IJANR
International Journal
of Agriculture
and Natural Resources

Int. J. Agric. Nat. Resour. 51(3):204-218. 2024
www.ijanr.cl

SOIL MANAGEMENT

DOI 10.7764/ijanr.v51i3.2627

RESEARCH ARTICLE

Copper bioavailability and phytotoxicity in Chilean agricultural soils: implications for sustainable fruit production

Rosanna Ginocchio[1,2], Humberto Aponte[3,4], Alexander Neaman[5], Luz María de la Fuente[1] and J. Tomás Schoffer[1,6]

[1] Center of Applied Ecology and Sustainability (CAPES), Pontificia Universidad Católica de Chile, Santiago, Chile.

[2] Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía y Sistemas Naturales, Pontificia Universidad Católica de Chile, Santiago, Chile.

[3] Institute of Agri-Food, Animal and Environmental Sciences (ICA3), Universidad de O'Higgins, San Fernando 3070000, Chile Agroalimentarias, Animales y Ambientales (ICA3), Universidad de O'Higgins, Rancagua, Chile.

[4] Centre of Systems Biology for Crop Protection (BioSaV), Institute of Agri-Food, Animal and Environmental Sciences (ICA3), Universidad de O'Higgins, San Fernando 3070000, Chile.

[5] Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Arica, Chile.

[6] Núcleo de Investigación en Sustentabilidad Agroambiental (NISUA), Escuela de Agronomía, Facultad de Medicina Veterinaria y Agronomía, Universidad de Las Américas.

An earlier version of the manuscript is available as a preprint on Research Square under the title 'Evaluation of Cu toxicity in agricultural topsoil contaminated by different sources in central Chile' (https://doi.org/10.21203/rs.3.rs-3937690/v1).

Abstract

R. Ginocchio, H. Aponte, A. Neaman, L. M. de la Fuente, and J. T. Schoffer. 2024. Copper bioavailability and phytotoxicity in Chilean agricultural soils: implications for sustainable fruit production. Int. J. Agric. Nat. Resour. Central Chile's lowland valleys constitute a major fruit-producing region, but they face soil copper (Cu) contamination originating from Cu mining in the nearby Andes Mountains as well as the historical and ongoing use of Cu-based pesticides. This study investigated the potential toxicity of Cu to plants (phytotoxicity) in a representative fruit-growing valley. To assess this risk, soil samples were collected from 12 agricultural sites with documented Cu contamination and one uncontaminated site to serve as a baseline. A short-term bioassay was conducted using perennial ryegrass (*Lolium perenne*) to evaluate the effects of Cu on plant growth. The results demonstrated that elevated Cu levels significantly reduced both the shoot length and dry mass of the ryegrass plants. However, other soil properties, such as the presence of soluble zinc, organic matter, available nitrogen, and clay content, could mitigate these negative effects. Interestingly, the estimated concentration of Cu causing a 50% reduction in plant growth (EC_{50}) was greater than the values reported in previous studies. This may be because Cu binds to soil organic matter (SOM), which reduces its

---

Received Jun 24, 2024. Accepted Nov 12, 2024
Corresponding author: jschoffer@udla.cl

bioavailability and immediate toxicity to plants. While this binding to SOM can initially reduce the negative effects of Cu, the eventual breakdown of SOM over time may release Cu back into the soil, posing long-term risks to both crops and the wider soil ecosystem. To fully understand these potential long-term impacts, further research is needed and should include studies with other soil organisms, such as earthworms and microorganisms, to gain a more comprehensive understanding of the ecological consequences of Cu contamination in these vital agricultural systems.

Keywords: Copper phytotoxicity, *Lolium perenne*, soil contamination, copper bioavailability, soil organic matter, effective concentration (EC_{50}), mining impacts, agricultural pollution, Chilean agriculture, soil health, environmental risk

Highlights

- High levels of copper (Cu) contamination in Chilean agricultural soils, stemming from mining activities and pesticide use, significantly reduce plant growth.

- Soil properties such as organic matter, soluble zinc, available nitrogen, and clay content mitigate Cu-induced plant toxicity.

- The study confirmed EC_{50} values for copper toxicity consistent with previous reports, providing further validation of these thresholds in real-world contaminated soils.

- Copper's binding to soil organic matter limits current bioavailability, but changes in agricultural management practices may result in copper solubilization in soil over time thus posing latent risks to crops and soil ecosystems.

- Results emphasize the importance of balancing Cu pesticide use with measures to maintain soil health and ensure sustainable agricultural practices.

Introduction

Central Chile, with its Mediterranean climate, is the country's primary intensive fruit-producing region (Schoffer et al., 2022). The O'Higgins Region, specifically its lowland valleys, is home to extensive fruit orchards covering 95,082 hectares. Stone fruits dominate production (46%), followed by table grapes (13%) and nuts (12%) (ODEPA-CIREN, 2021). However, this vital agricultural area faces a significant environmental challenge: copper (Cu) contamination in the soil. This pollution stems from two main sources. First, Cu mining operations in the nearby Andes Mountains release pollutants that can be carried by wind and water to the lowlands (Cacciuttolo & Cano, 2022). Second, the long-term use of Cu-based pesticides in fruit production has led to a legacy of Cu accumulation in the soil (Casanova et al., 2013).

The severity of this contamination is evident in the wide range of Cu concentrations found in the soil. Research by Badilla-Ohlbaum et al. (2001) in the Cachapoal Valley, an area downstream from the El Teniente copper mine and Caletones smelter, revealed total Cu concentrations ranging from 26 mg kg^{-1} to as high as 1,600 mg kg^{-1}. More recently, Schoffer et al. (2022) focused on fruit orchards within the O'Higgins Region and reported total Cu concentrations between 131 and 432 mg kg^{-1}, which were attributed primarily to the historical and ongoing use of Cu-based pesticides.

This Cu contamination poses significant environmental and public health risks, particularly in regions where mining and agriculture coexist (Tapia-Gatica et al., 2022). Copper is persistent in the environment and can accumulate in the food chain, affecting plant and soil health, with potential consequences for human health through the consumption of contaminated food (Georgopoulos et al., 2001). The widespread use of Cu-based pesticides, coupled with industrial emissions from mining, further elevates soil Cu levels. This problem is exacerbated by the tendency of Cu to bind strongly to organic matter (OM) in the soil (Brunetto et al., 2016), which prolongs its presence in the environment.

The solubility of Cu is a critical factor determining its mobility, bioavailability, and toxicity to plants (phytotoxicity) in soil (Sereni et al., 2023). These factors are influenced by specific soil characteristics (Kabata-Pendias, 2011). Importantly, the bioavailability of Cu varies depending on its source. Mining activities introduce Cu, mainly in the form of relatively insoluble minerals such as chalcopyrite ($CuFeS_2$) and chalcosine (Cu_2S) (Badilla-Ohlbaum et al., 2001; Clarkson et al., 2021). In contrast, smelters emit Cu primarily as copper sulfate ($CuSO_4$), a highly soluble compound (Skeaff et al., 2011; Richardson, 1997b).

In agricultural settings, Cu-based pesticides are a major contributor to soil contamination. The most commonly used pesticides in Chile include the Bordeaux mixture ($CuSO_4 + Ca(OH)_2$), copper hydroxide ($Cu(OH)_2$), and copper oxychloride ($CuCl_2 \cdot 3Cu(OH)_2$), which account for approximately 26%, 24%, and 20% of total Cu-based pesticide applications, respectively (SAG, 2019). These pesticides differ in their solubility and persistence in soil. The Bordeaux mixture, while initially highly soluble and bioavailable, degrades relatively quickly. In contrast, copper hydroxide and copper oxychloride have limited solubility and are less mobile in the soil environment (Richardson, 1997a; Paradelo et al., 2009; Paradelo et al., 2010).

Currently, product label instructions are the sole source of guidance for the application of Cu-based pesticides in Chile, as there are no national regulations defining maximum soil application rates (Neaman et al., 2024). This lack of regulatory oversight can lead to Cu applications exceeding limits set by other frameworks, such as those in the European Union.

The EU, while recognizing the environmental concerns associated with Cu use, still considers Cu a vital tool for managing fungal and bacterial diseases in agriculture due to the limited availability of equally effective alternatives. To balance this need with the risk of Cu accumulation in soils, the EU has implemented a restricted annual limit of 4 kg Cu ha^{-1} (Commission Implementing Regulation (EU) 2018/1981). However, studies in Chile have reported Cu application rates of 6.8 kg Cu ha^{-1} in plum orchards and 4.5 kg Cu ha^{-1} in cherry orchards (Schoffer et al., 2022), highlighting the potential for excessive Cu buildup in Chilean soils. This discrepancy underscores the urgent need for Chile to evaluate and potentially revise its Cu use practices in agriculture. A balanced approach is needed to ensure effective pest and disease control while safeguarding long-term soil health and environmental sustainability.

Adding another layer of complexity, research by Santa Cruz et al. (2021a, 2021b) has demonstrated that the toxicity of metals, including Cu, can differ significantly between soils artificially contaminated in a laboratory setting and those contaminated through real-world industrial activities. This difference arises because metal toxicity is influenced not only by concentration but also by the duration of exposure to the bioavailable fraction of the metal in the soil, a process known as ‘aging’ (McBride & Cai, 2016).

Considering the multiple sources of Cu contamination in the intensive fruit-producing valleys of the O’Higgins Region and the potential for variations in Cu bioavailability and phytotoxicity among these sources, this study aimed to assess the phytotoxic effects of accumulated Cu in the topsoil of a representative fruit-growing valley in central Chile.

Materials and Methods

Study sites and soil samples

To assess the phytotoxic effects of Cu in the O'Higgins Region, topsoil samples were collected from 12 heavily cultivated agricultural sites. These sites were strategically selected based on existing knowledge of the distribution of total Cu concentrations within the Cachapoal River Basin (Figures 1 and 2). To establish a baseline for comparison, an additional soil sample was collected from an agricultural area near Chimbarongo with a low Cu concentration (51 mg kg^{-1}).

All the soils included in this study are of alluvial origin and are classified as Entisols (Soil Survey Staff, 2022). Importantly, recent analyses have shown consistent climatic conditions across the O'Higgins Region (Navarro-Hasse et al., 2024), ensuring that climate factors do not influence variations in soil properties between sites. While the Chimbarongo sample provided a useful reference point, it is important to acknowledge that background Cu concentrations can vary across the Cachapoal Valley. Previous research by Badilla-Ohlbaum et al. (2001) reported background Cu levels ranging from 26 mg kg^{-1} in minimally impacted areas to 1,600 mg kg^{-1} in heavily impacted areas near the El Teniente Cu mine and the Caletones smelter. Based on this, a background Cu concentration range of 26 to 51 mg kg^{-1} can be considered representative of areas with minimal Cu input.

The sampling strategy aimed to capture a wide range of total Cu concentrations, including both background levels and those elevated due to agricultural practices (pesticide use) and industrial activities (mining). At each site, a substantial soil sample (40-60 kg) was collected from a 2-m² area to a depth of 20 cm to ensure sufficient material for the subsequent toxicity assays.

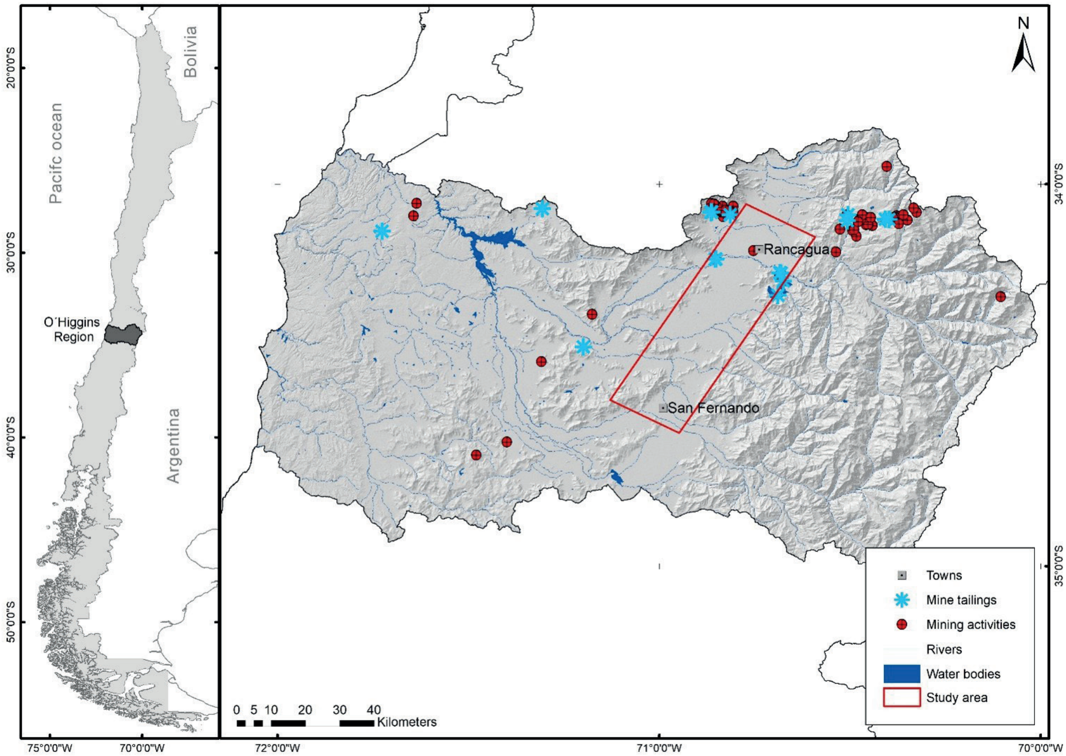

Figure 1. Location of the study area within the O'Higgins Region, central Chile (red square), showing copper mine tailings and active mining areas.

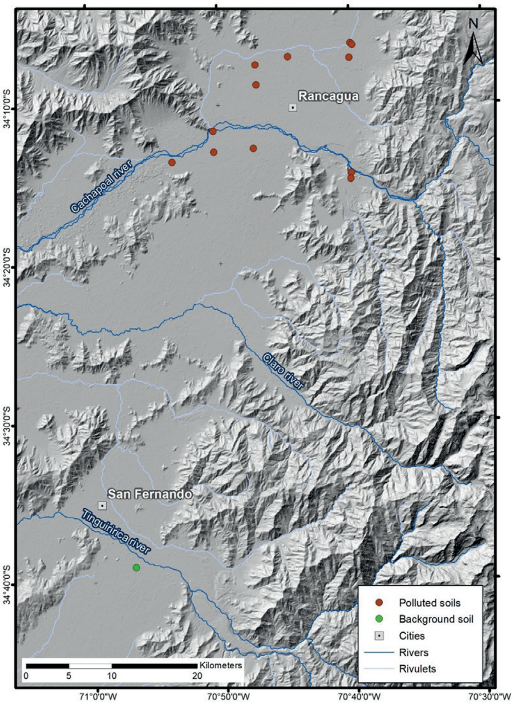

Figure 2. Distribution of topsoil sampling sites across agricultural lands in the O'Higgins Region, central Chile.

Analysis of soil physicochemical properties

To prepare the soil samples for analysis, they were first passed through a 2-mm nylon mesh sieve to remove any large debris and then dried at 40 °C until a constant weight was achieved. Standard methods (Sadzawka et al., 2006) were used to determine several key soil properties, including pH (KNO_3), electrical conductivity (EC), OM content, and the levels of available nutrients, specifically nitrogen (N), phosphorous (P), and potassium (K).

Soluble Cu and zinc (Zn) were extracted from the soil samples via a 0.1 M KNO_3 solution (Stuckey et al., 2008). To measure the total concentrations of Cu, Zn, lead (Pb), and arsenic (As) in the soil, a more rigorous digestion process was necessary. This involved digesting the soil samples in boiling nitric acid for 12 h, followed by the addition of perchloric acid. To prevent the loss of As due to volatilization during this digestion process, a Teflon stopper and a 30-cm glass reflux tube were used (Sadzawka et al., 2015).

The total concentrations of Cu, Pb, and Zn in the digested soil solutions were then measured via atomic absorption spectroscopy with a GBC SensAA system. The total As concentration was determined using a Thermo iCE 3000 series atomic absorption spectrometer equipped with a hydride vapor generator (model VP100). To ensure the accuracy of our elemental analyses, we included quality control measures in the form of duplicate digestions of certified reference materials: PACS-2 (from the National Research Council of Canada) and GRX-2 (from the United States Geological Survey). The results obtained for these reference materials were all within 10% of their certified values, confirming the reliability and accuracy of the analytical methods used in this study.

Toxicity bioassay with Lolium perenne

The phytotoxicity of the soil samples was assessed via a bioassay adapted from the procedure described by Verdejo et al. (2015). The experimental setup consisted of 1-L transparent plastic pots (11-cm lower diameter × 12-cm height × 15-cm upper diameter) wrapped in aluminum foil to prevent light-induced effects on root development (van Gelderen et al., 2018).

Each pot was filled with 480 g of the sieved (2 mm) soil sample and moistened to 70% of its water-holding capacity (WHC). To stabilize the soil chemically, the pots were kept in a greenhouse under controlled-temperature conditions (26 ± 2 °C) for three weeks. The soil moisture was meticulously monitored and adjusted daily to maintain a target WHC of 70%. Following this equilibration period, 60 mg of *Lolium perenne* seeds were sown in each pot. The specific variety used was 'Belinda', a commercial tetraploid Italian ryegrass obtained from ANASAC, a reputable Chilean agricultural company. Throughout the 28-d bioassay, which included a 7-d germination period for the *L. perenne* seeds, the temperature and soil moisture were carefully maintained at the established levels.

After 28 d, the *L. perenne* plants were harvested. Shoot length (SL) and shoot dry mass (SDM) were measured as indicators of plant response to potential soil pollutants. The entire procedure was replicated five times (quintuplicate) for each soil sample to ensure robust and reliable data.

Statistical analysis and data treatment

Multiple linear regression models were used to explain the variance in SL and SDM based on changes in the soil physicochemical properties. A sequential simplification process was performed, starting with a full model that included all physicochemical properties significantly correlated with each dependent variable. Highly correlated physicochemical properties were partially removed to avoid collinearity. The initial full model was then simplified by sequentially omitting insignificant variables until the final model contained only significant explanatory variables. The normality and homoscedasticity of the residuals for the final model were evaluated using the "nortest" and "lmtest" packages, respectively. Multicollinearity in the model was assessed using the "vif" function from the "vegan" package.

The effective concentrations of metals at 25% and 50% (EC_{25} and EC_{50}) were calculated via the Toxicity Relationship Analysis Program (TRAP) version 1.30a (US EPA, 2015). To compute these effective concentrations, a control was established as the mean of the responses obtained in the background soil.

Results and Discussion

Analysis of the soil samples revealed the following key physicochemical characteristics

The mean electrical conductivity (EC) value was 0.13 dS m^{-1}, with a maximum of 0.32 dS m^{-1} (Table 1). These values fall well below the threshold for saline soils (Gartley, 2011) and are notably lower than the 2.3 dS m^{-1} reported by Badilla-Ohlbaum et al. (2001) for a similar sampling area. These findings suggest that salinity was not a major factor influencing the soil conditions in this study.

Table 1. General physicochemical properties of background (n =1) and agricultural polluted (n =12) topsoils under study.

Soil property	Agricultural soils				Background soil[b]
	Unit	Median	Mean ± SD[a]	Range	
Electrical conductivity	dS m^{-1}	0.11	0.13±0.08	0.07-0.32	0.07
pH in KNO_3		6.3	6.4±0.49	5.7-7.2	5.3
Organic matter	%	2.2	2.5±1.09	1.3-4.4	1.5
Available N	mg kg^{-1}	21	23±10	9.9-45	9.1
Available P	mg kg^{-1}	30	37±28	9.4-100	24
Available K	cmol+kg^{-1}	0.63	0.71±0.44	0.25-1.4	0.29
Total Cu	mg kg^{-1}	472	523±296	160-1245	51
Total As	mg kg^{-1}	35	35±13	17-65	18
Total Zn	mg kg^{-1}	156	155±15	129-189	79
Total Pb	mg kg^{-1}	31	30±3.9	24-36	17
Soluble Cu	mg kg^{-1}	0.08	0.17±0.27	0-0.92	0
Soluble Zn	mg kg^{-1}	0.07	0.08±0.07	0-0.24	0.13
Sand	%	33	33±13	14-54	47
Clay	%	19	20±6.9	10-31	13
Silt	%	47	47±8.5	36-59	41

a SD = Standard deviation; b Average of duplicates

The soil pH ranged from 5.3 to 7.2, with an average of 6.3 (Table 1), indicating slightly acidic conditions. While Badilla-Ohlbaum et al. (2001) reported a relatively high average pH of 7.6 for the region, the average pH in this study aligns with the regional background value of 6.5 reported by Luzio (2010).

The mean soil organic matter (OM) content was 2.4%, ranging from 1.3% to 4.3% (Table 1). Only two sites exceeded the regional average OM content of 3.4% reported by Luzio (2010). Badilla-Ohlbaum et al. (2001) reported a slightly lower OM content of 1.8% in their study.

The concentrations of total soil Cu, Zn, and Pb (Table 1) were generally consistent with those reported by Badilla-Ohlbaum et al. (2001). The most significant variation was observed in total soil Cu levels, which were up to 24 times greater in some samples than in previous reports. However, soluble Cu levels showed minimal variation (less than 1× difference) and averaged 0.16 mg kg^{-1}, in agreement with findings by Schoffer et al. (2022).

Phytotoxicity assessment using L. perenne

The average SL was 12 ± 4.8 cm, with a range of 0–20 cm. The average SDM was 282 ± 171 mg, ranging from 0–739 mg. These variations likely reflect the diverse soil conditions and contamination levels across the sampling sites.

The presence of multiple metals (Cu, Zn, As, and Pb) in the topsoil samples confirms the influence of both Cu mining and pesticide use in the region, which is consistent with previous reports (Badilla-Ohlbaum et al., 2001; Schoffer et al., 2022). However, it is important to acknowledge the challenges in isolating the effects of individual metals on plants and other soil organisms in field-collected soils because of the complex interactions within the soil matrix (Yáñez et al., 2022).

The total soil Cu concentrations ranged from 51 to 1245 mg kg^{-1}. Considering that Santa Cruz et al. (2021a) reported an EC_{50} value of 987 mg kg^{-1} for Cu in plants grown in industrially contaminated soils, the levels observed in this study suggest the potential for Cu toxicity. This is supported by the significant effects of total soil Cu on both the SDM ($F = 16, p < 0.001, R^2 = 0.20$) and SL ($F = 53, p < 0.001, R^2 = 0.46$), although the relatively low determination coefficients indicate that other factors also contribute to the observed variation in plant growth.

The total Zn concentrations (79–189 mg kg^{-1}) were well below the reported EC_{50} of 1561 mg kg^{-1} (Santa Cruz et al., 2021a), suggesting that Zn toxicity was unlikely to be a major factor in this study.

No significant toxic effects on plant growth were observed for Pb ($p > 0.05$) at the measured total soil Pb concentrations (17–36 mg kg^{-1}). This is not unexpected, as there are no established reference thresholds for Pb toxicity in field-grown plants. Similarly, the As concentrations in the study area (17–65 mg kg^{-1}) were considerably lower than the EC_{50} of 407 mg kg^{-1} reported for *Triticum aestivum* (Mojsilovic et al., 2011), indicating that As likely had a minimal toxic impact on plant growth in this study.

To understand the factors influencing plant growth in copper-contaminated soils, we conducted a statistical analysis of the ryegrass bioassay data. This analysis revealed the following key relationships:

The following model best explained the variation in shoot length:

$$SL = 9.6 - 0.008\ (\text{total soil Cu}) + 19\ (\text{soluble Zn}) + 1.3\ (\text{SOM}) + 0.088\ (\text{available N})$$

where SL is measured in centimeters; total soil Cu, soluble Zn, and available N are in mg kg^{-1}; and SOM is expressed as a percentage.

This model, with an R^2 of 0.53 ($p < 0.001$), indicates that four factors — total soil Cu, soluble Zn, SOM, and available N — explained 53% of the observed variability in SL. Importantly, there was no evidence of collinearity among these predictor variables, as confirmed by variance inflation factors (VIFs) ranging from 1.08 to 1.13. This suggests that each variable makes an independent contribution to explaining SL.

The best-fit model for SDM was as follows:

$$\text{SDM} = -0.01 - 0.0001\ (\text{total soil Cu}) + 1.4\ (\text{soluble Zn}) + 0.05\ (\text{SOM}) + 0.006\ (\text{soil clay content})$$

where SDM is measured in grams; total soil Cu and soluble Zn are in mg kg^{-1}; and the SOM and soil clay contents are expressed as percentages.

This model had an R^2 of 0.58 ($p < 0.001$), indicating that the included factors accounted for 58% of the variation in the SDM. All variables in the model were statistically significant ($p < 0.05$), and the VIFs (1.12–1.25) confirmed the absence of collinearity.

These models provide valuable insights into the complex interactions that govern plant growth in soils contaminated with Cu. As expected, total soil Cu had a negative effect on both SL and SDM, underscoring its phytotoxic potential. However, the presence of Zn, N, and OM can help mitigate these negative effects.

The detrimental impact of excess Cu on plants primarily stems from the generation of reactive oxygen species (ROS). These ROS can damage essential cellular components such as proteins, lipids, and DNA, leading to oxidative stress and disrupting normal cellular functions (Adrees et al., 2015). This oxidative damage ultimately results in reduced plant growth and productivity (Behtash et al., 2022).

In contrast, soluble Zn exerts beneficial effects on plants through two primary mechanisms: Competition**:** Zn ions compete with Cu ions for binding sites on plant roots and within plant tissues, effectively reducing Cu uptake and its associated toxicity (Liu et al., 2014). Antioxidant activity**:** Zn promotes the activity of antioxidant enzymes in plants, which helps protect them from the oxidative damage caused by excess Cu (Cakmak, 2000; Upadhyay and Panda, 2010; Faizan et al., 2021; Behtash et al., 2022). Furthermore, Zn is an essential component in the production of photosynthetic pigments, which are crucial for capturing light energy and driving plant growth (Aravind et al., 2004).

The relationship between OM and Cu is complex. While Cu primarily exists in association with OM in soil (Brunetto et al., 2016), OM can influence Cu bioavailability in various ways. On the one hand, OM can bind Cu, reducing its release into the soil solution and thus its phytoavailability (Rachou et al., 2007; Stuckey et al., 2021). However, the effects of OM on Cu solubility and bioavailability also depend on the soil pH. In acidic soils (pH ~5.0), OM tends to hold Cu in the solid phase, limiting its solubility and availability to plants. Conversely, in neutral to alkaline soils (pH >7), OM can increase Cu solubility by forming complexes with dissolved organic carbon (DOC), potentially increasing the bioavailability of Cu (Sauvé et al., 1997; Tipping et al., 2003).

Our study encompassed a soil pH range of 5.3 to 7.2, spanning both acidic and neutral-to-alkaline conditions. Previous research by Schoffer et al. (2022) in soils with a similar pH range revealed little variation in soluble Cu concentrations, with an average of 0.16 mg kg^{-1}. This is consistent with our findings of consistently low soluble Cu concentrations across our soil samples. Considering the pH range in our study, it is likely that OM acts primarily to stabilize Cu in the soil by forming stable complexes with SOM rather than increasing its solubility by forming complexes with DOC. This would explain the consistently low concentrations of soluble Cu observed in our soils. Interestingly, we found a positive correlation between the OM content and soluble Cu ($r = 0.57$; $p < 0.05$), suggesting that OM may contribute to Cu solubility, possibly through the formation of DOC–Cu complexes, even within the observed pH range. However, the lack of a significant correlation between the OM content and pH indicates that the role of OM in Cu stabilization or solubilization may not be directly influenced by soil pH within the range encountered in our study.

As demonstrated in Equation (1), available N positively influences SL. This is because N is crucial for promoting the production of proline (Shabbir et al., 2020), an amino acid that functions as an important osmoregulator in plants. Proline helps maintain cell turgor and water balance, which are essential for plant growth and development. Furthermore, proline enhances plant tolerance to Cu-induced stress in several ways. It helps maintain the structural integrity of plant cells, preserves the NADP/NADPH ratio necessary for efficient carbon fixation (thereby mitigating the excess acidosis caused by Cu toxicity in the cytoplasm), and directly neutralizes ROS, protecting plant cells from oxidative damage (Nazir et al., 2019; Hayat et al., 2012; Tripathi et al., 2004).

Equation (2) reveals a positive relationship between the soil clay content and the SDM. This finding is supported by previous research showing that clay particles in the soil can effectively bind and immobilize Cu ions, particularly in soils with low OM contents. This binding capacity is attributed to the high density of negative charges on clay surfaces, which reduces the availability of Cu to plants (Wu et al., 1999; Rieuwerts, 2007; Kumar et al., 2021).

Our study determined the Cu toxicity thresholds (EC_{50}) for SL and SDM to be 1030 mg kg^{-1} and 1084 mg kg^{-1}, respectively (Figure 3). These values are consistent with the average EC_{50} value of 987 mg kg^{-1} reported by Santa Cruz et al. (2021a) for plants grown in anthropogenically contaminated soils, as well as the EC_{50} value of 1031 mg kg^{-1} for SL response reported by Verdejo et al. (2015) using a similar *L. perenne* bioassay in soils affected by Cu mining. This agreement between our findings and those of previous studies strengthens the validity of our results and highlights the consistency of Cu toxicity thresholds across different studies.

While our study indicates that Cu toxicity thresholds in soil may be as high as 1000 mg kg^{-1} without causing immediate, easily detectable harm to plant growth, this should not be misinterpreted as a complete absence of risk at lower concentrations. Importantly, the bioavailability and toxicity of metals in soil are not static; they are constantly influenced by a complex web of interactions between soil properties and environmental factors (Liu et al., 2017).

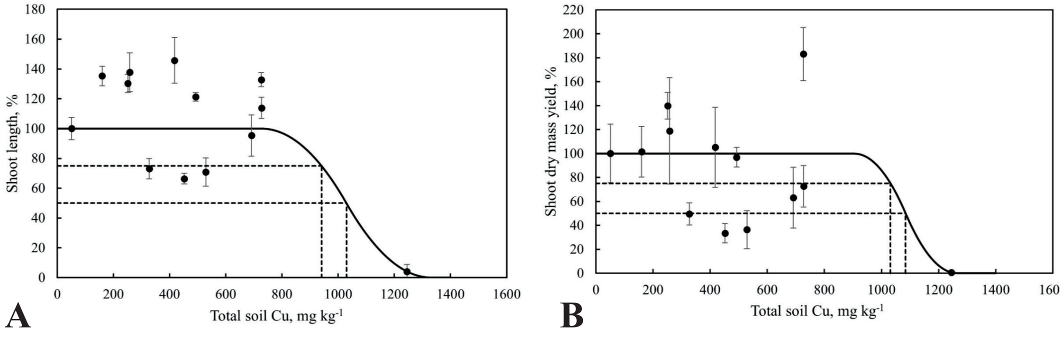

Figure 3. *Lolium perenne* shoot length response (A) and shoot dry mass response (B) as a function of total soil Cu. The responses of *Lolium perenne* were assessed by calculating the ratio of the average observed response in the studied soils to the average response in the background soil, which is expressed as a percentage. The effective concentrations at 25% (SL EC_{25}: 941 mg kg^{-1}; SDM EC_{25}: 1032 mg kg^{-1}) and 50% (SL EC_{50}: 1030 mg kg^{-1}; SDM EC_{25}: 1084 mg kg^{-1}) are also shown.

The concept of “chemical time bombs” (CTBs) highlights the potential for delayed risks associated with metals such as Cu (Held, 1998). When Cu is bound to SOM, it may seem harmless. However, as SOM decomposes into DOC (Tipping et al., 2003), or if environmental conditions change (Hekstra, 1992), this bound Cu can be released, increasing its bioavailability and posing a potential threat to soil organisms and crops. Furthermore, the increasing use of newer Cu-based pesticide formulations containing nanoparticles introduces additional challenges. These nanoparticles can increase Cu mobility within plant tissues (Tamez et al., 2019), potentially leading to Cu accumulation in plants and greater interactions with soil organisms. The heightened mobility and potential for accumulation have implications for both the health of the soil ecosystem and the safety of the food crops grown in these soils.

Adding another layer of complexity, it is important to consider that Cu toxicity in soil is not determined solely by its concentration; the duration of exposure, or ‘aging’, also plays a significant role. As the aging process occurs, the bioavailable fraction of metals such as Cu gradually decreases. This is because metals bind to more stable soil components, such as iron and manganese oxides, becoming less accessible to plants and other organisms (Guo et al., 2011). Consequently, freshly contaminated soils (such as those created in a laboratory setting) often display greater metal toxicity than soils contaminated over time through industrial activities or agricultural practices (Santa-Cruz et al., 2021b). While the aging process can reduce the immediate toxicity of Cu, the decreased bioavailability does not eliminate long-term risks. Shifts in soil properties, such as fluctuations in pH or declines in OM content, have the potential to remobilize Cu, leading to delayed adverse effects on the soil ecosystem and crops.

Therefore, while our findings offer valuable insights into Cu toxicity thresholds, it is crucial to maintain a broader perspective that considers the dynamic nature of Cu bioavailability and the potential for long-term risks. Continuous monitoring of soil conditions and the implementation of adaptive management strategies are essential for ensuring the sustainability of soil health and crop production in areas with Cu contamination.

Conclusions

Our study highlights the complex interplay of factors that influence metal toxicity in plants. This underscores the importance of considering multiple interacting factors when assessing the impacts of contamination by multiple metals (polymetallic contamination) on plant growth and development.

In our study, Cu emerged as the primary driver of the negative effects on the growth of *Lolium perenne*. However, we also observed that other metals, such as Zn, can mitigate Cu toxicity. This complex interaction between different metals warrants further investigation, potentially via the use of more sensitive bioindicators such as earthworms.

Our research established EC_{50} values for total soil Cu of 1030 mg kg^{-1} for SL and 1084 mg kg^{-1} for SDM. These findings are significant within the field of ecotoxicology, as they align with results documented in previous studies using naturally contaminated soils.

A key contribution of our study is the establishment of Cu toxicity thresholds for plants growing in soils contaminated under real-world conditions, where multiple sources of Cu contribute to the overall contamination. This provides a more realistic assessment of Cu toxicity in complex field environments, which has significant implications for both environmental risk assessment and the development of sustainable agricultural management practices.

Acknowledgment

We express our sincere gratitude to the Center of Applied Ecology and Sustainability (CAPES) for providing the opportunity to conduct this research and for the financial support received through projects ANID PIA/BASAL AFB240003 and ANID/FONDECYT 3220026. We also gratefully acknowledge the support of the ANID/FONDECYT 3210752 project.

Resumen

R. Ginocchio, H. Aponte, A. Neaman, L. M. de la Fuente, y J.T. Schoffer. 2024. Disponibilidad de cobre y fitotoxicidad en suelos agrícolas chilenos: implicancias para la producción sostenible de frutas. Int. J. Agric. Nat. Resour. 204-218. La producción frutícola intensiva en la zona central de Chile, concentrada en los valles de la depresión intermedia, se ve desafiada por la contaminación del suelo con cobre (Cu), originada tanto por la actividad minera en la Cordillera de los Andes como por la aplicación histórica y actual de pesticidas cúpricos. En este estudio, se evaluó la fitotoxicidad del Cu acumulado en el horizonte superficial del suelo de un valle representativo de cultivo frutícola intensivo. Se analizaron muestras de suelo de 12 sitios agrícolas con contaminación por Cu, contrastándolas con un sitio de referencia sin contaminación, mediante un bioensayo con *Lolium perenne*. Los resultados revelaron que, si bien las altas concentraciones de Cu impactan negativamente el crecimiento vegetal (longitud y masa seca del vástago), propiedades del suelo como el zinc soluble, la materia orgánica, el nitrógeno disponible y el contenido de arcilla ejercen un efecto mitigador sobre la toxicidad del Cu. La concentración efectiva 50 (CE50) para el Cu total en el suelo fue de 1030 mg kg^{-1} para la longitud del vástago y 1084 mg kg^{-1} para la masa seca del vástago, valores que superan a los reportados previamente en la literatura. Es crucial considerar que el Cu puede encontrarse mayormente asociado a la materia orgánica del suelo (MOS), lo que limita su biodisponibilidad. No obstante, la degradación de la MOS podría conllevar a la liberación de Cu, constituyendo un riesgo latente para los cultivos y la biota edáfica. Se propone la realización de estudios adicionales que incluyan otros organismos del suelo (e.g., lombrices de tierra, microbiota) para una evaluación integral de la toxicidad del Cu en estos agroecosistemas.

Palabras clave: Biodisponibilidad, CE$_{50}$, cobre, contaminación del suelo, fitotoxicidad, *Lolium perenne*, materia orgánica del suelo, minería, pesticidas.

References

Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Zia-ur-Rehman, M., Irshad, M. K. & Bharwana, S. A. (2015). The effect of excess copper on growth and physiology of important food crops: a review. *Environmental Science and Pollution Research*, *22*(11), 8148-8162. https://doi.org/10.1007/s11356-015-4496-5

Aravind, P. & Prasad, M. N. V. (2004). Zinc protects chloroplasts and associated photochemical functions in cadmium exposed *Ceratophyllum demersum* L., a freshwater macrophyte. *Plant Science*, *166*(5), 1321-1327. https://doi.org/10.1016/j.plantsci.2004.01.011

Badilla-Ohlbaum, R., Ginocchio, R., Rodríguez, P. H., Céspedes, A., González, S., Allen, H. E. & Lagos, G. E. (2001). Relationship between soil copper content and copper content of selected crop plants in central Chile. *Environmental Toxicology and Chemistry*, *20*(12), 2749-2757. https://doi.org/10.1002/etc.5620201214

Behtash, F., Abedini, F., Ahmadi, H., Mosavi, S.

B., Aghaee, A., Morshedloo, M. R. & Lorenzo, J. M. (2022). Zinc application mitigates vopper toxicity by regulating Cu uptake, activity of antioxidant enzymes, and improving physiological characteristics in summer squash. *Antioxidants*, *11*(9). https://doi.org/10.3390/antiox11091688

Brunetto, G., Bastos de Melo, G. W., Terzano, R., Del Buono, D., Astolfi, S,. Tomasi, N., Pii, Y., Mimmo, T. & Cesco, S. (2016). Copper accumulation in vineyard soils: Rhizosphere processes and agronomic practices to limit its toxicity. *Chemosphere*, *162*, 293-307. https://doi.org/10.1016/j.chemosphere.2016.07.104

Cacciuttolo, C. & Cano, D. (2022). 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*(19). https://doi.org/10.3390/w14193057

Cakmak, I. (2000). Tansley review No. 111 - possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytologist*. 146, 185-205. https://doi.org/10.1046/j.1469-8137.2000.00630.x

Casanova, M., Salazar, O., Seguel, O. & Luzio, W. (2013). *The Soils of Chile* (Hartemink, A.E. (ed.)). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-007-5949-7

Clarkson, A. H., Paine, S. W. & Kendall, N. R. (2021). Evaluation of the solubility of a range of copper sources and the effects of iron & sulphur on copper solubility under rumen simulated conditions. *Journal of Trace Elements in Medicine and Biology*, *68*, 126815. https://doi.org/10.1016/j.jtemb.2021.126815

Commission Implementing Regulation (EU) 2018/1981 of 13 December 2018 Renewing the Approval of the Active Substances Copper Compounds, as Candidates for Substitution, in Accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Co, L 317 Official Journal of the European Union 16 (2018).

Faizan, M., Bhat, J. A., Chen, C., Alyemeni, M. N., Wijaya, L., Ahmad, P. & Yu, F. (2021). Zinc oxide nanoparticles (ZnO-NPs) induce salt tolerance by improving the antioxidant system and photosynthetic machinery in tomato. *Plant Physiology and Biochemistry*, *161*, 122-130. https://doi.org/10.1016/j.plaphy.2021.02.002

Gartley, K. (2011). Recommended methods for measuring moluble salts in soils. In Horton, M. (Ed.), *Recommended Soil Testing Procedures for the Northeastern United States* (3rd ed., pp. 87-94). Northeastern Regional Publication Nº 493. http://extension.udel.edu/lawngarden/files/2012/10/CHAP10.pdf

Georgopoulos, P. G., Roy, A., Yonone-Lioy, M. J., Opiekun, R. E. & Lioy, P. J. (2001). Environmental copper: Its dynamics and human exposure issues. Journal of *Toxicology and Environmental Health* - Part B: Critical Reviews, 4(4), 341-394. https://doi.org/10.1080/109374001753146207

Guo, G.,Yuan, T. Wang, W. Li, D. & Wang, J. (2011). Effect of aging on bioavailability of copper on the fluvo aquic soil. *International Journal of Environmental Science & Technology*, 8(4), 715-722. https://doi.org/10.1007/BF03326256

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J. & Ahmad, A. (2012). Role of proline under changing environments: A review. *Plant Signaling and Behavior*, *7*(11). https://doi.org/10.4161/psb.21949

Hekstra, G. P. (1992). Can climate change trigger non-linear and time- delayed responses to pollutants stored in soils , sediments and sround water ? In N. H. Batjes & E. M. Bridges (Eds.), *World lnventory of Soil Emission Potentials. Proceedings of an International Workshop organized in the framework of the Netherlands National Research Programme on Global Air Pollution and Climate Change (NOP). WISE Report No. 2, International Soil Referen* (pp. 11-17). International Soil Reference and Information Centre.

Held, T. (1998). Heavy Metals: Chemical Time Bombs in Urban Soils. In U. O. Breuste J., Feldmann H. (Ed.), *Urban Ecology* (pp. 156-158). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-88583-9_27

Kabata-Pendias, A. (2011). Trace Elements in Soils and Plants (A. Kabata-Pendias (ed.); 4th editio). CRC Press. https://doi.org/10.1201/b10158

Kumar, V., Pandita, S., Singh Sidhu, G. P., Sharma, A., Khanna, K., Kaur, P., Bali, A. S. & Setia, R. (2021). Copper bioavailability, uptake, toxicity and tolerance in plants: A comprehensive review. *Chemosphere*, *262*, 127810. https://doi.org/10.1016/j.chemosphere.2020.127810

Liu, Y., Vijver, M. G. & Peijnenburg, W. J. G. M. (2014). 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. https://doi.org/10.1016/j.chemosphere.2014.04.077

Liu, Y., Du, Q., Wang, Q.,Yu, H., Liu, J., Tian, Y., Chang, C. & Lei, J. (2017). Causal inference between bioavailability of heavy metals and environmental factors in a large-scale region. *Environmental Pollution*, 226, 370-378. https://doi.org/10.1016/j.envpol.2017.03.019

Luzio, W. (2010). *Suelos de Chile*. Santiago: Universidad de Chile.

McBride, M. B. & Cai, M. (2016). Copper and zinc aging in soils for a decade: changes in metal extractability and phytotoxicity. *Environmental Chemistry*, *13*(1), 160-167. https://doi.org/10.1071/EN15057

Mojsilovic, O., McLaren, R. G. & Condron, L. M. (2011). Modelling arsenic toxicity in wheat: Simultaneous application of diffusive gradients in thin films to arsenic and phosphorus in soil. *Environmental Pollution*, *159*(10), 2996-3002. https://doi.org/10.1016/j.envpol.2011.04.022

Navarro-Hasse, E., Kelm, U., Jerez, O., Carolina, Y. & Neaman, A. (2024). Influence of parent rock on soil clay mineralogy and physicochemical properties: a case study from the O'Higgins region, central Chile. *Soil Research, 62*, SR24011. https://doi.org/10.1071/SR24011

Nazir, F., Hussain, A. & Fariduddin, Q. (2019). Hydrogen peroxide modulate photosynthesis and antioxidant systems in tomato (*Solanum lycopersicum* L.) plants under copper stress. *Chemosphere*, *230*, 544-558. https://doi.org/10.1016/j.chemosphere.2019.05.001

Neaman, A., Schoffer, J. T., Navarro-Villarroel, C., Pelosi, C., Peñaloza, P., Dovletyarova, E. A. & Schneider, J. (2024). Copper contamination in agricultural soils: A review of the effects of climate, soil properties, and prolonged copper pesticide application in vineyards and orchards. *Plant, Soil and Environment*, *70*(7), 407-417. https://doi.org/10.17221/501/2023-PSE

ODEPA-CIREN. (2021). *Catástro frutícola, Región de O'Higgins, Principales resultados*. https://bibliotecadigital.odepa.gob.cl/bitstream/handle/20.500.12650/71122/Ohiggins202109.pdf?sequence=1&isAllowed=y

Paradelo, M., Šimůnek, J., Novoa-Muñoz, J. C., Arias-Estevez, M. & Eugenio Lopez-Periago, J. (2009). Transport of copper oxychloride-based fungicide particles in saturated quartz sand. *Environmental Science and Technology*, *43*(23), 8860-8866. https://doi.org/10.1021/es901650g

Paradelo, M., Perez-Rodríguez, P., Arias-Estévez, M. & López-Periago, J. E. (2010). Effect of particle size on copper oxychloride transport through saturated sand columns. *Journal of Agricultural and Food Chemistry*, *58*(11), 6870-6875. https://doi.org/10.1021/jf100367b

Rachou, J., Hendershot, W. & Sauvé, S. (2007). Soil organic matter impacts upon fluxes of cadmium in soils measured using diffusive gradients in thin films. *Communications in Soil Science and Plant Analysis*, *38*(11-12), 1619-1636. https://doi.org/10.1080/00103620701380165

Richardson, H. W. (1997a). Copper fungicides/bactericides. In Richardson H. W. (Ed.), *Handbook of Copper Compounds and Aplications* (pp. 93-122). Marcel Dekker.

Richardson, H. W. (1997b). The manufacture of copper compunds. In Richardson H. W. (Ed.), *Handbook of Copper Compounds and Aplications* (pp. 53-92). Mercel Dekker.

Rieuwerts, J. S. (2007). The mobility and bioavailability of trace metals in tropical soils: A review. *Chemical Speciation and Bioavailability*, *19*(2), 75-85. https://doi.org/10.3184/095422907X211918

Sadzawka, A., Carrasco, M. A., Demanet, R., Flores, H., Mora, M. L., Neaman, A., Hernández, P. & Sandoval, M. (2015). *Métodos de análisis de lo-*

dos y de suelos. Sociedad Chilena de la Ciencia del Suelo. Universidad de Concepción.

Sadzawka, A., Carrasco, M., Grez, R., Mora, M., de la L. Flores, H. & Neaman, A. (2006). *Métodos de suelo recomendados para los suelos de Chile. Revisión 2006*. INIA.

SAG. (2019). *Declaración de ventas de plaguicidas de uso agrícola año 2019*. https://www.sag.gob.cl/sites/default/files/declaracion_de_ventas_de_plaguicidas_ano_2019_0.pdf

Santa-Cruz, J., Peñaloza, P., Korneykova, M. & Neaman, A. (2021a). Thresholds of metal and metalloid toxicity in field-collected anthropogenically contaminated Soils: A review. *Geography, Environment, Sustentability*, *14*(2), 6-21. https://doi.org/10.24057/2071-9388-2021-023

Santa-Cruz, J., Vasenev, I., Gaete, H., Peñaloza, P., Krutyakov, Y. A. & Neaman, A. (2021b). Metal ecotoxicity studies with artificially contaminated versus anthropogenically contaminated soils: Literature review, methodological pitfalls and research priorities. *Russian Journal of Ecology*, *52*(6), 479-485. https://doi.org/10.1134/S1067413621060126

Sauvé, S., McBride, M. B., Norvell, W. A. & Hendershot, W. H. (1997). Copper solubility ans speciation of in situ contaminated soils: Effects of copper level, pH and organica matter. *Water, Air, and Soil Pollutio*n, 10, 133-149. https://doi.org/10.1023/A:1018312109677

Schoffer, J. T., Aponte, H., Neaman, A., de la Fuente, L. M., Arellano, E. C., Gil, P. M. & Ginocchio, R. (2022). Copper content in soils and litter from fruit orchards in Central Chile and its relationship with soil microbial activity. *Plant, Soil and Environment*, *68*(3), 115-128. https://doi.org/10.17221/281/2021-PSE

Sereni, L., Guenet, B. & Lamy, I. (2023). Mapping risks associated with soil copper contamination using availability and bio-availability proxies at the European scale. *Environmental Science and Pollution Research*, *30*(8), 19828-19844. https://doi.org/10.1007/s11356-022-23046-0

Shabbir, Z., Sardar, A., Shabbir, A., Abbas, G., Shamshad, S., Khalid, S., Natasha Murtaza, G., Dumat, C. & Shahid, M. (2020). Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. *Chemosphere*, *259*, 127436. https://doi.org/10.1016/j.chemosphere.2020.127436

Skeaff, J. M., Thibault, Y. & Hardy, D. J. (2011). A new method for the characterisation and quantitative speciation of base metal smelter stack particulates. *Environmental Monitoring and Assessment*, *177*, 165-192. https://doi.org/10.1007/s10661-010-1627-9

Soil Survey Staff. 2022. *Keys to Soil Taxonomy*. 13th ed. Washington, DC: United States Department of Agriculture, Natural Resources Conservation Service.

Stuckey, J. W., Neaman, A., Ravella, R., Komarneni, S. & Martínez, C. E. (2008). Highly charged swelling mica reduces free and extractable Cu levels in Cu-contaminated soils. *Environmental Science and Technology*, *42*(24), 9197-9202. https://doi.org/10.1021/es801799s

Stuckey, J. W., Neaman, A., Verdejo, J., Navarro-Villarroel, C., Peñaloza, P. & Dovletyarova, E. A. (2021). Zinc Alleviates Copper Toxicity to Lettuce and Oat in Copper-Contaminated Soils. *Journal of Soil Science and Plant Nutrition*, *21*(2), 1229-1235. https://doi.org/10.1007/s42729-021-00435-x

Tamez, C., Morelius, E. W., Hernandez-Viezcas, J. A., Peralta-Videa, J. R. & Gardea-Torresdey, J. (2019). Biochemical and physiological effects of copper compounds/nanoparticles on sugarcane (Saccharum officinarum). *Science of the Total Environment*, 649, 554-562. https://doi.org/10.1016/j.scitotenv.2018.08.337

Tapia-Gatica, J., Selles, I., Bravo, M. A., Tessini, C., Barros-Parada, W., Novoselov, A., & Neaman, A. (2022). Global issues in setting legal limits on soil metal contamination: A case study of Chile. *Chemosphere*, 290. https://doi.org/10.1016/j.chemosphere.2021.133404

Tipping, E., Rieuwerts, J., Pan, G., Ashmore, M. R., Lofts, S., Hill, M. T. R., Farago, M. E., & Thornton, I. (2003). The solid-solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales. Environmental Pollution, 125(2), 213-225. https://doi.org/10.1016/S0269-7491(03)00058-7

Tripathi, B. N. & Gaur, J. P. (2004). Relationship between copper- and zinc-induced oxidative stress and proline accumulation in *Scenedesmus sp*. *Planta*, *219*(3), 397-404. https://doi.org/10.1007/s00425-004-1237-2

Upadhyay, R., Panda, S.K., 2010. Zinc reduces copper toxicity induced oxidative stress by promoting antioxidant defense in freshly grown aquatic duckweed Spirodela polyrhiza L. J. Hazard Mater. 175, 1081-1084. https://doi.org/10.1016/j.jhazmat.2009.10.016

US EPA. (2015). *Toxicity Relationship Analysis Program (TRAP) version 1.30a United States Environmental Protection Agency, Mid- Continent Ecology Division*. https://doi.org/http://www.epa.gov/med/Prods_ Pubs/trap.html

van Gelderen, K., Kang, C., & Pierik, R. (2018). Light signaling, root development, and plasticity. *Plant Physiology*, 176(2), 1049-1060. https://doi.org/10.1104/pp.17.01079

Verdejo, J., Ginocchio, R., Sauvé, S., Salgado, E. & Neaman, A. (2015). Thresholds of copper phytotoxicity in field-collected agricultural soils exposed to copper mining activities in Chile. *Ecotoxicology and Environmental Safety*, *122*, 171-177. https://doi.org/10.1016/j.ecoenv.2015.07.026

Wu, J., Laird, D. A. & Thompson, M. L. (1999). Sorption and desorption of copper on soil clay components. *Journal of Environmental Quality*, *28*(1), 334-338. https://doi.org/10.2134/jeq1999.00472425002800010041x

Yáñez, C., Verdejo, J., Moya, H., Donoso, P., Rojas, C., Dovletyarova, E. A., Shapoval, O. A., Krutyakov, Y. A. & Neaman, A. (2022). Microbial responses are unreliable indicators of copper ecotoxicity in soils contaminated by mining activities. *Chemosphere*, *300*, 134517. https://doi.org/10.1016/j.chemosphere.2022.134517