

Evaluation of Cu-toxicity in agricultural topsoil contaminated by different sources in central Chile

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Abstract

The primary fruit production zone in Chile lies in the lowland areas of the central region, which has a history of soil copper pollution due to both copper mining (in the highlands) and the use of copper-based pesticides in agriculture. This study assessed the phytotoxic effect of copper in agricultural topsoil contaminated by different sources in the region. A total of 13 agricultural sites, including polluted soils and a background unpolluted soil, were sampled, and a toxicity bioassay was conducted using *Lolium perenne*. Multiple linear regression models revealed that copper has a significant negative impact on plant growth, whereas soluble zinc, organic matter, available nitrogen, and clay have positive effects and mitigate copper toxicity ($p < 0.001$). The effective concentration at 50% (EC₅₀) of the total soil copper was 1030 mg kg⁻¹ for shoot length and 1084 mg kg⁻¹ for shoot dry mass. These results correspond with the EC₅₀ values reported in previous studies that also used real-world contaminated soils. Moreover, the findings are noteworthy as only six studies have estimated copper toxicity thresholds for plants grown in contaminated soils. Overall, the results highlight the complex nature of copper toxicity in plants grown in soils contaminated by different sources and underscore the importance of considering multiple factors when assessing the impact of contaminants on plant growth in soils contaminated by various metals besides copper.

Introduction

Central Chile—a Mediterranean climate-type region—is the primary hub for fruit production in Chile¹. Within the lowland areas of this region, the O'Higgins Region, which extends across 95,082 ha, is notable for its contribution to fruit production². However, this particular geographical region has a history of soil copper (Cu) pollution due to either metal pollution dispersal from Cu mining operations located in the highlands³ or the application of Cu-based pesticides⁴. Badilla-Ohlbaum et al.⁵ sampled agricultural soils downstream of the El Teniente Cu mine and the Caletones smelter and discovered a wide range of soil total Cu concentrations in the Cachapoal valley, varying between 26 mg kg⁻¹ and 1,600 mg kg⁻¹. Meanwhile, Schoffer et al.¹ conducted a soil study in the fruit tree orchards of the O'Higgins Region, and detected total Cu concentrations of 131–432 mg kg⁻¹, which were attributed solely to the application of Cu-based pesticides.

Cu solubility indicates the mobility of Cu in soil and, consequently, its potential bioavailability and phytotoxicity⁶. The bioavailability of Cu in soil contaminated by different sources varies significantly owing to the distinct chemical properties of each source⁷. Badilla-Ohlbaum et al.⁵ found several Cu minerals, such as chalcopyrite (CuFeS₂), chalcosine (Cu₂S), coveline (CuS), and enargite (Cu₃AsS₄), that are associated with mining waste materials, such as tailings. These minerals are highly insoluble in water⁸. In contrast, Skeaff et al.⁹ revealed that the predominant Cu emission from smelters is CuSO₄—a highly soluble and hygroscopic compound⁸. Considering Cu-based pesticides, the primary pesticides used in the O'Higgins Region include Bordeaux mixture (CuSO₄ + Ca(OH)₂), copper hydroxide (Cu(OH)₂), and copper oxychloride (CuCl₂·3Cu(OH)₂), which account for approximately 26%, 24%, and 20%, respectively, of the total Cu-based pesticide usage in the region¹⁰. Richardson⁸ referred to copper hydroxide and copper oxychloride as "fixed copper" owing to their limited solubility. Specifically, copper oxychloride pesticide is typically applied as a colloidal water suspension¹¹. Consequently, it is transported within the soil matrix as colloidal particles, which are characterized by their limited solubility¹². In contrast, owing to its pronounced solubility, the Bordeaux mixture exhibits high bioavailability; however, it lacks persistence in the field¹³.

Studies on Cu toxicity in plants often employ artificially contaminated soils—an approach that has several limitations. Santa-Cruz et al.¹⁴ demonstrated that toxic metal concentrations in artificially contaminated soils are significantly

higher than those in industrially contaminated soils. This is because metal toxicity depends on the duration of metal presence in the soil, which is referred to as "aging"¹⁵. In their review, Santa-Cruz et al.¹⁶ identified only six studies on Cu toxicity in plants that used industrially contaminated soils, with three of them conducted in the Valparaíso Region of Chile, which is known for its mining-related Cu contamination^{17–19}. To the best of our knowledge, there are no studies evaluating the effects of Cu on plants growing in soils in the O'Higgins Region, which is contaminated by Cu from both mining and Cu-based pesticides. Thus, the objective of this study is to evaluate the effect of Cu on plants using field-collected agricultural soils from the O'Higgins Region, which is polluted by different Cu sources.

Materials and Methods

Study sites and soil samples

Topsoil from 13 agricultural sites in the O'Higgins Region, central Chile, were sampled. The sampling sites of the polluted soils were selected based on prior knowledge of the spatial distribution of soil total Cu concentration in the Cachapoal River basin (Fig. 1 and Fig. 2). One extra sample was taken from agricultural soil near Chimbarongo town, which has a low level of soil total Cu (51 mg kg^{-1}), to represent a background soil sample. The sites were chosen such that a wide range of total Cu concentrations could be obtained. At each site, the soil sample was taken from a continuous surface of 2 m^2 to a depth of 20 cm. A total of 40–60 kg of soil was obtained and used in further toxicity assays.

Physical–chemical characterization of soil

Each soil sample was sieved through a 2 mm nylon mesh and dried at 40°C until a constant mass was obtained. The soil pH (KNO_3), electric conductivity (EC), organic matter (OM), and available nitrogen (N), phosphorus (P), and potassium (K) were determined through routine methods²⁰. The soluble Cu and zinc (Zn) were extracted using a 0.1 M KNO_3 solution²¹. To determine the total concentrations of Cu, Zn, lead (Pb), and arsenic (As) in the soil, the samples were subjected to a digestion process for 12 h involving boiling nitric acid, followed by the addition of perchloric acid. To prevent the volatilization of As during the digestion process, a Teflon stopper was employed along with a 30 cm glass reflux tube²². The total soil concentrations of Cu, Pb, and Zn were subsequently quantified using atomic absorption spectroscopy with the GBC SensAA system. The total As concentration was determined using an atomic absorption spectrophotometer—Thermo iCE 3000 series AA spectrometer (USA)—in conjunction with a hydride vapor generator (model VP100). To ensure the precision and accuracy of our analysis, duplicate digestion procedures were carried out on certified reference samples, namely, PACS-2 from the National Research Council of Canada and GRX-2 from the United States Geological Survey. The results obtained using these certified reference samples were accurate to within 10% of the certified values, thereby validating the reliability of our analytical approach.

Toxicity bioassay with *Lolium perenne*

The toxicity bioassay was based on the procedure outlined by Verdejo et al. (2015). The experimental unit consisted of 1 L plastic pots (11 cm lower diameter $\times$ 12 cm height $\times$ 15 cm upper diameter) wrapped in aluminum foil. The pots were filled with 480 g of sieved sample soils (2 mm) and moistened to 70% of their water-holding capacity (WHC), respectively. The soils were chemically stabilized by maintaining them in a greenhouse under controlled temperature conditions ($26 \pm 2^\circ\text{C}$) for three weeks; the soil humidity was monitored and controlled on a daily basis to adjust the WHC to 70%. After three weeks, 60 mg of *Lolium perenne* seeds were sown in each pot. The temperature and soil moisture levels were consistently maintained during the 28-day bioassay period. After 28 days of incubation, the *L.*

perenne plants were harvested and their shoot length (SL) and shoot dry mass (SDM) were determined as indicators of the plant's responses to soil pollutants. The procedure was performed in quintuplicate for each soil sample.

Statistical analysis

Multiple linear regression models were used to explain the variance of SL and SDM with the changes in the soil physicochemical properties. To achieve this, a sequential simplification was performed from a full model. The physicochemical properties that showed significant correlations with each dependent variable were considered for the full model. Highly correlated physicochemical properties were partly removed to avoid collinearity. The first model (i.e., the full model) was built using the variables obtained after evaluating correlations and collinearity. The selected variables were obtained after the sequential omission of insignificant variables based on insignificant results obtained with the model so as to create a model that only comprises significant explanatory variables. The normality and homoscedasticity of residuals was evaluated for the final model using the packages "nortest" and "lmtest", respectively. The multicollinearity of the model was evaluated using the function "vif" from the package "vegan". The effective concentrations of the metals at 25% and 50% (EC₂₅ and EC₅₀) were calculated using the Toxicity Relationship Analysis Program (TRAP) version 1.30a²³. To compute the effective concentration values, a control was established as the mean of the responses obtained in the background soil.

Results and Discussion

The mean and maximum EC soil values were 0.13 dS m⁻¹ and 0.32 dS m⁻¹ (Table 1), respectively, which is considered non-saline soil²⁴. These values are significantly lower than that recorded by Badilla-Ohlbaum et al.⁵ (2.3 dS m⁻¹) in a similar sampling area. The pH of the sampled soils varied from acidic (5.3) to neutral (7.2), averaging 6.3, which indicates slight acidity (Table 1). Badilla-Ohlbaum et al.⁵ reported a pH value of 7.6, which is higher than ours. However, the average pH value obtained in this study is in accordance with the background value for the region of 6.5, as reported by Luzio²⁵. The mean soil OM content was 2.4%, ranging from 1.3–4.3% (Table 1). Only two soils exhibited values exceeding the OM content reported by Luzio²⁵ for the region (3.4%). In contrast, Badilla-Ohlbaum et al.⁵ reported an OM content of 1.8%, which is slightly lower than our value. Considering the total soil Cu, Zn, and Pb (Table 1) concentrations, they are consistent with those reported by Badilla-Ohlbaum et al. (2001). Notably, only the total soil Cu concentration exhibited a wide range of variation (up to 24× higher). However, the soil soluble Cu content exhibited a negligible variation (less than 1×), with a mean value of 0.16 mg kg⁻¹, which is the same value obtained in a previous study by Schoffer et al. 2022 that was conducted in the same region.

Table 1
General physicochemical properties of background and agricultural polluted
topsoils under study (n = 13).

Soil property	Unit	Median	Mean $\pm$ SD*	Range
Electrical conductivity	dS m ⁻¹	0.10	0.13 $\pm$ 0.07	0.07–0.32
pH in KNO ₃		6.3	6.3 $\pm$ 0.57	5.3–7.2
Organic matter	%	2.2	2.4 $\pm$ 1.08	1.3–4.3
Available N	mg kg ⁻¹	20	22 $\pm$ 11	9.1–45
Available P	mg kg ⁻¹	30	36 $\pm$ 27	9.4–100
Available K	cmol + kg ⁻¹	0.61	0.68 $\pm$ 0.44	0.25–1.4
Total Cu	mg kg ⁻¹	452	487 $\pm$ 312	51–1245
Total As	mg kg ⁻¹	33	34 $\pm$ 13	17–65
Total Zn	mg kg ⁻¹	156	149 $\pm$ 26	79–189
Total Pb	mg kg ⁻¹	31	29 $\pm$ 5.2	17–36
Soluble Cu	mg kg ⁻¹	0.06	0.16 $\pm$ 0.25	0–0.92
Soluble Zn	mg kg ⁻¹	0.09	0.08 $\pm$ 0.07	0–0.24
Sand	%	34	34 $\pm$ 13	14–54
Clay	%	18	20 $\pm$ 6.9	10–31
Silt	%	46	46 $\pm$ 8.3	36–59
*SD, standard deviation				

As discussed previously, the topsoil in the study region is influenced by Cu-mining activity, as indicated by the polymetallic (Cu, Zn, As, and Pb) pollution detected in the soil, which is in agreement with corresponding literature related to the study area²⁶. However, we were unable to discriminate between mining and agricultural sources for the total Cu concentration. An inherent challenge in toxic assessments involving field-collected soils lies in determining the specific metal responsible for inducing the observed effects²⁷. Santa-Cruz et al.¹⁶, provided a comprehensive summary of the mean effective concentrations (EC₅₀) of Cu and Zn pertaining to the detrimental effects observed in plants grown in industrially contaminated soils. They reported EC₅₀ values of 987 mg kg⁻¹ for Cu and 1561 mg kg⁻¹ for Zn. In this study, the total soil Cu concentration was 51–1245 mg kg⁻¹, whereas the total Zn concentration was 79–189 mg kg⁻¹ (Table 1). Thus, the total Cu is expected to be toxic, whereas the total Zn is not. Notably, the total soil Cu exhibited a significant and negative effect on SDM ($F = 11$, $p = 0.002$, $R^2 = 0.15$) and SL ($F = 32$, $p < 0.001$, $R^2 = 0.33$), albeit with relatively low coefficients of determination. We were unable to find reference literature relating to a toxicity threshold for Pb (EC₅₀) pertaining to plants grown in field-contaminated soils. The total soil Pb was 17–36 mg kg⁻¹ and did not affect the plant responses ($p > 0.05$). Mojsilovic et al.²⁸ reported an EC₅₀ for As of 407 mg kg⁻¹, considering *Triticum aestivum* grown in field-contaminated soils—a toxicity threshold that is significantly higher than

the maximum concentration of arsenic found in our soil samples ($17\text{--}65\text{ mg kg}^{-1}$). Thus, we do not expect As to have toxic effects.

The SL of *L. perenne* is well explained by the total soil Cu concentrations, soil organic matter, soluble Zn, and available N, with all variables being significant ($p < 0.05$). Specifically, we found that the total soil Cu has a negative effect, whereas soluble Zn, soil organic matter, and available N have a positive effect on SL, as described by the following regression equation:

$$SL = 9.6 - 0.008\text{totalsoilCu} + 19\text{solubleZn} + 1.3\text{soilorganicmatter} + 0.088\text{availableN}; (R^2 = 0.53; p < 0.001) \quad (1)$$

where SL is in cm; total soil Cu, soluble Zn, and available N are in mg kg^{-1} ; and soil organic matter is in %. Notably, there was no collinearity among the independent variables according to the calculated variance inflation factors ($1.08\text{--}1.13$). Considering the SDM of *L. perenne*, the model that best fits the observed response is:

$$SDM = -0.01 - 0.0001\text{totalsoilCu} + 1.4\text{solubleZn} + 0.05\text{soilorganicmatter} + 0.005\text{soilclaycontent}; (R^2 = 0.58; p < 0.001) \quad (2)$$

where SDM is in g; total soil Cu and soluble Zn are in mg kg^{-1} ; and soil organic matter and soil clay content are in %. All the variables were significant ($p < 0.05$). The calculated variance inflation factors ($1.12\text{--}1.25$) indicated no collinearity among the independent variables.

From a biological perspective, both models are suitable for elucidating the response of *L. perenne*. In this context, the influence of excessive Cu on plants is primarily linked to the production and exposure of reactive oxygen species, which can interact with proteins, lipids, and DNA, resulting in oxidative harm and perturbing typical cellular functions²⁹. These disruptions ultimately translate into reduced plant yield and productivity³⁰. The beneficial impacts of soluble zinc on plants is twofold: first, Zn^{2+} competes with Cu^{2+} for binding sites on plant biotic ligands, as demonstrated by Liu et al.³¹; second, it protects plants from Cu-induced oxidative degradation by promoting antioxidant enzyme activity, as recently evidenced by Faizan et al.³² and Behtash et al.³⁰. Moreover, Zn enhances the production of photosynthetic pigments, thereby stimulating plant growth³³. The primary form of Cu in the soil is associated with soil OM³⁴. However, the rate at which soil elements are released from the solid phase into the soil solution decreases as the concentration of soil OM increases³⁵. Consequently, reducing the copper flux from the solid phase to the soil solution can reduce Cu phytoavailability³⁶. As shown in Eq. (1), the available N has a positive effect on SL. This is because N serves as a proline promoter³⁷. Proline—an osmoregulator amino acid—plays a crucial role in enhancing plant tolerance to Cu-induced oxidative stress by helping sustain plant sub-cell structures³⁸. Furthermore, proline has a pivotal function in maintaining the proper NADP/NADPH ratio to ensure effective carbon fixation, thereby alleviating the excess acidosis caused by Cu toxicity in the cytoplasm³⁹. Additionally, proline can also safeguard plant cells from Cu-induced oxidative damage by neutralizing reactive oxygen species⁴⁰. Soil clay content has a positive effect on SDM (Eq. (2)). Wu et al.⁴¹ established that Cu^{2+} is sequestered by clay components in soil after the removal of soil OM, highlighting its significant affinity for Cu^{2+} . This affinity can be primarily ascribed to the higher intensity of surface negative charges⁴², which results in low Cu bioavailability⁴³.

The calculated copper toxicity thresholds (EC_{50}) for SL and SDM were 1030 mg kg^{-1} and 1084 mg kg^{-1} , respectively (Fig. 3). These thresholds are in agreement with the average EC_{50} value for plants reported by Santa-Cruz et al.¹⁶ (987 mg kg^{-1}) in anthropogenically contaminated soils for various plant responses (Table 2). Additionally, this value is

consistent with the EC₅₀ value documented by Verdejo et al.¹⁷, thereby reinforcing the validity of our findings. Specifically, Verdejo et al.¹⁷ reported an EC₅₀ of 1031 mg kg⁻¹ for SL response using a similar *L. perenne* bioassay method in soils affected by copper mining operations (Table 2).

Table 2

Effective concentration at 50% (EC₅₀) of copper that causes a toxic effect in plants. We focused on studies that used anthropogenically contaminated soils rather than artificially contaminated (metal-spiked) soils.

Adapted from Santa-Cruz et al.¹⁶

Study	Soil origin	Copper source	Specie tested	Test duration (days)	Endpoint	Total Cu EC ₅₀ (mg kg ⁻¹)
Hamels et al. ⁴⁴	Sweden	Timber treatment (CuSO ₄)	<i>Hordeum vulgare</i> (Barley)	14	TDM	1260
Kolbas et al. ⁴⁵	France	Timber treatment (CuSO ₄)	<i>Helianthus annuus</i> (Sunflower)	28	CC	759
					ChITot	691
					LA	954
					RDM	677
					SDM	717
Verdejo et al. ¹⁷	Chile	Mining activities	<i>Lolium perenne</i> (Ryegrass)	21	SL	1031
					RL	1144
Verdejo et al. ¹⁹	Chile	Mining activities	<i>Lactuca sativa</i> (Letucce)	21	SL	1805
Mondaca et al. ¹⁸	Chile	Mining activities	<i>Avena sativa</i> (Oat)	21	SDM	1230
					SL	1802
				62	RDM	853
					SDM	798
					SL	889
			<i>Brassica rapa</i> (Turnip)	21	SDM	452
					SL	526
				42	RL	809
					SDM	506
					SL	616
					NSP	480
Kolbas et al. ⁴⁶	France	Timber treatment (CuSO ₄)	<i>Helianthus annuus</i> (Sunflower)	28	ChITot	329
					RDM	203
					SDM	333
					SL	407

CC: carotenoid content; ChITot: total chlorophyll; LA: leaf asymmetry; NSP: number of seed pods; RDM: root dry mass; RL: root length; SDM: shoot dry mass; SL: shoot length; TAC: total antioxidant capacity; TDM: total dry mass; TLA: total leaf area.

Study	Soil origin	Copper source	Specie tested	Test duration (days)	Endpoint	Total Cu EC ₅₀ (mg kg ⁻¹)
This study	Chile	Mining activities and Cu-based pesticide	<i>Lolium perenne</i> (Ryegrass)	26	TAC	301
					TLA	335
					SL	1030
					SDM	1084
CC: carotenoid content; ChlTot: total chlorophyll; LA: leaf asymmetry; NSP: number of seed pods; RDM: root dry mass; RL: root length; SDM: shoot dry mass; SL: shoot length; TAC: total antioxidant capacity; TDM: total dry mass; TLA: total leaf area.						

Conclusions

Our results underscore the intricate nature of metal toxicity in plants and emphasizes the importance of considering multiple factors for assessing its impact on plant growth and development in polymetallic contaminated soils. Cu is the primary driver of adverse growth responses in *Lolium perenne*. Notably, contrasting effects were observed between contaminants, such as between Cu and Zn, with the latter acting as a mitigator of Cu toxicity. Considering this finding, further studies are required to evaluate the alleviating effect of Zn on Cu toxicity in field-contaminated soils using a more sensitive bioindicator, such as earthworms. The EC₅₀ for total soil Cu concentration was determined to be 1030 mg kg⁻¹ and 1084 mg kg⁻¹ for SL and SDM, respectively. This finding is important in the field of ecotoxicology, as the obtained EC₅₀ value aligns with results documented in previous studies using naturally contaminated soils. Furthermore, the unique contribution of this study is emphasized by the fact that few prior investigations have attempted to establish copper toxicity thresholds for plants grown in real-world contaminated soils that are polluted by different copper sources (Table 2).

Declarations

Data availability

The datasets used and/or analyzed during the study can be obtained from the corresponding author upon reasonable request.

Author contribution

J. T. S.: Conceptualization, methodology, formal analysis, writing—original draft, funding acquisition. H. A.: Writing—review and editing, formal analysis. A. N.: Conceptualization, writing—review and editing, supervision, funding acquisition. L. M. F.: Investigation. R. G.: Conceptualization, methodology, formal analysis, writing—review and editing, supervision, funding acquisition.

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Competing interests

The authors declare no competing interests.

References

1. Schoffer, J. T. *et al.* Copper content in soils and litter from fruit orchards in Central Chile and its relationship with soil microbial activity. *Plant, Soil Environ.* 68, 115–128 (2022).
2. ODEPA-CIREN. Catástro frutícola, Región de O'Higgins, Principales resultados. 55 <https://bibliotecadigital.odepa.gob.cl/bitstream/handle/20.500.12650/71122/Ohiggins202109.pdf?sequence=1&isAllowed=y> (2021).
3. Cacciuttolo, C. & Cano, D. Environmental Impact Assessment of Mine Tailings Spill Considering Metallurgical Processes of Gold and Copper Mining: Case Studies in the Andean Countries of Chile and Peru. *Water (Switzerland)* 14, (2022).
4. Casanova, M., Salazar, O., Seguel, O. & Luzio, W. *The Soils of Chile*. (Springer Netherlands, 2013). doi:10.1007/978-94-007-5949-7.
5. Badilla-Ohlbaum, R. *et al.* Relationship between soil copper content and copper content of selected crop plants in central Chile. *Environ. Toxicol. Chem.* 20, 2749–2757 (2001).
6. Sereni, L., Guenet, B. & Lamy, I. Mapping risks associated with soil copper contamination using availability and bio-availability proxies at the European scale. *Environ. Sci. Pollut. Res.* 30, 19828–19844 (2023).
7. Clarkson, A. H., Paine, S. W. & Kendall, N. R. Evaluation of the solubility of a range of copper sources and the effects of iron & sulphur on copper solubility under rumen simulated conditions. *J. Trace Elem. Med. Biol.* 68, 126815 (2021).
8. Richardson, H. W. The manufacture of copper compounds. in *Handbook of Copper Compounds and Applications* (ed. Richardson, H. W.) 53–92 (Mercel Dekker, 1997).
9. Skeaff, J. M., Thibault, Y. & Hardy, D. J. A new method for the characterisation and quantitative speciation of base metal smelter stack particulates. *Environ. Monit. Assess.* 177, 165–192 (2011).
10. SAG. Declaración de ventas de plaguicidas de uso agrícola año 2019. (2019).
11. Paradelo, M., Perez-Rodríguez, P., Arias-Estévez, M. & López-Periago, J. E. Effect of particle size on copper oxychloride transport through saturated sand columns. *J. Agric. Food Chem.* 58, 6870–6875 (2010).
12. Paradelo, M., Šimůnek, J., Novoa-Muñoz, J. C., Arias-Estévez, M. & Eugenio Lopez-Periago, J. Transport of copper oxychloride-based fungicide particles in saturated quartz sand. *Environ. Sci. Technol.* 43, 8860–8866 (2009).
13. Richardson, H. W. Copper fungicides/bactericides. in *Handbook of Copper Compounds and Applications* (ed. Richardson, H. W.) 93–122 (Marcel Dekker, 1997).
14. Santa-Cruz, J. *et al.* Metal ecotoxicity studies with artificially contaminated versus anthropogenically contaminated soils: Literature review, methodological pitfalls and research priorities. *Russ. J. Ecol.* 52, 479–485 (2021).
15. McBride, M. B. & Cai, M. Copper and zinc aging in soils for a decade: changes in metal extractability and phytotoxicity. *Environ. Chem.* 13, 160–167 (2016).

16. Santa-Cruz, J., Peñaloza, P., Korneykova, M. & Neaman, A. Thresholds of metal and metalloid toxicity in field-collected anthropogenically contaminated Soils: A review. *Geogr. Environ. Sustainability* 14, 6–21 (2021).
17. Verdejo, J., Ginocchio, R., Sauv , S., Salgado, E. & Neaman, A. Thresholds of copper phytotoxicity in field-collected agricultural soils exposed to copper mining activities in Chile. *Ecotoxicol. Environ. Saf.* 122, 171–177 (2015).
18. Mondaca, P., Catrin, J., Verdejo, J., Sauv , S. & Neaman, A. Advances on the determination of thresholds of Cu phytotoxicity in field-contaminated soils in central Chile. *Environ. Pollut.* 223, 146–152 (2017).
19. Verdejo, J., Ginocchio, R., Sauv , S. & Neaman, A. Thresholds of copper toxicity to lettuce in field-collected agricultural soils exposed to copper mining activities in Chile. *J. Soil Sci. Plant Nutr.* 16, 154–158 (2016).
20. Sadzawka, A. *et al. M todos de suelo recomendados para los suelos de Chile. Revisi n 2006.* (INIA, 2006).
21. Stuckey, J. W., Neaman, A., Ravella, R., Komarneni, S. & Mart nez, C. E. Highly charged swelling mica reduces free and extractable Cu levels in Cu-contaminated soils. *Environ. Sci. Technol.* 42, 9197–9202 (2008).
22. Sadzawka, A. *et al. M todos de an lisis de lodos y de suelos. Sociedad Chilena de la Ciencia del Suelo.* (Universidad de Concepci n, 2015).
23. US EPA. Toxicity Relationship Analysis Program (TRAP) version 1.30a United States Environmental Protection Agency, Mid-Continent Ecology Division. (2015) doi:http://www.epa.gov/med/Prods_Pubs/trap.html.
24. Gartley, K. Recommended Methods for Measuring Soluble Salts in Soils. in *Recommended Soil Testing Procedures for the Northeastern United States* (ed. Horton, M.) 87–94 (Northeastern Regional Publication N  493, 2011).
25. Luzio, W. *Suelos de Chile.* (Universidad de Chile, 2010).
26. Badilla-Ohlbaum, R. *et al.* Relationship between soil copper content and copper content of selected crop plants in central Chile. *Environ. Toxicol. Chem.* 20, 2749–2757 (2001).
27. Y  ez, C. *et al.* Microbial responses are unreliable indicators of copper ecotoxicity in soils contaminated by mining activities. *Chemosphere* 300, 134517 (2022).
28. Mojsilovic, O., McLaren, R. G. & Condon, L. M. Modelling arsenic toxicity in wheat: Simultaneous application of diffusive gradients in thin films to arsenic and phosphorus in soil. *Environ. Pollut.* 159, 2996–3002 (2011).
29. Adrees, M. *et al.* The effect of excess copper on growth and physiology of important food crops: a review. *Environ. Sci. Pollut. Res.* 22, 8148–8162 (2015).
30. Behtash, F. *et al.* Zinc application mitigates copper toxicity by regulating Cu uptake, activity of antioxidant enzymes, and improving physiological characteristics in summer squash. *Antioxidants* 11, (2022).
31. Liu, Y., Vijver, M. G. & Peijnenburg, W. J. G. M. Comparing three approaches in extending biotic ligand models to predict the toxicity of binary metal mixtures (Cu-Ni, Cu-Zn and Cu-Ag) to lettuce (*Lactuca sativa* L.). *Chemosphere* 112, 282–288 (2014).
32. Faizan, M. *et al.* Zinc oxide nanoparticles (ZnO-NPs) induce salt tolerance by improving the antioxidant system and photosynthetic machinery in tomato. *Plant Physiol. Biochem.* 161, 122–130 (2021).
33. Aravind, P. & Prasad, M. N. V. Zinc protects chloroplasts and associated photochemical functions in cadmium exposed *Ceratophyllum demersum* L., a freshwater macrophyte. *Plant Sci.* 166, 1321–1327 (2004).
34. Brunetto, G. *et al.* Copper accumulation in vineyard soils: Rhizosphere processes and agronomic practices to limit its toxicity. *Chemosphere* 162, 293–307 (2016).
35. Rachou, J., Hendershot, W. & Sauv , S. Soil organic matter impacts upon fluxes of cadmium in soils measured using diffusive gradients in thin films. *Commun. Soil Sci. Plant Anal.* 38, 1619–1636 (2007).
36. Stuckey, J. W. *et al.* Zinc Alleviates Copper Toxicity to Lettuce and Oat in Copper-Contaminated Soils. *J. Soil Sci. Plant Nutr.* 21, 1229–1235 (2021).

37. Shabbir, Z. *et al.* Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. *Chemosphere* 259, 127436 (2020).
38. Nazir, F., Hussain, A. & Fariduddin, Q. Hydrogen peroxide modulate photosynthesis and antioxidant systems in tomato (*Solanum lycopersicum* L.) plants under copper stress. *Chemosphere* 230, 544–558 (2019).
39. Hayat, S. *et al.* Role of proline under changing environments: A review. *Plant Signal. Behav.* 7, (2012).
40. Tripathi, B. N. & Gaur, J. P. Relationship between copper- and zinc-induced oxidative stress and proline accumulation in *Scenedesmus* sp. *Planta* 219, 397–404 (2004).
41. Wu, J., Laird, D. A. & Thompson, M. L. Sorption and Desorption of Copper on Soil Clay Components. *J. Environ. Qual.* 28, 334–338 (1999).
42. Rieuwerts, J. S. The mobility and bioavailability of trace metals in tropical soils: A review. *Chem. Speciat. Bioavailab.* 19, 75–85 (2007).
43. Kumar, V. *et al.* Copper bioavailability, uptake, toxicity and tolerance in plants: A comprehensive review. *Chemosphere* 262, 127810 (2021).
44. Hamels, F., Malevé, J., Sonnet, P., Kleja, D. B. & Smolders, E. Phytotoxicity of trace metals in spiked and field-contaminated soils: Linking soil-extractable metals with toxicity. *Environ. Toxicol. Chem.* 33, 2479–2487 (2014).
45. Kolbas, A., Marchand, L., Herzig, R., Nehnevajova, E. & Mench, M. Phenotypic seedling responses of a metal-tolerant mutant line of sunflower growing on a Cu-contaminated soil series: potential uses for biomonitoring of Cu exposure and phytoremediation. *Plant Soil* 376, 377–397 (2014).
46. Kolbas, A., Kolbas, N., Marchand, L., Herzig, R. & Mench, M. Morphological and functional responses of a metal-tolerant sunflower mutant line to a copper-contaminated soil series. *Environ. Sci. Pollut. Res.* 25, 16686–16701 (2018).

Figures

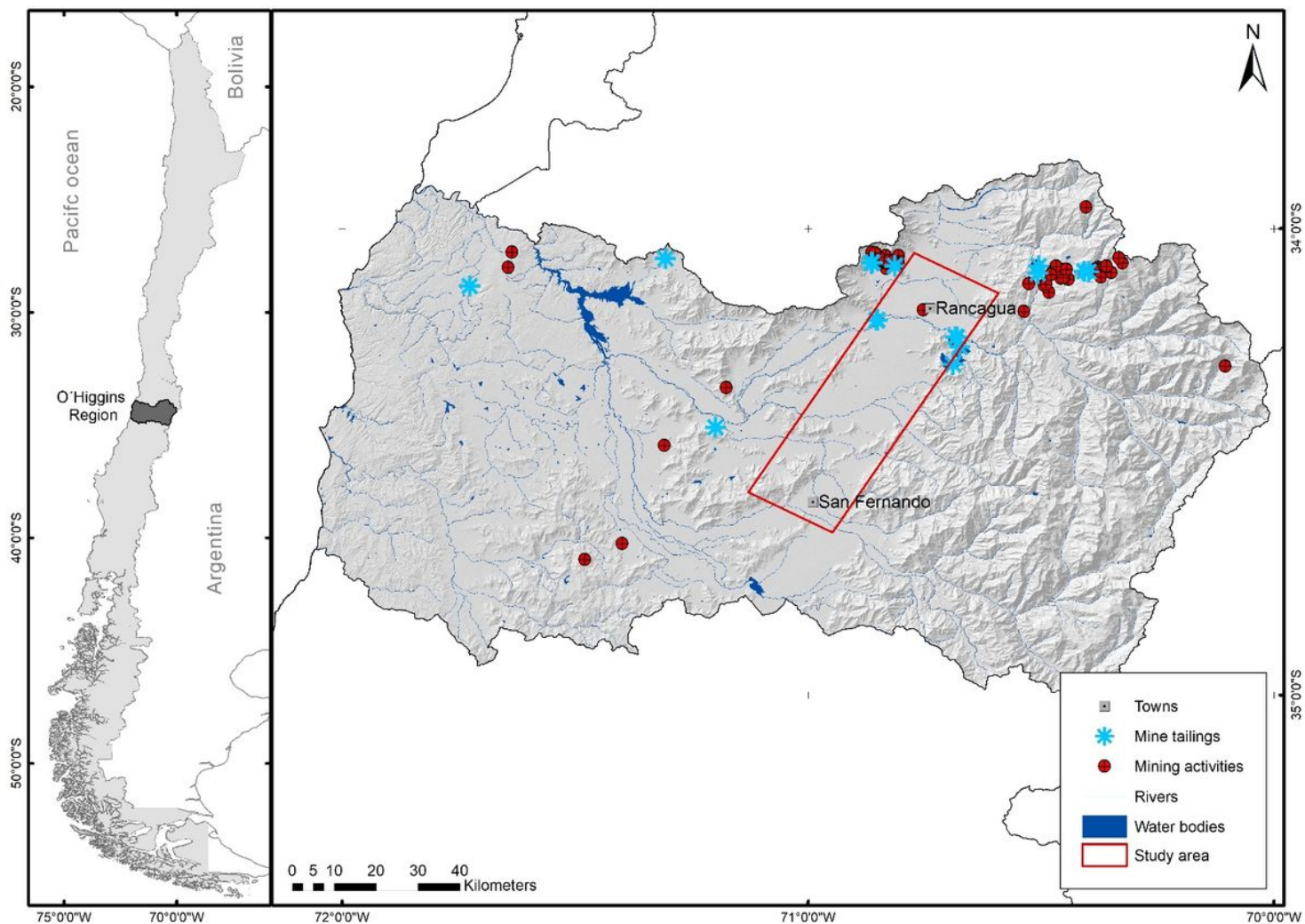


Figure 1

General location of the study area in the O'Higgins Region, central Chile (red square). Location of copper mine pollution sources (mine tailings and mine activities) inside the region are shown as well.

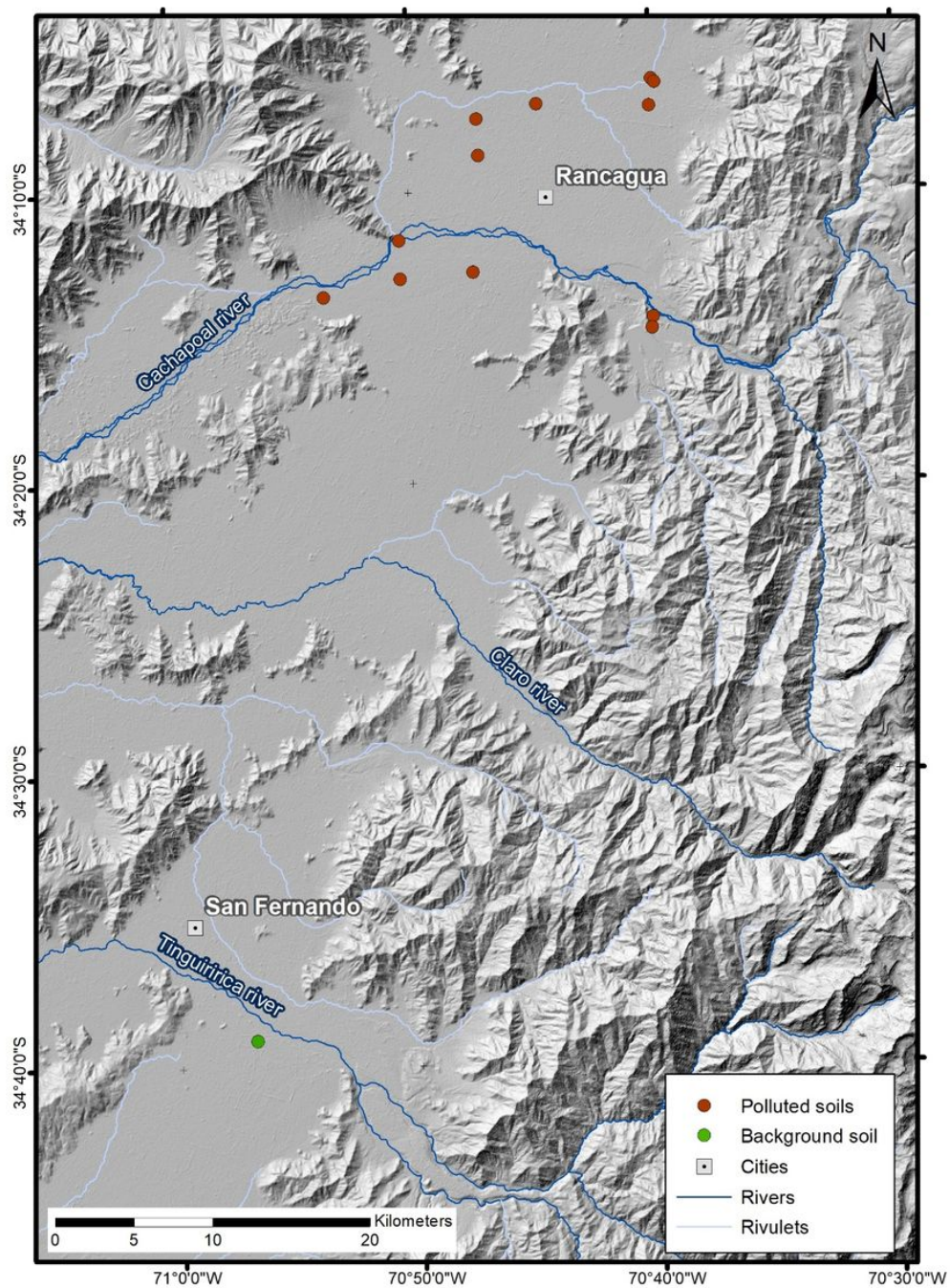


Figure 2

Topsoil sampling locations in agricultural lands located in the lowlands of the study area (O'Higgins Region, central Chile).

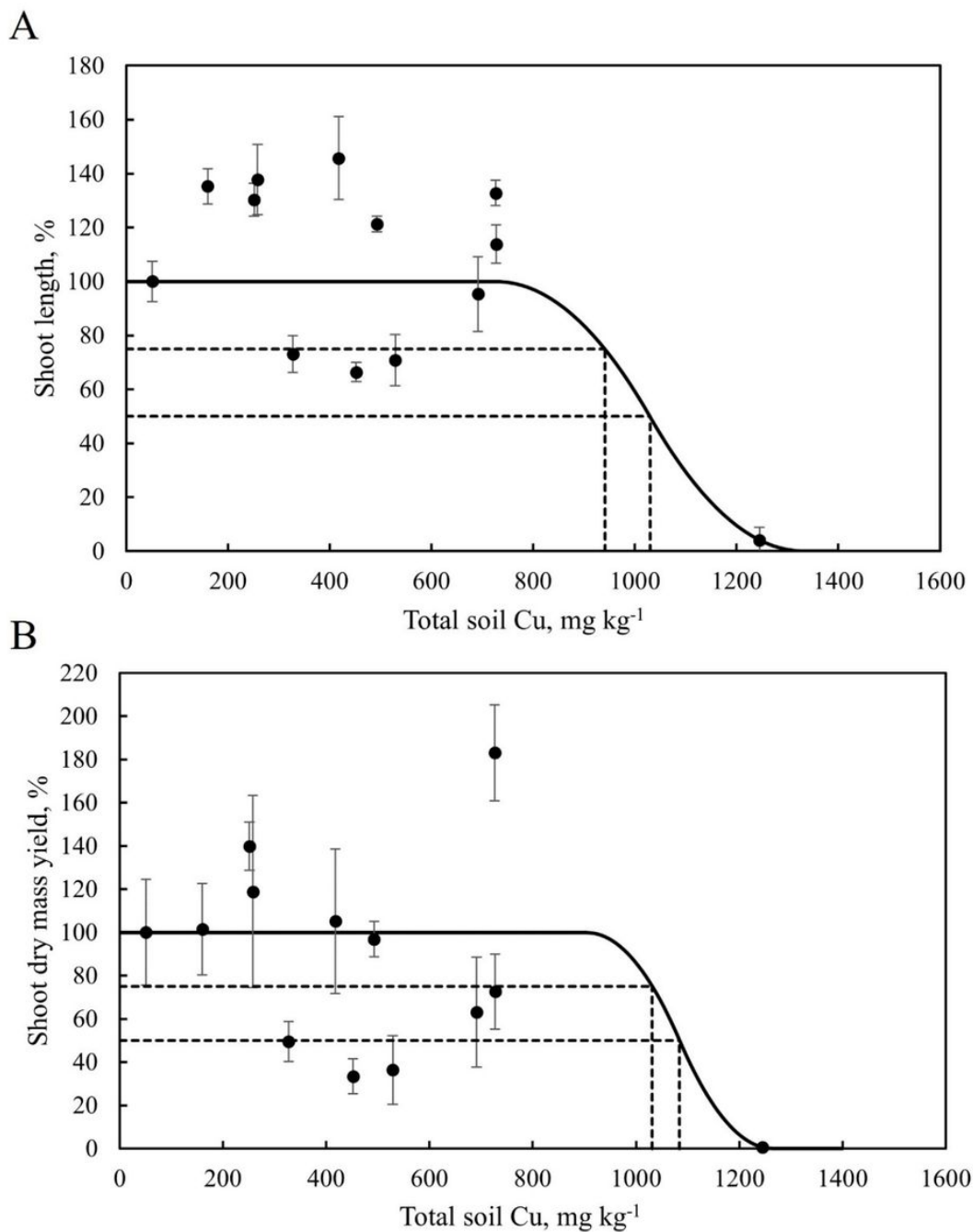


Figure 3

Lolium perenne shoot length response (A) and shoot dry mass response (B) as a function of total soil Cu. The responses of *Lolium perenne* were assessed by calculating the ratio of the average observed response in the studied soils to the average response in the background soil, which is expressed as a percentage. The effective concentrations at 25% (SL EC₂₅: 941 mg kg⁻¹; SDM EC₂₅: 1032 mg kg⁻¹) and 50% (SL EC₅₀: 1030 mg kg⁻¹; SDM EC₂₅: 1084 mg kg⁻¹) are also shown.