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Thresholds of copper phytotoxicity in field-collected agricultural soils exposed to copper mining activities in Chile



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ABSTRACT

It has been argued that the identification of the phytotoxic metal thresholds in soil should be based on field-collected soil rather than on artificially-contaminated soils. However, the use of field-collected soils presents several difficulties for interpretation because of mixed contamination and unavoidable covariance of metal contamination with other soil properties that affect plant growth. The objective of this study was to estimate thresholds of copper phytotoxicity in topsoils of 27 agricultural areas historically contaminated by mining activities in Chile. We performed emergence and early growth (21 days) tests (OECD 208 and ISO 11269-2) with perennial ryegrass (*Lolium perenne* L.). The total Cu content in soils was the best predictor of plant growth and shoot Cu concentrations, while soluble Cu and pCu^{2+} did not well correlate with these biological responses. The effects of Pb, Zn, and As on plant responses were not significant, suggesting that Cu is a metal of prime concern for plant growth in soils exposed to copper mining activities in Chile. The effects of soil nutrient availability and shoot nutrient concentrations on ryegrass response were not significant. It was possible to determine EC_{10} , EC_{25} and EC_{50} of total Cu in the soil of 327 mg kg^{-1} , 735 mg kg^{-1} and 1144 mg kg^{-1} , respectively, using the shoot length as a response variable. However, the derived 95% confidence intervals for EC_{10} , EC_{25} and EC_{50} values of total soil Cu were wide, and thus not allowing a robust assessment of metal toxicity for agricultural crops, based on total soil Cu concentrations. Thus, plant tests might need to be performed for metal toxicity assessment. This study suggests shoot length of ryegrass as a robust response variable for metal toxicity assessment in contaminated soils with different nutrient availability.

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1. Introduction

Chile is the first among the producers of copper in the world (Comisión Chilena del Cobre, www.cochilco.cl). Environmental problems associated with copper mining in Central Chile are widely known, particularly in relation to the historical contamination of agricultural soils with metals, such as Cu, Zn, Pb, and Cd, and metalloids (As) that will be henceforth referred to as “metals” for convenience (De Gregori et al., 2003; Ginocchio, 2000; González et al., 1984). Copper is the main contaminant in the soils of copper mining areas in Chile (Goecke et al., 2011). Copper is an essential micronutrient to all organisms but becomes toxic above a certain threshold (McBride, 1994; Adriano, 2001).

Total metal concentration in soil is not sufficient to predict its potential phytotoxicity (ISO 17402, 2008; McBride, 1994; Sauvé et al., 1998). Metal toxicity to plants has often been better related to the bioavailable fraction of metal in soil that, in turn, is related to its chemical form in the soil (Adriano, 2001). The National Research Council (2003) defines bioavailability as the fraction of the total element that is available to the receptor organism. Bioavailability may be assessed in two complementary ways (ISO 17402, 2008): (1) chemical methods which determine a fraction of a metal in the soil, (2) biological methods which expose organisms to soils in order to monitor effects.

With regards to chemical methods, it is generally considered that metal soluble fractions—extracted by chemically-non-aggressive neutral salts—are useful for assessing metal phytotoxicity in contaminated soils (Kabata-Pendias, 2004; McBride et al., 2009). An alternative approach for assessing toxicity of metals to plants is to use the soil solution free metal activities (Sauvé et al.,

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1996, 1998). Nevertheless, metal toxicity depends not only on the activity of free metals in the soil solution, but also on competition with other cations (e.g., H^+ , Ca^{2+} and Mg^{2+}), as explained by the Terrestrial Biotic Ligand Model (Antunes et al., 2007; Thakali et al., 2006; Wang et al., 2012). Moreover, intrinsic soil properties greatly influence the expression of metal toxicity to plants (Rooney et al., 2006). Thus, soluble metal concentrations and/or free metal activities in a soil extract are not suitable indexes for metal phytotoxicity, and one should determine more soil physicochemical properties in order to model metal phytotoxicity.

With regards to biological methods for assessing metal phytotoxicity in contaminated soils, ISO 11269-2 (2005) and OECD 208 (2006) propose the use of artificial soils (composed of a mixture of peat, clay, and sand) or natural non-contaminated soils spiked with solutions of metals at increasing concentrations. However, it is well known that the solubility of metals, and thus their potential phytotoxicity, is greater in artificially-contaminated soils in comparison to field-collected soils (Hamels et al., 2014; McBride et al., 2009; Smolders et al., 2009). This difference in solubility is explained by aging processes in soils, which are very slow and occur during several years (Ma et al., 2006; Martínez and McBride, 2000). Furthermore, chemical forms of metal contaminants released to the environment by mining and smelting operations may be very different from metal salts used on standard metal phytotoxicity studies in soils. Thus, metal-spiked soils cannot adequately represent real environmental conditions (Davies et al., 2003).

Thus, we argue that metal-spiked soils have limited relevance from an environmental point of view and emphasize the importance of using field-collected soils for phytotoxicity tests. However, the use of these soils presents several difficulties. First, in areas near copper mining activities in Chile, soils have high concentrations of several metals (Cu, Pb, Zn, Cd and As, among others) (De Gregori et al., 2003; Ginocchio et al., 2004). In this case, it might be difficult to distinguish between the effects of different metals on plant responses. Second, the intrinsic physicochemical characteristics of the soil, such as pH, texture and organic matter content, among others, also affect the degree of toxicity of the metals present in the soil (Adriano, 2001; McBride, 1994; Rooney et al., 2006). Third, secondary effects of mine contaminants may occur such as soil acidification (Ginocchio et al., 2009). Finally, differences in nutrient availability in the soil and its physical properties may also affect the plant responses, in addition to metal toxicity (Verdejo et al., under review).

In the present study, we hypothesized that a detailed characterization of soil properties and of plant tissue concentrations of metals and nutrients would allow separating the confounding factors from metal-toxicity factors, permitting estimation of thresholds of copper phytotoxicity. Thus, the objective of the present study was to estimate thresholds of copper phytotoxicity in field-collected agricultural soils exposed to copper mining activities.

2. Materials and methods

2.1. Study sites

Topsoils of 27 agricultural areas historically contaminated by mining activities in Chile were used in this study. Sampling points (Supplementary Table 1) were chosen based on prior knowledge on the spatial distribution of Cu in the soils of the Aconcagua River basin (Aguilar et al., 2011) and the Puchuncaví Valley (González et al., 2014). The sites and sampling points were chosen with the aim of obtaining a wide range of total metal concentrations. All soils were classified as Entisols (Soil Survey Staff, 2003).

Table 1

General physicochemical properties of studied soils.

Soil property	Unit	Median	Mean \pm SD	Range
Electrical conductivity	dS m ⁻¹	2.0	2.6 \pm 2.2	0.2–10.4
pH in KNO ₃		7.2	7.0 \pm 0.5	5.7–7.6
pCu ²⁺ in KNO ₃		8.6	8.6 \pm 0.7	6.8–9.8
Organic matter	%	3.3	3.1 \pm 1.3	0.7–5.8
Available N	mg kg ⁻¹	25	33 \pm 26	4–134
Available P	mg kg ⁻¹	32	48 \pm 35	8–123
Available K	mg kg ⁻¹	255	302 \pm 250	78–1143
Total Cu	mg kg ⁻¹	355	418 \pm 285	82–1295
Total As	mg kg ⁻¹	21	20 \pm 9	7–41
Total Zn	mg kg ⁻¹	147	160 \pm 59	86–345
Total Pb	mg kg ⁻¹	43	46 \pm 17	25–97
Soluble Cu	mg L ⁻¹	0.14	0.22 \pm 0.17	0.04–0.71
Soluble As	mg L ⁻¹	0.02	0.04 \pm 0.05	0.002–0.18
Sand	%	52	54 \pm 21	21–95
Clay	%	17	18 \pm 9	5–37
Silt	%	30	28 \pm 13	0–44

SD: Standard deviation.

2.2. Physical-chemical characterization of soils

Each soil sample was taken in a continuous surface area of 2 m² and to a depth of 20 cm, collecting approximately 50 kg of soil. Samples were sieved through a 2 mm mesh and then homogenized in a cement mixer with an inner plastic cover. The mixer was washed between samples to avoid cross contamination. In order to verify the homogeneity of the mixed soils, 4 subsamples were analyzed from each sample. Each subsample was dried at 40 °C for 48 h. All concentrations in the following text are expressed on a dry weight basis.

The general physicochemical characteristics of the soils were determined using routine methods (Table 1) (Sheldrick and Wang, 1993; Sparks et al., 1996). Concentrations of soluble Cu, Pb, Zn and As were determined using a solution of 0.1 M KNO₃ as extractant (Stuckey et al., 2008). In order to determine total Cu, Pb, Zn and As, the samples were digested in boiling nitric acid followed by perchloric acid addition (Maxwell, 1968). Approximately 1 g of soil was finely ground in an agate mortar and weighed in an Erlenmeyer flask. Concentrated nitric acid (25 mL) was then added, and a Teflon stopper with a 30 cm-long glass reflux tube was used to prevent the volatilization of As during the digestion process (adapted from Verfinden, 1982). The sample was digested at 60 °C overnight and then at 120 °C for 1 h. The stopper was removed, and the nitric acid was evaporated to obtain a volume of approximately 5 mL. The sample was cooled, and 5 mL of concentrated perchloric acid was added. Again, a Teflon stopper with a 30-cm-long glass reflux tube was used, and the sample was digested at 220 °C for 30 min; the nitric acid was volatilized during this stage. The sample was then cooled and filtered into a 100-mL volumetric flask. Quality was assured by similarly digesting in duplicate the following certified reference samples: PACS-2 obtained from the National Research Council Canada, and GRX-2 obtained from the United States Geological Survey. The obtained values were within 10% of the certified value. Spikes of Cu, Pb, Zn and As were performed on every 10th sample and recovery was 100% \pm 7%.

Total and soluble concentrations of Cu, Pb, Zn and As were determined by atomic absorption spectroscopy. The activity of Cu²⁺ was determined in the 0.1 M KNO₃ extract with an ion selective electrode (Rachou et al., 2007). The results were expressed as pCu²⁺, which is $-\log$ (activity of the free Cu²⁺ ion). Available K was determined by atomic emission spectrophotometry after extraction with 1 mol L⁻¹ CH₃COONH₄ at pH 7.0. Available P was determined by the Olsen method (extraction with 0.5 mol L⁻¹ NaHCO₃ at pH 8.5) and measured colorimetrically with

molybdenum blue method. Available N (the sum of N-NO_3^- and N-NH_4^+) was extracted by 2 mol L^{-1} KCl and determined by titration following NH_3 distillation. The water holding capacity (WHC) was determined by the saturation and gravity drainage method (ISO 11269-1, 1993).

2.3. Biotesting of early plant development

Perennial ryegrass (*Lolium perenne* L.), variety Nui, was used for the assessment of Cu phytotoxicity. Ryegrass is recommended by the ISO and OECD methods for testing toxicity of compounds in soils (ISO 11269-2, 2005; OECD 208, 2006) and has been used often as a toxicity bioindicator for metals in soils contaminated by mining activities (Arienza et al., 2004; Goecke et al., 2011; Stuckey et al., 2009). For determining seed quality, prior to testing, a germination test was conducted using standard methods (ISTA, 2004) obtaining a germination percentage of 82%.

A $200 \text{ cm} \times 100 \text{ cm} \times 200 \text{ cm}$ (4 m^3) growth chamber was used to grow plants with 16 h of light (at an intensity of $30,000 \pm 1,000 \text{ lx}$ and active photosynthetic radiation of $366 \pm 13 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Relative humidity was $50 \pm 5\%$ during the day and $70 \pm 5\%$ during the night; diurnal temperature was $25 \pm 1^\circ\text{C}$ while nocturnal temperature was $20 \pm 1^\circ\text{C}$. Plastic containers were used, measuring $9 \text{ cm} \times 9 \text{ cm} \times 9.5 \text{ cm}$ (width \times length \times height; 770 cm^3) holding 400 g of soil per container.

Biotesting was carried out with four replicates. The containers were placed in the growth chamber using a fully randomized design. Ten seeds were sown per container, which were thinned out on day seven to leave only five plants in each container. The total length of the test period was 21 days, including the germination period.

Soils in the containers were watered by spray with distilled water up to 70% of WHC during the first week and were then fertilized with a nutrient solution diluted 10 times more than the standard Hoagland nutrient solution: MgSO_4 0.2 mM, $\text{Ca}(\text{NO}_3)_2$ 0.5 mM, KNO_3 0.5 mM and K_2HPO_4 0.1 mM. The purpose of diluting this nutrient solution was to provide minimal nutrients yet avoid precipitation of metals as phosphates and metal co-precipitation with Ca and/or Mg phosphates (Harper et al., 1997). Irrigation was carried out daily during the testing period.

2.4. Plant responses

Upon completion of the biotesting period, the length and weight of the shoot and longest root of each individual plant were recorded. Shoot and root lengths were measured from the root neck to the distal ends of the last leaf and the longest root, respectively. The biomass of shoots and roots were determined after drying for 48 hours in an oven at 70°C .

Concentrations of metals (Table 2) and nutrients (Table 3) were measured at the end of the testing period using standard methods (Kalra, 1998). A standard reference material (NIST SRM 1570a) was taken through the entire plant analysis process with experimental values being within 10% of the certified value. Spikes were performed on every 10th sample and recovery was $100\% \pm 10\%$.

Table 2

Shoot concentration of Cu, Zn, Pb and As (in mg kg^{-1}). Median values are in parenthesis.

Crop	Cu	Zn	Pb	As
Ryegrass	23 ± 7 (22)	75 ± 51 (57)	13 ± 5 (13)	1.0 ± 0.7 (0.86)
Plant normal values	5–20 ^a	25–150 ^a	0.2–20 ^b	0.02–7 ^b

^a Mortvedt et al. (1983).

^b Alloway (1995).

Table 3

Shoot nutrient concentrations of ryegrass. Nitrogen concentrations were not determined due to lack of biomass for the analysis.

Nutrient	Unit	Normal values ^a	Range	Median	Mean \pm SD
N	%	2.0–3.5		not determined	
P	%	0.2–0.6	0.2–0.5	0.3	0.3 ± 0.1
K	%	1.5–3.5	0.9–3.3	2.3	2.2 ± 0.8
Ca	%	0.4–0.8	0.1–1.5	0.5	0.6 ± 0.3
Mg	%	0.1–0.30	0.1–0.4	0.3	0.3 ± 0.1
Zn	mg kg^{-1}	15–60	21–206	57	75 ± 51
Mn	mg kg^{-1}	30–300	11–206	104	95 ± 57
Fe	mg kg^{-1}	100–200	55–235	100	104 ± 38
Cu	mg kg^{-1}	3–15	9–37	22	23 ± 7

^a Whitehead (2000); SD: Standard deviation.

2.5. Statistical analysis

Simple and multiple regressions were carried out between the biological responses and the physicochemical characteristics of the soils. Normal distribution and homogeneity of residuals were verified (Kutner et al., 2004). Statistical analyses were carried out using Minitab 16 (2010). Effective concentrations (EC_x) were determined by the Toxicity Relationship Analysis Program (TRAP) version 1.22 (US EPA, 2013). Total copper concentrations in the range of 70–155 mg kg^{-1} was considered as background concentrations in the soils of the Aconcagua River basin (Aguilar et al., 2011), while the range of 16–115 mg kg^{-1} was considered as background concentrations in the soils of the Puchuncaví Valley (González et al., 2014). Thus, for the determination of the EC_x values, a control response was established as the average of the responses obtained in the soils with total Cu concentration $< 155 \text{ mg kg}^{-1}$.

3. Results and discussion

3.1. General soil properties

The physicochemical characteristics of soils are presented in Table 1. The ranges for pH (5.7–7.6), pCu^{2+} (6.8–9.8) and organic matter (0.7–5.8%) in soils were narrower than in other studies (Ivezić et al., 2012; Sauvé et al., 1997; Tipping et al., 2003; Unamuno et al., 2009). On the other hand, wide ranges were seen for all other variables. The distribution of most of the variables was not normal. Thus, we presented median values in the Table 1, along with means and standard deviations.

The median value for pCu^{2+} (8.6) suggests low Cu toxicity in the studied soils, considering the EC_{50} threshold value for pCu^{2+} of 6.8 ± 1.7 (Sauvé et al., 1998). In the present study, the activity of free Cu^{2+} ion showed a significant relationship with total Cu content and pH, with both variables being significant ($p < 0.001$)

$$\text{pCu}^{2+} = 1.14 - 0.953 \log \text{Cu}_T + 1.40 \text{ pH}$$

$$(R^2 = 0.90; p < 0.001)$$

where Cu_T is total copper (mg kg^{-1}).

This is consistent with the theory on the formation of metallic complexes by McBride et al. (1997). In particular, the values for pH and pCu^{2+} were highly correlated ($R^2 = 0.74; p < 0.001$), showing the direct effect of pH on Cu activity in the soils, as was also reported by other authors (Sauvé et al., 1997; Tipping et al., 2003). The increase in the activity of the free Cu^{2+} ion is likely to be caused by a reduction in proton competition on binding with dissolved organic carbon (Luo et al., 2006).

The concentration of soluble Cu was correlated with total Cu content ($R^2 = 0.35; p < 0.001$). On the other hand, pH and organic matter content were not significant in explaining Cu solubility in

soils, unlike the results of [Sauvé et al. \(2000\)](#). [Mondaca et al. \(2015\)](#) reported that dissolved organic carbon was the best variable explaining Cu solubility in the soils of central Chile. However, dissolved organic carbon was not determined in the present study.

3.2. Emergence rate as an indicator of metal toxicity

The emergence rate of ryegrass was determined solely by soil texture ($R^2=0.27$ for sand, $R^2=0.23$ for clay, $R^2=0.25$ for silt; $p < 0.01$) with no statistically significant relationship to metal concentration ($p > 0.05$). The percentage of emergence was in the range of 50–90% in the studied soils. Specifically, emergence was higher in sandy soils, in comparison to clayey and silty soils. Thus, emergence rate was not considered a good indicator of metal toxicity in contaminated soils.

3.3. Effects of different metals on plant responses

In order to distinguish between toxicity effects of different metals to ryegrass, we used simple and multiple regressions between plant response variables and free, soluble and total soil metal fractions. The effects of soil Pb, Zn, and As on plant responses were not significant ($p > 0.05$). Likewise, we used simple and multiple regressions between plant response variables and shoot metal concentrations. The effects of shoot Pb, Zn, and As on plant responses were not significant ($p > 0.05$), which is consistent with the findings that shoot concentrations of Zn, Pb and As were within the range considered normal for plant grown in soils without metal contamination ([Table 2](#)). On the other hand, regressions revealed that effects were caused by Cu, which is consistent with the findings that shoot concentrations of Cu were above the range considered normal for plant grown in soils without metal contamination ([Table 2](#)). As discussed in the following sections, Cu was a metal of prime concern for *Lolium perenne* in soils affected by Cu mining activities. However, we are aware that other metals might have secondary effects, but these effects were not significant in this soil set.

3.4. Effect of different Cu fractions on ryegrass responses

Generally, it is considered that total metal content is not a good indicator of soil metal toxicity ([Adriano, 2001](#); [McBride, 1994](#)), while metal soluble fractions are more useful for assessing metal phytotoxicity in contaminated soils ([Kabata-Pendias, 2004](#); [McBride et al., 2009](#)). Furthermore, the free Cu^{2+} ion is considered the main bioavailable form of copper in soils and the best indicator of copper phytotoxicity ([Oliver et al., 2004](#); [Sauvé et al., 1998](#)). However, according to the Terrestrial Biotic Ligand Model, other ions, principally H^+ , Ca^{2+} and Mg^{2+} , compete with Cu^{2+} and, therefore, affect its toxicity ([Thakali et al., 2006](#)). Toxicity is correlated only to the fraction of the total biotic ligand sites occupied by Cu^{2+} . For this reason, soluble copper concentrations and/or free Cu^{2+} activities in a soil extract are not suitable indexes for Cu phytotoxicity ([Zhao et al., 2006](#)) and Cu uptake by plants ([Zhang et al., 2001](#)).

Consistent with these arguments, in the present study, total Cu concentration was a better predictor of ryegrass responses, in

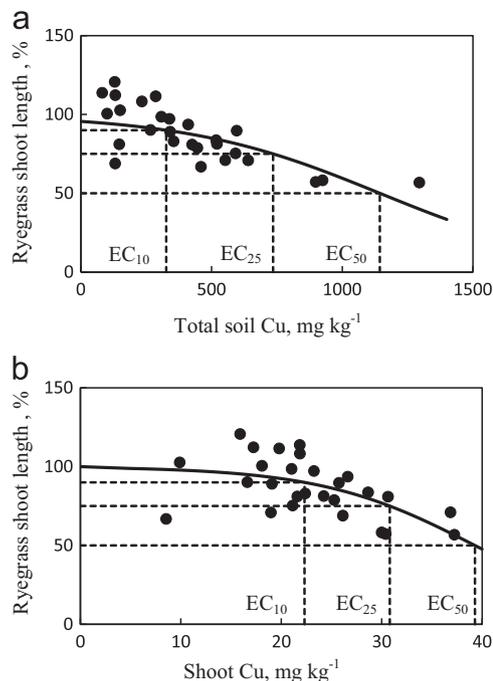


Fig. 1. Ryegrass shoot length as a function of (a) total soil Cu content, and (b) shoot Cu content. EC_{10} , EC_{25} and EC_{50} are indicated.

comparison to soluble Cu and pCu^{2+} ([Table 4](#) and [Fig. 1](#)). Likewise, the shoot Cu concentration was correlated with total Cu concentration in the soils ($R^2=0.39$; $p < 0.001$), while soluble copper and pCu^{2+} were not significant in explaining shoot Cu concentration. These findings are also consistent with arguments of [Zhang et al. \(2001\)](#) that resupply from solid phase due to local depletion is the dominant controlling process for Cu uptake by plants. In other words, the process of Cu uptake by roots depends on the buffering capacity of the soil to resupply pCu^{2+} ([Sauvé, 2002](#); [Zhao et al., 2006](#)). For these reasons, Cu uptake by ryegrass and Cu toxicity to ryegrass in the present study depended on the total soil Cu pool that was capable of supplying Cu to the soil solution at the same time as plant roots locally depleted the ions through active uptake.

The technique of diffusive gradients in thin films (DGT) was shown to be superior for predicting Cu phytotoxicity and Cu uptake by plants, in comparison to soluble copper concentrations and/or free Cu^{2+} activities in a soil extract ([Zhang et al., 2001](#); [Zhao et al., 2006](#)). The authors demonstrated that plant bioavailability of Cu in soil depends on Cu speciation, interactions with protective ions (particularly H^+), and the resupply from the solid phase. They concluded that the DGT measurement provides an integrated measurement of both intensity (free activities of Cu^{2+} and H^+) and resupply. One can argue that the DTG-measured Cu would be a better predictor of plant responses, in comparison to total soil Cu; however, the DGT technique was not used in the present study.

Table 4
Determination coefficients (R^2) of regressions between different Cu fractions in the soil and ryegrass responses ($p < 0.05$).

Shoot length			Root length			Dry shoot mass			Dry root mass		
CuT	CuS	pCu^{2+}	CuT	CuS	pCu^{2+}	CuT	CuS	pCu^{2+}	CuT	CuS	pCu^{2+}
0.58	0.24	ns	0.40	0.40	0.33	0.34	ns	ns	0.35	ns	ns

CuT: total soil Cu concentration; CuS: soluble Cu; pCu^{2+} : $-\log[\text{Cu}^{2+}]$; ns: not significant.

3.5. Effect of nutrient availability on ryegrass responses

The relationships between shoot nutrient concentrations and ryegrass responses (shoot length, root length, dry shoot mass, dry root mass) were insignificant ($p > 0.05$), consistent with the insignificant effect of soil nutrient availability on ryegrass responses ($p > 0.05$). In addition, shoot nutrient concentrations (Table 3) were within normal values (Whitehead, 2000) despite the wide ranges of soil nutrient availability (Table 1). Therefore, we suggest that ryegrass is a good bioindicator of Cu toxicity in contaminated soils with different nutrient availability.

Several crops are recommended by the ISO and OECD methods for testing toxicity of compounds in soils (ISO 11269-2, 2005; OECD 208, 2006). In our previous study with the same set of soils (Verdejo et al., under review), we found that the responses of lettuce (shoot length, root length, dry shoot mass, dry root mass) were best explained by Cu toxicity and P deficiency. Likewise, responses of maize and tomato in the same set of soils were best explained by nutrient deficiencies, rather than Cu toxicity (unpublished results). Thus, lettuce, maize and tomato have a limited applicability for metal toxicity assessment in metal-contaminated soils with different nutrient availability, due to sensitivity of their responses to nutrient deficiencies.

3.6. Ryegrass responses to Cu toxicity

Based on regression analysis between total soil Cu and ryegrass responses, it was concluded that shoot and root lengths are better indicators of Cu toxicity, in comparison to shoot and root biomass (Table 4). This result is concordant with the study by Füleky and Barna (2013), who found that the reduction in shoot length in ryegrass is a sensitive indicator of soil contamination by metals.

The EC₁₀, EC₂₅ and EC₅₀ values of total soil Cu for shoot length were similar to the EC₁₀, EC₂₅ and EC₅₀ values for root length in ryegrass (Table 5), suggesting that both response variables can be useful as indicators of Cu toxicity in contaminated soils. Certainly, the 50% inhibition represents a drastic impact on the agricultural productivity. Although this might be deemed acceptable for an industrial site, it would certainly not be acceptable from an agricultural perspective where even 10% yield reductions would generate serious financial difficulties.

3.7. Relationship between plant tissue Cu concentrations and ryegrass responses

Shoot Cu concentration was a weak indicator of the ryegrass response (Fig. 1), explaining 26% and 15% of the variance in the case of shoot length and shoot biomass, respectively, while being insignificant in the case of root length and root biomass. Ryegrass is known to exhibit greater absorption and accumulation of Cu in the roots than in the shoots (Füleky and Barna, 2013; Jarvis and Whitehead, 1981; Jarvis, 1978; Santibáñez et al., 2008). Therefore,

shoot Cu concentration of ryegrass was not a good indicator of metal toxicity for the plant within our experimental setup.

Keeping in mind the above-mentioned limitations, we still derived the EC₁₀, EC₂₅ and EC₅₀ values for Cu shoot concentrations in ryegrass, using only shoot length as response variable (Table 5), because root length did not significantly correlate with Cu shoot concentration. The derived values are higher than the Cu concentrations considered normal for ryegrass (11 mg kg⁻¹) (Davis and Beckett, 1978). Similarly, the EC₁₀ found in our study (22 mg kg⁻¹, Table 5) is very similar to the lowest observed effect concentration of foliar Cu of 21 mg kg⁻¹ reported for ryegrass by these authors.

4. Conclusions

Total Cu content in soils was the best predictor of plant growth and shoot Cu concentrations, while soluble Cu and pCu²⁺ did not well correlate with the biological responses of ryegrass. The effects of Pb, Zn, and As on plant responses were not significant, suggesting that Cu is a metal of prime concern for plant growth in soils exposed to copper mining activities in Chile.

The response of ryegrass was determined by total soil Cu concentration, while the effects of soil nutrient availability and nutrient shoot concentrations were not significant. Thus, ryegrass is a good bioindicator of Cu toxicity in contaminated soils with different nutrient availability. It was possible to determine EC₁₀, EC₂₅ and EC₅₀ of total Cu in the soil of 327 mg kg⁻¹, 735 mg kg⁻¹ and 1144 mg kg⁻¹, respectively, using the shoot length of ryegrass as a response variable. However, the derived 95% confidence intervals for EC₁₀, EC₂₅ and EC₅₀ values of total soil Cu were wide, not allowing a robust assessment of metal toxicity for agricultural crops, based on total soil Cu concentrations. Thus, plant tests might need to be performed for metal toxicity assessment. This study suggests shoot length of ryegrass as a robust response variable for metal toxicity assessment in contaminated soils with different nutrient availability.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.ecoenv.2015.07.026>.

Table 5

Effective concentration (EC₁₀, EC₂₅ and EC₅₀) of total Cu content in soil (mg kg⁻¹) and in plant tissues (mg kg⁻¹) for responses of shoot length and root length in ryegrass, along with the 95% confidence intervals.

	Shoot length			Root length		
	EC ₁₀	EC ₂₅	EC ₅₀	EC ₁₀	EC ₂₅	EC ₅₀
Soil	327 (94–559)	735 (575–896)	1144 (874–1413)	500 (268–732)	765 (600–931)	1031 (813–1248)
Tissue	22 (16–28)	31 (27–35)	39 (32–47)	ns	ns	ns

ns: Not significant.

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