 45060 Orléans Cedex, France*

**Corresponding author.*

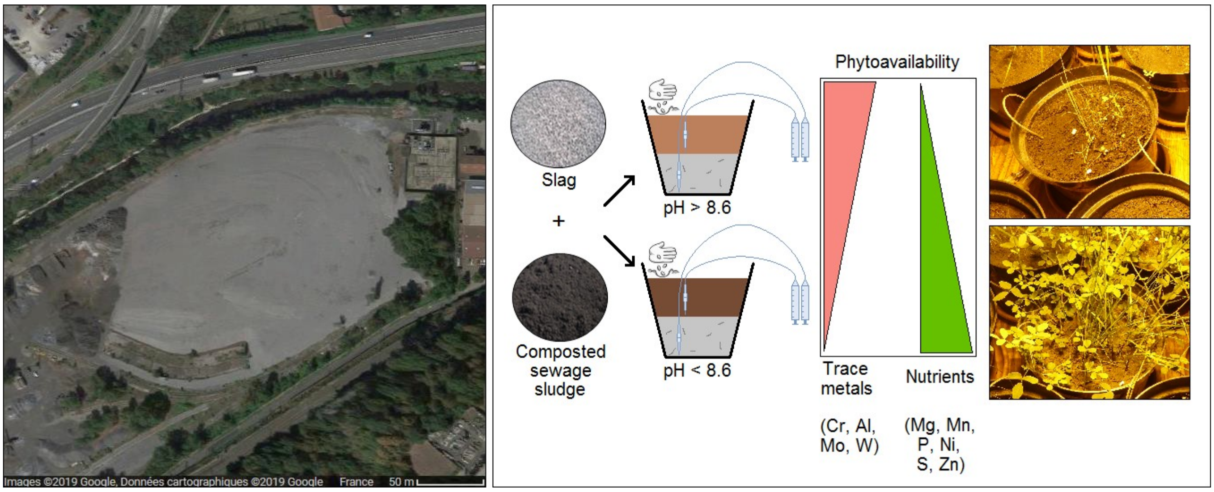
Olivier Faure

Mines Saint-Etienne, Univ Lyon, Univ Jean Moulin, Univ Lumière, Univ Jean Monnet, ENTPE, INSA Lyon, ENS Lyon, CNRS, UMR 5600 EVS, Centre SPIN, Département PEG, 158 cours Fauriel, F - 42023 Saint-Etienne Cedex2, France

Email: ofaure@mines-stetienne.fr

Phone: +33 477 420 206

Graphical abstract



Abstract

Because of their high content in toxic metals, steel slag dumps are potential threats for the environment and public health. Among management methods that could mitigate their hazard, aided-phytostabilization is a relevant, though challenging, option. Indeed, steel slags are very unfavorable for plant growth, due to metal toxicity and very alkaline pH (>10). In this work, we investigated how composted sewage sludge could alleviate slag's toxicity while improving its nutritional status. A pot experiment was performed to study biomass production and leaf ionome composition of five herbaceous species (*Achillea millefolium*, *Bromus erectus*, *Festuca arundinacea*, *Melilotus officinalis* and *Medicago sativa*), in relation to soil pore water's pH, concentration of trace and major elements and their chemical speciation. Results showed that pH had a clear-cut effect on plant development. Above pH 8.6, plant biomass was severely affected, due to accumulation of Cr above toxic threshold and deficiencies in Mn, Zn and P. Below pH 8.6, biomass increased significantly, together with a decrease in leaf Cr below toxic level, and an increase in Mn, Zn and P above deficiency levels. Thus, these results bring new insights into the causes of slag phytotoxicity and allow considering aided-pytostabilization as a realistic and efficient approach for the remediation of steel slag dumps, provided soil pH is carefully monitored before seeding.

Keywords

Phytomanagement; Toxic metals; Nutrient deficiency; Chemical speciation; Plant ionome

1. Introduction

Steel slags are ubiquitous wastes generated by metallurgical industries during the purification of iron and its alloys. Although their precise composition depends on the process used for steel production, these slags are generally highly alkaline materials (pH >10) mainly composed of Al, Ca, Fe, Mg and Si, associated in various mineralogical forms such as lime, silicates and metal oxides (Piatak *et al.*, 2015). They also contain various levels of potentially harmful trace elements (TEs), such as Cr, Cu, Mn, Mo, Pb, V, W and Zn.

Since the industrial revolution, the massive steel demand led to the production of huge quantities of slags, that were mainly stored as open dumps, often left bare without any protective measures. Consequently, most slagheaps are exposed to wind and rainfalls, causing them to weather and leach. They are therefore a source of hazard, due to potential metal transfers toward the atmosphere, surrounding soils, surface and ground waters, and ultimately human populations or other biological targets. Indeed, several studies have pointed out a degradation of water quality in the vicinity of slag dumps, due to high levels of Al, Cr, Mo, V, Ni, Pb, and/or Zn in slag's leachates (Bayless and Schulz, 2003; Roadcap *et al.*, 2005; Mayes *et al.*, 2008; Gomes *et al.*, 2016). Thus, slag deposits and their leachates are requiring the development of sustainable ways of management for limiting their environmental impact. In this respect, plant-based approaches, *i.e.* phytostabilization (Kidd *et al.*, 2015; Wang *et al.*, 2017) and constructed wetlands (Gomes *et al.*, 2019) could constitute relevant options.

The main goal of phytostabilization is to mitigate pollutants transfers, by implementing a homogenous plant cover without bringing an extra layer of clean soil (Mendez and Maier, 2008; Vamerali *et al.*, 2010; Kidd *et al.*, 2015). This approach has at least three advantages. Firstly, vegetated soils are far less prone to mechanical erosion than bare ones, thereby limiting dispersion of contaminated dusts. Second, plants intercept a significant amount of water, thereby limiting leaching of pollutants towards groundwater. Third, root exudates released by plants in their rhizosphere promote the development of complex communities of microbes (bacteria and fungi), which are key players in many soil functions (Nannipieri *et al.*, 2003), including organic matter dynamics and nitrogen and phosphorus cycling. These microorganism-driven transformations speed up the so-called soil aging effect (Alexander, 2000), which generally decreases the (bio)availability (Semple *et al.*, 2004; AFNOR, 2008) of organic and metal pollutants in the soil (Lock and Janssen, 2003; Remon *et al.*, 2013).

During the last decade, several studies have addressed the issue of phytostabilization of slags derived from various metallurgical processes, *e.g.* zinc smelting (Luo *et al.*, 2018, 2019, 2020), lead smelting (Ramos da Silva *et al.*, 2017), copper smelting (Agnello *et al.*, 2018), and iron and steel smelting (Bouchardon *et al.*, 2014; Oustriere *et al.*, 2016). All these studies emphasized that plant growth on metallurgical slags must overcome severe edaphic constraints. Indeed, besides the potential toxicity of metal elements, slags are almost devoid of organic matter and major nutrients, such as nitrogen and potassium. They also have a sandy texture, leading to a very low water holding capacity that impairs seeds germination and plantlets development. Moreover, an additional feature of steel slag, as compared to Zn, Pb and Cu slags, is its highly alkaline pH (> 10) that is very unfavorable for plant establishment.

However, Bouchardon *et al.* (2014) and Oustriere *et al.* (2016) demonstrated that bringing composted sewage sludge (CSS) (at an amount of 120 T/ha and mixed with the 10 top centimeters of the slag), was a cheap and efficient treatment to promote plant development on steel slags, provided that an assemblage of adapted species (drought tolerant, calcicolous, pseudo-metallophyte species) was sown. By contrast, application of an inorganic NPK fertilization was ineffective on plant growth, as compared to control plots. Although these authors hypothesized that the alleviation of slag's toxicity by CSS could be due to the lowering of soil pore water (SPW) pH and the improvement of the plants' nutritional status, these mechanisms were not further investigated. Yet, a comprehensive characterization of the main parameters governing plant establishment on steel slags is mandatory for the successful implementation of phytostabilization at a large scale.

Actually, whether soil elements are toxic or essential for plants, their uptake by roots (*i.e.* their phytoavalaibility), and thus their inhibitory or beneficial effects on growth and development, depends on their solubility, *i.e.* their partitioning between the soil solid phases and the pore water (Ge *et al*, 2000). This is, in turn, strongly influenced by soil properties (*e.g*. pH, redox potential, cation exchange capacity) and by interactions between chemical species in the SPW (*e.g.* precipitation *vs* dissolution).

Therefore, in this study, we hypothesized that the beneficial effect of CSS amendment, in addition to its role in macronutrient supply, could be primarily due to a pH-induced change of elements' solubility in SPW. This could decrease the mobility of potentially toxic metal species, while increasing the availability of essential nutrients. To test this hypothesis, we performed a pot experiment using a gradient of CSS amendment and we studied element concentration and speciation in the soil pore water and their accumulation in plants, in relation to slag's pH.

2. Materials and methods

2.1 Slag and CSS characteristics

The slag used in this study was collected from a metallurgical landfill (45°26′ N, 4°17′ E) located at Châteauneuf (France). This slagheap had received the by-products of a neighboring steel and iron smelting plant from ca. 1860 to 2001, and contained approx. 500,000 m^3 of slags, spread over an area of about 5 ha and a height of 10 m. About 300 kg of slag was taken at the surface of the slagheap (0 - 10 cm depth).

The CSS used for slag amendment was purchased from a specialized producer (Terralys Inveko, Les Allerys, St Priest la Roche, France).

For plant culture experiments, both slag and CSS were sieved to 5 mm, and air-dried until constant weight. Slag was then thoroughly mix with a ciment mixer.

For the determination of the main agronomic characteristics of slag and CSS, dry sub-samples (approx. 1 kg) were sieved to 2 mm. The selected following parameters were analyzed: cation exchange capacity (CEC) (NF X31-130), total organic carbon (TOC) (NF ISO 14235), total nitrogen (NF ISO 11261), pH in water (NF ISO 10390), particle size distribution (NF X31-107), total $CaCO_3$ (NF ISO 10693), active $CaCO_3$ (NFX 31-106) and exchangeable phosphorus (NF ISO 11263). All analyses were performed at the "Laboratoire Agronomique de Normandie" (LANO, St-Lô, France), according to the standardized working procedure NF EN ISO/CEI 17025.

For the determination of elemental composition of slag and CSS, samples (approx. 1 g) were finely powdered with an agate mortar and then calcinated at 960°C for 2 hours in platinum crucibles, before digestion by alkali fusion. Fusion beads were made using a fusion machine (xrFuse 2, XRF Scientific Ltd, Australia), by fusing 200 mg of calcinated sample with 1.5 g $LiBO_2$ plus a few drops of LiBr. Beads were then dissolved in 100 ml 2M HNO_3, before ICP-OES analysis (Horiba Jobin Yvon, Activa).

The selected characteristics of slag and CSS are given in Table 1.

2.2 Plant culture experiments

A pot experiment was performed in order to better understand the influence of slag amendment with CSS, on soil pore water pH, element speciation, and plant growth. For this purpose, plastic pots (20 cm ⌀ x 20 cm) were first filled with 3 kg of dry slag (approx. 10 cm in high), and then completed with a 10 cm layer of slag mixed with CSS. This two-layers system was used to mimics field conditions as close as possible. Two levels of CSS were used: 60 T/ha (*i.e.* 180 g CSS/pot) and 120 T/ha (*i.e.* 360 g CSS/pot). Control pots were filled with 5 kg of slag and did not received any amendment. During pot preparation, two soil pore water samplers (Rhizon flex®, Rhizosphere Research Products, The Netherlands) were inserted with an angle of 45° in both layers of each pot. Pots were then watered with deionized water (2 MΩ.cm), up to 70% of the substrate water holding capacity (WHC); they were left in equilibrium with the atmosphere for two weeks at room temperature. During this period, pots were watered with deionized water every two days, taking care to avoid leaching.

Sowing was then performed two weeks after pots' preparation, using the same seed assemblage as recommended by Bouchardon *et al.* (2014), *i.e*: *Achillea millefolium* L., *Bromus erectus* Huds, *Festuca arundinacea* Schreb., *Melilotus officinalis* (L.) Lam., and *Medicago sativa* L.. These species belonged to three botanical families (*Asteraceae*, *Fabaceae* and *Poaceae*) and were selected because they were known as drought tolerant, calcicolous, pseudo-metallophyte species. The seed density was 6 g/m² (*i.e.* 200 mg per pot) with the following mass proportions for each species: 45% for *B. erectus*, 25% for *F. arundinacea*, 12.5% for *M. officinalis* and *M. sativa,* and 5% for *A. millefolium*.

Pots were placed in the greenhouse for 75 days (photoperiod 14h light: 10h dark, 700µmol photons/m²/s, temperature 25°C day-20°C night). They were watered with deionized water about three times a week, to maintain substrate's humidity at 70% WHC while avoiding leaching of the substrate. The experiment was performed as a completely randomized design with ten replicates per treatment.

2.3 Soil pore water sampling and analysis

SPW was sampled (approx. 5 mL) from both rhizons of each pot at the day of sowing (day-0). The pH of each SPW sample was immediately measured using a Consort C832 pH-meter equipped with a combined glass electrode. For trace element determination, SPW samples were diluted 5 times in ultrapure water (> 18 MΩ.cm). Analyses were performed by ICP-MS (Agilent Technology 7800 ICP MS) using calibration curves made with certified multi-element standards (PlasmaCAL, SCP Science). Limits of quantification were 1 nM for Cd, As, and W; 5 nM for Pb and V; 10 nM for Mn and Cr; 50 nM for Ni, Mo, Cu and Zn; 200 nM for P and Al; 500 nM for Mg and Fe; 1 µM for Na and K; 1.5 µM for Ca; 9 µM for S. The relative standard deviation between replicate samples was less than 5%. For each pot, data presented in both text and figures are the average of the two rhizons.

2.4 Plant harvest and analysis

After 75 days of culture, shoots from all plants present in each pot were harvested. They were thoroughly washed with tap water, rinsed with deionized water, and dried at 60°C until constant weight. Total shoot dry weight (DW) per pot was then recorded for each species. Additionally, leaves from each species were taken for ionome analysis. For this purpose, 100 mg dry leaf samples were first pre-digested in 1 mL HNO_3 (65%, sub-boil distilled analytical grade) at 60°C during 1 hour; 200 µL H_2O_2 (35%, analytical grade) was then added and pre-digestion was continued at 90°C for 1 hour. After the pre-digestion step, 1 mL HNO_3 was added again and samples were heated at 125°C for 24h. Finally, 200 µL HF (analytical grade, 5% V/V in ultrapure water) was added, and samples were evaporated to dryness at 95°C. The final residue was then diluted in 25 mL HNO_3 (1%, V/V in ultrapure water). Digested samples were analyzed by ICP-MS (Agilent Technology 7800 ICP MS). Limits of quantification were 0.2 mg/kg for Mo and W; 0.5 mg/kg for Mn and Ni; 1 mg/kg for Cr; 7 mg/kg for Zn; 10 mg/kg for Mg and Al; 100 mg/kg for P; 200 mg/kg for S. For the quality assurance of plant mineralization and analysis, the certified reference material CTA-OTL1 (Oriental Tobacco Leaves), ERM-CD81 (Rye Grass) and BCR-482 (Lichen) were used in each digestion series. Mean percent recovery were the following: Al 108%; Cr 77%; Mg 98%; Mn 99%; Mo 87%; Ni 94%; P 96%; S 92%; W (not certified for the CRM used) and Zn 97%.

2.5 Geochemical modelling of elements' speciation in SPW

The speciation and chemical equilibrium of elements in SPW was determined using the Arxim code (Moutte, 2009). Calculations were performed by considering median chemical composition of the SPW for different pH ranges ([8.1-8.6],]8.6-9.6],]9.6-10.6] and]10.6-11.6]), as measured at day-0; physical parameters for the model were fixed at: T = 25°C, P = 10^5 Pa, oxidizing conditions (that were those of experimental plant growing conditions). Formation constants for minerals and aqueous species were computed using the SUPCRT approach (Johnson *et al*., 1992) based on data recently updated by Zimmer *et al*. (2016). This database was completed by equilibrium constants of the molybdate and chromate species (aqueous complexes and salts) extracted from the MINTEQ database (Allison, 1991).

2.6 Data exploration and statistical analyses

Visual exploration of univariate data was performed using the box-and-whiskers method. In every case, boxplots were drawn according to Tukey's approach (Tukey, 1977) [*i.e.* the bottom and the top of the box are the 1^{st} (Q1) and 3^{rd} (Q3) quartile of data distribution, while the line inside the box shows the median value. Whiskers are drawn to reach the lower (top whisker) and higher (bottom whisker) point of data distribution lying within the ranges (Q3 + 1.5 IQR) and (Q1 – 1.5 IQR), respectively (with IQR = interquartile range = Q3 – Q1). Points outside the whiskers are considered as outliers].

The effects of CSS addition and/or SPW's pH on plant biomass production and leaf metal contents were compared using Kruskall-Wallis' one-way ANOVA on ranks. When necessary, pairwise analyses were performed using the Wilcoxon rank sum test, with Holm's *p*-adjust method for multiple comparisons. Significance levels and associated symbols in graphical plots were the following: $p > 0.1$, non-significant (NS); $0.05 < p < 0.1$, poorly significant (*); $0.01 < p < 0.05$, significant (**); $p < 0.01$, highly significant (***).

Multi-elemental composition of SPW was explored using principal component analysis (PCA) on standardized data. Relationships between individual elements in SPW and plant biomass production were explored by calculating Spearman's rank correlation coefficients and associated *p*-values.

All statistical calculations and plot drawings were performed using R software (R core Team, 2017) and the FactoMineR (Lê *et al*., 2008) package.

3. Results

3.1 CSS addition decreases SPW's pH

The pH of SPW, as measured at the time of sowing (day-0) in amended and non-amended slag, is given in Fig. 1a. Results showed that median values of SPW's pH had sharply decrease with CSS addition in slag, ranging from 11.2 for bulk slag (CSS0), to 9.1 at 60 T/ha CSS (CSS60), and up to 8.5 for slag with 120 T/ha CSS (CSS120). This decrease in SPW's pH was highly significant when comparing CSS0 with CSS60 or CSS120 ($p = 5\ 10^{-4}$) but was non-significant between CSS60 and CSS120 ($p = 0.27$). It is noteworthy that, for a given experimental condition, SPW's pH was quite heterogeneous, ranging from 10.7 to 11.6 in CSS0, from 8.3 to 10.3 in CSS60 and from 8.1 to 9.6 in CSS120. Accordingly, sorting the overall pots by an ascending pH order (Fig. 1b) revealed an almost continuous curve of pH values within the range 8.1 to 11.6. Thus, observing points distribution on this curve allowed discriminating four pH ranges for experimental pots: 8.1 to 8.6 (including 5 pots of CSS60 and 5 of CSS120), 8.7 to 9.6 (including 5 pots of CSS120), 9.7 to 10.6 (including 5 pots of CSS60) and 10.7 to 11.6 (including the 10 pots of CSS0).

3.2 Plant biomass is strongly reduced when SPW pH is above 8.6

Fig. 2a shows plant biomass, expressed as the total shoot dry weight (DW) per pot at the time of harvesting (day-75), in relation to CSS level in slag. Seeds sown on bulk slag (CSS0) did not germinated; consequently, no biomass was obtained at d-75. By contrast, CSS addition allowed seed germination and plant development, but no significant difference was evidenced between CSS60 and CSS120 (p = 0.35). Median values of shoot biomass production in amended slag was about 6 g DW/pot but, for both CSS levels, a very high variability was observed between pots, with biomass values ranging from less than 1g to close to 15 g.

When shoot biomasses were plotted against SPW's pH ranges (Fig. 2b), a highly significant difference between groups was evidenced ($p = 5.10^{-6}$). Plants grown in pots with SPW's pH ≤ 8.6 were well-developed and reached about 13 g DW/pot. By contrast, the development of plants grown in pots with SPW's pH > 8.6 was strongly reduced: within the pH range 8.7-9.6, total shoot DW per pot was less than 1.3 g, while for pots with SPW pH between 9.7 and 10.6, shoot biomass was about 0.2 g DW.

When shoot dry weight was computed for each individual species, the same trend was noticed (data not shown). For *B. erectus*, *F. arundinacea* and *M. sativa* , growth was observed from pH 8.1 to 10.6, but was significantly reduced above pH 8.6, while *M. officinalis* was severely affected within the range 8.7-9.6, and did not grow at all when SPW's pH was above 9.6 . For *A. millefolium*, growth was very few and was observed in only two pots whose pH was below 8.6.

3.3 Plant biomass is related to elemental composition of SPW

Possible links between pH, elemental composition of SPW and plant biomass were explored using correlation analyses.

As a preliminary exploratory approach, a PCA was performed on elemental composition of SPW at the time of sowing (Fig. 3). The first two principal components caught 69% of data variance; the loading plot for the two first axis is given in Fig. 3a. The first PC axis accounted for 44.3% of total variance of the data set; its major loadings were As, Cu, Fe, K, Ni, and P and, negatively-correlated to the former, Cr, Mo, V and W. The second axis explained 24.7% of variance and was mainly driven by S, Mg and Mn on one hand, and Al, on the other hand. It is noteworthy that total shoot biomass (added on the loading plot as a supplementary variable), was anticorrelated to soluble Al, Cr, Mo and W in SPW (Fig. 3a). The scatter plot of PC scores distinctly separated experimental pots when grouped according to SPW's pH measured at d-0 (Fig. 3b). Pots with pH below 8.6 were distinctly separated from those within the range 8.7-9.6 and 9.7-10.6 on the PC2 (*i.e.* they had overall higher soluble contents in Mg, Mn and S, and lower contents in soluble Al, Cr and W).

To go a step further in this analysis, Spearman correlations between shoot dry weight production and element concentrations in the SPW were calculated for each species (except for *A. millefolium*, whose number was too small). Results showed (Fig. 4) that soluble contents in Mg, Mn, and P, were strongly and positively correlated with biomass production for the four studied species; likewise, Ni, S and Zn were positively correlated to plant biomass for three out the four studied species. Conversely, SPW contents in Al, Cr, Mo and W were strongly and negatively correlated to plant growth for all species, but *Medicago sativa*.

3.4 Element speciation in SPW depends on pH

More than their total concentration in SPW, the phytoavailability of soluble elements, and thus their impact on plant development, depends on their chemical speciation. Therefore, we conducted a geochemical modelling of SPW composition within the studied pH ranges, to characterize the dominant chemical species of elements which were either negatively or positively correlated with plant growth, *i.e.* Al, Cr, Mo and W, on one hand and Mg, Mn, Ni, P, S and Zn on the other hand. Results are given in Fig. 5.

For Al, Cr and W (Fig. 5a, b and d), dominant species in SPW within the studied pH ranges were almost exclusively the oxyanions aluminate ($Al(OH)_4^-$), chromate (CrO_4^{2-}) and tungstate (WO_4^{2-}). However, their total content in SPW was dependent on the pH range and was minimal when pH was below 8.6 or above 10.7. This was particularly noticeable for $Al(OH)_4^-$, whose concentration was about 30-fold lower within the pH range 8.1-8.6, as compared to the range 9.7-10.6 (Fig. 5a). Likewise, SPW contents in CrO_4^{2-} (Fig. 5b) and WO_4^{2-} (Fig. 5d) were about 3-fold lower at pH 8.1-8.6, as compared to the ranges 8.7-9.6 or 9.7-10.6, respectively. Concerning Mo (Fig. 5c), two major chemical species were evidenced: the molybdate anions (MoO_4^{2-}) and aqueous calcium molybdate ($CaMoO_4$(aq)); the level in MoO_4^{2-} was almost constant within the studied pH range, but the level in $CaMoO_4$(aq) slightly decreased when pH was below 8.6.

Mg (Fig. 5e) mostly occurred as free magnesium cations (Mg^{2+}); however total Mg^{2+} amount was much higher when SPW pH was below 8.6: within the ranges 8.7-9.6 and 9.7-10.6, its total level was respectively 4.6- and 111-fold lower than within the range 8.1-8.6.

For Mn (Fig. 5f), three chemical species were identified: the free manganese cations (Mn^{2+}), and the manganese oxyde (MnO) and the monohydroxy manganese (II) cations ($Mn(OH)^+$). Total levels and respective concentrations of these Mn species were closely dependent on SPW pH. Mn^{2+} was the dominant chemical species within the pH range 8.1-8.6 where it accounted for 92% of total soluble Mn; at the opposite, MnO/$Mn(OH)^+$ were only present within the pH range 10.7-11.6, *i.e.* in non-amended slag. Within the pH range 9.7-10.6, no soluble Mn was detected.

For Ni (Fig. 5g), two major species were evidenced: the nickel cation (Ni^{2+}) and the hydroxy(oxo)nickel anion ($HNiO_2^-$). The latter was almost exclusively found in non-amended slag within the pH range 10.7-11.6, where it accounted for 88% of total soluble Ni. By contrast, Ni^{2+} was the dominant species in CSS amended slag and its content in SPW clearly increased when pH decreased.

For P (Fig. 5h), two main chemical species were present within the studied pH range: the hydrogen phosphate anion (HPO_4^{2-}) and the calcium orthophosphate anion ($CaPO_4^-$). Within the pH range 10.7-11.6, soluble P was quite high (about 30 μM), but was exclusively as $CaPO_4^-$. Decreasing pH led to a sharp decrease in $CaPO_4^-$ and a concomitant increase in HPO_4^{2-}. Thus, within the pH range 8.1-8.6, the main P species was HPO_4^{2-}, which represented close to 70% of total soluble P, and reached about 10 μM.

S (Fig. 5i) mainly occurred as three chemical species: the sulfate anion (SO_4^{2-}), aqueous magnesium sulfate ($MgSO_4$(aq)) and aqueous calcium sulfate ($CaSO_4$(aq)). However, within all studied pH ranges the dominant species was SO_4^{2-}, which represented between 70 and 80% of total soluble S. The concentration of SO_4^{2-} in SPW increased when pH decreased, and reached about 3 mM within the pH range 8.1-8.6.

Lastly, soluble Zn (Fig. 5j) appeared as three main chemical species: the free zinc cation (Zn^{2+}), the zinc hydroxyde cation ($ZnOH^+$), and the hydrogen zincate anion ($HZnO_2^-$). The Zn^{2+} and $ZnOH^+$ cations were virtually absent at pH higher than 9.7, but their content in SPW increased when pH decreased to the range 8.1-8.6, reaching about 0.04 μM and 0.08 μM, respectively. Conversely, the $HZnO_2^-$ anion was quite high within the pH range 10.7-11.6 (0.16 μM), but sharply decreased with pH.

3.5 Plant nutritional status depends on SPW pH

Leaf ionome of the four plant species that grew in CSS-amended slag was studied in order to evaluating the influence of SPW's pH on the accumulation of toxic or essential elements. For comparison purposes, reference values for "usual" levels, "excessive or toxic" levels, and "deficient" levels for leaf element concentrations were taken from the literature, when available (Market, 1992; Kabata-Pendias, 2011; Marshner, 2012).

Fig. 6 shows foliar concentrations of elements whose SPW contents were negatively correlated to plant growth *(i.e*. Al, Cr, Mo and W).

Median Al concentration in leaves of the four studied species (Fig. 6a) was very close or slightly lower than "usual" concentration in plants (≈80 mg/kg), when pH was in the ranges 8.1-8.6 and 8.7-9.6. However, for *F. arundinacea* and *B. erectus* leaf Al significantly increased (up to 500 mg/kg) when pH was in the range 9.7-10.6 (no data was available for the *M. officinalis* and *M. sativ*a within this pH range).

Cr in leaves (Fig. 6b) significantly increased with SPW's pH. Within the range 8.1-8.6, leaf Cr was 4- to 11-fold higher than "usual" content in plants (≈1.5 mg/kg), but remained lower than "excessive or toxic" levels (≈30 mg/kg). By contrast, within the pH ranges 8.7-9.6 and 9.7-10.6, Cr in leaves sharply increased, reaching up to 20-times the threshold value for "excessive or toxic" concentration within the highest pH range.

In the same way as Cr, leaf content in Mo (Fig. 6c) and W (Fig. 6d) significantly increased with SPW's pH. Within the pH range 8.1-8.6, leaf Mo was about 50- to 100-fold higher than usual level (≈0.5 mg/kg) and was just below or slightly higher than "excessive or toxic" level (≈50 mg/kg). At higher pH, Mo in leaves noticeably increased, reaching more than 3 times the threshold for "excessive or toxic" concentration. For W, leaf content was close to usual level (≈0.5 mg/kg) when SPW pH was within the range 8.1-8.6, and rose up to approx. 15-times the usual level within the range 9.7-10.6. Note that for W no toxicity threshold was found in the literature.

Fig. 7 shows foliar concentrations of elements whose SPW contents were positively correlated to plant growth (*i.e.* Mg, Mn, Ni, P, S and Zn).

In the four studied species, median content in leaf Mg (Fig. 7a) was higher than usual content in plants (≈2,000 mg/kg), and slightly decreased when pH increased. Note that leaf Mg was lower in the two *Poaceae* species (*F. arundinacea* and *B. erectus*), than in the two *Fabaceae* species (*M. officinalis* and *M. sativa*), ranging from 2,200 to 4,300 mg/kg and from 10,000 to 12,000 mg/kg, respectively.

Mn in leaves (Fig. 7b) was below usual content in plants (≈200 mg/kg), but remained higher or very close to deficiency threshold (≈20 mg/kg). According to the species, leaf Mn content was comprised between 50 and 100 mg/kg, when SPW's pH was in the range 8.1-8.6. Increasing pH of SPW from 8.1-8.6 to 8.7-9.6, significantly decreased leaf Mn content, at least in the two *Poaceae* species.

Leaf Ni (Fig. 7c) was close to usual level in plants (≈1.5 mg/kg) and varied between 0.7 and 4.1 mg/kg, according to the species and pH range. Although *F. arundinacea* accumulated significantly higher Ni when SPW's pH was in the range 9.7-10.6, in any case leaf Ni remained far below toxicity threshold (≈50 mg/kg) and above deficiency level (≈0.1 mg/kg).

P in leaves (Fig. 7d) varied according to species and pH range. When SPW's pH was within 8.1-8.6, leaf P of *F. arundinacea* and *M. officinalis* was close to usual value in plants (≈2,000 mg/kg); by contrast, within the same pH range leaf P was close to deficiency threshold (≈1,000 mg/kg) for *B. erectus* and *M. sativa*. When SPW's pH was higher than 8.6, leaf P significantly decreased to values close or below deficiency level.

For S content in leaves (Fig. 7e), no clear relationship between pH and/or species was observed. For instance, in *B. erectus* leaf S was almost the same within the different pH ranges, while for *M. officinalis* it significantly decreased when pH increased. However, in any case, leaf S was above deficiency threshold.

Lastly, Zn in leaves (Fig. 7f) varied according to species and pH. For *F. arundinacea* and *M. sativa* grown within the pH range 8.1-8.6, leaf Zn reached respectively 66 and 53 mg/kg, that was very close to usual level in plants (≈50 mg/kg). In the same pH range, leaf Zn in *B. erectus* and *M. officinalis* was respectively 27 and 36 mg/kg; this was slightly lower than usual level, but still higher than deficiency threshold (≈15 mg/kg). In every case, plants accumulated significantly lower Zn when SPW's pH was >8.6.

4. Discussion

4.1 Seed germination onto unamended slag is hampered by high pH

The results obtained in this study confirm (Bouchardon *et al*., 2014; Oustrière *et al*., 2016) that plants cannot grow on bare metallurgical slag. Indeed, germination percentages for all five studied species were nil when sown onto unamended slag. It is worth to note that even at the studied slag dump, which has yet been derelict since several years, no plant was observed. In fact, germination (*i.e.* early events of plantlet development, beginning with seed imbibition, and ending with radicle emergence) is very sensitive to environmental conditions, such as drought, temperature, oxygen and light, as well as other abiotic factors, such as high pHs, high salinity (Llanes *et al.*, 2016) and soil content in organic and inorganic pollutants (You *et al.*, 2019).

The studied metallurgical slag had a high content in potentially toxic metals and a high pH, that could both affect seed germination. However, our data do not bring evidence that the high metal content in unamended slag was the primary cause of germination failure. Indeed, albeit SPW of unamended slag contained relatively high content in soluble Al, Cr, Mo and W, these amounts (Fig. 5) were not higher, or were even lower, than those measured in CSS-amended slag where seed germination and plantlet development was yet observed. In fact, the inhibitory effect of metal elements on seed germination has been seldom studied to date, and a great variability in responses has been reported according to the element, its concentration, the plant species and even the cultivar chosen (Marquez-Garcia *et al*., 2013; Mot *et al*., 2019).

However, it is generally admitted that metal toxicity mostly affects root development and seedling growth during plantlet establishment, rather than germination *sensu stricto* (Li *et al*., 2005; Kranner and Colville, 2011).

By contrast, the extreme slag's pH (>11), is a severe constraint germinating seeds have to face. Although mechanisms involved in the inhibition of seed germination in highly alkaline soils are still debated, it is likely (Ma *et al.*, 2015) that the key-inhibiting factor for germination at high pH is the alkaline stress induced by high concentration in bicarbonate and/or carbonate ions. This triggers an imbalance of the water status of the germinating seed, leading to a severe physiological drought (Liu *et al*., 2010) at a stage where water uptake is highly critical for successful germination. Such a water deficit dramatically increases in substrates with very low water retention capacity, as metallurgical slags, and is probably one of the main factors causing germination to fail on bare slag.

4.2 Decreasing pH below 8.6 is a key-factor for plant growth and development

In this study we used CSS as an organic amendment to decrease slag's pH. Although this allowed seeds to germinate, plant growth was highly heterogeneous between experimental pots, and this was clearly related to the SPW pH of each pot. Indeed, regardless of the CSS level, when pH equilibrated to values below 8.6, all sown species, but *A. millefolium,* developed and produced high biomass; conversely, when pH was above 8.6, seeds germinated but plant growth was severely affected (Fig. 2). Thus we concluded that the main limiting factor for plant development onto CSS-amended slag was the SPW pH value, which should be mandatory less than 8.6 for an adequate growth.

It is well known that high pH affects root activity and disrupts water uptake, leading to plant growth reduction (Zhang *et al*., 2013; Zhang and Zwiazek, 2016a and b). These perturbations of root hydraulic properties and plant water relations are primarily due to an inhibition of root aquaporins (Javot and Maurel, 2002; Gambetta *et al.*, 2017). However, aquaporins are known to be sensitive to both pH and mineral nutrient availability (Wang *et al.*, 2016; Kapilan *et al.*, 2018; Singh *et al.*, 2020). Consequently, it is not clear whether plant growth reduction and associated physiological perturbations at high pH are directly due to pH toxicity or to a pH-induced imbalance of nutrients and toxic metals in the soil pore water. This topic has been recently adressed by Zhang and Zwiazek (2016a), who concluded that "the impaired growth and physiological performances at high pH are more likely caused by nutrient deficiencies than by direct or indirect pH-induced toxicity". In line with this result, the PCA performed on SPW compositions revealed that elements were indeed differently partitioned according to pH (Fig. 3). Overall, plant biomass was negatively correlated with SPW concentrations in Al, Cr, Mo and W, while it was positively correlated with soluble Mg, Mn, Ni, P, S and Zn. This suggested that plant growth and development on CSS-amended slag could be impaired due to an excess of potentially toxic elements as well as a lack in essential nutrients, both being pH-dependent.

4.3 Decreasing pH below 8.6 alleviates chromium toxicity and improves manganese, phosphorus and zinc phytoavailability

Among those elements that could negatively affect plant growth on slag, Al showed the highest level in SPW (Fig. 5a), varying from ca. 40 µM up to 1300 µM, according to the pH range. Speciation analyses showed that, within the whole pH range considered (*i.e.* 8.6-11.6), soluble Al was exclusively aluminate anions [$Al(OH)_4^-$]. Plant analyses (Fig. 6a) revealed that leaf Al concentrations were about 4- to 5-fold higher than usual levels when SPW pH was within the range 9.7-10.6; that is, a pH range where plant growth was severely affected. However, when pH was in the range 8.7-9.6, Al in leaves was very close to usual levels, but plant growth was also very weak. This suggests that high Al content in leaves was not the primary cause for plant growth reduction.

The second potentially toxic element which was in high level in SPW was Cr; its concentration was approx. 80 μM when pH was in the range 8.1-8.6 and rose up to ca. 300 μM when pH was within the range 8.7-10.6 (Fig. 5b). Chromium in SPW was almost exclusively present as chromate ion (CrO_4^{2-}), the Cr most labile and toxic form (Shahid *et al.*, 2017; Sinha *et al.*, 2018). In fact, due to its high positive redox potential, hexavalent chromium triggers oxidative stress inducing deleterious effects on plant cells, such as DNA breakages, lipid peroxidation and enzyme inhibition (Signh *et al.*, 2013; Shahid *et al.*, 2017). Plants exposed to Cr toxicity exhibit a lower germination rate, as well as root growth inhibition and reduced shoot development (Tiwari *et al.*, 2009). Although Cr toxicity or tolerance can vary between species, its usual level in leaves is about 1.5 mg/kg DW, and toxicity symptoms can occur at levels in excess of 30 mg/kg DW (Market, 1992; Kabata Pendias, 2011; Marshner, 2012). Analysis of plants grown on CSS-amended slags showed that, whatever the species studied, leaf Cr was higher than usual levels in plants, and sharply increased with SPW pH (Fig. 6b). However, when SPW pH was below 8.6, leaf Cr remained below toxic levels. By contrast, when pH was above 8.6, leaf Cr significantly exceeded toxicity level. Consequently, there was a clear relationship between the pH of SPW, the concentration in soluble chromate ion, the chromium level in plant leaves, and, finally, plant biomass production. This strongly suggests that the pH-dependent decrease in chromate level in SPW was a key factor for plant growth and development on slag.

Mo occurred in SPW as two main species: MoO_4^{2-} and $CaMoO_4$(aq), only the former being available to plants (Barker and Pilbeam, 2015). The level in molybdate anion in SPW slightly increased when pH increased (Fig. 5c). As a result, leaf Mo content increased with pH (Fig. 6c) and varied between ca. 30 to 180 mg/kg, according to the plant species and the pH range considered. Although leaf Mo levels above 50 mg/kg are considered as "excessive", when compared to the usual content of 0.5 mg/kg (Market, 1992; Kabata Pendias, 2011; Marshner, 2012), Mo is actually poorly toxic for plants and numerous species can accumulate Mo levels in leaves as high as 500 mg/kg and up to 2000 mg/kg, without any deleterious effect on growth (Barker and Pilbeam, 2015). Consequently, in our experimental conditions, it is very unlikely that Mo toxicity was a limiting factor for plant development.

The fourth element negatively correlated with plant growth was W. In CSS-amended slag, W was exclusively present as tungstate anion and its concentration in SPW slightly increased with pH (Fig. 5d). Likewise, leaf W content increased with pH (Fig. 6d), ranging from ca. 0.5 to 8 mg/kg, according to the plant species and pH value. Usual W concentration in plants leaves is about 0.5 mg/kg (Market, 1992; Kabata Pendias, 2011; Marshner, 2012), but toxic concentrations remained to be defined. However, studies performed on plants grown on naturally W rich soils (Quin and Brooks, 1974), or abandoned mining area (Pratas *et al*., 2005; Pé-Leve Santos *et al*., 2013), commonly reported W concentrations in leaves higher than 10 mg/kg, without toxicity symptoms. Consequently, in our experimental conditions, it is likely that W levels did not reach toxicity threshold and was not a key-factor limiting plant development on slag.

Among elements that positively correlated with plant growth were three micronutrients (Mn, Ni and Zn) and three macronutrients (Mg, P and S).

Mn content and its chemical speciation in SPW was highly pH-dependent (Fig. 5f). At pH > 8.6, Mn in SPW was very low (< 0.1 μM); as a result, leaf Mn (Fig. 7b) was close to deficiency level. When pH was < 8.6, soluble Mn increased; thus, Mn in leaves increased significantly, although it remained below usual levels in plants. Due to its redox properties, Mn acts as an enzyme activator or cofactor for many enzymes involved in the detoxification of reactive oxygen species (*e.g.* peroxidase and catalase); it is thus closely involved in the prevention of cell damages caused by free radicals (Frausto da Silva and Williams, 2001). Mn is also part of photosystem II, where it is necessary for the photolysis of water and the production of dioxygen during photosynthesis. Consequently, when slag's pH is above 8.6, Mn-deficiency could alter the photosynthetic efficiency as well as the mechanisms involved in the detoxification of reactive oxygen species. This, coupled with the oxidative stress induced by chromate ions, could be a key-factor limiting plant development on slag at pH higher than 8.6.

Ni in SPW was mostly as the phytoavailable form Ni^{2+} and markedly decreased when pH increased (Fig. 5g). However, within the pH range 8.1 - 10.6, leaf Ni (Fig. 7c) remained close to usual value and was not below deficiency threshold. This suggests that Ni was not an element of concern for plant growth restriction on slag.

Soluble Zn in CSS amended pots was very low (< 0.15 µM) and significantly decreased when pH increased (Fig. 5j). Accordingly, leaf Zn (Fig. 7f) varied with SPW pH and, for pH higher than 8.6, it was close or below deficiency level. By contrast, at pH < 8.6, leaf Zn was close to usual level, except for *B. erectus* where it remained very low. Owing that more than 1200 proteins contain or act with Zn, a deficiency in this element may obviously affect numerous metabolic pathways. However, like Mn, Zn is involved both in photosynthesis, as a cofactor of carbonic anhydrase (Escudero-Almanza *et al*., 2012), and in the detoxification of reactive oxygen species, as part of the Cu-Zn superoxyde dismutase (Obata *et al*, 1999). Thus, at pH above 8.6, Zn deficiency could be a key factor, strengthening the effect of Mn deficiency in plant growth prevention.

Mg in SPW was almost exclusively as the plant-available Mg^{2+} species and sharply decreased when pH increased (Fig. 5e). However, within the whole pH range of CSS-amended slag, leaf Mg was above usual levels (Fig. 7a). Thus, Mg availability and accumulation was not a limiting factor for plant growth onto slag.

P in SPW of CSS amended slag was partitioned between two main chemical species (Fig. 5h): HPO_4^{2-} , which is easily available to plants, and $CaPO_4^-$, which is not available for plant uptake (Marshner, 2012). The former was the major form at pH< 8.6, while the latter was the major form at pH>8.6. Accordingly, except for *M. officinalis*, plants grown at pH>8.6 had significantly lower leaf P content (approx. 1000 $mg.kg^{-1}$) than those grown at pH<8.6 (approx. 2000 mg/kg) (Fig. 7d). It is generally admitted that the P requirement for optimal growth lies between 2000 and 5000 mg/kg (Barker and Pilbeam, 2015), while deficiency level is below 1000 mg/kg. Thus, plants grown on CSS amended slag where very close to deficiency threshold when pH was >8.6, and decreasing pH below 8.6 partly improved their P nutritional status.

Lastly, S in SPW was mainly as the SO_4^{2-} phytoavailable form and increased when pH decreased (Fig. 5i). Although S concentrations in plant tissues depends on many factors, and especially the species considered and its growth stage (Withers, 1995; Hitsuda *et al.*, 2005) it is admitted that usual levels in leaves are approx. 3000 mg/kg and deficiency occurs at less than 1000 mg/kg. Whatever the pH of SPW, plants grown on amended slag had leaf S level between 1500 and 3200 mg/kg, meaning that plants were not subject to S-deficiency (Fig. 7e).

5. Conclusions

The results of this study showed that pH was indeed a key-factor for the success or failure of aided-phytostabilization at steel slag dumps. When pH was above 8.6, plant growth and development were severely restricted. This was due to both Cr(VI) toxicity, inducing oxidative injuries, and to deficiencies in Mn, Zn and P, that are essential elements closely involved in antioxidatives defenses and plant energetics. By contrast, decreasing pH below 8.6 triggered changes in the chemical speciation of most elements, leading to a decrease in phytoavailable Cr(VI) and to a concomitant increase in phytoavailable Mn, Zn and P. As a result, Cr(VI) mobility and toxicity were mitigated, while plant nutrient status was improved, allowing efficient growth. Consequently, when implementing an aided-pytostabilisation project at a steel slagheap scale, care should be taken to carefully monitor soil pH before seeding; this should allow predicting how vegetation cover will install and survive, and how Cr(VI) mobility and transfer would be mitigated.

CRediT authorship contribution statement

Mathieu Scattolin: Conceptualization, Formal analysis, Investigation, Writing – Original draft. **Steve Peuble:** Methodology, Software, Formal analysis. **Fernando Pereira:** Project administration, Funding acquisition. **Frédéric Paran:** Resources. **Noureddine Menad:** Supervision, Funding acquisition. **Jacques Moutte:** Methodology, Software, Formal analysis. **Olivier Faure:** Conceptualization, Validation, Writing – Review & Editing, Visualization.

Declaration of Competing Interest

The authors declare they have no competing financial interest

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References

AFNOR (2008). Soil quality - Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials. *International Standard ISO 17402:2008*.

Agnello, A.C., Potysz, A., Fourdrin, C., Huguenot, D. and Chauhan, P.S. (2018). Impact of pyrometallurgical slags on sunflower growth, metal accumulation and rhizosphere microbial communities. *Chemosphere*, 208:626-639.

Alexander, M. (2000). Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environmental Science & Technology*, 34:4259-4265.

Allison, J.D. (1991). Geochemical assessment model for environmental systems: version 3.0, user's manuel. *British Library, Document Supply Center.*

Barker, A.V. and Pilbeam, D.J. (2015). Handbook of Plant Nutrition – Second Edition. *CRC Press, Taylor and Francis Group,* 743 pp.

Bayless, E. R. and Schulz, M. S. (2003). Mineral precipitation and dissolution at two-slag-disposal sites in northwestern Indiana, USA. *Environmental Geology*, 45:252-261.

Bouchardon J.L., Faure O., Lespagnol G., Moutte J., Guy B., Mimoun D., Graillot D., Paran F., Croze V., Athenol, J.D., Ferrando B., Boisson J., Perret S., Hitmi A., Verney P., Moussard Gauthier C., Ledoigt G., Goupil P., Sac C. and Mench M. (2014). Physafimm: La phytostabilisation, méthodologie applicable aux friches industrielles, métallurgiques et minières. *Rapport final, convention ADEME n° 0872C0119, ADEME Ed.*, 158 pp.

Escudero-Almanza1, D.J., Ojeda-Barrios, D.L., Hernandez-Rodriguez, O.A., Sanchez Chavez, E., Ruiz-Anchondo1, T. and Sida-Arreola1, J.P. (2012). Carbonic anhydrase and zinc in plant physiology. *Chilean Journal of Agricultual Research*, 72 (1):141-146.

Frausto da Silva, J. J. R. and Williams, R. J. P. (2001). The Biological Chemistry of Elements, The Inorganic Chemistry of Life. Second Edition. *Oxford university Press, Oxford*, 561 pp.

Gambetta G.A., Knipfer T., Fricke W., McElrone A.J. (2017). Aquaporins and root water uptake. *In:* Chaumont F., Tyerman S. (eds), Plant Aquaporins. Signaling and Communication in Plants. Springer, Cham. https://doi.org/10.1007/978-3-319-49395-4_6

Ge, Y, Murray, P. and Hendershot, W.H. (2000). Trace metal speciation and bioavailability in urban soils. *Environmental Pollution*, 107(1):137-144.

Gomes, H. I., Mayes, W. M., Rogerson, M., Stewart, D. I. and Burke I. T. (2016). Alkaline residues and the environment: a review of impacts, management practices and opportunities. *Journal of Cleaner Production*, 122:3571-3582.

Gomes, H. I., Mayes, W. M., Whitby, P. and Rogerson, M. (2019). Constructed wetlands for steel slag leachate management: partitioning of arsenic, chromium, and vanadium in waters, sediments, and plants. *Journal of Environmental Management*, 243:30-38.

Hitsuda, K., Yamada, M. and Klepker, D. (2005). Sulfur requirement of eight crops at early stages of growth. *Agronomy Journal*, 97:155-159.

Javot, H. and Maurel, C. (2002). The role of aquaporins in root water uptake. *Annals of Botany*, 90:301-313.

Johnson, J.W., Oelkers, E.H. and Helgeson, H.C. (1992). SUPCRT92: A software package for calculating the standard molal thermodynamic properties of minerals, gases, aqueous species, and reactions from 1 to 5000 bars and 0 to 1000°C. *Computers and Geosciences*, 18(7):899-947.

Kabata-Pendias, A. (2011). Trace elements in soils and Plants – Fourth Edition. *CRC Press, Taylor and Francis GroupBoca Raton, Fl*, 505 pp.

Kapilan, R. Vaziri, M. and Zwiazek, J.J. (2018). Regulation of aquaporins in plants under stress. *Biological Research*, 51:4.

Kidd, P., Mench, M., Alvarez-Lopez, V., Bert, V., Dimitriou, I., Friesl-Hanl, W., Herzig, R., Janssen, J. O., Kolbas, A., Müller, I., Neu, S., Renella, G., Ruttens, A., Vangronsveld, J. and Puschenreiter, M. (2015). Agronomic practices for improving gentle remediation of trace element-contaminated soils. *International of Phytoremediation*, 17:1005-1037.

Kranner, I. and Colville, L. (2011). Metals and seeds: biochemical and molecular implications and their significance for seed germination. *Environmental and Experimental Botany*, 72:93-105.

Lê, S., Josse, J. and Husson, F. (2008). FactoMineR: A package for multivariate analysis. *Journal of Statistical Software*, 25(1):1–18.

Li, W., Khan, M.A., Yamaguchi, S. and Kamiya, Y. (2005). Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*. *Plant Growth Regulation,* 46:45-50.

Liu, J., Guo, W.Q. and Shi, D.C. (2010). Seed germination, seedling survival, and physio-logical response of sunflowers under saline and alkaline conditions. *Photosynthetica*, 48(2):278-286.

Llanes, A., Andrade, A., Masciarelli, O., Alemano, S. and Luna, V. (2016). Drought and salinity alter endogenous hormonal profiles at the seed germination phase. *Seed Science Research*, 26:1-13.

Lock, K. and Janssen, C.R. (2003). Influence of aging on copper bioavailability in soils. *Environmental Toxicology and Chemistry*, 22:1162-1166.

Luo, Y., Wu, Y., Xing, R., Yao, C, Shu, J. and Wu, Z. (2018). Effects of plant litter decomposition on chemical and microbiological characteristics of artisanal zinc smelting slag using indigenous methods. *Journal of Geochemical Exploration*, 190:292-301.

Luo, Y., Wu, Y., Shu, J. and Wu, Z. (2019). Effect of particulate organic matter fractions on the distribution of heavy metals with aided phytostabilization at a zinc smelting waste slag site. *Environmental Pollution*, 253:330-341.

Luo, Y., Wu, X., Sun, H. and Wu, Y. (2020). Root-induced changes in aggregation characteristics and potentially toxic elements (PTEs) speciation in a revegetated artificial zinc smelting waste slag site. *Chemosphere*, 243: https://doi.org/10.1016/j.chemosphere.2019.125414

Ma, H., **Yang, H., Lü, X., Pan, Y., Wu, H., Liang, Z. and Ooi, M.K.J. (2015)**. Does high pH give a reliable assessment of the effect of alkaline soil on seed germination? A case study with *Leymus chinensis* (*Poaceae*). *Plant and Soil*, 394:35-43.

Markert, B. (1992). Establishing of "reference plant" for inorganic characterization of different plant species by chemical fingerprint. *Water, Air, and Soil Pollution*, 64:533-538.

Marquez-Gracia, B., Marquez, C., Sanjosé, I., Nieva, F.J.J., Rodriguez-Rubio, P. and Munoz-Rodriguez, A.F. (2013). The effects of heavy metals on germination and seedling characteristics in two halophyte species in Mediterranean marshes. *Marine Pollution Bulletin,* 70:119-124.

Marschner, P. **(2012)**. Marschner's Mineral Nutrition of Higher Plants – Third Edition. *Academic Press, Elsevier*, 651 pp.

Mayes, W. M., Younger, L. and Aumônier, J. (2008). Hydrogeochemistry of alkaline steel slag leachates in the UK. *Water, Air, and Soil Pollution*, 195:35-50.

Mendez, M. and Maier, R. (2008). Phytostabilization of mine tailings in arid and semiarid environments - An emerging remediation technology. *Environmental Health Perspectives*, 116:278-283.

Mot, A., Madjar, R., Badulescu, L. and Mihalache, M. (2019). The effects of heavy metals on seed germination and seedling growth of *Lactuca sativa* L. and *Spinacia oleracea* L. *Research Journal of Agricultural Science*, 51(3):124-132.

Moutte, J. (2009). Arxim, a library for thermodynamic modeling of fluid-rock systems. https://www.emse.fr/~moutte/arxim/ (site visited July 2020).

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G. and Renella, G. (2003). Microbial diversity and soil functions. *European Journal of Soil Science*, 54:655-670.

Obata, H., Kawamura, S., Senoo, K. and Tanaka, A. (1999). Changes in the level of protein and activity of Cu/Zn-superoxide dismutase in zinc deficient rice plant, *Oryza sativa* L. *Soil Science and Plant Nutrition*, 45(4):891-896.

Oustriere, N., Marchand, L., Bouchardon, J. L., Faure, O., Moutte, J. and Mench, M. (2016). Aided phytostabilization of a trace element-contaminated technosol developed on steel mill wastes. *Journal of Hazardous Materials*, 320:458-468.

Pé-Leve Santos, S.C., Cruz, M.E., Barroso, A.M.E., Fonseca, C.P.S., Guerra, M., Carvalho, M.L. and Santos, J.P. (2013). Elemental characterization of plants and soils in Panasqueira tungsten mining region. *Journal of Soils and Sediments*, 14 (4):778-784.

Piatak, N. M., Parsons, M. B. and Seal, R. R. (2015). Characteristics and environmental aspects of slag: a review. *Applied Geochemistry*, 57:236-266.

Pratas, J., Prasad, M.N.V., Freitas, H. and Conde, L. (2005). Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. *Journal of Geochemical Exploration*, 85:99-107.

Quin, B.F. and Brooks, R.R. (1974). Tungsten concentrations in plants and soils as a mean of detecting scheelite-bearing ore-bodies in New Zealand. *Plant and Soil,* 41:177-188.

R Core Team (2017). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*. URL https://www.R-project.org/.

Ramos da Silva, W., Vieira da Silva, F.B., Muniz Araujo, P.R. and Araujo do Nascimento, C.W. (2017). Assessing human health risks and strategies for phytoremediation in soils contaminated with As, Cd, Pb, and Zn by slag disposal. *Ecotoxicology and Environmental Safety*, 144: 522-530.

Remon, E., Bouchardon, J.L., Le Guedard, M., Bessoule, J.J., Conord, C. and Faure O. (2013). Are plants useful as accumulation indicators of metal bioavailability? *Environmental Pollution*, 175:1-7.

Roadcap, G. S., Walton, R. K. and Bethke, C. M. (2005). Geochemisty of extremely alkaline (pH > 12) ground water in slag-fill aquifers. *Ground Water*, 43:806-816.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A. and Harms, H. (2004). Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. *Environmental science and technology*, 38(12):228A – 231A.

Shahid, M., Shashad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N. K., Dumat, C. and Rashid, M. I. (2017). Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: A review. *Chemosphere*, 178:513-533.

Signh, H. P., Priyanka, M. Kaur, S., Batish, D. R. and Kohli, R. K. (2013). Chromium toxicity and tolerance in plants. *Environmental Chemistry Letters*, 11:229-254.

Singh, R.K., Deshmukh, R., Muthamilarasan, M., Rani, R. and Prasad, M. (2020). Versatile roles of aquaporins in physiological processes and stress tolerance in plants. Plant *Physiology and Biochemistry*, 149:178-189.

Sinha, V., Pakshirajan, K. and Chaturvedi, R. (2018). Chromium tolerance, bioaccumulation and localization in plants: An overview. *Journal of Environmental Management*, 206:715-730.

Tiwari, K.K., Dwivedi, S., Singh, N.K. Rai, U.N. and Tripathi, R.D. (2009). Chromium (VI) induced phytotoxicity and oxidative stress in pea (*Pisum sativum L.*): Biochemical changes and translocation of essential nutrients. *Journal of Environmental Biology*, 30:389-394.

Tukey, J.W. (1977). Exploratory data analysis. *Pearson, Ed.,* 712 pp.

Vamerali, T., Bandiera, M. and Mosca, J. (2010). Field crops for phytoremediation of metal-contaminated land. A review. *Environmental Chemistry Letters*, 8:1-17.

Wang, L., Bin, J., Yuehua, H., Runqing, L. and Sun, W. (2017). A review on in situ phytoremediation of mine tailings. *Chemosphere*, 184:594-600.

Wang, M., Ding, L., Gao, L., Li, Y., Shen, Q. and Guo, S. (2016). The interactions of aquaporins and mineral nutrients in higher plants. *International Journal of Molecular Sciences*, 17:1229.

Withers, P.J.A., Tytherleigh, A.R.J. and O'Donnell, F.M. (1995). Effect of sulphur fertilizers on the grain yield and sulphur content of cereals. *The Journal of Agricultural Science*, 125:317-324.

You, R., **Dominguez, C., Matamoros, V., Bayona, J.M. and Diez, S. (2019)**. Chemical characterization and phytotoxicity assessment of peri-urban soils using seed germination and root elongation tests. *Environmental Science and Pollution Research*, 26:34401-34411.

Zhang, W., Calvo-Polanco, M., Chi Chen, Z. and Zwiazek, J.J. (2013). Growth and physiological responses of trembling aspen (*Populus tremuloides*), white spruce (*Picea glauca*) and tamarack (*Larix laricina*) seedlings to root zone pH. *Plant and Soil*, 373:775-786.

Zhang, W. and Zwiazek, J.J. (2016a). Responses of reclamation plants to high root zone pH: effects of phosphorus and calcium availability. *Journal of Environmental Quality*, 45:1652-1662.

Zhang, W. and Zwiazek, J.J. (2016b). Effects of root pH on root water transport and apoplastic pH in red-osier dogwood (Cornus sericea) and paper birch (Betula papyrifera) seedlings. *Plant Biology*, 18:1001-1007.

Zimmer, K., Zhang, Y., Lu, P., Chen, Y, Zhang, G., Dalkilic, M. and Zhu, C. (2016). SUPCRTBL: A revised and extended thermodynamic dataset and software package of SUPCRT92. *Computers and Geosciences*, 90:97-111.

Tables

Table I: Selected pedological and geochemical characteristics of the studied slag and composted sewage sludge (CSS)

Category	Parameter	Slag	CSS
Physical	pH	10.5	8.0
	CEC [cmol(+)/kg]	5.9	38.9
Grain size (%)	< 2000 µm to 50 µm	96.1	36.2
	< 50 µm to 2 µm	2.2	49.5
	< 2 µm to 0 µm	1.7	14.3
Fertility (g/kg)	N	0.1	25.7
	TOC	43	295
	Active $CaCO_3$	20	480
	Total $CaCO_3$	304	136
Exchangeable macronutrients (g/kg)	P_2O_5	0.010	1.092
	K_2O	0.070	5.526
	CaO	9.84	13.43
	MgO	0.741	1.713
Bioavailable micronutrients (mg/kg)	Cu	1.2	7.3
	Fe	9.5	134.7
	Mn	7.4	66.0
	Zn	11.9	85.0
Major elements (g/kg)	Al_2O_3	127	92
	CaO	131	202
	Fe_2O_3	194	95
	K_2O	3	41
	MgO	29	16
	MnO	20	10
	Na_2O	6	14
	P_2O_5	2	64
	SiO_2	437	462
Trace elements (mg/kg)	Cr	6861	157
	Cu	476	378
	Mo	209	< LQ
	Ni	308	36
	Pb	< LQ	< LQ
	V	288	56
	W	97	< LQ
	Zn	524	746

CEC: Cation-Exchange Capacity
TOC: Total Organic Carbon

Figures with captions

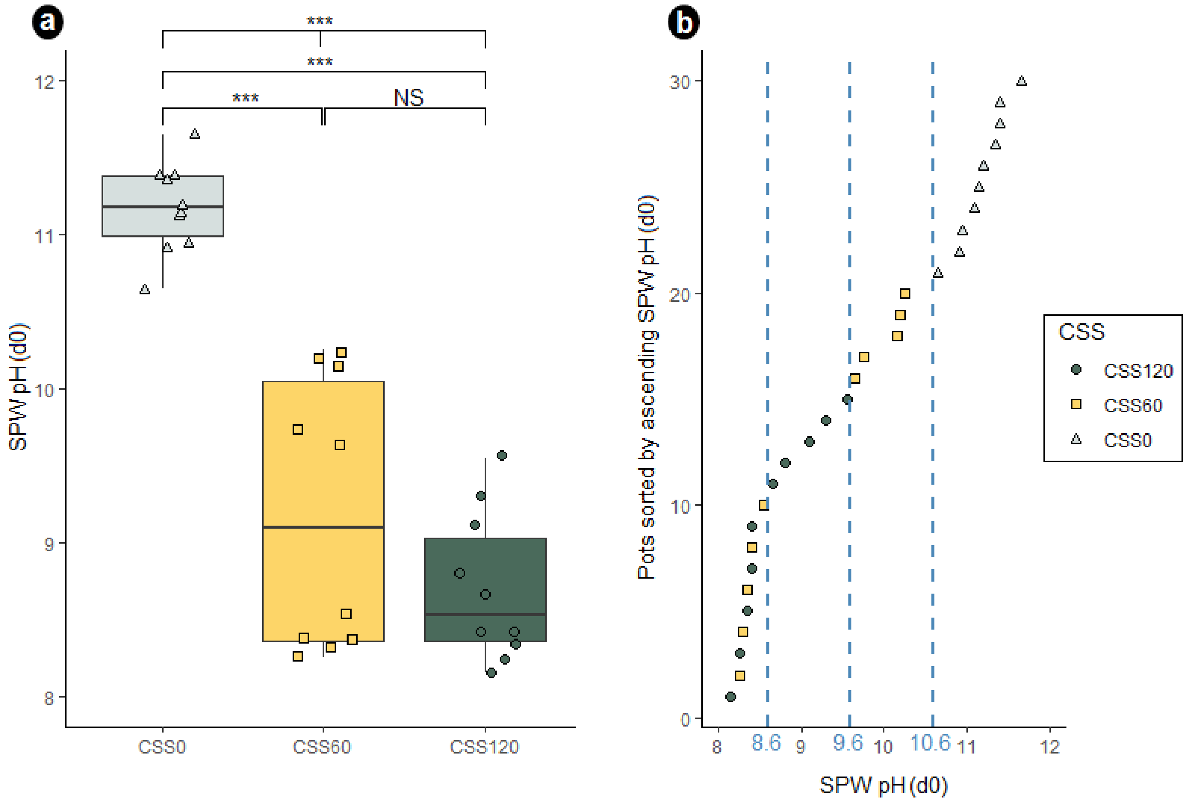

Figure 1: Distribution of soil pore water (SPW) pH of bare slag, and of slag amended with composted sewage sludge (CSS), at the day of sowing (d0). (a) Effect of CSS addition on SPW pH (CSS0: bare slag, n=10; CSS60: slag with 60 T/ha CSS, n=10; CSS120: slag with 120 T/ha CSS, n=10), (b) pH values of the experimental pots (n=30), sorted by an ascending order.

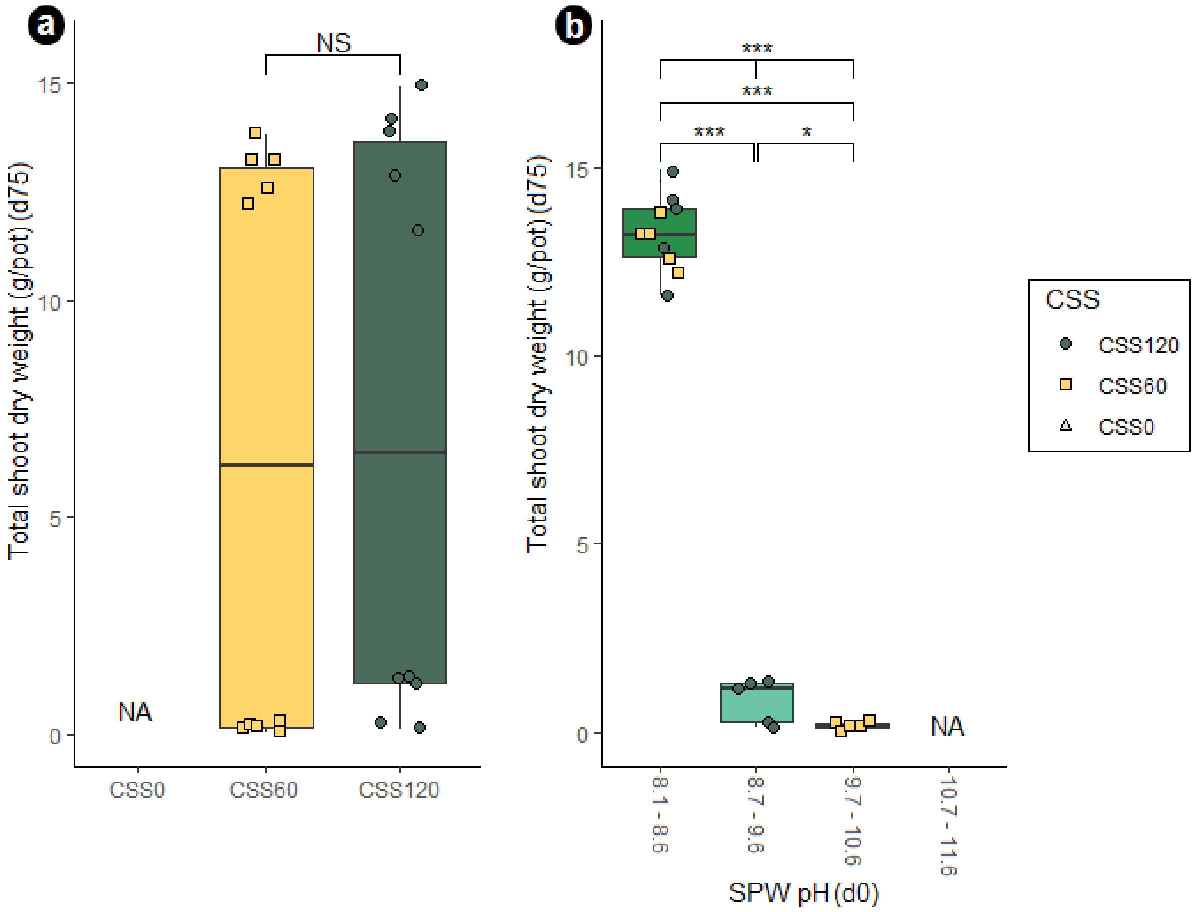

Figure 2: Total shoot dry weight (g/pot) of a plant assemblage made of 5 species (*Achillea millefolium*, *Bromus erectus*, *Festuca arundinacea*, *Melilotus officinalis* and *Medicago sativa*), sown on bare slag and on slag amended with composted sewage sludge (CSS), harvested after 75 days of culture. (a) effect of CSS amount on biomass production (CSS0: bare slag, n=10; CSS60: slag with 60 T/ha CSS, n=10; CSS120: slag with 120 T/ha CSS, n=10), (b) effect of SPW pH at the day of sowing (d0), on biomass production.

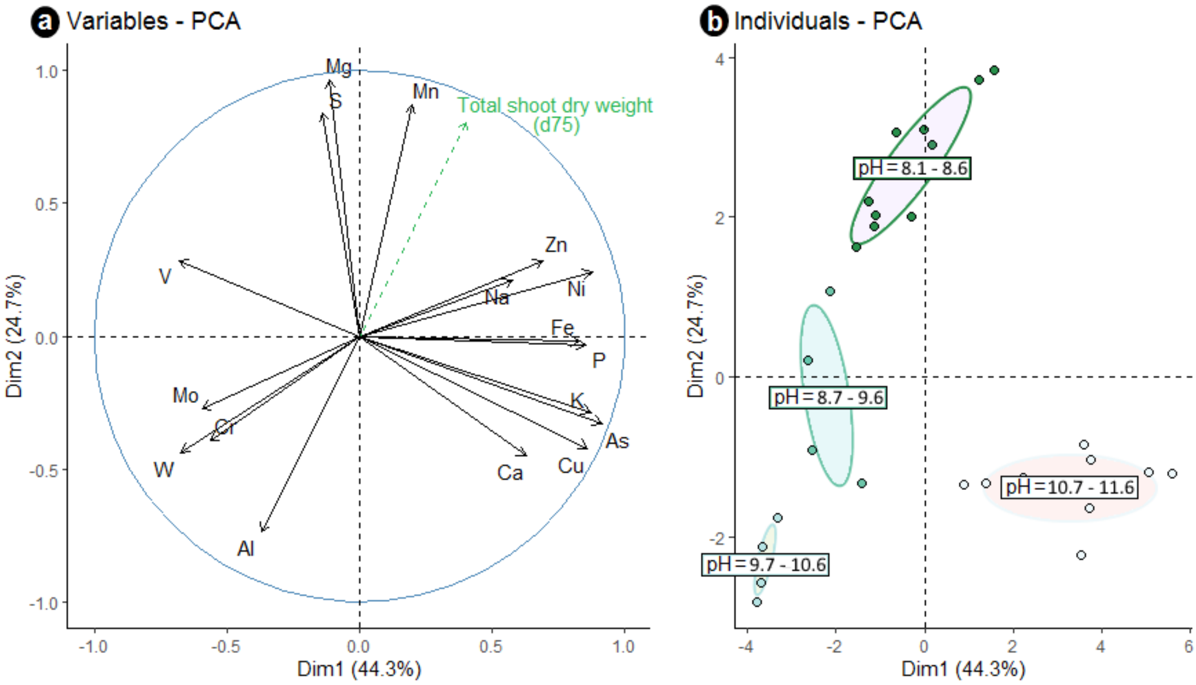

Figure 3: Principal component analysis (PCA) of elemental composition of soil pore water (SPW) of the 30 experimental pots at day 0. (a) Correlation circle of the 17 measured elements (Total Shoot dry weight at d75 was added as a supplementary variable), (b) scatter plot of PC scores of each pot, grouped as a function of SPW pH.

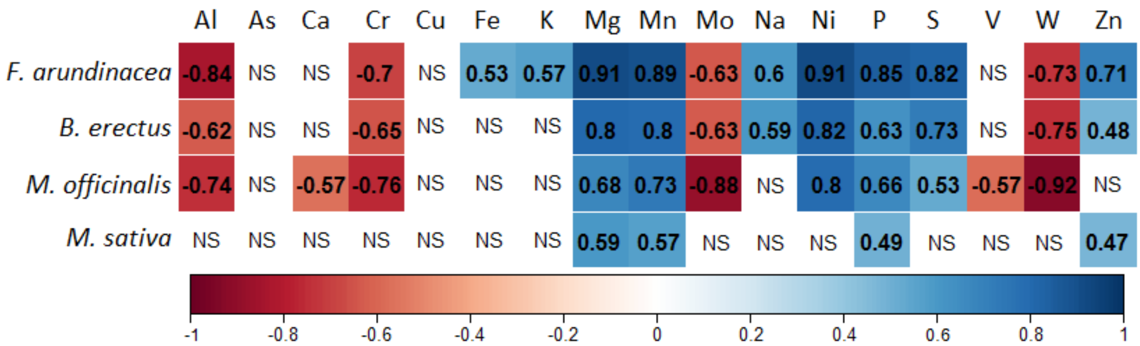

Figure 4: Spearman correlation coefficients between shoot dry weights of individual plant species (measured after 75 day of culture), and element contents in soil pore water at the day of sowing.

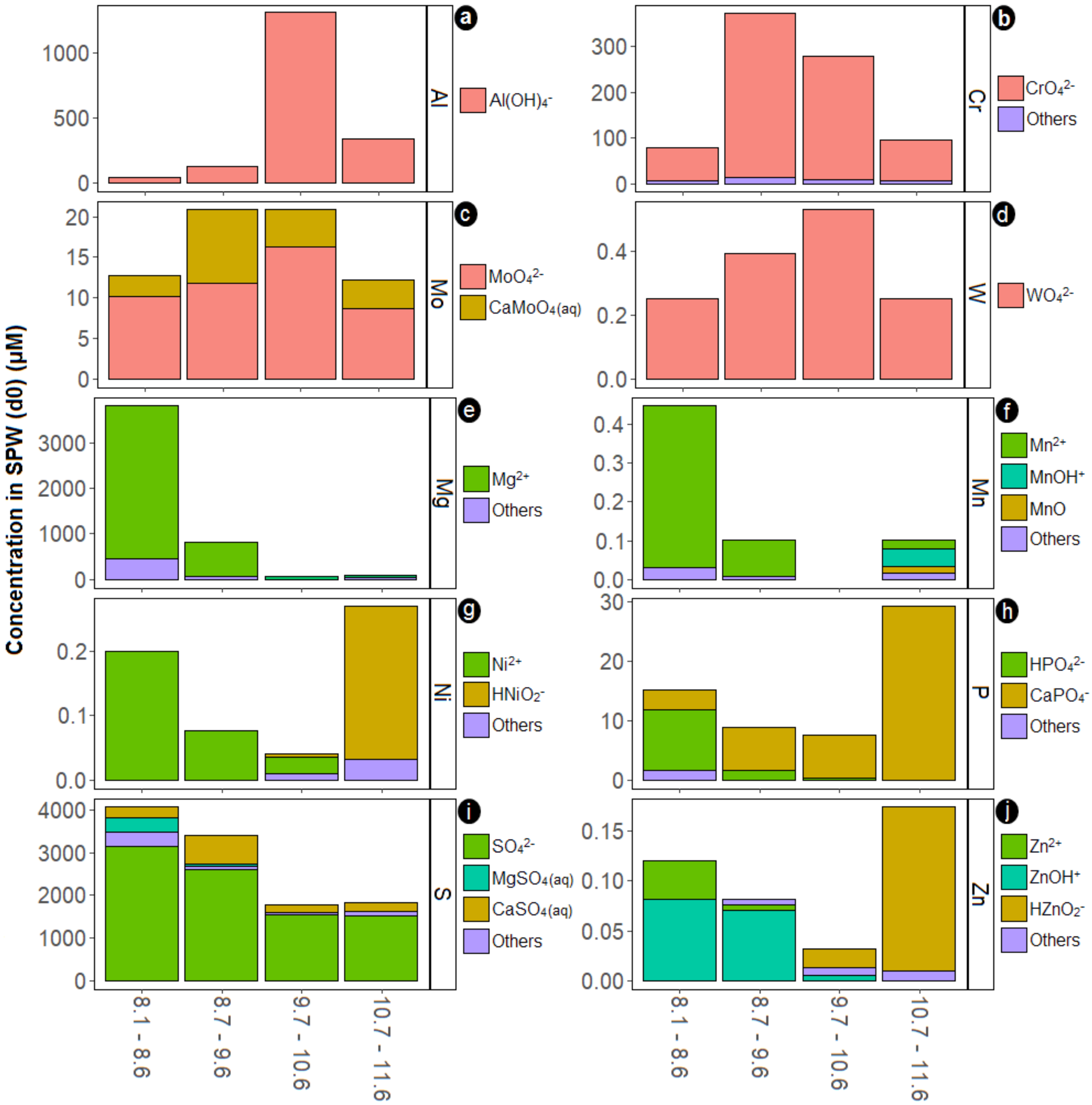

Figure 5: Distribution of soluble chemical species as a function of soil pore water (SPW) pH at the day of sowing (d0). (a to d) elements negatively correlated to plant growth; (e to j) elements positively correlated to plant growth.

Chemical species grouped as "others" are those species that together accounted for less than 15% of total chemical species.

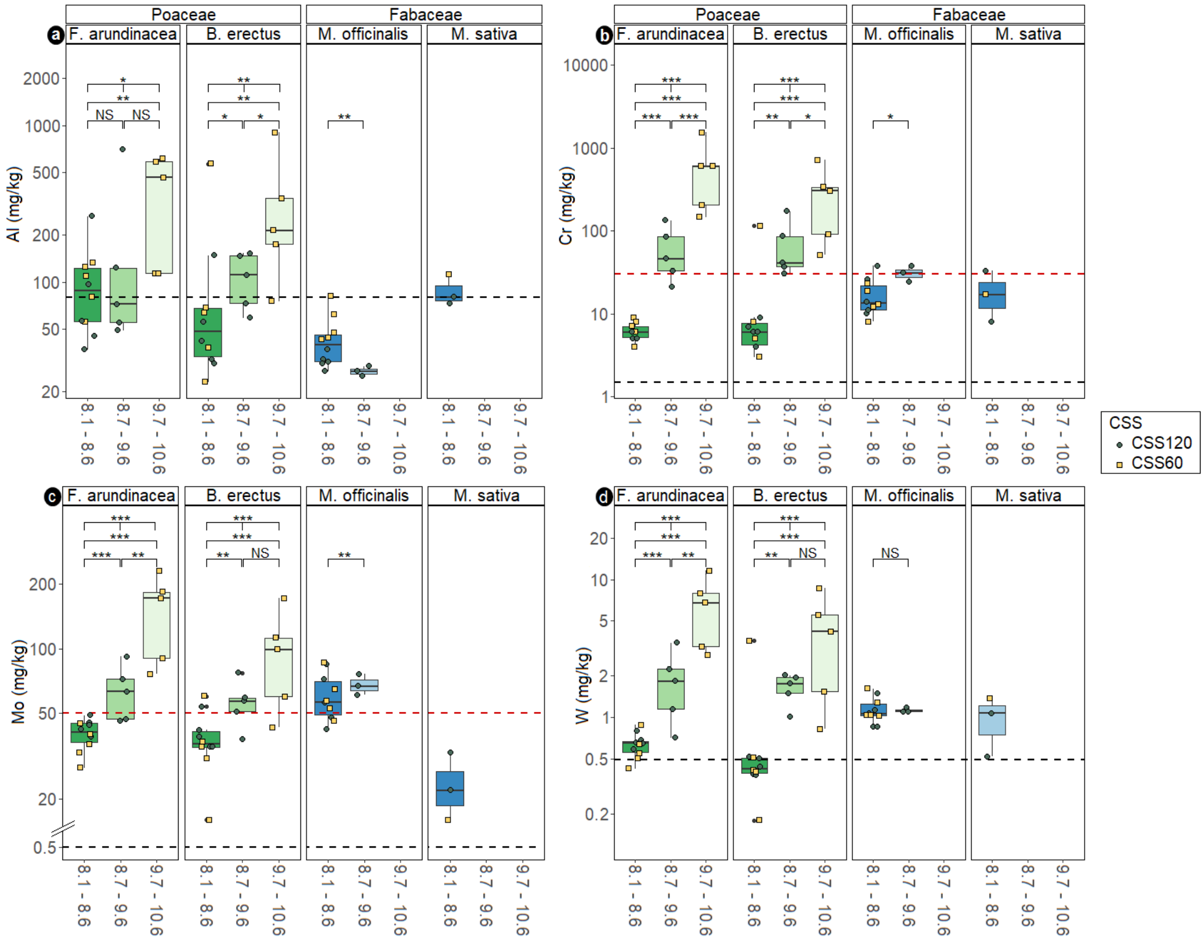

Figure 6: Leaf contents (after 75 days of culture) in elements negatively correlated to plant growth on steel slag amended with composted sewage sludge, as a function of soil pore water pH at the day of sowing.

Black lines indicate usual or sufficient concentrations in plants leaves while red lines are excessive or toxic levels.

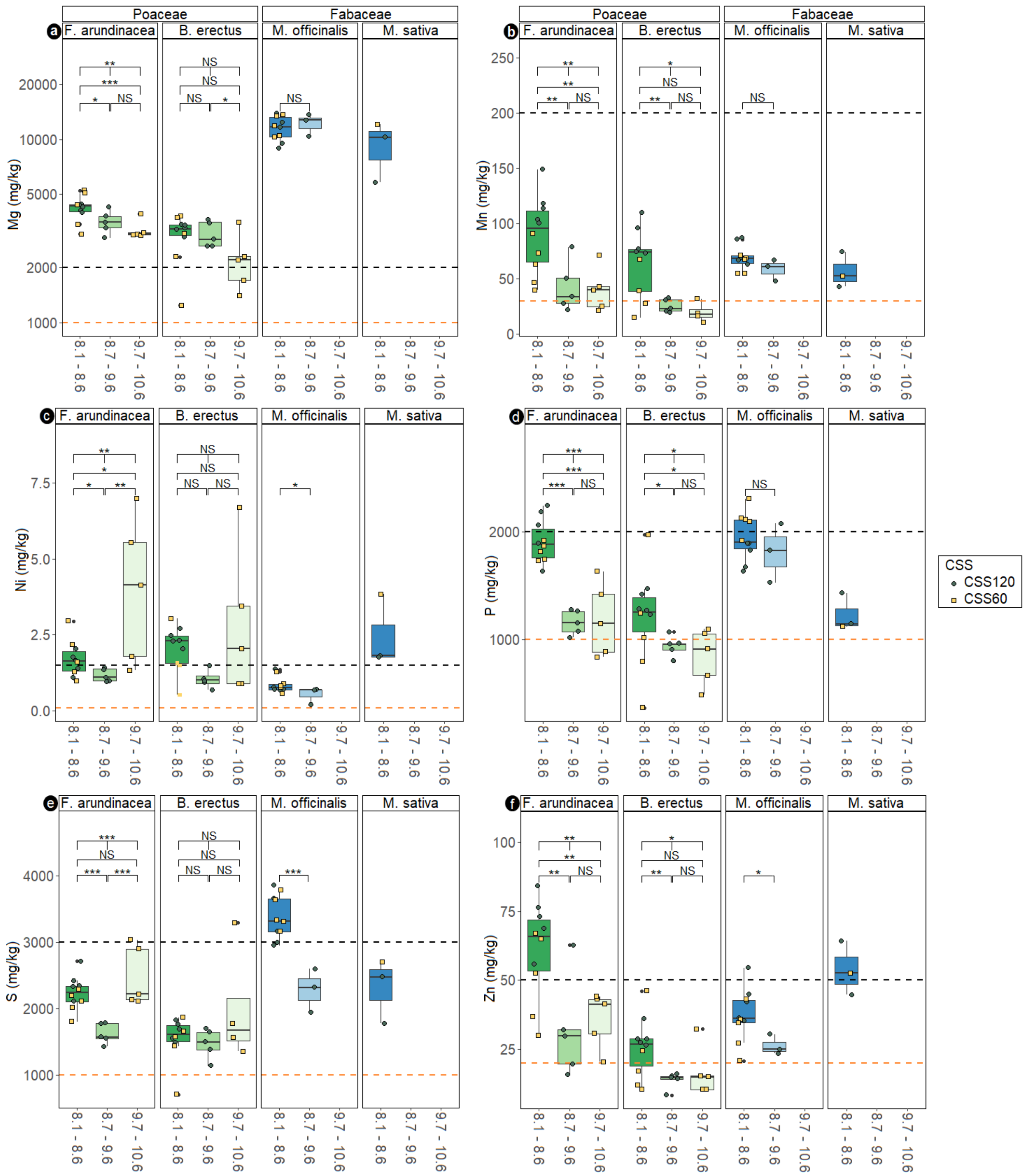

Figure 7: Leaf contents (after 75 days of culture) in elements positively correlated to plant growth on steel slag amended with composted sewage sludge, as a function of soil pore water pH at the day of sowing.

Black lines indicate usual or sufficient concentrations in plants leaves while orange lines are deficiency levels.