AGRICULTURAL SOILS SPIKED WITH COPPER MINE WASTES AND COPPER CONCENTRATE: IMPLICATIONS FOR COPPER BIOAVAILABILITY AND BIOACCUMULATION

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Abstract—A better understanding of exposure to and effects of copper-rich pollutants in soils is required for accurate environmental risk assessment of copper. A greenhouse experiment was conducted to study copper bioavailability and bioaccumulation in agricultural soils spiked with different types of copper-rich mine solid wastes (copper ore, tailing sand, smelter dust, and smelter slag) and copper concentrate. A copper salt (copper sulfate, $CuSO_4$) that frequently is used to assess soil copper bioavailability and phytotoxicity also was included for comparison. Results showed that smelter dust, tailing sand, and $CuSO_4$ are more likely to be bioavailable and, thus, toxic to plants compared with smelter slag, concentrate, and ore at equivalent total copper concentrations. Differences may be explained by intrinsic differences in copper solubilization from the source materials, but also by their capability to decrease soil pH (confounding effect). The copper toxicity and bioaccumulation in plants also varied according to soil physicochemical characteristics (e.g., pH and total organic carbon) and the available levels of plant nutrients, such as nitrogen, phosphorus, and potassium. Chemistry/mineralogy of mine materials, soil/pore-water chemistry, and plant physiological status thus should be integrated for building adequate models to predict phytotoxicity and environmental risk of copper.

Keywords—Metal bioavailability   Metal solubility   Mine pollution   Risk assessment   Copper toxicity

INTRODUCTION

Copper is a natural element normally found at low concentrations (50–70 mg/kg) in soils [1]. Although it can reach very high levels in specific sites, such as outcrops and naturally mineralized soils (up to 10,000 mg/kg), background soil copper levels of the world normally are in the range of 0.3 to 250 mg/kg (see, e.g., [1,2]). Several anthropogenic activities have caused important increases in soil copper levels in urban areas as well as in wild and agricultural lands. However, mining and smelting have been the most important anthropogenic sources of soil copper through atmospheric and particulate emissions as well as liquid and solid wastes [3–6].

High copper levels in soils may pose risks to humans and ecosystems [1]. For instance, exposure of crops and vegetables to elevated copper levels in soils may result in direct plant toxicity, whereas chronic exposure to moderate copper levels may impact reproduction rates and vegetative growth (e.g., biomass of aerial and underground structures) [1,7–9].

Regulatory agencies in Canada, Europe, and the United States as well as metal industry associations and scientists worldwide are assessing the environmental hazards of metals in soils and are developing methods to establish protective soil guidelines and quality criteria. Although some progress has been made in understanding the factors that determine exposure and effects of copper in terrestrial environments, major gaps in knowledge remain.

Most studies of copper in soils have evaluated the effects of synthetic copper salts (e.g., $CuSO_4$) through standardized test methods (see, e.g., [1,9,10]). Rarely have actual copper-based materials, such as wood-preservation chemicals, agrochemicals, antifouling paint, and mine wastes, been used as sources of copper in controlled assays, although they constitute important sources of soil copper pollution in areas with a history of human disturbance, such as mining and smelting (see, e.g., [4,6,11,12]). The extent to which complex, copper-based compounds can trigger toxic effects cannot be extrapolated from results obtained using simple copper salts, because copper bioavailability may vary greatly in the former while being at maximum in the latter [11–14]. Copper bioavailability in soil (and, thus, toxicity) varies significantly with the chemical and mineralogical properties of the copper compound and its speciation in the soil [1,13].

Most research studies involving metal-rich mine wastes have focused mainly on soil profile distribution, mineralogical properties, chemical partitioning, and leaching (see, e.g., [15]). The aim of the present study was to evaluate the extent of soil copper solubility and bioavailability, as well as its bioaccumulation and toxicity in plants, when added to soil under the form of various copper-mining materials.

MATERIALS AND METHODS

Copper-rich mine materials

Different types of copper-rich mine wastes and copper concentrate were selected based on their potential as soil-polluting sources near mining and smelting areas. A bulk sample of all materials was obtained from a large-scale porphyry copper

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Table 1. General physicochemical characteristics of copper-rich mine materials[a]

Cu-rich material	pH (1:1 water)	CEC (meq/100 g)	Sulfate sat. ext. (mg/L)	Total Cu (mg/kg)	Total Zn (mg/kg)	Total Fe (mg/kg)	Total Pb (mg/kg)	Particle size <2 μm (%)	Particle size 2–50 μm (%)	Particle size 50–2,000 μm (%)
Cu ore	7.51	3.70	1,303	32,150	61	48,670	29	10	10	80
Concentrate	8.03	8.64	1,411	284,600	1,033	171,900	148	8	47	45
Tailing sand	3.70	14.23	7,736	3,996	107	38,370	51	43	57	0
Smelter dust	2.24	23.35	219,264	214,000	23,540	25,860	482	78	19	3
Smelter slag	7.35	2.60	75	9,044	2,950	302,700	159	4	49	47
$CuSO_4$	2.81									

[a] CEC = cation exchange capacity; sat. ext. = saturation extract.

mine plant and smelter located in central Chile. These materials were sulfide copper ore, copper concentrate, tailing sand from the upper oxidation zone (depth, 0–20 cm) of an abandoned tailing deposit, smelter dust, and smelter slag. Copper ore and smelter slag were crushed to obtain a material with particle sizes of less than 2 mm before being used in the plant-cultivation studies described below. General physicochemical characteristics of the study mine materials are shown in Table 1.

Agricultural soils

Three agricultural soils of central Chile were selected for the present study (Table 2) to represent a range of soil properties, such as pH and total organic carbon (TOC) content. As shown in Table 2, soils differed in terms of pH, TOC, inorganic carbon, cation-exchange capacity, and available macronutrients (nitrogen, phosphorus, and potassium). Total copper was low in all soils (Table 2) and represented background levels for central Chile. A large-scale soil sample (~40 kg) from the upper horizon (depth, 0–20 cm) was collected at each site, stored in polyethylene plastic bags, and carried to the laboratory. Collected soils were air dried, homogenized, and passed through a 2-mm nylon mesh before use.

Plant-cultivation studies

Study soils were spiked with increasing amounts of selected copper-rich materials to prepare mixtures with a range of total copper concentrations (Table 3). Because copper salts often have been used in standard soil toxicity assays, $CuSO_4 \times 5H_2O$ Suprapur (Merck, Darmstadt, Germany) was included in the present study as an additional source of copper. To avoid soil dilution with increasing addition of copper-rich materials, soils were first mixed with up to 20% of acid-washed sand, which was then replaced with the required amount of copper source material when the experimental mixtures were prepared.

Two 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 deionized water, homogenized for 7 d in an automatic roller, air-dried for 7 d, rehydrated with deionized water, and mixed again for 7 d to allow mixture equilibration. The same procedure was followed with batches of unspiked soils (controls). Approximately 500 g of each experimental mixture and control soils were used to determine total copper as described below. The remaining mixture was distributed into four 320-ml, acid-washed, polyethylene plastic pots with drainage at their base. Method 1638 of the U.S. Environmental Protection Agency (U.S. EPA; 1996; http://www.epa.gov/cgi-bin/claritgw?op-display&document=clserv:OW:0569;&rank=4&template=epa) was used for the acid-washing of all polyethylene containers (24 h with 0.5% Extran MA O_2 neutral [Merck], 24 h with 1 N HNO_3, 24 h with 1 N HCl, and four washings with high-purity [>18 MΩ/cm] deionized water).

Lettuce seedlings (*Lactuca sativa* var. *capitata*; 31 d since germination) were used for the plant studies. Lettuce seeds (Vilmorin, La Verpillière, France) were germinated, and seedlings were grown on aerated hydroponics culture with one-fifth strength (micronutrients) Hoagland nutrient solution (0.5 mmol of KNO_3, 0.5 mmol of $Ca(NO_3)_2 \times 4H_2O$, 0.2 mmol of $MgSO_4 \times 7 H_2O$, 0.1 mmol of KH_2PO_4, 10 μmol of H_3BO_3, 2 μmol of $MnCl_2 \times 4 H_2O$, 0.2 μmol of $CuSO_4 \times 5 H_2O$, 0.2 μmol of $ZnSO_4 \times 7H_2O$, 0.1 μmol of MoO_3, and 10 μmol of iron-ethylenediaminedi(*o*-hydroxyphenylacetic) acid) under controlled conditions (23 ± 2°C; light intensity, 47 μmol/s/m²; 12-h light:dark photoperiod). One seedling was transplanted to each experimental pot (four replicates/treatment) and irrigated to field capacity with deionized water (no leaching). Experimental pots were kept in a plant growth room under controlled conditions (23 ± 2°C; light intensity, 47 μmol/s/m²; 12-h light:dark photoperiod) and randomly relocated once a week. The soil moisture of every pot was checked daily, and deionized water was added when necessary (no leaching) to

Table 2. General physicochemical properties of study agricultural soils of Chile[a]

Soil	Site (country location)	pH (water)	TOC (%)	IC (%)	CEC (meq/100 mg)	Texture Clay (%)	Texture Sand (%)	Texture Silt (%)	Available macronutrients N (mg/kg)	Available macronutrients P (mg/kg)	Available macronutrients K (mg/kg)	Total Cu (mg/kg)
IV-R	Quilimari Valley (32°06′34″S, 71°26′32″W)	7.7	0.8	0.1	14.8	18.5	66.3	15.2	62	81	86	52.2
V-R	Puchuncavi Valley (32°47′26″S, 71°31′20″W)	5.5	0.9	0.1	17.5	13.0	70.7	16.3	19	29	267	171.5
VII-R	Itahue-Camarico (35°08′33″S, 71°20′45″W)	6.4	3.6	1.0	22.8	14.0	61.4	24.7	287	42	677	21.3

[a] CEC = cation exchange capacity; IC = inorganic carbon; TOC = total organic carbon.

Table 3. Copper fractions and pH (water) of study mixtures of agricultural soils and copper-rich materials as well as regression models relating copper in pore water (log-transformed) with total copper (log-transformed)[a]

Soil and Cu-rich material (n)	Total Cu range (mg/kg)	Cu pore-water range (μg/L)	pH (water) range	$Log[Cu]_{pore} = A + B \cdot log[Cu]_{total}$		
				r^2	β	SE
IV-R						
Smelter dust (28)	41–5,054	33–74,053	5.2–7.7	0.86*	1.39 a	0.39
Tailing sand (36)	41–2,196	33–944,635	4.2–7.7	0.76*	2.11 a	0.70
Smelter slag (32)	41–1,777	33–306	7.5–7.9	0.69*	0.41 b	0.15
Cu ore (32)	41–1,386	33–106	7.3–7.7	0.60*	0.25 b	0.09
Concentrate (32)	41–1,865	33–246	7.5–7.8	0.87*	0.47 b	0.10
CuSO$_4$ (32)	41–287	33–277	7.1–7.8	0.83*	1.09 a	0.11
V-R						
Smelter dust (28)	197–5,118	164–2,890,000	4.4–5.4	0.99*	2.93 a	0.15
Tailing sand (36)	197–1,911	164–594,571	4.1–5.4	0.97*	3.71 a	0.22
Smelter slag (24)	197–764	164–383	5.3–5.5	0.76*	0.43 b	0.05
Cu ore (24)	197–585	164–492	4.8–5.1	0.42*	0.09 c	0.04
Concentrate (24)	197–782	164–258	5.3–5.6	0.14 NS	0.11 c	0.06
CuSO$_4$ (40)	197–3,966	164–9,810,000	4.1–5.9	0.97*	3.59 a	0.36
VII-R						
Smelter dust (28)	30–4,114	17–11,498	4.9–6.3	0.94*	1.20 a	0.24
Tailing sand (36)	30–2,130	17–956,351	4.3–6.3	0.77*	1.99 a	0.69
Smelter slag (32)	30–1,984	17–79	5.7–6.3	0.69*	0.25 b	0.15
Cu ore (32)	30–1,071	17–52	5.6–6.3	0.41*	0.25 b	0.16
Concentrate (32)	30–1,757	17–98	5.6–6.3	0.83*	0.38 b	0.11
CuSO$_4$ (32)	30–324	17–199	5.4–6.3	0.89*	1.18 a	0.13

[a] n = replicate number; r^2 = coefficient of determination; β = slope; SE = standard error of estimate; *A* and *B* are constants; NS = not significant.
* $p < 0.01$; different lowercase letters indicate significant differences among materials within soil type.

maintain each substrate at a minimum of 80% field capacity throughout the experiment. On day 15 of the assay, aliquots of soil pore water (5–7 ml) were taken from three randomly selected pots per treatment with acid-washed Rhizon Soil Pore Water Samplers (length, 5 cm; Rhizosphere Research Products, Wageningen, The Netherlands) following the methodology described by Vulkan et al. [16]. Soil pore-water samples were kept in acid-washed polyethylene plastic vials (15 ml), and pH determinations were done up to 10 min after sample collection with a combination pH electrode (Sensorex 120C, Garden Grove, CA, USA). Pore-water samples were then acidified with HNO$_3$ Suprapur (Merck) and analyzed for total copper using an inductively coupled plasma–mass spectrometer (ICP-MS; ELAN 6100 with auto sampler AS90; Perkin-Elmer, Uberlinger, Germany) following U.S. EPA method SW-486 [17].

After 35 d of growth, aerial tissues (shoots) of lettuce plants were harvested. Shoots were washed with high-purity (>18 MΩ/cm) deionized water [18] to eliminate soil particles that were adsorbed on plant tissue surfaces. Shoots were then air-dried at 45°C to a constant weight and weighed. Copper content in shoots was determined as described below.

Chemical analysis

All mixtures and control soil samples (substrates) were dried in a forced air-drying cabinet at a temperature of approximately 30°C, and agglomerates were broken by hand using a wooden mallet. Substrates were then sieved through a 2-mm nylon mesh and subjected to microwave acid digestion (1200 Mega Microwave System; Milestone Microwave System, Monroe, CT, USA) and extraction (modified U.S. EPA method 3052 [19]). Digested samples were analyzed for total copper content by flame atomic absorption using an atomic absorption spectrometer (AAnalyst 300; Perkin-Elmer, Shelton, CT, USA) under U.S. EPA methods SW-486 [17] and 7210 [20]. Background nonatomic absorption was corrected with a

deuterium continuous lamp. The atomic absorption analytical device was housed in a class 1000 clean-room laboratory, and the loading of the autosampler tray was done in a class 100 laminar flow cabinet. The calibration standard was prepared with high-purity (>18 MΩ/cm) deionized water and acidified with HNO$_3$ Suprapur (Merck) to 0.2%. For performance control of the atomic absorption spectrometer, a certified multi-element standard was used (Spectrascan® Certified; Teknolab, Drobak, Norway). The quality-assurance and -control criteria were satisfied when the measured parameter of the standard reference material (Loam-B, catalog no. CRM-LO-B; High-Purity Standard, Charleston, SC, USA) and the quality-control sample (a previously characterized soil sample with a known concentration of metals) differed by no more than 10%.

Dried lettuce shoots were finely crushed in the Retsch (Newtown, PA, USA) S100 agate ball mill and digested after the same modified U.S. EPA protocol used for soils [19]. Every digestion batch included one blank sample, one standard reference material (SRM) sample (SRM 1573a tomato leaves or SRM 1570a spinach leaves; National Institute of Standards and Technology, Gaithersburg, MD, USA), one duplicate sample, and one quality-control sample for the quality-assurance and -control criteria. Copper was analyzed by ICP-MS (ELAN 6100 with auto sampler AS9; Perkin-Elmer, Uberlinger, Germany).

Data analysis

Simple linear regressions [21] were used to evaluate statistical relationships between chemical characteristics of soil mixtures and plant toxicity endpoints (e.g., shoot biomass and copper content in shoot). Analysis of covariance was used for slope comparisons within study soils or copper-rich materials, whereas the multiple-comparison procedure was used for a posteriori tests [22]. One-way analysis of variance was used to test for copper effects on shoot biomass, and the Tukey test
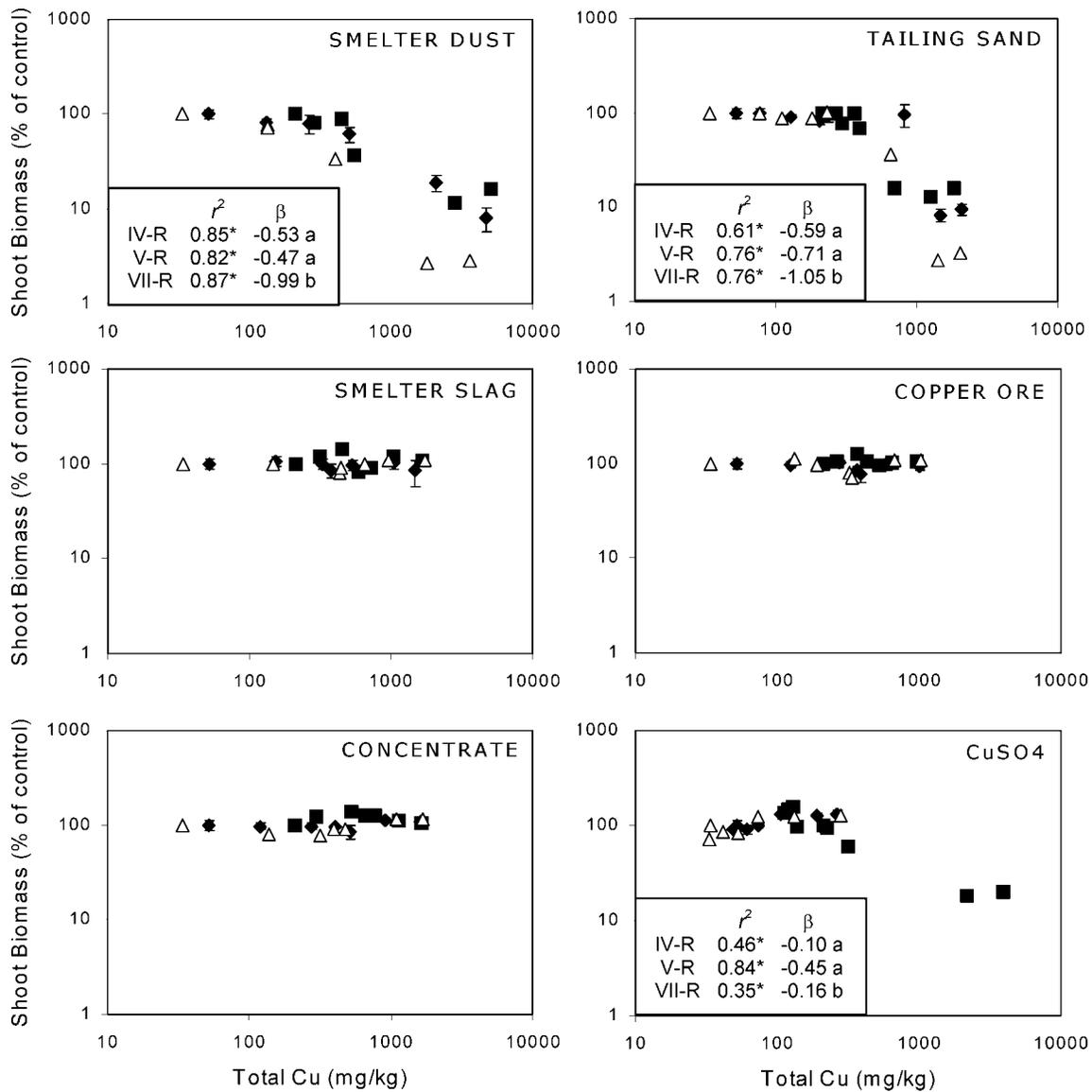
Fig. 1. Variation of shoot biomass of lettuce plants, as a percentage of control soils, with total soil copper of agricultural soils (◆ = IV-R; ■ = V-R; △ = VII-R) spiked with copper-rich materials (mean and one standard deviation). Significant coefficients of determination, r^2, and estimated slope (β) values for simple regression models are given for each copper-rich material and soil. Different letters indicate significant differences within soils.

was used for a posteriori comparisons [22]. The total soil copper content, copper in soil pore water, shoot biomass, and shoot copper content were corrected for nonnormality using logarithmic transformations ($x' = \log_{10}[x]$; [22]) before one-way analysis of variance and regression analyses. All statistical analyses were performed using the SPSS® statistical software package (Ver 11.0.1; SPSS, Chicago, IL, USA).

Toxicological parameters for shoot biomass of lettuce plants were estimated based on total soil copper levels using the REGTOX macro Excel™ software (E. Vindimian, 2001, Ministry for Ecology and Sustainable Development, Paris, France; http://eric.vindimian.9online.fr/en_index.html) for dose–response modeling. The 50% effective concentrations (EC50s) were estimated for all experimental soil mixtures using the Hill equation of REGTOX from effect and exposure variables. Confidence intervals for EC50s were estimated by the software using a nonparametric bootstrap method (Monte Carlo simulation).

RESULTS AND DISCUSSION

General properties of experimental soil mixtures

Total copper concentration of experimental soil mixtures varied from normal levels for central Chile (30–197 mg/kg up to between 3- and 137-fold the concentration in control soils) (Tables 2 and 3). As shown in Table 3, copper-rich materials showed a broad range of copper solubilization potentials, with smelter dust and tailing sand exhibiting the highest variability (as much as a 3,000-fold range for the latter). Unfortunately, because of the characteristics of the experimental setting, it is not possible to estimate the fraction of soil copper represented by the pore-water copper in each case. Nonetheless, smelter dust, tailing sand, and $CuSO_4$ have, by far, the highest potential to leach copper into solution. Indeed, copper solubilization has been related to chemical and mineralogical characteristics of materials themselves (see, e.g., [5,15,23]). Preliminary results from optical mineralogical analysis of sulfidic ore, concentrate,

Table 4. Median effective concentrations (EC50s) for shoot biomass (dry wt) of lettuce plants grown on experimental mixtures of copper-rich materials and agricultural soils according to total soil copper[a]

Cu-rich material and soil	EC50 (average)	Confidence intervals	
		$\alpha < 5\%$	$\alpha > 5\%$
Smelter dust			
IV-R	703	549	833
V-R	536	512	554
VII-R	270	233	320
Tailing sand			
IV-R	1,302	876	1,401
V-R	546	411	652
VII-R	522	433	623
$CuSO_4$			
IV-R	NC	—	—
V-R	877	632	1,143
VII-R	NC	—	—

[a] NC = not calculable.

and smelter slag used in the present study indicated that copper is mainly present in insoluble mineral forms, such as chalcopyrite, coveline, and bornite (unpublished data). Smelter dust and tailing sand have high contents of nonmineral copper phases, specifically chalcocyanite ($CuSO_4$) in the smelter dust (J. Skeaff, Environmental Laboratory, Natural Resources Canada, Ottawa, ON, Canada, personal communication) and chalcanthite ($CuSO_4 \times 5H_2O$) in the oxidized tailing sand [24].

The addition of smelter dust, tailing sand, and $CuSO_4$ to the experimental soils resulted in their acidification (data not shown) because of the acidic characteristics of these materials in solution (Table 1). For instance, the pore-water pH of control soil IV-R was 7.7, which dropped to 4.2 when copper content was raised from 41 to 2,196 mg/kg by adding tailing sand. Likewise, the pore-water pH of control soil VII-R was 6.3, which dropped to 4.9 when the copper content was raised from 30 to 4,114 mg/kg by adding smelter dust. However, the magnitude of this effect was highly variable, depending on the material and the soil type. This effect is very relevant, because free ionic copper species and dissolved organic carbon–bound copper forms are dominant in acidic environments [25–27]. Indeed, a small decrease in pH can increase copper solubility by several orders of magnitude, as reported previously for metal-polluted soils (see, e.g., [12,16,26–30]). Other confounding factors, such as increased ionic strength of soil solution, also have been associated with spiking soils using soluble synthetic copper salts at high doses [28]. It is interesting to note that $CuSO_4$ showed somewhat comparable copper solubilization and soil acidification effects versus the sulfate-rich mine materials used in the present study (smelter dust and tailing sand) (Table 3), even though the latter are complex and heterogeneous mixtures.

Maximum pore-water copper levels varied among soils, irrespective of the copper-rich material used as a spike (Table 3). As expected, copper solubilization was higher in the V-R soil than in the VII-R soil because of its lower values for pH, TOC, and cation-exchange capacity (Table 2). These results underline the importance of soil chemical characteristics on soil copper solubility [12,25–27]. However, total soluble copper in soils is not necessarily a good estimation of bioavailable copper [10,14,16]. Currently, no metal-toxicity predictive models for terrestrial environments are equivalent to the biotic

ligand model developed to assess acute toxicity of metals in freshwaters [30]. Therefore, biological testing was required to evaluate the potential toxicity of the copper-rich materials under study.

Plant studies

Increasing levels of smelter dust and tailing sand spiked into all soil types had clear adverse effects on the growth of lettuce plants (Fig. 1), reducing it by as much as 95% at the highest levels of exposure. No such effect was observed for slag, ore, and copper concentrate. In the case of copper sulfate, only two observations in soils found copper levels higher than 1,000 mg/kg (for soil V-R), and in both cases, shoot growth decreased to approximately 20% of the control. This adverse effect was reflected in typical metal-toxicity symptoms, such as stunted growth with chlorotic and reddish leaves. The same results have been described for other vegetables grown on soils spiked with $CuSO_4$ (see, e.g., [9]) and mine tailings (see, e.g., [8,23]). Significant reduction in shoot biomass caused by smelter dust, tailing sand, and $CuSO_4$ allowed estimation of the EC50 for total soil copper (Table 4). In general, lower EC50s were found for smelter dust than for tailing sand and $CuSO_4$, thus suggesting higher toxicity for smelter dust than for the other copper-rich materials, irrespective of the soil type.

The EC50s also were significantly lower on the VII-R soil than on the other soils (Table 4), irrespective of the copper-rich material used. Actual toxicity and uptake of metals by plants depends not only on their environmental availability but also on plant physiology, nutritional status, capacity for active regulation of internal metal concentration, and plant size [31,32]. Control plants grown in VII-R soil were more vigorous and had much larger shoots (1,232 mg dry wt) compared with plants grown in unspiked IV-R and V-R soils (771 and 247 mg dry wt, respectively), which may result from differences in the physical and chemical characteristics of soils, such as the availability of macronutrients (nitrogen, phosphorus, and potassium) (Table 2). At this point, however, we do not have a hypothesis to account for this difference in sensitivity.

Toxicity effect of $CuSO_4$ on lettuce shoots was similar to effects produced by smelter dust and tailing sand (Fig. 1), suggesting that results from standard test methods performed with synthetic copper salts may represent a worst-case scenario for real mine pollutants. Therefore, $CuSO_4$-spiked soils may be useful for testing acute toxicity effects of certain copper-rich materials on plants, at least in the short term (excluding aging effects).

Copper concentration in shoots (bioaccumulation) was significantly related to copper in soil, expressed either as total copper or as pore-water copper, for smelter dust– and tailing sand–spiked soils (Fig. 2). No such correlation was observed for slag, ore, and concentrate. Copper bioaccumulation in shoots varied among soils for the same source material (see slopes or β values in Fig. 2), being largest in the acidic V-R soil. Low pH and organic matter content in the V-R soil may account for the higher levels of shoot copper, as shown previously for this vegetable in other metal-polluted soils [8,12]. Previous studies also have found that copper concentration in the shoot correlate positively with soil solution copper (see, e.g., [9,12,14,33–35]). Lettuce plants accumulate metals at relatively high internal levels compared with other vegetables, because they efficiently translocate metals from roots to shoots [33]. However, they seem to keep quite constant copper concentrations in shoots between 9 and 24 mg/kg (Fig. 2) for a
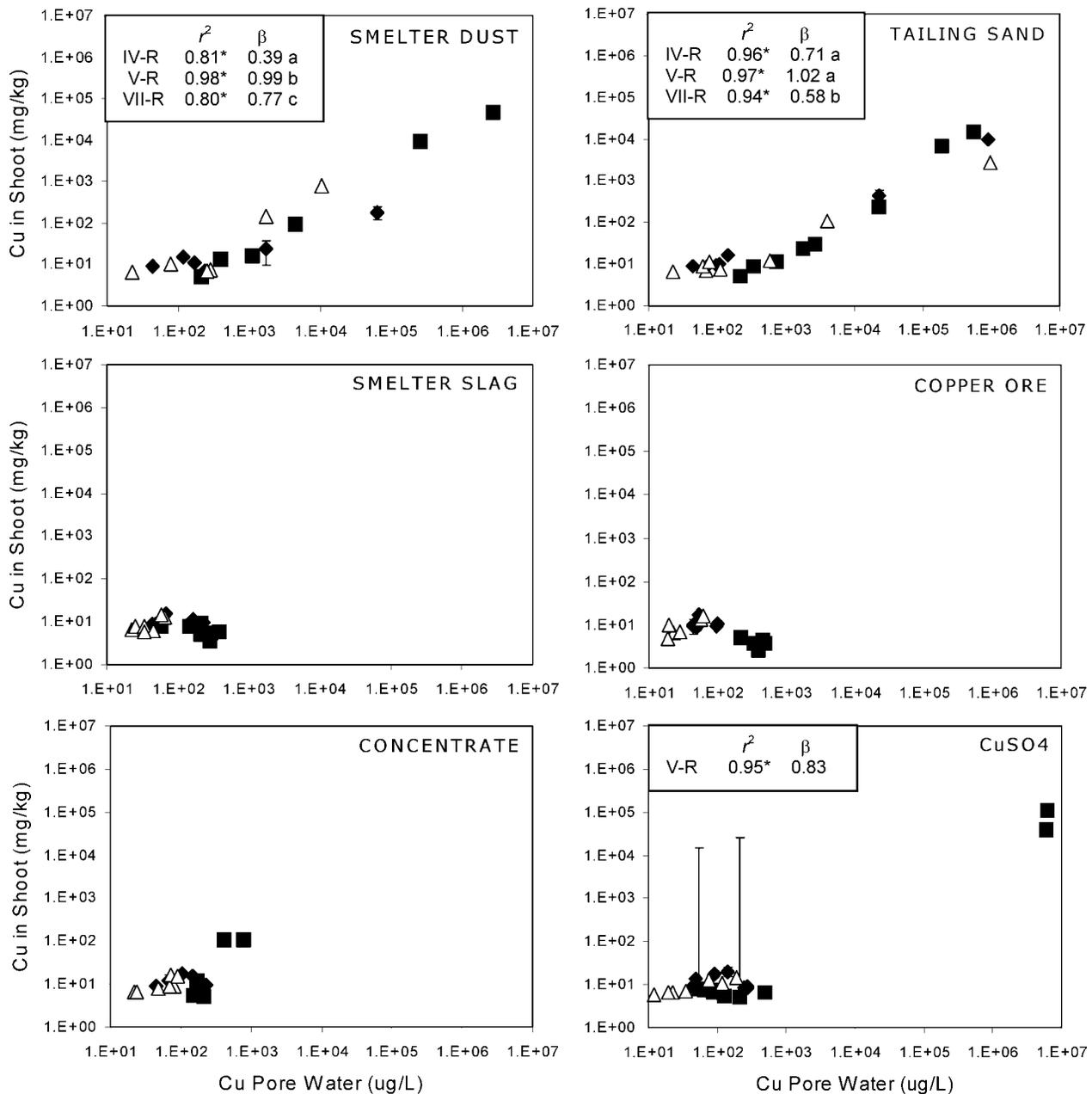
Fig. 2. Variation of copper levels in lettuce shoots (as dry wt) with soil pore-water copper of agricultural soils (◆ = IV-R; ■ = V-R; △ = VII-R) spiked with copper-rich materials (mean and one standard deviation). Significant coefficients of determination, r^2, and estimated slope (β) values for simple regression models are given for each copper-rich material and soil. Different letters indicate significant differences within soils.

range of low-to-medium pore-water copper levels (up to 500–600 μg/L), as shown in Figure 2. Above these values, significant increases in shoot-copper levels are observed. These results are in agreement with the critical toxicity level of 20 to 30 mg/kg for copper in shoots (dry matter) described for most plant species [34].

Our results show that for the group of copper sources used in the present study, copper solubilization to soil pore water is a useful variable to predict potential toxicity effects of the materials. However, the spike materials clearly introduce multiple sources of stress to the plants, such as relevant changes in pH, which may affect the bioavailability of copper as well as that of other stressors and nutrients. The final effect observed may be a complex function of these variables.

In soils contaminated with these materials, however, total soil copper clearly is not an appropriate indicator for risk of plant toxicity. At the least, the mineralogical nature of the source of the metal, its impact on pore-water chemistry, and the physiological condition of the plants should be taken into account.

CONCLUSION

Copper-rich mine materials pose quite different levels of risk to plants when incorporated into agricultural soils at a same total copper concentration. Specifically, the following general tendency for copper solubility, bioavailability, and plant toxicity may be suggested, from high to low as follows: $CuSO_4$, smelter dust, acidic tailing sand ≫ sulfidic copper ore,

copper concentrate, smelter slag. However, the intensity of the effect varies among soil types.

Copper sulfate–spiked soils may be useful for testing acute toxicity effects of copper on plants, because they represent the worst-case scenario for real copper-rich mine materials, at least in the short term (no aging considered).

Mine materials have a broad range of potential for copper release to the aqueous soil environment because of their different chemical/mineralogical characteristics. Further studies are required to develop predictive tools that ensure protection or remediation measures when they are actually required.

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