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RESEARCH ARTICLE

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

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Abstract

Several previous studies highlighted the importance of using field-collected soils instead of artificially spiked contaminated soils for phytotoxicity tests. However, the use of field-collected soils presents several difficulties for interpretation of results, due to presence of various contaminants in the soil and unavoidable differences in the physicochemical properties of the tested soils. 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 standard emergence and early growth (21 days) tests (OECD 208 and ISO 11269-2) with lettuce. The response of lettuce was best explained by Cu toxicity and P deficiency. Growth of lettuce was related to soil total Cu concentration and Olsen-P and was not affected by soluble Cu (extractable by 0.1 M KNO_3) or Cu^{2+} free ion activity of the soil solution. Thus, lettuce has a limited applicability for metal toxicity assessment in metal-contaminated soils, due to sensitivity of its response to P deficiency. However, it was possible to determine toxic thresholds for shoot concentrations of Cu in lettuce for responses of shoot and root length, suggesting that shoot concentrations of Cu in lettuce can be useful as indicators of Cu toxicity even in soils with a wide range of nutrient concentrations.

Keywords: Bioavailability, copper, EC_{50}, lettuce, toxicity

1. Introduction

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) (De Gregori *et al.*, 2003; Ginocchio, 2000; González *et al.*, 1984). It is well known that soil contamination with metals diminishes its quality and presents a risk for soil functioning (Adriano, 2001). Lettuce (*Lactuca sativa* L.) is recommended as an indicator organism in bioassays for assessment of soil quality (ISO 11269-2, 2005; OECD 208, 2006).

Several previous studies highlighted the importance of using field-collected soils – and not artificially-contaminated soils – for phytotoxicity tests for assessment of soil quality of metal-contaminated soils (Hamels *et al*., 2014; McBride *et al*., 2009; Smolders *et al*., 2009). Likewise, the latest version of the protocol of soil quality tests with plants (ISO 11269-2, 2012) also considers field-collected soils, instead of artificially-contaminated soils. However, there might be several difficulties in interpreting the results of bioassays in field-collected soils. 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). Finally, differences in nutrient availability in the soil and its physical properties may also affect the plant responses, in addition to metal toxicity.

Recognizing the above-mentioned difficulties in interpreting the results of plant tests in field-collected soils, raises the question if a detailed characterization of soil properties and metal concentrations in plant tissues allows differentiating the metal-related effects and other confounding effects in field-collected agricultural soils exposed to copper mining activities? If the responses were affirmative, it would be possible to estimate thresholds of metal toxicity in soil. Thus, in the present study we aimed to: (1) differentiate confounding factors from metal-toxicity factors affecting lettuce growth in field-collected agricultural soils exposed to copper mining activities, (2) estimate thresholds of metal toxicity to plants in these soils.

2. Materials and Methods

A detailed description of methodology can be found in our previous study (Verdejo *et al*., 2015) and in the Online Supplementary Material (www.webcitation.org/6bEciJFes). Topsoils of 27 agricultural areas historically contaminated by mining activities in Chile were used in this study (See Online Suplementary Table 1). The general physicochemical characteristics of the soils, total and soluble concentrations of Cu, Pb, Zn and As, and the activity of free Cu^{+2} were determined (See Online Suplementary Table 2 and 3). Fertility analysis was conducted on soils by determining the available nitrogen, phosphorus and potassium.

The biotesting was based on ISO 11269-2 (2005) and OECD 208 (2006). Lettuce (*Lactuca sativa* L.), variety Winter Gallega, was used for the assessment of metal phytotoxicity. A growth chamber was used for the test. 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 was 21 days, including the germination period. Upon completion of the biotesting, a record was made of the length of each individual plant (shoot and longest root) along with the dry biomass of shoots and roots. Metal concentrations (Cu, Pb, Zn and As) and nutrient concentrations (See Online Suplementary Table 4) were measured at the end of the testing.

Simple and multiple regressions were carried out between the biological responses and the physicochemical characteristics of the soils. Statistical analyses were carried out using Minitab 16. Effective concentrations (EC_x) were determined by the Toxicity Relationship Analysis Program (TRAP) version 1.22 (US EPA, 2013).

3. Results and Discussion

Total soil concentrations of Cu, Zn, Pb, and As were weak indicators of the lettuce response; the same was true for soluble concentrations of the elements and free Cu^{2+} activity (See Online Suplementary Table 5). However, shoot metal concentrations were good predictors of the lettuce response (See Online Suplementary Table 6). Also, the lettuce responses were determined by soil available P (Olsen-P) and P shoot concentration (See Online Suplementary Table 7). Despite the fact that Olsen-P was high (>15 mg kg^{-1}) in most of the studied soils (See Online Suplementary Table 8) shoot P concentrations were below the normal value for lettuce (See Online Suplementary Table 9). Shoot P concentrations were correlated with Olsen- P ($R^2 = 0.21$, $p < 0.05$).

Stepwise regression analysis revealed that lettuce responses were best explained by Cu and P, with both variables being significant ($p<0.05$) in the following equations:

Shoot length = 8.5 - 0.003 total soil Cu + 0.042 available soil P (Olsen-P)
($R^2 = 0.58$; $p<0.001$) (1)

Shoot length = 8.5 - 0.263 shoot Cu + 17 shoot P (2)
($R^2 = 0.69$; $p<0.001$),

where shoot length is in cm, while total soil Cu, available soil P, shoot Cu, and shoot P are in mg kg^{-1}.

Importantly, there was no correlation between total soil Cu and available soil P (Olsen-P), nor between shoot Cu and P concentration ($p>0.05$). At mean Olsen-P (48 mg kg^{-1}), the equation (1) predicts that the shoot length would be reduced by 10%, 25% and 50% at soil total Cu concentrations of 445 mg kg^{-1}, 955 mg kg^{-1} and 1805 mg kg^{-1}, respectively. The 95% confidence intervals of these EC_x thresholds were also estimated using the 95% confidence intervals of the mean values of Olsen-P (See Online Suplementary Table 10).

It has been reported that Cu toxicity reduced P uptake by crops (Reuther, 1957; Rhoads *et al*., 1992). However, a factorial regression revealed that the interaction between Cu and P was not statistically significant ($p>0.05$), thus suggesting that these two factors are independent of each other in the present study.

These findings differ from results of our previous study with the same set of soils (Verdejo *et al*., 2015), in which we reported that the effects of soil nutrient availability on the ryegrass responses were not significant. Thus, lettuce has a limited applicability for metal toxicity assessment in field–collected metal-contaminated soils, due to sensitivity of its response to P deficiency often observed in non-agricultural areas.

Despite the above-mentioned confounding effects, it was possible to determine effective Cu shoot concentration (EC_X) for lettuce responses. We derived the EC_{10}, EC_{25} and EC_{50} values for Cu shoot concentrations in lettuce, using shoot and root length as response variables (Table 1 and Online Supplementary Figure 1). The EC_X values for the shoot length response were similar to the EC_X values for the root length response, suggesting that both responses can be useful as indicators of Cu toxicity in field contaminated soils.

Table 1. Effective concentration (EC_{10}, EC_{25} and EC_{50}) of shoot Cu (mg kg^{-1}) for responses of shoot length and root length in lettuce, along with the 95% confidence intervals.

	Effective concentration of shoot Cu in lettuce (mg kg^{-1})		
	EC_{10}	EC_{25}	EC_{50}
Shoot length	11 (7 - 16)	16 (13 - 20)	21 (17 - 26)
Root length	12 (10 - 15)	17 (15 - 19)	21 (20 - 23)

The derived EC_{10} values are higher than the shoot Cu concentration of 10 mg kg^{-1}, which is considered as normal for lettuce (Davis and Beckett, 1978; Lamb *et al*., 2012). Similarly, Lamb *et al*. (2012) reported an EC_{50} of 19 mg kg^{-1} for shoot Cu concentration in lettuce, which is equal to the EC_{50} found in our study (Table 1). This shoot Cu threshold was exceeded in the soils 14 and 23, in which the total soil Cu concentrations were 898 mg kg^{-1} and 549 mg kg^{-1}, respectively. The soil number 13 was a soil with high total soil Cu but where high total Olsen-P masked the Cu effects and which resulted in rather good growth and lower shoot Cu (See Online Supplementary Tables 3 and 4). These findings suggest that lettuce shoot Cu concentrations can be useful as indicators of Cu toxicity, even in soils with a wide range of nutrient concentrations.

4. Conclusions

Growth of lettuce was related to soil total Cu concentration and Olsen-P and was not affected by soluble Cu (extractable by 0.1 M KNO_3) or Cu^{2+} free ion activity of the soil solution. Thus, lettuce has a limited applicability for metal toxicity assessment in metal-contaminated soils, due to sensitivity of its response to P deficiency. However, it was possible to determine toxic thresholds for shoot concentrations of Cu in lettuce for responses of shoot and root length, suggesting that shoot concentrations of Cu in lettuce can be useful as indicators of Cu toxicity even in soils with a wide range of nutrient concentrations.

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