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Amendment application in a multicontaminated mine soil: Effects on trace element mobility.

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ABSTRACT

Several amendments were tested for their effectiveness in aiding plant growth and immobilising contaminants in pots containing soil from an arsenopyrite mine contaminated with arsenic and heavy metals. Trace element solubility in pore water was monitored using Rhizon samplers for five weeks. Results showed that amendments containing ferrous sulphate and ferrous sulphate combined with paper mill limited arsenic mobilisation and increased metal solubility. However, ferrous sulphate in combination with calcium carbonate was effective in reducing arsenic and metal solubilisation. Plant biomass of both the grass species used during the experiment, *Arrhenatherum elatius* and *Festuca curvifolia*, was a sensitive indicator of the comparative efficiency of the amendments, although metal(loid)s concentration in pore water did not correlate with plant uptake.

Keywords

Arsenic; Metals; Amendments; Pore water; Remediation

1 Introduction

Mining activities are one of the main anthropogenic sources of arsenic and heavy metals in the environment. Large amounts of arsenic (up to 3%) and metals can be found in the surroundings of a mining dump, decreasing rapidly with the distance from the mine tailings (Zhang and Selim 2008). One of the strategies to remediate those soils is phytostabilisation, combining plants and agricultural practices to reduce mobilisation and transfer of contaminants to other environmental compartments (Chaney et al. 1997). Plants can accumulate contaminants in their roots, limiting trace element mobilisation and their transfer through food chain (Madejón et al. 2009). However, some authors have reported the limitations of this technique, showing that arsenic availability was not affected by the presence of woody plants on the tailings (King et al. 2008). Stabilisation of contamination by adding soil amendments may reduce inorganic contaminant mobility in soils through trace element adsorption, (co)precipitation and binding (Kumpiene et al. 2008). On the other hand, soil amendments may also enhance trace element solubility and increase their plant uptake (Lombi et al. 1998).

Iron-bearing additives are one of the most studied materials used in arsenic and metals attenuation in soils (Hartley and Lepp 2008). Recent studies have used red-mud and furnace slag containing a high percentage of iron to reduce arsenic mobility in soils (Lee et al. 2011). Common soil amendments used are compost, manure, sewage sludge (Walker et al. 2003; Antoniadis and Damalidis, 2012; Branzini and Zubillaga, 2012) and lime (Hartley et al. 2004; Bade et al. 2012). Alternative organic materials, such as paper industry waste materials or biochar, have been reported to successfully retain metals from water solutions (Calace et al. 2003; Méndez et al. 2009; Beesley et al. 2011).

The bioavailable fraction is the one which is susceptible of interacting with the organisms present in soils and it is considered the most significant fraction when assessing the potential ecotoxicological risks. Nowadays, there is not a consensus within the scientific community about how to evaluate bioavailability, but it is well reported that total concentrations of trace elements are a poor indicator of metal(loid) toxicity and mobility, since they exist in different solid-phase forms that can vary considerably in terms of bioavailability (Temminghoff et al. 1998; Allen, 2001; Nolan et al. 2003). Thus, the bioavailable fraction of trace elements should be included in the current guidelines as an alternative to the total metal concentration (Mench et al. 2009). The knowledge of the chemical composition of the interstitial pore water of soil, or soil solution, is important in order to provide an insight into the behaviour and fate of contaminants and provides more realistic information for assessing environmental impact than total element concentration. Water soluble metals are the fraction most readily available to plants, so it could be used as an indicator of bioavailability to the receptor organism (Séguin et al. 2004). The soluble fraction of trace elements in soils has been evaluated using several extraction methods in ecotoxicity assessments. One of those consists in soil solution extraction with Rhizon samplers. Rhizon samplers are a non-destructive method for extracting soil water from soil pores and has been reported elsewhere to effectively monitor labile fractions of trace elements (Clemente et al. 2008; Beesley and Dickinson 2011; Moreno-Jiménez et al. 2011).

The aim of this work was to evaluate the efficiency of several amendments in assisting the phytostabilisation of a multi-element contaminated soil from a former arsenopyrite mine. Efficiency was assessed by monitoring contaminant solubility (using Rhizon pore water samplers), plant growth and metal(oid) uptake by two species of herbaceous plants naturally growing in the surroundings of the mine.

2 Materials and methods

Samples were collected from an ancient mine located in Bustarviejo, NW Madrid (Spain). It is an arsenopyrite mine where silver has been extracted from the 18th century until the 1980's. Abandoned tailing dumps are the only source of contamination, containing the highest concentrations of arsenic and metals. The contaminated material was collected from the tailings and uncontaminated soil from an area close to the mine. Both materials were air-dried for one week and sieved to 4 mm. Uncontaminated soil plus dumping material were mixed in a 60:40 proportion (w/w) in order to obtain an homogeneous contaminated substrate in which plants could grow. Each amendment used was mixed with the soil prior to being added to pots. Pots (2 L) were filled with 2 kg of the respective mixtures and left to equilibrate for one month at 70% water holding capacity (previously determined). The different amendments used were as follows (treatment codes used in brackets):

Not amended ('NA')

FeSO₄ 1% w/w ('Fe')

CaCO₃ 1% w/w ('Ca')

FeSO₄ 1% w/w + CaCO₃ 1% w/w ('Fe+Ca')

Paper mill 1% w/w ('PM')

FeSO₄ 1% w/w + paper mill 1% w/w ('Fe+PM')

FeSO₄ and CaCO₃ were obtained from Sigma Aldrich and paper mill was supplied by Holmen Paper (Madrid, Spain).

After one month, the equilibrated pots were divided into two sections. 100 seeds of *Arrhenatherum elatius* subsp. *Bulbosum* and 100 seeds of *Festuca curvifolia* were sown in each half of each pot. Both gramineae plants present fasciculate, abundant and

similar roots, spreading over the surface and assisting soil stabilisation. They have lateral buds so that their growth extends in parallel to the surface of the soil, forming dense stem clusters.

The experiment was carried out in a greenhouse situated in the Universidad Autónoma de Madrid (Spain) under controlled conditions (10-28 °C and 60-80 % relative humidity) for five weeks after seed planting. Four replicates for each treatment were used.

2.1 Experiment monitoring

Every day pots were watered with tap water to their maximum water holding capacity. Pore water was sampled every week using a Rhizon soil sampler (Eijkelkamp, Agrisearch Equipment, The Netherlands) made of a hydrophilic porous polymer (10 cm long, Ø 2.5 mm) inserted horizontally into the pots. Pore water was obtained by placing the sampler under vacuum with a vacuum tube. Five fractions were collected over the five weeks of the experiment, the first fraction taken just after the seeds were sown. Pore water samples taken were acidified with HNO₃ (0.1% v/v) to preserve them before analysis.

At the end of the experiment, above ground tissues were collected. Fresh weights were recorded and shoots were dried for five days at 60 °C before analysis.

2.2 Determination of element concentrations

Pseudo-total concentration of metal(oids) in the materials were determined by autoclave digestion of 0.5 g material with HNO₃:H₂O₂ for 30 min at 1.5 kg/cm²

(Wenzel et al. 2001). Extractable elements were determined by shaking soil with 0.1M $(\text{NH}_4)_2\text{SO}_4$ in a soil to solution ratio 1:10 for four hours at 25 °C; the suspension was then filtered, acidified with HNO_3 (0.1% v/v) to preserve samples and the filtrate analysed (Vázquez et al. 2008). Plant material was digested by autoclaving to 0.25 g of dried plant material $\text{H}_2\text{O}:\text{HNO}_3:\text{H}_2\text{O}_2$ 1:1.5:1 (v/v/v) for 30 minutes at 1.5 kg/cm² (Lozano-Rodríguez et al. 1995). Arsenic and metals in pore water and plant material were determined by ICP-MS (Sciex Elan 6000, Perkin Elmer). Total and extractable arsenic and metals in soils were analysed by atomic fluorescence spectroscopy (Millenium Excalibur, PS Analytical) and atomic absorption respectively (AA800, Perkin Elmer). Characterisation of soils and paper mill are shown in Table 1.

pH measurements in soil was carried out with a Crison electrode 5014 in a 1:2.5 soil to deionised water suspension, shaken for 30 min (MAPA, 1994). Electric conductivity was determined in a soil to deionised water ratio 1:5 with a Crison electrode CE5070.

pH and electric conductivity of the paper mill amendment was analysed in a 1:10 paper mill to deionised water solution. Organic matter was determined by loss on ignition at 550 °C for 5 h.

2.3 Data analysis

SPSS 15.0 was used for all statistical analysis. Statistical tests performed include one-way ANOVA followed by Duncan's test to determine whether there were any significant differences between the means of each treatment, assuming a normally distribution of the dependent variable data and homogeneity of variances. Kruskal-Wallis tests alongside with Mann-Whitney post hoc tests were performed when the

assumption of normality was rejected. Robust tests of equality of means (Welch or Brown-Forsythe tests followed by Games-Howell post hoc tests) were run when homogeneity of variances assumptions was violated and the use of the one-way ANOVA was inappropriate. Bivariate correlations were analysed using Pearson's coefficient.

3 Results

3.1 Initial soil parameters

Table 1 shows the initial parameters of all the materials used in this experiment: uncontaminated soil; mine tailings; the soil and tailings mixture; and paper mill. Initial pH in the uncontaminated soil (~6) was higher than for the tailings (~4). When both materials were mixed, pH values reached 5. One month after equilibration, at the time of seed sowing, soil pH was measured again (Table 2). Compared to the initial pH of the mixture (Table 1), there was a significant increase in soil pH with the incorporation of the amendments 'Ca', 'Fe+Ca', 'PM' and 'Fe+PM', while FeSO₄ addition ('Fe') led to a decrease in pH by one and a half units, reaching values below 4.

Total arsenic and metals concentration in the tailings was high (up to 4000 mg·kg⁻¹ total As) so the mine tailings and uncontaminated soil mixture resulted in a substrate also containing large levels of metal(oid)s. Paper mill was the only amendment which contained Mn, Cu and Zn, but in lower concentrations than those of the mixture.

3.2 Pore water analysis

Pore water pH values were quite similar during the experiment across most treatments, apart from 'PM', which showed a progressive increase along the experiment, and 'Fe+Ca', which suffered an increase from the first to the second sampling (Fig. 1). The lowest pH values (Table 2) corresponded to the 'Fe' treatment, while 'Ca', 'Fe+Ca' and 'PM' treatments gave the highest values. Pore water samples obtained from 'Fe+PM' presented similar pH values to those obtained in the 'NA' pots ($p < 0.05$).

Paper mill and CaCO_3 incorporation significantly increased As concentrations in pore water (Fig. 2) with a 290 and 400 % increase respectively compared to 'NA', while 'Fe+PM' and 'Fe+Ca' treatments significantly decreased As concentrations with a 88 and 70% reduction respectively compared to 'NA'. These treatments were the most effective amendments in reducing soluble As concentrations along the five pore water collections. There were not significant differences in pore water As in 'Fe' compared to the unamended treatment.

An extreme increase of metals concentration in pore water with the incorporation of 'Fe' and 'Fe+PM' was observed (Fig. 2). Metals concentrations were well above that of the unamended treatment (in the 'Fe' treatment: 360 for Mn, 5000 for Cu and 450 for Cd times higher than 'NA'; in 'Fe+PM': 135 for Mn, 380 for Cu and 145 for Cd times higher than 'NA'). 'Ca' and 'PM' efficiently reduced Mn and Zn leaching compared to 'NA'. However, all the amendments had a negligible effect on the reduction of Cu pore water concentration ($p < 0.05$).

Amendments can be classified from major to minor according to their effectiveness for reducing metal(oid)s pore water concentration as follows. The unamended treatment is included in the list ($p < 0.05$):

Cu: NA>Fe+Ca>Ca~PM>Fe+PM>Fe

Mn: Ca~PM>NA~Fe+Ca>Fe+PM~Fe

Zn: Ca~Fe+Ca~PM>NA~Fe+PM~Fe

Cd: Ca~Fe+Ca~PM> NA>Fe~Fe+PM

Bivariate correlations among trace element concentrations and pH values in each sampling showed a significant linear dependent relation between the two variables along all the pore water collections, being positive for As ($p < 0.05$) and negative for Cu, Mn, Zn and Cd ($p < 0.01$). Pearson's coefficient accounted for between 40 and 70% of the dependent variable variance (data not shown).

3.3 Biomass and metal(oid) concentration in plants

Seeds germinated properly and the majority of seedlings grew satisfactorily excepting those in 'Fe' treatment, where seed germination percentage was very low and the seedlings died some days after emergence.

Arrhenatherum elatius fresh weight was higher than *F. curvifolia* on average at the end of the experiment (Table 3). Both herbaceous plants had different growing rates. *Arrhenatherum elatius* grew rapidly whereas *F. curvifolia* growth rate was lower and was less spread out over the soil surface. In both species, the lowest fresh weight was recorded in the unamended treatment, while the highest fresh biomass was found with 'Ca', 'Fe+Ca' and 'PM'. None of the plants presented chlorosis or brownish leaf ends, but plants in the unamended pot presented lower height than in the rest of treatments.

Amendments were effective in reducing As concentration in the aerial part of both species, except in 'Ca' treatment where there were no significant differences in comparison to the non-amended treatment. Further, Zn concentration in plant tissue

decreased with the incorporation of the amendments, except in *F. curvifolia* growing in 'Fe+Ca' treatment (Table 4). 'Ca' and 'PM' reduced Mn concentration but it increased significantly in 'Fe+PM' followed by 'Fe+Ca'. Significant differences were observed for Cu concentration only for *A. elatius*, being 'Ca' and 'PM' the treatments which reached larger Cu concentration. Amendments addition did not produce any clear pattern in the Cu concentration found in *F. curvifolia*.

4 Discussion

4.1 Soil

Arsenic has high affinity for iron, manganese and aluminium oxides surfaces (Fitz and Wenzel, 2002; De Brouwere et al. 2004). Arsenate can form inner-sphere complexes with iron (Cheng et al. 2009). Previous studies on soils treated with iron-bearing amendments have reported that the addition of Fe as goethite or iron grit reduced arsenic and cadmium leaching (Hartley et al. 2004). However, in the present study, FeSO₄ by itself ('Fe' treatment) did not decrease As concentration in pore water in comparison to the soils that did not receive any amendment (Fig. 1). Furthermore, it promoted the increase of Cu, Mn, Zn, Cd and Fe concentrations enormously. The addition of iron (II) sulphate produces ferric oxides spontaneously in the presence of oxygen and would lead to the formation of very insoluble iron arsenate forms and cause a drop in the soil solution pH (Artiola et al. 1990). The activity of Fe³⁺ maintained by Fe (III) oxides and amorphous Fe(OH)₃ in the soil is higher as pH decreases. Thus, the probability to form iron oxihydroxides at pH 2.5 is lower than at higher values of pH (Lindsay, 1979). Additionally, when pH decreases below 2.5, arsenic can be found as

uncharged molecule (Zhang and Selim 2008). Taking into account these two approaches and that the amount of iron (II) sulphate triggered a great drop of pH, arsenic may not be retained by the soil particles.

'PM' and 'Ca' were the amendments which furthest increased As concentrations in pore water, as well as reduced Zn, Mn and Cd concentrations, according to the increase in pH values observed. Adding FeSO₄ to paper mill and calcium carbonate, ('Fe+PM', 'Fe+Ca') decreased soluble As concentrations, probably because of the formation of iron oxihydroxides at pH values higher than those obtained when only iron (II) sulphate was applied. A similar effect was observed by Hartley et al. (2004). The 'Fe+PM' treatment was not as successful as expected, since the pH was around 4.3 and metals were mobilised in soils in a similar way as 'Fe'. The higher pH values reached by the 'Fe+Ca' treatment contributed to a reduction in metals solubility including iron, despite the fact that iron sulphate was added in the same proportion in 'Fe' and 'Fe+PM'. Larger doses of paper mill in combination with iron should be tested in order to obtain higher pH values and lower metal mobility.

Bivariate correlations indicated pH is a parameter which accounted for up to 70% of the variability in metals and 60% of the As concentration in pore water (data not shown). Fitz and Wenzel (2002) reported that arsenate solubility increases in soils within the pH interval 3-8. Accordingly, pore water pH in the 'PM' treatment increased significantly along the five samplings, as arsenic soluble concentration did.

Many reports have shown that liming a soil contributes to metal immobilisation due to a pH increase, being a common practice in soil management where metals are present (Madejón et al. 2011). Metals are less soluble in alkaline conditions (Adriano, 2001). According with this fact, alkaline amendments such as calcium oxide and sugar beet sludge decreased CaCl₂-extractable Cu, Pb and Zn (Alvarenga et al. 2008) and the

incorporation of organic material plus lime increased pH and immobilised partially Cu, Zn, Mn and Cd (Clemente et al. 2008). In agreement with these findings, 'Ca', 'PM' and 'Fe+Ca' raised the pore water pH more than 2 units (up to 7) compared to the non-amended treatment, being the amendments which contributed to the greater metal retention.

4.2 Plant

None of the seedlings were able to emerge in the 'Fe' treatment. It is known that aluminium solubility is enhanced by low pH, being a major factor limiting plant production in acid soils (Delhaize and Ryan, 1995). Aluminium concentration in pore water found in 'Fe' treatment was high enough to trigger toxicity with concentrations (from 140 to 1200 mg L⁻¹ in 'Fe' compared to 0.012 to 0.800 mg L⁻¹ in 'NA'). Plant growth was also limited within the pH interval 4-5, but to a lower extent, as was observed in the 'Fe+PM' treatment. This latter amendment did not improve plant growth as paper mill alone ('PM'), probably because the paper mill amount added together with iron sulphate was not able to enhance pH above 5. Some authors have reported a negligible effect on plant growth with lime and lime with added iron (II) sulphate, being similar or worse than plants growing in unamended controls (Hartley et al. 2008). However, the addition of CaCO₃ to FeSO₄ increased pH, contributing to improved plant growth as indicated by a biomass yield of 65 and 92% for *A. elatius* and *F. curvifolia* respectively compared to the control 'NA'. The rest of amendments, 'Ca' and 'PM' enhanced biomass yield, showing that liming is a beneficial practice.

Adding an organic material to a soil where the percentage of organic matter is very low, such as degraded soils, is one way to encourage vegetal cover development

and also favours a positive effect on soil physical properties. Phytostabilisation practices can create a long term vegetal cover able to sustain by itself and hinder contaminant dispersion (Robinson et al. 2006). Paper mill incorporation (containing 25% of organic matter) appears to create a favoured environment for improved *A. elatius* and *F. curvifolia* biomass yield, significantly above the control 'NA'.

The large soluble As released with CaCO₃ and paper mill incorporation did not increase As uptake by plants above the As uptake in the controls. The same trend was observed for Zn and Cu despite the large levels of both metals in 'Fe+PM'. Soluble Mn correlated well with Mn concentration in *A. elatius*, but not in the case of Zn and Cu. Adriano et al. (2004) reported that amendments decreased the soluble fraction of trace elements but were not effective in changing trace element plant uptake. Beesley and Dickinson (2009) reported that the addition of amendments can involve a rapid mobilisation and vertical transport of trace elements, so plant uptake might be restricted by their fast mobilisation. Furthermore, both species were in the first stages of their growth, with low root area and length. These two factors, the rapid mobilisation of elements and lack of root development, may have brought about the non-homogeneous plant response to the changes of the soluble fraction of trace elements, although differences in plant uptake cannot be explained by higher plant biomass.

Arrhenatherum elatius and *F. curvifolia* are native to the region of the mine where the contaminated and uncontaminated materials were collected and the establishment of vegetation with good biomass yield is preferred as the primary objective over metal(loid) accumulation. Focus must be on successful natural attenuation after incorporating the amendments. As Clemente et al. (2006) reported, soil amendments contributed to natural attenuation in soil contaminated with metals and arsenic. *Arrhenatherum elatius* and *F. curvifolia* are caespitose grasses with fasciculate roots

which fix the soil and allow for proper establishment of vegetation. Contaminants can be stabilised, making them less susceptible of being eroded and dispersed via water or air. Plant growth and long-term survival are crucial, since plants function as a contaminant barrier to other environment compartments, such as the atmosphere. Soil health may also be improved, contributing to promote soil microbial activity.

5 Conclusions

Dealing with multi-element contaminated sites requires widening the focus not only to metals, but also to metalloid behaviour. Based on the findings of the current study, changes in arsenic and metals leaching cannot be considered independently when choosing a suitable amendment for successful assisted phytostabilisation in a multicontaminated mine soil recovery program.

Rhizon samplers have been demonstrated to be valid devices to assess the potential mobility of trace elements in soils. The effects on metal(loid) solubility by amendment incorporation have been appropriately reflected in the soil solution extracts measured over the course of the experiment.

Due to arsenic's anionic character, it has more similarities with phosphorus than with metals when considering the mechanism of adsorption on soil particles. This may explain why the addition of amendments had opposite effects on arsenic and metal mobilisation, in addition to being mainly dependent on pH changes and Fe addition. Plant growth was improved by the amendments able to increase soil and pore water pH to values around 6-7. Plant uptake was not influenced by trace elements in pore water, although some differences were found in plant absorption among treatments.

According to our results, the 'Fe+Ca' treatment would be the most suitable amendment, as it reduced arsenic mobility and did not increase soluble metal concentrations in comparison to the unamended soil. It also promoted plant growth, favouring the establishment of vegetation.

The post-amendment incorporation followed by a natural attenuation phase must be monitored over time to assure the success of the management practices. Future work should consider remediation monitoring through chemical methodologies as the ones described in this paper alongside with biological approaches. Assessing these different approaches for site monitoring of a remediation strategy will help evaluate long term risks and highlight any changes in the effectiveness of amendment addition for assisted phytostabilisation.

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Figure captions

Fig. 1 pH values of the five pore water collections (mean±SE, n=4). Vertical bars represent the soil treatments in each sampling fraction: not amended ('NA'), FeSO₄ ('Fe'); CaCO₃ ('Ca'); FeSO₄+CaCO₃ ('Fe+Ca'); paper mill ('PM'); and FeSO₄+paper mill ('Fe+PM'). Statistical differences among treatments were identical in each sampling fraction, being as follows: 'Ca', 'Fe+Ca', 'PM' > 'NA' > 'Fe+PM' > 'Fe' (p < 0.05).

Fig. 2 As, Cu, Mn, Zn, Cd and Fe in pore water for sampling fraction 1 and 5 (mean±SE, n=4). Only fractions 1 and 5 are shown as no significant changes were observed over time. Vertical bars represent the soil treatments in each sampling fraction: not amended ('NA'), FeSO₄ ('Fe'); CaCO₃ ('Ca'); FeSO₄+CaCO₃ ('Fe+Ca'); paper mill ('PM'); and FeSO₄+paper mill ('Fe+PM'). Different letters indicate significant differences among treatments for each pore water fraction (p < 0.05).

Table 1

Uncontaminated soil, dumping material, mixture 60:40 (uncontaminated soil+dumping material) and paper mill characterisation (mean±SE; n=3).Extractable elements in paper mill was not determined. (n.d.=not detected).

	Uncontaminated soil	Dumping material	Mixture 60:40	Paper mill
pH	6.02±0.01	4.1±0.01	4.9±0.03	7.7 ± 0.01
Organic matter (%)	3.9±0.06	0.6±0.01	3.3±0.04	29.3± 0.40
Electric Conductivity (µS·cm⁻¹)	14.2±1.2	46.3±6.2	37.0±0.1	253.0±9.6
Total element (mg·kg⁻¹)				
As	17.3±4.8	4265.8±506.1	1595.9±33.9	0.31±0.07
Cu	27.9±1.9	758.1±178.1	327.1±35.3	151.8±6.3
Mn	267.1±62.8	219.9±19.6	207.5±19.9	85.4±18.0
Zn	42.5±8.3	2455.1±430.2	910.9±133.6	41.1±5.9
Cd	n.d.	40.3± 3.7	19.3±0.3	8.67±0.6
Extractable element (mg·kg⁻¹)				
As	0	7.4±0.3	2.1±0.04	
Cu	n.d.	10.0±0.1	1.9±0.05	
Mn	2.7±0.06	4.5±0.5	3.9±0.1	
Zn	0.7±0.1	21.1±0.3	8.7±0.05	
Cd	n.d.	1.3±0.5	0.06±0.01	

Table 2

pH values obtained in (1) soil (mixture of uncontaminated soil+ contaminated material) one month after pots equilibration; (2) first pore water sampling; (3) last pore water sampling. Different letters indicate significant differences between the first and the last pore water fraction in each treatment (mean±SE, n=4, p < 0.05).

Treatment	(1)	(2)	(3)
‘NA’	5.0±0.03	4.7±0.10 a	4.7±0.06 a
‘Fe’	3.4±0.02	2.7±0.06 a	3.0±0.04 a
‘Ca’	8.2±0.07	7.2±0.2 a	7.4±0.06 a
‘Fe+Ca’	7.4±0.04	6.8±0.04 b	7.1±0.06 a
‘PM’	7.2±0.08	6.7±0.07 b	7.2±0.05 a
‘Fe+PM’	6.6±0.10	4.0±0.4 a	4.2±0.2 a

Table 3

Fresh weight biomass of *Arrhenatherum elatius* and *Festuca curvifolia* (g·pot⁻¹).

Different letters indicate significant differences among treatments for each plant species

(mean±SE, n=4, p < 0.05).

Treatment	<i>A. elatius</i>	<i>F. curvifolia</i>
‘NA’	1.5±0.1 b	0.4±0.05 c
‘Fe’	-	-
‘Ca’	2.5±0.04 a	0.8±0.05 a
‘Fe+Ca’	2.3±0.06 a	0.8±0.07 a
‘PM’	2.4±0.09 a	0.8±0.05 a
‘Fe+PM’	1.5±0.07 b	0.6±0.05 b

Table 4

Trace element concentrations in plants ($\mu\text{g}\cdot\text{g}^{-1}$). Different letters indicate significant differences among treatments for each plant species (mean \pm SE, n=4, p < 0.05).

	‘NA’	‘Ca’	‘PM’	‘Fe+Ca’	‘Fe+PM’
As					
<i>A. elatius</i>	17.4 \pm 1.8 a	15.5 \pm 1.9 a	11.6 \pm 0.8 b	9.5 \pm 0.7 b	9.9 \pm 0.6 b
<i>F. curvifolia</i>	18.7 \pm 4.9 a	14.9 \pm 2.5 a	13.6 \pm 1.1 b	12.9 \pm 0.7 b	14.0 \pm 2.5 b
Cu					
<i>A. elatius</i>	7.1 \pm 1.1 b	13.5 \pm 0.7 a	17.5 \pm 9.3 a	10.5 \pm 1.5 b	9.1 \pm 1.6 b
<i>F. curvifolia</i>	23.9 \pm 5.9	31.9 \pm 11.7	14.4 \pm 2.9	23.6 \pm 3.8	19.3 \pm 2.1
Mn					
<i>A. elatius</i>	410.9 \pm 22.5 b	146.4 \pm 7.7 c	210.8 \pm 4.5 c	439.1 \pm 13.9 b	630.4 \pm 58.9 a
<i>F. curvifolia</i>	71.2 \pm 8.1 c	80.2 \pm 6.3 bc	128.9 \pm 6.9 c	369.4 \pm 11.9 a	282.3 \pm 32.6 b
Zn					
<i>A. elatius</i>	101.6 \pm 20.8 a	31.5 \pm 2.5 b	34.7 \pm 4.2 b	41.7 \pm 1.8 b	43.2 \pm 5.1 b
<i>F. curvifolia</i>	88.7 \pm 8.9 a	56.4 \pm 2.4 b	55.2 \pm 2.8 b	86.6 \pm 7.1 a	63.8 \pm 5.5 b

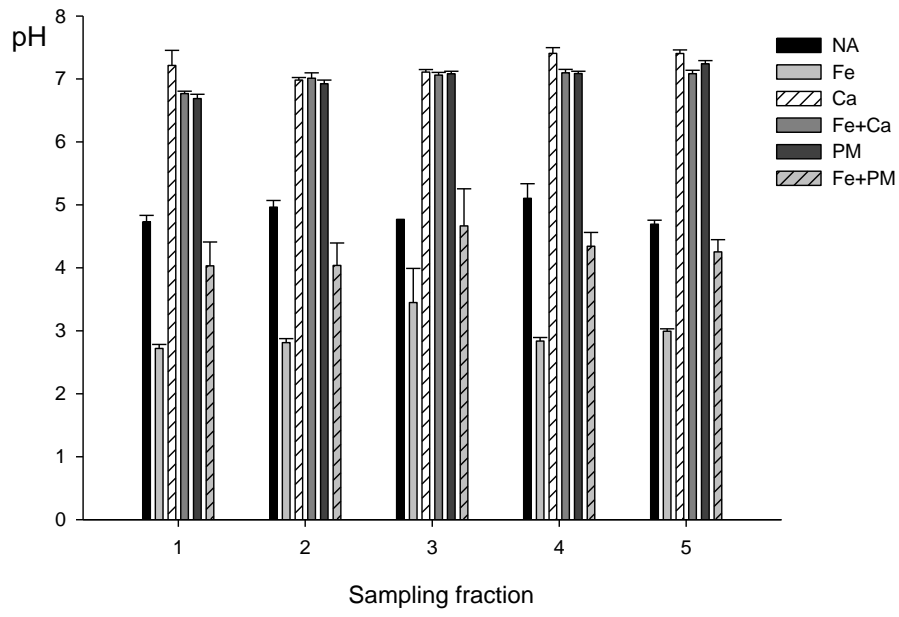


Fig.1.

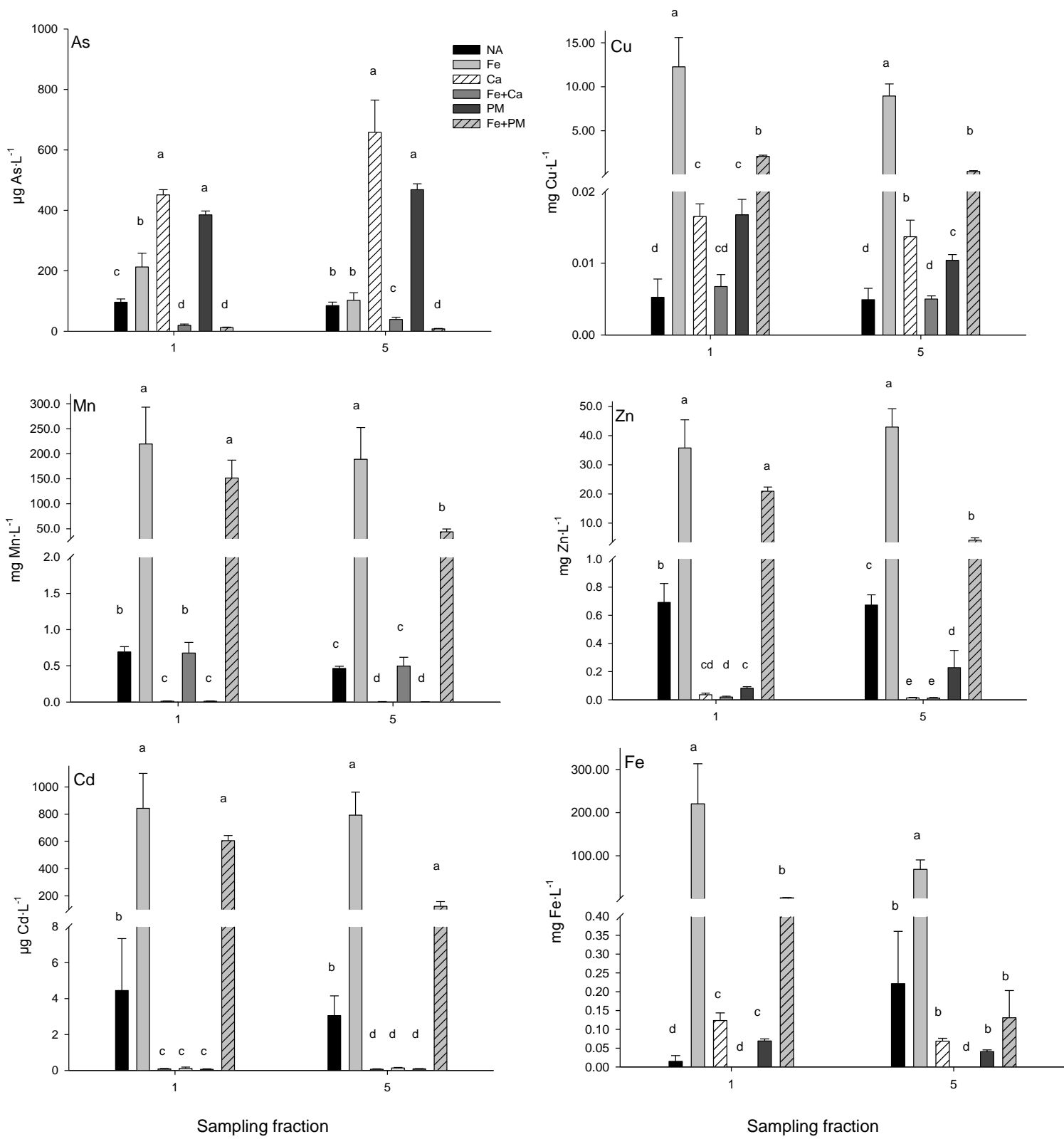


Fig.2.