

SOIL ACIDIFICATION AS A CONFOUNDING FACTOR ON METAL PHYTOTOXICITY  
IN SOILS SPIKED WITH COPPER-RICH MINE WASTES

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**Abstract**—Pollution of soil with mine wastes results in both Cu enrichment and soil acidification. This confounding effect may be very important in terms of phytotoxicity, because pH is a key parameter influencing Cu solubility in soil solution. Laboratory toxicity tests were used to assess the effect of acidification by acidic mine wastes on Cu solubility and on root elongation of barley (*Hordeum vulgare* L.). Three contrasting substrates (two soils and a commercial sand) and two acidic, Cu-rich mine wastes (oxidized tailings [OxT] and smelter dust [SmD]) were selected as experimental materials. Substrates were spiked with a fixed amount of either SmD or OxT, and the pH of experimental mixtures was then modified in the range of 4.0 to 6.0 and 7.0 using PIPES (piperazine-1,4-bis(2-ethanesulfonic acid)), MES (2-(*N*-morpholino)ethanesulfonic acid), and MOPS (3-(*N*-Morpholino)-propanesulfonic acid) buffers. Chemical (pore-water Cu and pH) and toxicological (root length of barley plants) parameters were determined for experimental mixtures. Addition of SmD and OxT to substrates resulted in acidification (0.11–1.16 pH units) and high levels of soluble Cu and Zn. Neutralization of experimental mixtures with MES (pH 6.0) and MOPS (pH 7.0) buffers resulted in a marked decrease in soluble Cu and Zn, but the intensity of the effect was substrate-dependent. Adjustment of soil pH above the range normally considered to be toxic to plants (pH in water extract, >5.5) significantly reduced metal toxicity in barley, but phytotoxicity was not completely eliminated. The present results stress the importance of considering confounding effects on derivation of toxicity thresholds to plants when using laboratory phytotoxicity tests.

**Keywords**—Soil acidification Tailings Smelter dust Copper toxicity Barley

## INTRODUCTION

Several anthropogenic activities have caused important increases in Cu levels of agricultural soils, which in some cases have resulted in plant toxicity and reduction in productivity [1–4]. In agricultural soils, Cu and other metals may come from application of sewage sludge as fertilizer, irrigation water, fertilizers and pesticides, natural minerals, industrial wastes and emissions, or a combination thereof. However, Cu mining and smelting have been the most important sources of soil Cu contamination through atmospheric and particulate emissions as well as through dispersal of liquid and solid wastes [5–7].

Copper-rich mine wastes and materials pose very different levels of risk to plants. For example, smelter dust (SmD) and oxidized tailings (OxT) are much more toxic to plants than smelter slag and Cu concentrate when spiked into agricultural soils at a same range of total Cu concentrations [4]. This results mainly from differences in Cu solubility for these mine materials because of their different chemical/mineralogical characteristics [7–9]. Indeed, preliminary results from optical mineralogical analysis of mine materials derived from a large-scale porphyry Cu mine plant and smelter in central Chile [4,10] have shown that Cu is present mainly as insoluble mineral forms in concentrate and smelter slag (i.e., chalcocopyrite, coveline, and bornite) but as highly soluble, nonmineral phases in SmD (i.e., chalcocyanite or  $\text{CuSO}_4$ ) and OxT (i.e., chalcantite or  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ).

Pollution of agricultural soils with some Cu-rich mine wastes, such as SmD or OxT, also may result in soil acidification [4]. For example, a decrease in soil solution pH by up to three units was reported by Ginocchio et al. [4] in a laboratory study in which soils were spiked with either SmD or OxT to reach total soil Cu concentrations of up to 5,000 mg/kg, because SmD and OxT are very acidic materials (pH 2.2 and 3.9, respectively). Laboratory tests of the toxicity of metals to plants and other soil organisms also have shown that spiking soils with highly soluble metal salts (e.g.,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and  $\text{CuCl}_2$ ) not only increases the total metal content in soils but also decreases the pH of the soil solution [11–14]. Indeed, Oorts et al. [14] reported a decrease in soil solution pH by up to two units with addition of a high Cu dose (9,600 mg/kg, nominal), whereas Lock et al. [15] reported a drop in soil solution pH by as much as three units or more when experimental soils were spiked at high Pb concentrations (>3,877 mg/kg, nominal). These changes reflect the replacement of protons and exchangeable alkaline earth cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and  $\text{Na}^+$ ) from the exchange complex in the soil by the added metal cations [15].

This confounding effect of highly soluble metal salts and acidic, Cu-rich mine wastes on soil pH normally has been ignored, even though it may be very relevant for assessing the effects of toxicity in plants. On one hand, pH is definitely the main factor affecting both metal speciation/adsorption in soils and metal phytotoxicity. Experimental studies with soils spiked using soluble metal salts demonstrated that desorption of metals like Cu, Zn, and Cd increases in soil solution at decreasing pH [3,16–17], whereas precipitation, adsorption [3,17,18], and fixation (slow

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Table 1. General chemical characteristics of acidic, Cu-rich mine wastes and chemical characteristics of the same samples under saturation extracts (sat. ext.)<sup>a</sup>

Mine waste	pH (1:1 water)	TOC (%)	CEC (mEq/100 mg)	Total Cu (mg/kg)	Total Zn (mg/kg)	EC (mS/cm <sup>2</sup> )	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	Cl <sup>-</sup> (mg/L)	Cu sat. ext. (mg/kg)
Smelter dust	2.58	1.12	23.35	220,334	40,047	58.6	424	782	2,048	41	44,896 (20%) <sup>b</sup>
Oxidized tailings	3.68	0.03	14.23	2,493	65	5.6	600	369	96	12	2,051 (82%) <sup>b</sup>

<sup>a</sup> CEC = potential cation-exchange capacity at pH 7.0; EC = electric conductivity; TOC = total organic carbon.

<sup>b</sup> Percentage from total Cu.

immobilization [19–21]) dominates with increasing soil solution pH. Furthermore, pH also impacts metal phyto-availability by affecting ion competition on metal binding to the biological receptor in plant roots (the biotic ligand); ions in solutions (e.g., H<sup>+</sup> and Ca<sup>2+</sup>) also may form complexes with the biotic ligand in roots and occupy sites that otherwise would be available to metal cations, thus alleviating metal toxicity to plants [14,22–24]. On the other hand, secondary chemical changes in metal-spiked soils, such as soil pH, also can pose indirect toxic effects to plants and other soil organisms. For instance, a decrease in soil pH leads to a reduction in the solubility of P and Mo and increases the concentration of Al ions in the soil solution as a consequence of H<sup>+</sup>-buffered processes [25]. Elevated levels of Al in acidic soil solutions are very toxic to plants [3,26,27].

Deriving the toxicity effects of Cu in plants from tests with Cu salts and acidic, Cu-rich mine waste-spiked soils therefore is problematic, because adverse effects often are confounded as a result of covariance of direct Cu toxicity with other changes in soil properties that both indirectly increase Cu solubility and phytotoxicity in soil and directly increase Al toxicity to plants. To our knowledge, no study has evaluated experimentally the importance of the confounding effect of pH on Cu solubility and plant toxicity of acidic, metal-rich mine wastes when spiked into agricultural soils. Therefore, a laboratory assay was carried out to evaluate the effect of acidic, Cu-rich mine wastes on soil solution pH and the importance of soil solution pH on Cu solubility and phytotoxicity.

## MATERIALS AND METHODS

### Acidic, Cu-rich mine wastes

A bulk sample of SmD and OxT from the upper oxidation zone (depth, 0–20 cm) of an abandoned tailing storage facility was obtained from a large-scale porphyry Cu mine plant and smelter in central Chile. General physicochemical characteristics of these materials are given in Ginocchio et al. [4] and in Table 1. Both were acidic materials (pH < 4.0) and had elevated total and water-extractable Cu contents, but they differed in cation-exchange capacity (CEC; i.e., potential CEC measured by the method of ammonium acetate buffered at

pH 7.0) and Ca, Mg, Na, and Cl<sup>-</sup> contents in saturation extracts (Table 1).

### Experimental substrates

Bulk samples from two agricultural soils of central Chile (topsoils; depth, 0–20 cm) with contrasting soil properties were considered in the present study (Tables 2 and 3) (for further information about these soils, see Ginocchio et al. [4]). In addition, a sample of acid-washed commercial sand [28] (<http://www.epa.gov/cgi-bin/claritgw?op-display&document=clserv:OW:0569;rank=4&template=epa>) was included to encompass the range of soil properties under evaluation, particularly total organic C, CEC (at pH 7.0), and clay content (Tables 2 and 3). Selected substrates were slightly acidic (V-R soil and VII-R soil) or acidic (sand) and had low total and water-extractable Cu and Zn contents (Tables 2 and 3). Substrates differed in their buffering capabilities as expected from their physicochemical characteristics. Indeed, they differed in terms of particle size distribution, pH, total organic C, inorganic C, dissolved organic C, CEC (at pH 7.0), electric conductivity (EC), hardness and contents of Ca, Mg, Na, and Cl<sup>-</sup> in pore water (Tables 2 and 3). All three substrates were air-dried, homogenized, and sieved to pass through a 2-mm nylon mesh before use.

### Preparation of buffer solutions

The noncomplexing tertiary amine buffers (Good's *N*-substituted aminosulfonic acids) PIPES (piperazine-1,4-*bis*(2-ethanesulfonic acid)), MES (2-(*N*-morpholino)ethanesulfonic acid), and MOPS (3-(*N*-Morpholino)-propanesulfonic acid) were selected for the present study, because they are increasingly being used in biological and chemical applications, including ecotoxicity testing [29]. The PIPES (20 mM), MES (0.1 M), and MOPS (0.5 M) buffer solutions were prepared from the acid salts of the buffers (purchased from Merck) and ultrapure water (>18 MΩ/cm) as indicated by Kandedgara and Rorabacher [30]. The pH of solutions was adjusted with either H<sub>2</sub>SO<sub>4</sub> (1 N) or KOH (1 N) to 4.0 (PIPES), 6.0 (MES), and 7.0 (MOPS). These buffer solutions were used to buffer experimental soils spiked with either SmD

Table 2. General physicochemical properties of study substrates<sup>a</sup>

Substrate	pH (water)	TOC (%)	IC (%)	CEC (mEq/100 mg)	Total Cu (mg/kg)	Total Zn (mg/kg)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Particles (%)		
										<2 μm	2–50 μm	51–2,000 μm
V-R soil	5.35	0.83	0.11	14.7	130	133	19	29	267	12	18	71
VII-R soil	5.96	4.63	1.10	27.9	24	68	287	42	677	8	26	66
Sand	4.45	<0.02	ND	7.8	41	55	7	32	21	6	0	94

<sup>a</sup> CEC = potential cation-exchange capacity at pH 7.0; IC = inorganic carbon; ND = not determined; TOC = total organic carbon.

Table 3. Chemical characteristics of saturation extracts (upper) and pore water (lower) of study substrates<sup>a</sup>

Substrate	Saturation extracts						
	pH	EC (mS/cm <sup>2</sup> )	Cu (mg/L)	Zn (mg/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)
V-R soil	4.4	0.41	0.05	0.07	24.8	4.0	15.0
VII-R soil	5.4	2.18	0.01	0.03	255.2	61.2	9.0
Sand	5.1	0.18	0.01	0.04	23.2	3.2	12.4

Substrate	Pore water									
	pH	EC (mS/cm <sup>2</sup> )	DOC (mg/L)	Cu (mg/L)	Zn (mg/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	Cl <sup>-</sup> (mg/L)	Hardness (mg/L as CaCO <sub>3</sub> )
V-R soil	4.49	1.35	11.2	0.08	0.51	151	24	48	18	478
VII-R soil	5.23	2.27	183.4	0.04	0.60	264	74	8	15	963
Sand	4.30	0.14	5.5	0.02	0.6	12	8	19	29	65

<sup>a</sup> DOC = dissolved organic carbon; EC = electric conductivity.

or OxT from control pH (Table 1) to pH 4.0 (PIPES), pH 6.0 (MES), and pH 7.0 (MOPS).

#### Plant toxicity assays

A short-term laboratory assay was set up to evaluate the effect of pH on Cu solubility and phytotoxicity in selected substrates spiked with acidic, Cu-rich mine wastes. Experimental factors of the study are shown in Table 4. Substrates (V-R soil, VII-R soil, and sand) were spiked with a fixed amount of either SmD (0.16 g/100 g substrate) or OxT (5.59 g/100 g substrate). These doses are known for their phytotoxic effect from previous studies [4]. To avoid any soil dilution with addition of Cu-rich wastes, mixtures of 80% soil and 20% acid-washed sand were prepared; the appropriate weight of acid-washed sand was first replaced with the required amount of each mine waste before mixing with experimental soils.

Two to three kilograms of each experimental mixture were prepared in acid-washed polyethylene plastic containers. Care

was taken in sampling and manipulation to prevent cross-contamination between mixtures. Mixtures were hydrated with the buffer solutions and homogenized over a 17-d period to allow mixture equilibration until the desired pH (4.0, 6.0, or 7.0) was reached. Addition of H<sub>2</sub>SO<sub>4</sub> (1 N) or KOH (1 N) sometimes was necessary to reach the target pH on experimental mixtures. The same procedure was followed with batches of both unspiked substrates (control) and mine waste-spiked substrates, but with hydration using ultrapure water (unbuffered mixture). After the spiking and equilibration process, experimental substrates and mixtures were used to fill 18 acid-washed polyethylene plastic containers (internal diameter, 40 mm; height, 145 mm) with drainage at their base. The remaining substrates and mixtures were used to determine total Cu and pH as described below. Method 1638 of the U.S. Environmental Protection Agency (EPA) [28] (<http://www.epa.gov/cgi-bin/claritgw?op-display&document=clserv:OW:0569;rank=4&template=epa>) was used for acid-

Table 4. Experimental factors to assess the effect of soil pH on Cu phytotoxicity in substrates spiked with acidic, Cu-rich mine wastes

Factor	Sand	V-R soil	VII-R soil
1. S <sup>a</sup>	Control	Control	Control
2. S + mine waste	OxT <sup>b</sup> SmD <sup>c</sup>	OxT SmD	OxT SmD
3. S + B <sup>d</sup>	PIPES <sup>e</sup> MES <sup>f</sup> MOPS <sup>g</sup>	PIPES MES MOPS	PIPES MES MOPS
4. S + B + pH adjustment	H <sub>2</sub> SO <sub>4</sub> + PIPES KOH <sup>h</sup> + MES KOH + MOPS	H <sub>2</sub> SO <sub>4</sub> + PIPES KOH + MES KOH + MOPS	H <sub>2</sub> SO <sub>4</sub> + PIPES KOH + MES KOH + MOPS
5. S + OxT + B + pH adjustment	H <sub>2</sub> SO <sub>4</sub> <sup>i</sup> + PIPES KOH + MES KOH + MOPS	H <sub>2</sub> SO <sub>4</sub> + PIPES KOH + MES KOH + MOPS	H <sub>2</sub> SO <sub>4</sub> + PIPES KOH + MES KOH + MOPS
6. S + SmD + B + pH adjustment	KOH + PIPES KOH + MES KOH + MOPS	H <sub>2</sub> SO <sub>4</sub> + PIPES KOH + MES KOH + MOPS	H <sub>2</sub> SO <sub>4</sub> + PIPES KOH + MES KOH + MOPS
Treatments (n)	15	15	15
Experimental units (n) <sup>j</sup>	270	270	270

<sup>a</sup> S = substrate.

<sup>b</sup> OxT = oxidized tailings.

<sup>c</sup> SmD = smelter dust.

<sup>d</sup> B = buffer.

<sup>e</sup> PIPES = piperazine-1,4-bis(2-ethanesulfonic acid) (20 mM).

<sup>f</sup> MES = 2-(N-morpholino)ethanesulfonic acid (0.1 M).

<sup>g</sup> MOPS = 3-(N-Morpholino)-propanesulfonic acid (0.5 M).

<sup>h</sup> KOH 1 N.

<sup>i</sup> H<sub>2</sub>SO<sub>4</sub> 1 N.

<sup>j</sup> 18 replicates were used for each treatment.

washing of all polyethylene containers (24 h with 0.5% Extran MA O<sub>2</sub> Neutral [Merck], 24 h with 1 N HNO<sub>3</sub>, 24 h with 1 N HCl, and four washings with ultrapure water [ $>18$  M $\Omega$ /cm]).

The effect of soil pH and Cu toxicity from Cu-rich mine wastes on plants was assessed through the short-term barley root elongation test (International Organization for Standardization Method 11269-1 [31]). Barley (*Hordeum vulgare* L.) is sensitive to both excess soluble soil Cu [24] and excess soluble or exchangeable Al in acidic soils with pH less than approximately 5.5 [32,33]. Some modifications were introduced to the International Organization for Standardization method: Container size was reduced in diameter to 40 mm; seed number per container was reduced to three; a local barley variety (*H. vulgare* L. var. Acuario-INIA-CCU, developed by Instituto de Investigaciones Agropecuarias in 1978) was used; and experimental time was reduced from 5 to 4 d for both agricultural soils (V-R and VII-R) but not for sand, because the roots reached 80% of container length more rapidly in the soils than in sand. Experimental substrates and mixtures were hydrated with ultrapure water up to 60% (V-R soil) or 70% (VII-R soil and sand) of maximum water-holding capacity and sown with three pregerminated barley seeds. Experimental containers were kept in a plant growth room under controlled conditions ( $23 \pm 2^\circ\text{C}$ , light intensity, 122.5  $\mu\text{mol/s/m}^2$ , and 12-h light:dark photoperiod) for 4 d (V-R and VII-R soils) or 5 d (sand). Soil moisture of every container was checked daily, and ultrapure water was added (no leaching) to keep experimental substrates and mixture at either 60 or 70% of maximum water-holding capacity throughout the experiment (no leaching).

Aliquots (5–7 ml) of soil pore water were taken daily from 6 of 18 replicated containers per treatment with acid-washed Rhizon<sup>®</sup> Soil Pore Water Samplers (length, 5 cm; Rhizosphere Research Products) following the methodology described by Vulkan et al. [34]. Soil pore-water samples were kept in acid-washed polyethylene plastic vials (15 ml), and pH and EC determinations were done up to 10 min after sample collection with a combination pH electrode (120C, Sorex) and a conductivity meter (CG 858, Schott Gerate), respectively. A composite soil pore-water sample was obtained per treatment after the 4- to 5-d experimental period. Every sample was acidified with 5% HNO<sub>3</sub> Suprapur (Merck) for Cu, Zn, and Ca determinations as described below. After 4 d (V-R and VII-R soils) or 5 d (sand), the length of the longest root of barley plant was determined as indicated by International Organization for Standardization Method 11269-1 [31].

#### Chemical analysis

Experimental mixtures and control substrate samples were dried in a forced air-drying cabinet at a temperature of approximately 30°C. The pH was determined in 1:1 soil:water according to a protocol of the U.S. Department of Agriculture [35]. Total Cu and Zn were determined after microwave acid digestion (1200 Mega Microwave System; Milestone Microwave System) and extraction (modified U.S. EPA Method 3052 [28]). Digested samples were analyzed for total Cu and Zn by flame atomic absorption using an atomic absorption spectrometer (AAAnalyst 300; PerkinElmer) under U.S. EPA methods SW-486 [36], 7210 [37], and 7950 [38].

#### Soil pore-water analysis

Total dissolved Cu, Zn, and Ca in soil pore-water samples were determined using an inductively coupled plasma–mass spectrometer (ICP-MS; ELAN 6100 with autosampler AS90;

PerkinElmer) following U.S. EPA Method SW-486 [34]. The standard calibration solution used was ICP-MS multielements, 99.99% purity, high-purity, and reference material ION-20 (National Water Research Institute). Sample, calibration standards, and reference material were in 0.2% HNO<sub>3</sub>.

#### Data analysis

Simple correlations and simple linear regression analysis [39] were performed to determine significant relationships among chemical characteristics of experimental substrates and mixtures (i.e., metals in pore water and pore-water pH, and metals in pore water and total metals). One-way analysis of variance was used to test for effects of experimental treatments on root elongation of barley plants per soil, and the Tukey honestly significant difference test was used for a posteriori comparisons [40]. Stepwise multiple regression analysis [39] was used to determine significant relationships among root elongation data and soil/pore-water properties of experimental substrates and mixtures. Soil/pore-water properties (with the exception of pH) and root elongation data were logarithmically transformed ( $x' = \ln(x + 1)$ ) to correct for nonnormality [40] before statistical analysis. All statistical analyses were performed using the SPSS<sup>®</sup> statistical package (Ver 11.0.1; SPSS).

## RESULTS AND DISCUSSION

#### General properties of experimental mixtures

Addition of OxT or SmD into selected substrates increased total Cu levels up to 15.7-fold and total Zn levels up to 2.2-fold (Fig. 1), because Zn concentration is one to two orders of magnitude lower than Cu in both mine wastes (Table 1). Total Cu and Zn levels of SmD-spiked substrates were approximately twofold higher than those in OxT-spiked substrates, ranging from 167 to 473 mg/kg for Cu and from 53 to 195 mg/kg for Zn (Fig. 1). These metal concentrations are not very high compared with the maximum additions to soils normally used in assays of metal toxicity to plants (e.g., [14,24]) but, as shown in a previous study [4], are high enough to produce phytotoxicity. Significant ( $p < 0.05$ ) positive correlations were found among total Cu and total Zn levels across experimental treatments ( $n = 15$ ) in all selected substrates ( $r_{\text{sand}} = 0.61$ ,  $r_{\text{V-R soil}} = 0.78$ , and  $r_{\text{VII-R soil}} = 0.76$ ) (Fig. 1).

The mean reduction in pH (water) of substrates spiked with either OxT or SmD was 0.4 unit (Fig. 2). However, acidification differed among substrates, being lower in the VII-R soil (0.11–1.16 pH units) than in the other two substrates (0.49–0.59 pH unit) (Fig. 2), probably because of the higher buffering capability of this substrate (see general substrate properties in Table 2) and the rather low addition of mine wastes used in the present study. Acidification resulting from addition of soluble metal salts to soils is well documented in laboratory toxicity testing, particularly in soils that are low in clay and organic matter [4,13–15,41]. For example, Rooney et al. [24] found that soils spiked with CuCl<sub>2</sub> to a much higher total Cu concentration than that used in the present study (up to 1,600 vs 167–473 mg/kg) also decreased soil pH up to 1.28 units, stressing the high acidification capability of both Cu-rich mine wastes. Even though evidence shows that acidification in pore-water pH normally is much pronounced than soil pH values determined in water extracts (see, e.g., [14]), we did not find this trend. The same mean reduction value of 0.4 unit was found in pore-water pH of the sand substrate spiked with either OxT or SmD, and no acidification was detected in pore

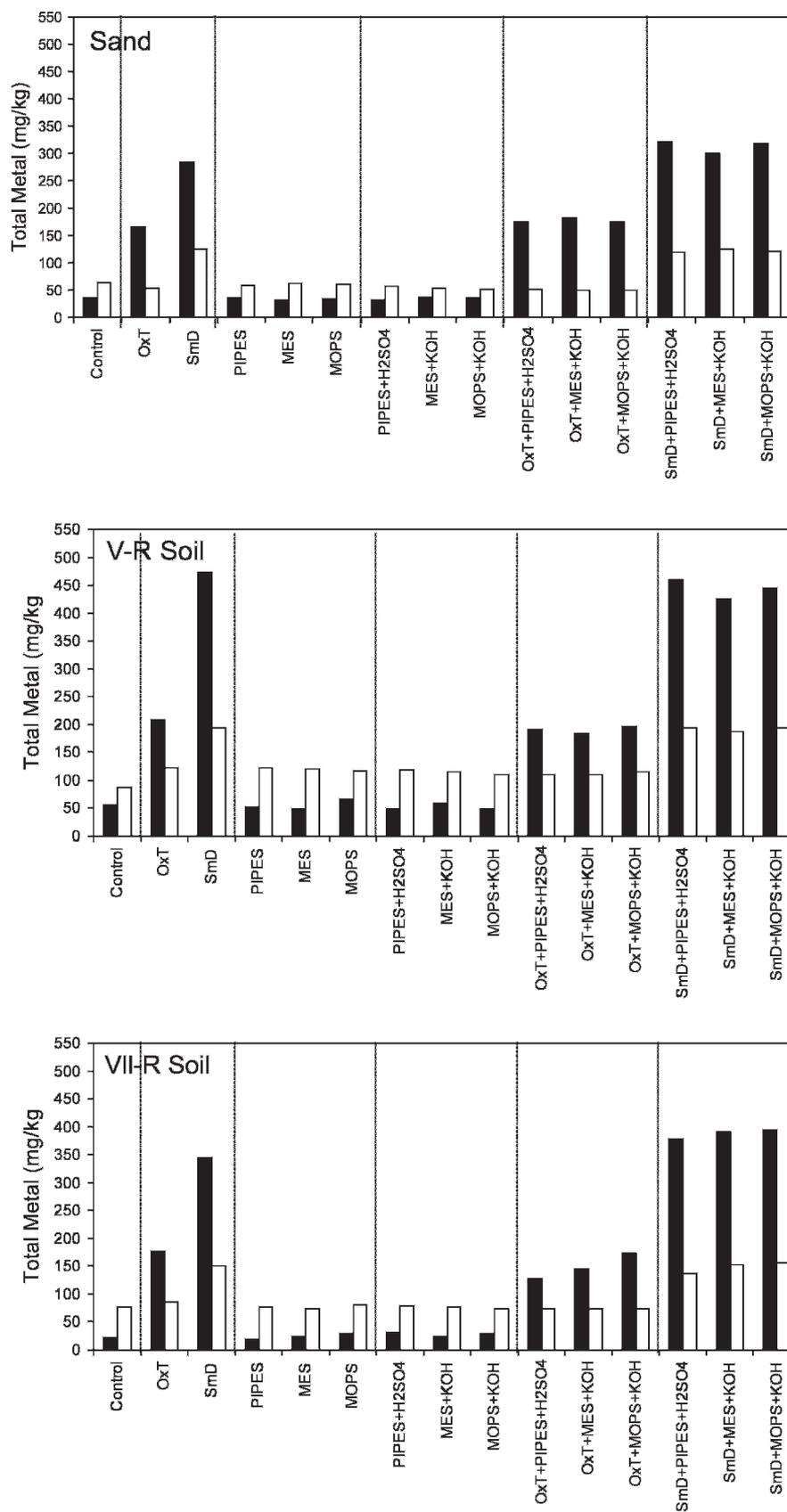


Fig. 1. Total Cu (■) and total Zn (□) of control substrates (unspiked) and all experimental treatments according to substrate type. For definitions of abbreviations, see Table 4.

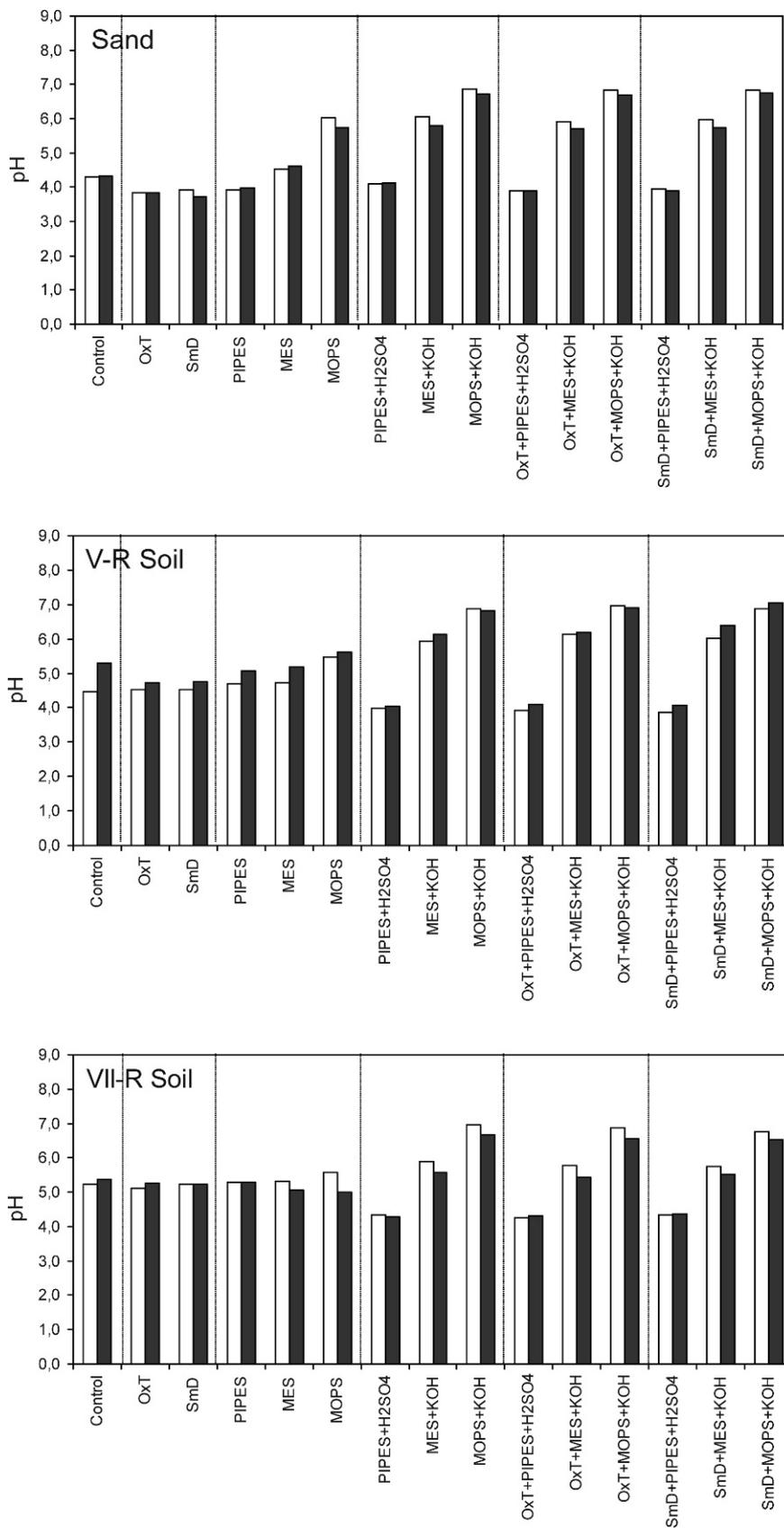


Fig. 2. Soil pH (water; ■) and pore-water pH (□) of control substrates (unspiked) and all experimental treatments according to substrate type. For definitions of abbreviations, see Table 4.

water of the other two substrates when spiked with both mine wastes (Fig. 2). However, significant ( $p < 0.05$ ) positive correlations were found among pore-water pH and soil pH (water) across experimental treatments ( $n = 15$ ) in all selected substrates ( $r_{\text{sand}} = 1.0$ ,  $r_{\text{V-R soil}} = 0.98$ , and  $r_{\text{VII-R soil}} = 0.96$ ) (Fig. 2).

Good evidence indicates that metal salt-spiked soils that are not leached have larger soil solution cationic strength and lower pH than nonspiked soils [11–14] and that both changes can affect metal partitioning [15]. Even though all experimental substrates were spiked with a fixed amount of either SmD (0.16 g/100 g substrate) or OxT (5.59 g/100 g substrate), total dissolved Cu and Zn measured in pore-water samples of the same treatment varied among substrates (Fig. 3). For example, sand showed 45- and 18-fold more Cu solubility than the V-R soil for the OxT and SmD treatments, respectively, and the V-R soil showed 8- and 15-fold more Cu solubility than the VII-R soil for the OxT and SmD treatments, respectively. However, Zn solubility was high and rather similar in sand and V-R soil but decreased 17- and 280-fold in the VII-R soil for the OxT and SmD treatments, respectively (Fig. 3). Even so, significant ( $p < 0.05$ ) positive correlations were found among total dissolved Cu and total dissolved Zn in pore water across experimental treatments ( $n = 15$ ) in all selected substrates ( $r_{\text{sand}} = 0.84$ ,  $r_{\text{V-R soil}} = 0.92$ , and  $r_{\text{VII-R soil}} = 0.78$ ) (Fig. 3). These differences follow variations in pore-water pH (Fig. 2), particularly in sand and the V-R soil, and significant ( $p < 0.05$ ) negative correlations were found among total dissolved Cu and pore-water pH across experimental treatments ( $n = 15$ ;  $r_{\text{sand}} = -0.53$  and  $r_{\text{V-R soil}} = -0.54$ ). No significant ( $p > 0.05$ ) correlation, however, was found among total dissolved Cu in pore water and pore-water pH in the VII-R soil ( $r_{\text{VII-R soil}} = -0.08$ ). Low total dissolved Cu and Zn concentrations of the VII-R soil may be the result of high adsorption of metal cations by the organic C of this substrate [3,21,42,43] (Table 2). Good evidence also exists for the influence of clay content, CEC, and organic C on sorption and availability of metals in soils [3,17,44].

Both EC and total dissolved Ca in pore water of all substrates increased when spiked with mine wastes (Fig. 4), but the intensity of variation varied according to substrate type. It was high for sand (up to 12- and 222-fold for EC and total Ca, respectively), intermediate for the V-R soil (up to 2.2- and 3.4-fold for EC and total Ca, respectively), and low for the VII-R soil (up to 1.7- and 2.4-fold for EC and total Ca, respectively), irrespective of the mine waste used. Mean Ca values, however, were greatly elevated in the case of the VII-R soil (605 mg/L) and the V-R soil (505 mg/L) compared with sand (324 mg/L), irrespective of the mine waste spiked. These results are in agreement with the different buffering capabilities of experimental substrates, as seen from the general physicochemical properties given in Table 2.

#### *Effect of buffer addition with and without pH adjustment*

Addition of MES and MOPS buffer solutions (without pH adjustment) to sand increased pore-water pH by 0.2 and 1.7 units, respectively, whereas an increase of 1 and 0.3 unit occurred on the V-R and VII-R soils, respectively, when MOPS buffer solution was added (Fig. 2). The pH adjustments that followed addition of buffer solution were very effective in changing pore-water pH to the desired values of 4.0 (PIPES), 6.0 (MES), and 7.0 (MOPS), irrespective of the substrate type (Fig. 2). However, increases in pore-water EC values occurred in all substrates for the treatments PIPES plus

H<sub>2</sub>SO<sub>4</sub> (range, 1.8–4.1), MES plus KOH (range, 1.6–5.9), and MOPS plus KOH (range, 2.4–20), particularly for the MOPS plus KOH treatment in sand, in which a 20-fold increase in EC was detected (Fig. 4). This likely is related to effects of pH adjustments on soil solution ionic strength.

Higher solubilization of Cu into pore water occurred after pH adjustment in all selected substrates (range, 1.2- to 9.7-fold) (Fig. 3) with no mine wastes added, but particularly under the MOPS plus KOH treatment in sand (9.7-fold increase) and V-R soil (3.0-fold increase). However, total dissolved Cu concentrations in pore water of all these treatments were less than 0.25 mg/L (Fig. 3).

#### *pH effect on metal solubility of experimental substrates*

Addition of buffer solutions to experimental mixtures of substrates and mine wastes, followed by pH adjustments with KOH or H<sub>2</sub>SO<sub>4</sub>, had an impact on both substrate (water) and pore-water pH values. Both parameters followed the targeted pH levels (4.0 for PIPES, 6.0 for MES, and 7.0 for MOPS), irrespective of the substrate and the mine waste (Fig. 2), and were significantly and positively correlated (Table 5). Therefore, the experimental design developed to modify the pH of substrates was very effective.

Copper and Zn concentrations in pore water were not correlated to total Cu and Zn concentrations of experimental mixtures, irrespective of the substrate and the mine waste (Table 5). However, increasing neutralization of experimental mixtures to pH 6.0 and 7.0 with MES and MOPS, respectively, dramatically decreased Cu and Zn levels in pore water (Fig. 3), particularly in the low-buffering-capacity substrates (sand and V-R soil). Acidification of experimental mixtures to pH 4.0 with PIPES buffer resulted in high Cu and Zn solubility in pore water (Fig. 3). Indeed, pore-water Cu and Zn both were negatively and significantly correlated with pore-water pH (Table 5). The exception to this pattern was found in the VII-R soil, in which no significant correlations were found among pore-water pH and pore-water Cu for both mine wastes (Table 5). Low total dissolved Cu concentrations found on the VII-R soil may be the result of high adsorption of this metal cation by the organic C contained in this soil (4.63%) (Table 2), because Cu is preferentially sorbed by soil organic matter [3,2,42,43].

Even though total Zn concentrations were one to two orders of magnitude lower than total Cu concentrations in experimental mixtures (Fig. 1), Zn solubility was much higher than Cu solubility (Fig. 3), particularly in the substrates with organic matter (V-R and VII-R soils). This result may be caused by differences in the mineral/chemical form of these metals in study mine wastes, but it seems clear that the Zn forms are more soluble than the Cu forms. Indeed, the solubility of metal from mine wastes has been related to chemical and mineralogical characteristics of the materials themselves [4,7–9].

Buffer addition to experimental mixtures of substrates and Cu-rich mine wastes increased the EC of pore-water samples, particularly with the MES and MOPS buffers (Fig. 4), irrespective of both the substrate and mine waste. However, no significant correlations were found among pore-water EC and either pore-water Cu, pore-water Zn, or pore-water Ca (Table 5). The exception was a significant ( $p < 0.01$ ) and positive relationship found among pore-water Ca and pore-water EC in sand for both mine wastes (Table 5). These results are in agreement with those of other studies that have shown

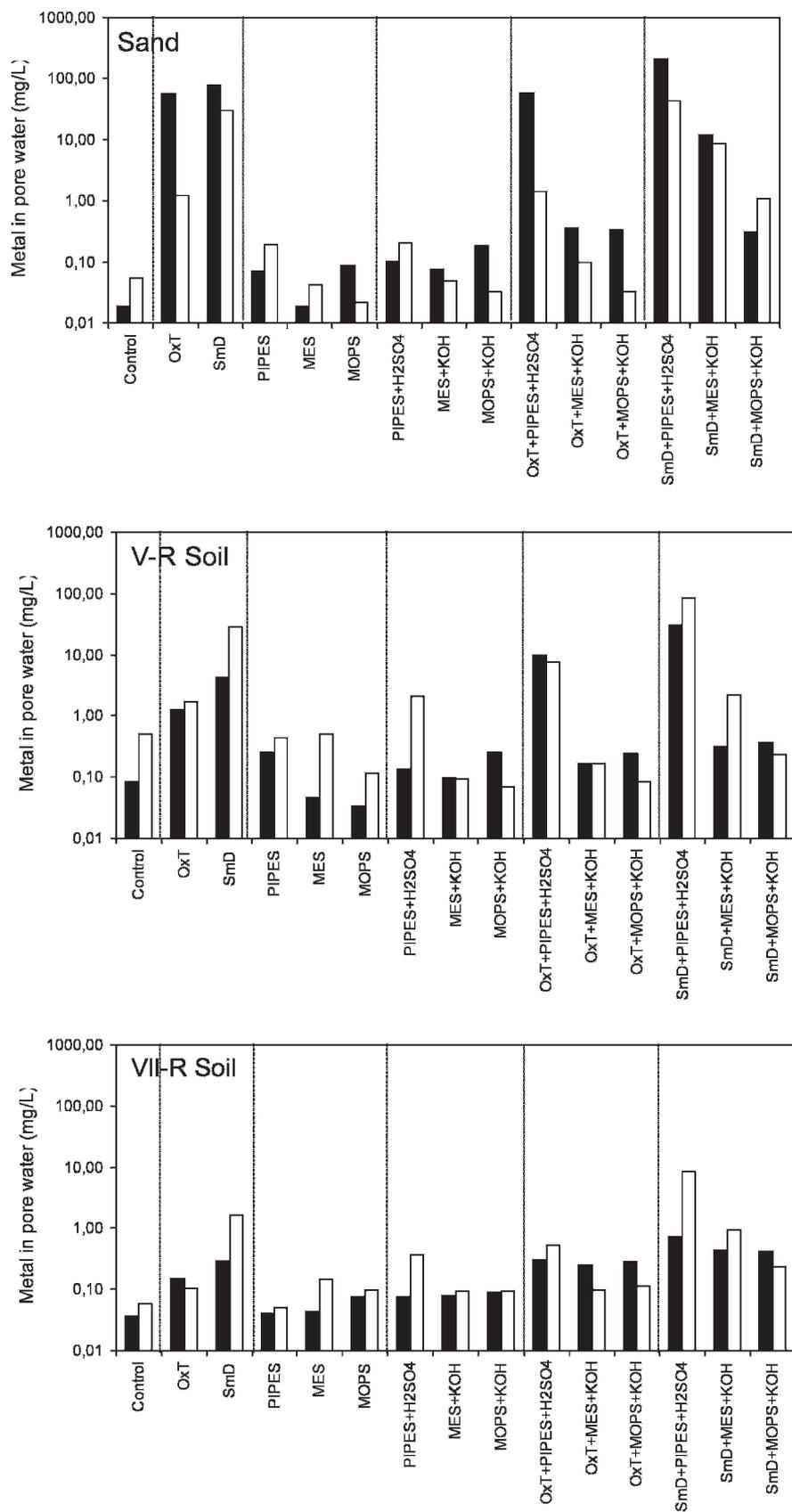


Fig. 3. Total dissolved Cu (■) and Zn (□) in pore-water samples of control substrates (unspiked) and all experimental treatments according to substrate type. For definitions of abbreviations, see Table 4.

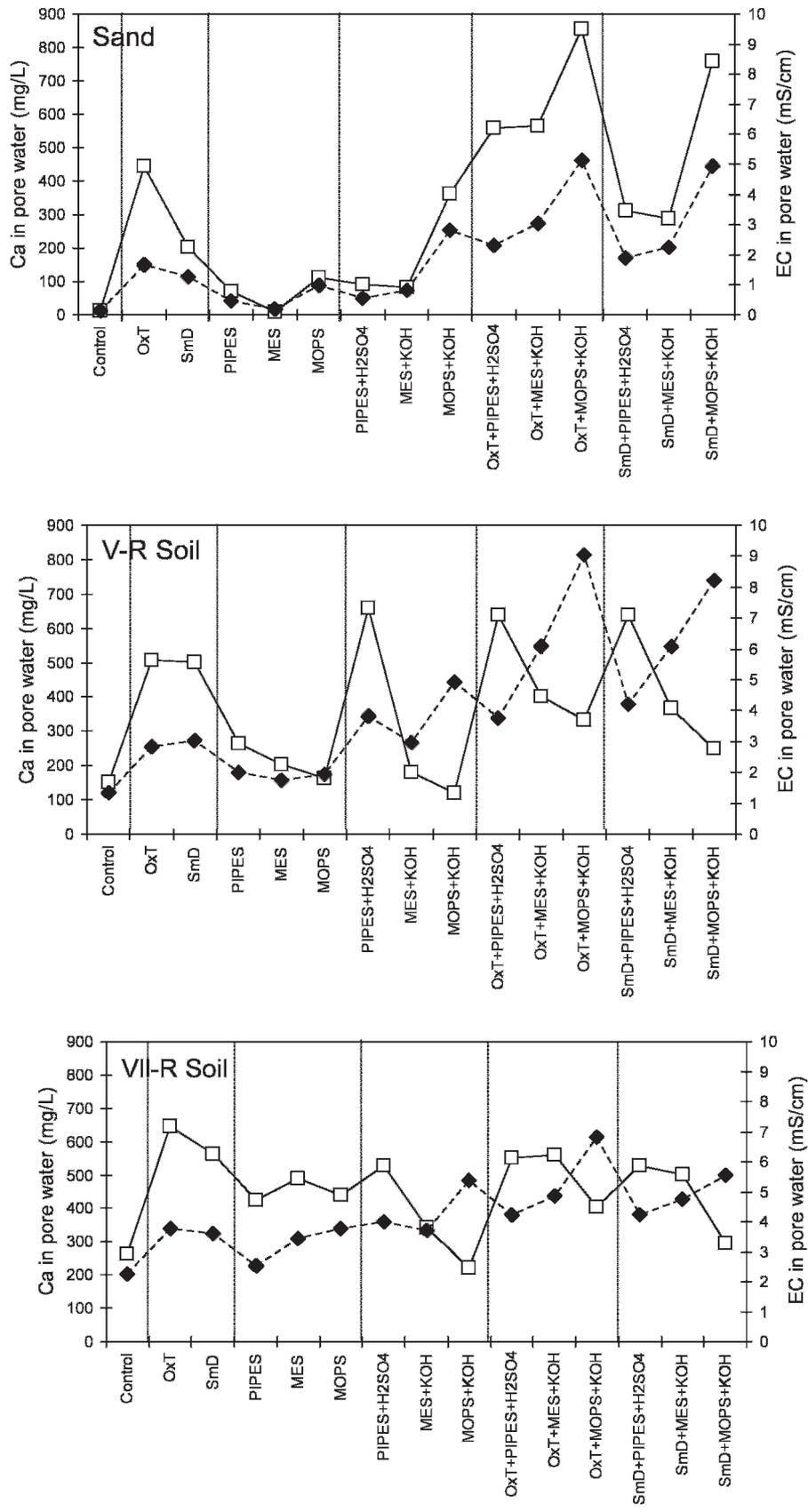


Fig. 4. Electric conductivity (EC;  $\blacklozenge$ ) and total dissolved Ca ( $\square$ ) in pore-water samples of control substrates (unspiked) and all experimental treatments according to substrate type. For definitions of abbreviations, see Table 4.

Table 5. Coefficients of determination ( $r^2$ ) and estimated slope ( $\beta$ ) from simple regression models for chemical parameters of experimental mixtures of substrates and acidic, Cu-rich mine wastes with and without adjustment to pH 4.0, 6.0, and 7.0<sup>a</sup>

Correlations	Sand		V-R soil		VII-R soil	
	$r^2$	$\beta$	$r^2$	$\beta$	$r^2$	$\beta$
<b>Oxidized tailings</b>						
pH <sub>pore water</sub> – pH <sub>substrate</sub>	0.998*	0.999*	0.936*	0.967*	0.956*	0.978*
Cu <sub>pore water</sub> – pH <sub>pore water</sub>	0.542*	-0.736*	0.351*	-0.592*	0.017	0.131
Cu <sub>pore water</sub> – EC <sub>pore water</sub>	0.052	-0.228	0.036	-0.190	0.622*	0.789*
Cu <sub>pore water</sub> – Cu <sub>total</sub>	0.129	0.359	0.076	0.275	0.489*	0.699*
Zn <sub>pore water</sub> – pH <sub>pore water</sub>	0.543*	-0.737*	0.441*	-0.664*	0.422*	-0.650*
Zn <sub>pore water</sub> – EC <sub>pore water</sub>	0.052	-0.227	0.036	-0.274	0.001	0.032
Zn <sub>pore water</sub> – Zn <sub>total</sub>	0.091	-0.301	0.011	0.104	0.077	-0.277
Ca <sub>pore water</sub> – EC <sub>pore water</sub>	0.920*	0.959*	0.003	0.053	0.040	0.199
<b>Smelter dust</b>						
pH <sub>pore water</sub> – pH <sub>substrate</sub>	0.995*	0.997*	0.954*	0.977*	0.978*	0.989*
Cu <sub>pore water</sub> – pH <sub>pore water</sub>	0.369*	-0.608*	0.390*	-0.624*	0.066	-0.256
Cu <sub>pore water</sub> – EC <sub>pore water</sub>	0.028	-0.167	0.013	-0.115	0.423*	0.650
Cu <sub>pore water</sub> – Cu <sub>total</sub>	0.162	0.402	0.114	0.337	0.637	0.798
Zn <sub>pore water</sub> – pH <sub>pore water</sub>	0.434*	-0.658	0.474*	-0.688*	0.561*	-0.749*
Zn <sub>pore water</sub> – EC <sub>pore water</sub>	0.048	-0.219	0.035	-0.187	0.006	0.075
Zn <sub>pore water</sub> – Zn <sub>total</sub>	0.208	0.456	0.135	0.367	0.021	0.146
Ca <sub>pore water</sub> – EC <sub>pore water</sub>	0.986*	0.993*	0.001	-0.018	0.022	0.149

<sup>a</sup> EC = electric conductivity;  $n = 5$  per substrate and mine waste, \* $p < 0.01$ .

EC is one of several controlling factors, but not the most important one, for metal solubility in soils [16,45].

#### Plant toxicity assays

Barley seedlings grown in control substrates were healthy when harvested and displayed no visual signs of stress; no significant differences were found among substrates in root lengths of plants ( $F = 4.25$ ,  $p = 0.62$ ,  $n = 18$ ). In general, addition of buffer solutions (PIPES, MES, and MOPS) to substrates (not spiked) did not affect root growth of barley plants (Fig. 5), but pH adjustment (PIPES plus H<sub>2</sub>SO<sub>4</sub>, MES plus KOH, or MOPS plus KOH) resulted in significant changes of root elongation when compared to control in a substrate-dependent way (Fig. 5). Specifically, a significant increase of 1.1-fold in root length was found under the treatments PIPES plus H<sub>2</sub>SO<sub>4</sub> and MES plus KOH for sand, whereas significant reductions in root length occurred in the treatments MOPS plus KOH and PIPES plus H<sub>2</sub>SO<sub>4</sub> for the V-R soil (1.7-fold) and VII-R soil (1.2-fold), respectively (Fig. 5). From these results, it is possible to conclude that both buffer solutions and the range of pH values used in the present study (pH 4.0–7.0) are, in general, nontoxic to barley seedlings.

Almost all seedlings from experimental treatments with OxT and SmD appeared stunted on visual examination. Specifically, addition of OxT ( $F = 626$ ,  $p < 0.01$ ,  $n = 18$ ) and SmD ( $F = 368$ ,  $p < 0.01$ ,  $n = 18$ ) significantly reduced root elongation of barley plants, but the intensity of root growth inhibition was substrate-dependent, being stronger in sand and V-R soil than in VII-R soil (Fig. 5), although all substrates were spiked with the same amount of mine waste. This result may be explained by differences in pore-water Cu, Zn, and Ca levels among substrates (Figs. 3 and 4). Copper and Zn concentrations in pore water of VII-R soil were lower than those in the other substrates because of that soil's high dissolved organic C (183 mg/L) and hardness (963 mg/L as CaCO<sub>3</sub>), as shown in Table 3, but also because of its higher values of total dissolved Ca (Fig. 4).

The increase in soil solution pH of experimental mixtures of substrates and acidic, Cu-rich mine wastes from 4.0 to 6.0 and 7.0 resulted in significant improvements in root elongation of barley plants, irrespective of the mine waste (Fig. 4). However, the intensity of the response was substrate- and pore water pH-dependent. When soil solution pH of experimental mixtures was increased from 4.0 to 6.0, roots of barley plants increased approximately eight-, five-, and onefold in length for V-R soil, sand, and VII-R soil, respectively, whereas roots increased zero-, one-, and twofold in length for V-R soil, VII-R soil, and sand, respectively, when pH increased from 6.0 to 7.0 (Fig. 4). These results are in agreement with the fact that the pH threshold for yield reduction for *H. vulgare* because of Al toxicity is at a value of approximately 5.5 (pH in water extract) [32,33]. On the other hand, they also followed changes in Cu/Zn solubility measured in pore water of experimental mixtures (Fig. 3); for example, pore-water Cu decreased by one to two orders of magnitude when pH increased from 4.0 to 6.0, but no further reduction resulted when soil solution pH was increased to 7.0. A synergistic effect is suggested for Cu/Zn and Al phytotoxicity below pH 6.0, but this needs to be further studied.

Significant relationships among root elongation data and some pore-water chemical properties of experimental treatments (control, SmD/SmD, OxT/SmD plus PIPES plus H<sub>2</sub>SO<sub>4</sub>, OxT/SmD plus MES plus KOH, and OxT/SmD plus MOPS plus KOH) were found after stepwise multiple regression analysis performed per mine waste (Table 6). Total dissolved Cu in pore water predicted 79% of changes detected in root length of barley plants grown on substrates spiked with OxT, whereas total dissolved Cu and Zn, along with pore-water pH, explained 94% of changes in root length on substrates spiked with SmD (Table 6). Differences in the main chemical properties explaining changes in root length among OxT and SmD treatments may be caused by the higher Zn content of SmD than OxT and other differences in the chemical/mineralogical composition of these mine wastes (Table 1). This result stresses the importance of changes in pore-water pH on metal solubilization and, thus, on phytotoxicity.

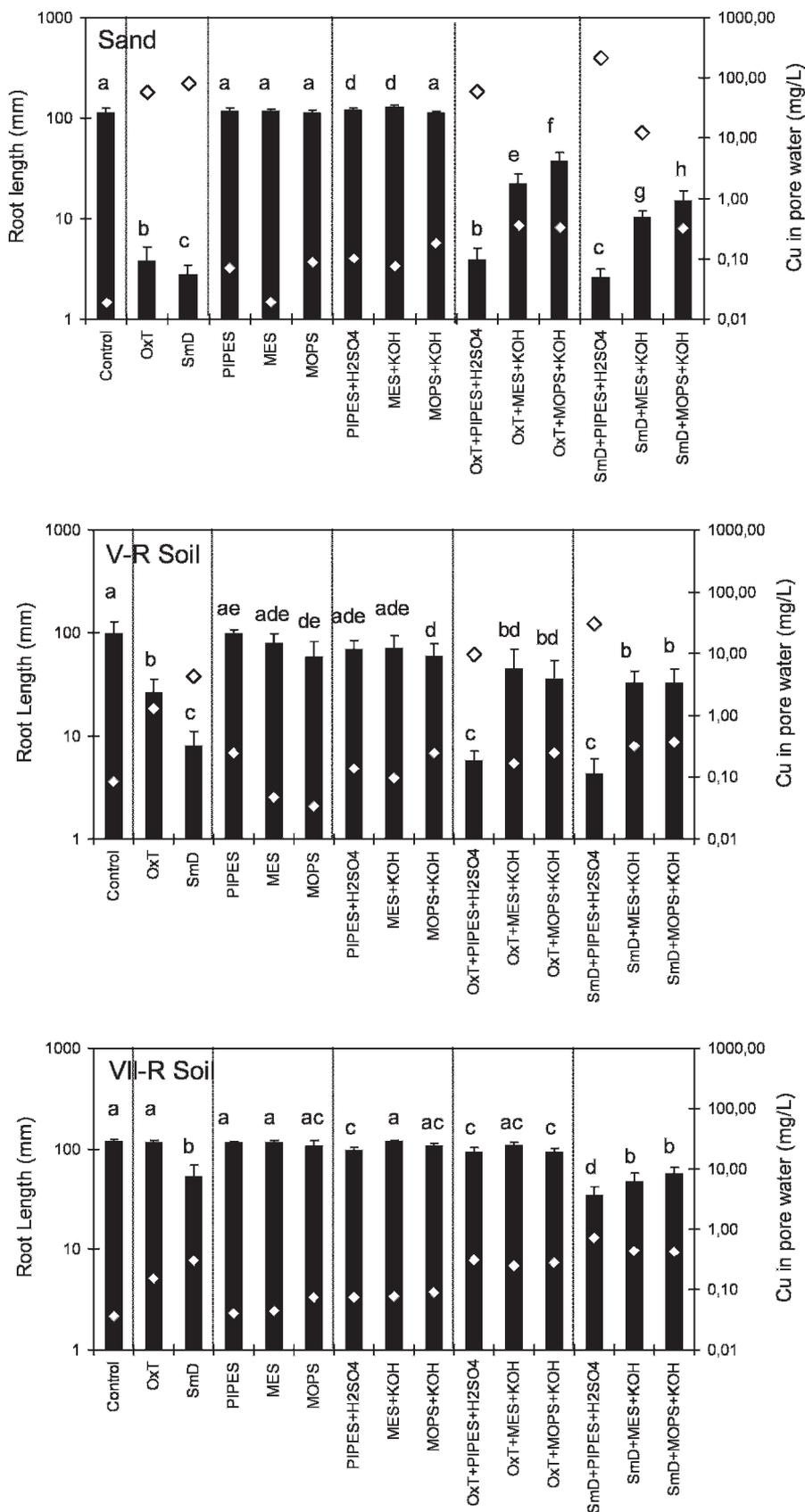


Fig. 5. Root length (■) of barley (*Hordeum vulgare* L.) plants grown on control substrates (unspiked) and all experimental treatments according to substrate type. Total dissolved Cu (◇) is given for comparison. Means and standard deviations are given for root length data ( $n = 18$ ). Different lowercase letters indicate significant differences ( $p < 0.01$ ) according to one-way analysis of variance per substrate ( $F_{\text{sand}} = 1,208, df = 14$ ;  $F_{\text{V-R soil}} = 94, df = 14$ ;  $F_{\text{VII-R soil}} = 135, df = 14$ ). For definitions of abbreviations, see Table 4.

Table 6. Relationship between (ln-transformed) root length (RL) of barley plants and chemical parameters of soil pore water (pw) of experimental mixtures of substrates and acidic, Cu-rich mine wastes with and without adjustment to pH 4.0, 6.0, and 7.0<sup>a</sup>

	Regression equation	$r_{adj}^2$	F	SE	p
OxT	ln RL = 4.3 - 0.7 ln [Cu] <sub>pw</sub>	0.79	53.4	0.29	<0.01
SmD	ln RL = 6.8 - 0.3 ln [Cu] <sub>pw</sub> - 0.6 ln [Zn] <sub>pw</sub> - 0.4pH <sub>pw</sub>	0.94	75.3	0.09	<0.01

<sup>a</sup> Best-fit multiple regression models are shown ( $n = 5$  per substrate and mine waste). OxT = oxidized tailings; SE = standard error of the estimate; SmD = smelter dust;  $r_{adj}^2$  = adjusted  $r^2$ .

It is interesting to note that even though neutralization of experimental mixtures of substrates with mine wastes allowed significantly longer roots in barley plants compared with acidic mixtures, these roots were still significantly smaller than those in control substrates, substrates with buffer solutions, and substrates with pH adjustment (Fig. 5), irrespective of the substrate and the mine waste. This finding indicates that increasing soil solution pH above 5.5 results in improved plant performance because of elimination of pH-dependent Cu/Zn solubility (and, possibly, elimination of Al toxicity to barley plants, a parameter not measured in the present study). In other words, substrate acidification resulting from addition of acidic, metal-rich mine wastes poses stronger metal toxicity effects to plants than nonacidic mine wastes pose. However, we were not able to assess the relative importance of direct (Al toxicity) and indirect (greater Cu/Zn solubility) effects of pH on barley plants, because we did not measure Al concentration in soil pore-water samples. Furthermore, we did not evaluate changes in other cations (e.g., H<sup>+</sup> and Ca<sup>2+</sup>), which may pose protective effects on the biological receptors [14,22–24], and other ligands, such as dissolved organic C and organic matter, which adsorb metal ions in a pH-dependent form [46].

We also may have overestimated metal toxicity on mine waste-spiked substrates (SmD/SmD, OxT/SmD plus PIPES plus H<sub>2</sub>SO<sub>4</sub>, OxT/SmD plus MES plus KOH, and OxT/SmD plus MOPS plus KOH), because fresh additions of these materials with soluble metal salts could cause direct toxic effects of salinity on plant growth [47,48]. This effect likely occurred in the barley tests, because pore-water EC values were all increased, particularly in the sand substrate (range, 9–37 mS/cm in sand, 2–7 mS/cm in the V-R soil, and 2–3 mS/cm in the VII-R soil) (Fig. 4). Metal toxicity and the potential salinity effect generally decrease in field-aged soils because of the leaching of excess solutes in the soil solution and the shift of metals from more-soluble to less-soluble phases, an effect called aging [46,47]. It has been reported in the literature that soil acidification and salinization resulting from addition of highly soluble metal salts under laboratory conditions is an artifact, because leaching through rainfall will continually remove the excess salts from the topsoil over the period of metal accumulation under field conditions [11–14,41]. Therefore, it has been recommended that soils should be leached after contamination to achieve a more realistic exposure situation for assessing metal toxicity to plants, as would be found under field conditions [11–14,41]. The same suggestions should be followed in assessing effects of acidic metal-mine wastes on plants and soil organisms, which will require some modifications to the standard laboratory ecotoxicity tests.

Irrespective of the criticisms regarding the experimental methods used in the present study, our results support the fact that the confounding effect of acidification on Cu solubility in

soils spiked with acidic, Cu-rich mine wastes is very relevant for determining metal bioavailability and toxicity to plants and soil organisms, particularly in soils with low organic C and clay contents [16,44,45].

## CONCLUSION

The experimental design developed in the present study to test the effect of pH on metal solubility and phytotoxicity of substrates spiked with acidic, Cu-rich mine wastes was very effective. Acidification of soils resulting from the addition of acidic mine wastes has an effect on metal solubility (i.e., Cu and Zn). However, soil organic matter seems to have a higher influence on Cu solubility, but not on Zn solubility, compared with that of pore-water pH, because Cu is preferentially sorbed by organic matter.

Barley seedlings grown in acidic soils or soils with low pH had higher metal toxicity effects than those grown in slightly acidic and neutral soils, particularly soils with low buffering capability (i.e., low organic matter and clay contents). That this effect occurred at low rates of addition of mine wastes (0.16 g SmD/100 g substrate or 5.59 g OxT/100 g substrate) stresses the importance of considering these confounding effects on derivation of toxicity thresholds to plants, particularly under standard laboratory phytotoxicity tests.

The present results suggest that mitigation of metal toxicity in barley plants by soil neutralization was an indirect effect (i.e., the reduction of acidity reducing Cu/Zn solubility and bioavailability through increasing partitioning coefficient [ $K_d$ ] values), but it also could be a direct effect (i.e., the reduction of Al toxicity because of decreasing soil acidity). Therefore, soil acidification on addition of acidic mine wastes is sufficient to significantly affect soil biochemical processes and, therefore, complicates the derivation of toxicity thresholds for metals. However, this is dependent on the soil type and the mine waste used.

It is recommended that soils be leached after contamination with acidic, metal-rich mine wastes to achieve a more realistic exposure situation for assessing metal toxicity to plants, as is regularly done when assessing metal phytotoxicity with highly soluble metal salts.

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