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Impact of mining contamination source on copper phytotoxicity in agricultural soils from central Chile

Impacto de la fuente de contaminación minera sobre la fitotoxicidad del cobre en suelos agrícolas de Chile central

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ABSTRACT

Chile is the largest producer of copper in the world. Mining operations have led to the contamination of neighboring agricultural soils through the deposition of smelter dust or spilling of mine tailings. However, the impacts of mining contamination source on copper phytotoxicity in soils are poorly resolved. Here we show that perennial ryegrass (*Lolium perenne* L.) exhibits different phytotoxicity responses when grown in agricultural topsoils contaminated by smelter dust than in those contaminated by tailing sand. Specifically, shoot length of ryegrass decreased as a function of total soil copper concentration in soils contaminated with smelter dust. In contrast, no correlation was observed between shoot length of ryegrass and total copper concentration in soils contaminated with tailing sand. A potential explanation for the differential trends in copper phytotoxicity may be attributed to zinc. The tailing sand-contaminated soils had significantly higher total zinc concentrations than did the smelter-impacted soils (201 mg kg^{-1} versus 126 mg kg^{-1}; $p < 0.05$). Shoot length significantly increased with increasing foliar zinc concentrations for ryegrass grown in tailing sand-contaminated soils ($n = 23$, $R^2 = 0.76$, $p < 0.001$), whereas no significant correlation between shoot length and foliar zinc was observed for ryegrass grown in smelter-impacted soils. Thus, zinc seems to alleviate copper phytotoxicity in tailing sand-contaminated soils but not in smelter-impacted soils, which show clear copper phytotoxicity.

RESUMEN

Chile es el principal productor del cobre en el mundo. Las operaciones mineras han provocado contaminación de suelos agrícolas adyacentes mediante deposición de polvo de fundición o derrames de relaves mineros. Sin embargo, el impacto de la fuente de contaminación minera sobre la fitotoxicidad del cobre en suelos está poco estudiado. En este estudio, se demuestra que ballica inglesa (*Lolium perenne* L.) exhibe diferentes respuestas de fitotoxicidad cuando se cultiva en suelos agrícolas contaminados por una fundición, en comparación con aquellos contaminados por relaves mineros. Específicamente, la longitud del vástago de ballica disminuyó en función de la concentración total de cobre en suelos contaminados con polvo de fundición. En contraste, tal correlación no se observó en suelos contaminados con relaves mineros. Una posible explicación de esta diferencia en la fitotoxicidad del cobre puede atribuirse al zinc. Los suelos contaminados por relaves mineros tenían concentraciones totales de zinc significativamente más altas, en comparación con los suelos impactados por la fundición (201 mg kg^{-1} versus 126 mg kg^{-1}; $p < 0,05$). La longitud del vástago aumentó significativamente con concentraciones foliares de zinc de ballica cultivada en suelos contaminados por relaves mineros ($n = 23$, $R^2 = 0,76$, $p < 0,001$), mientras que no se observó tal correlación en suelos impactados por la fundición. Por lo tanto, zinc parece aliviar la fitotoxicidad del cobre en los suelos contaminados por relaves mineros, pero no en los suelos afectados por la fundición, que muestran clara fitotoxicidad del cobre.

Palabras clave: toxicidad, Cu, Zn, ballica, suelo, contaminación.

INTRODUCTION

Copper mining and smelting activities may lead to the deposition of potentially bioavailable forms of copper onto neighboring soils (Lillo-Robles *et al.*, 2020). In particular, two common forms of Cu-rich particles are smelter dust and tailing sand (Ginocchio *et al.*, 2006). Previous work showed that smelter dust was more toxic to plants than was tailing sand. Specifically, soils spiked with smelter dust produced a lower effective concentration leading to a 50% decrease in shoot biomass (EC_{50}) for lettuce (*Lactuca sativa* var. *capita-*

ta) than did soils spiked with tailing sand (Ginocchio *et al.*, 2006), suggesting a higher phytotoxicity of smelter dust. However, measurements of metal toxicity to plants grown in artificially spiked soils do not reflect the phytotoxicity observed in field-contaminated soils (Neaman *et al.*, 2020). For instance, plants exhibit greater toxicity symptoms to Cu and Zn when grown in spiked soils than in field-contaminated soils (Hamels *et al.*, 2014). The impact of Cu contamination source (i.e., smelter dust versus tailing sand) on Cu phytotoxicity in field-contaminated soils is poorly resolved.

Further, the extent to which aging of the Cu contaminant form controls the bioavailability of Cu within soils is unknown. Here we compare the phytotoxicity of copper in field-collected soils from two field areas: one contaminated with smelter dust and the other contaminated by tailing sand. The contamination in each site occurred decades ago, until 1992 for the smelter area (González *et al.*, 2014; Tapia-Gatica *et al.*, 2020) and in 1965 (due to an earthquake) for the mine tailing area (Aguilar *et al.*, 2011; Villavicencio *et al.*, 2014). Previous work showed that dissolved organic carbon and not source of pollution explain Cu solubility (Mondaca *et al.*, 2015). However, Cu bioavailability is difficult to predict from soluble Cu pools (Verdejo *et al.*, 2016; Mondaca *et al.*, 2017) and therefore ought to be assessed through plant uptake studies. Thus, the objective of this study was to assess the impact of Cu contaminant source (smelter dust versus tailing sand) in field-collected soils on Cu phytotoxicity after decades of deposition. Since field studies are complex and time consuming, we decided to perform experiments under laboratory conditions.

MATERIALS AND METHODS

Soil Sampling and Processing

Topsoils (0-20 cm) were collected based on prior knowledge of the spatial distribution of Cu in agricultural soils from central Chile (Aguilar *et al.*, 2011; González *et al.*, 2014). Specifically, 24 agricultural soils in the smelting area and 19 agricultural soils in the mine tailing area were collected (Table 1). The copper contamination of both areas occurred decades ago. The soils from both areas were of alluvial origin and classified as Entisols (Soil Survey Staff, 2003).

Physicochemical Characterization of the Soils

Physicochemical characteristics (electrical conductivity, pH, organic matter content, and texture) were determined by using routine methods (Sheldrick and Wang, 1993; Sparks *et al.*, 1996). In order to determine total Cu, Cd, Pb, Zn and As, the samples were digested in boiling nitric acid followed by perchloric acid addi-

Table 1. Sampling points of soils under study.

Cuadro 1. Puntos de muestreo de los suelos en estudio.

Sample	Coordinates	
	W	S
1	71°12’3.97”	32°39’22.11”
2	71°12’39.27”	32°39’57.81”
3	71°12’38.48”	32°40’3.02”
5	71°11’55.18”	32°39’45.80”
7	71°11’46.60”	32°39’10.40”
8	71°12’42.23”	32°39’31.66”
9	71°12’36.45”	32°39’32.12”
10	71°12’47.85”	32°40’22.11”
11	71°12’16.20”	32°39’18.58”
12	71°12’25.24”	32°39’11.90”
13	71°12’24.39”	32°39’28.79”
14	71°12’3.84”	32°39’54.57”
15	71°12’53.56”	32°40’12.24”
18	70°56’37.10”	32°47’55.88”
19	70°56’8.14”	32°47’57.01”
20	70°57’26.56”	32°47’44.23”
22	70°57’19.23”	32°46’50.88”
23	70°56’59.65”	32°47’25.53”
24	70°55’46.90”	32°46’50.52”
25	71°12’34.22”	32°40’21.99”
26	71°12’26.62”	32°40’18.78”
27	71°12’18.89”	32°39’54.70”
28	71°12’20.98”	32°39’33.46”
29	71°12’27.73”	32°39’36.49”
30	71°12’41.63”	32°40’12.61”
31	71°13’1.21”	32°39’44.67”
32	71°12’45.40”	32°39’37.22”
33	71°12’52.82”	32°39’26.93”
34	71°28’55.64”	32°44’41.64”
36	71°27’11.35”	32°44’16.17”
37	71°27’2.88”	32°45’46.71”
38	71°26’48.84”	32°45’40.33”
39	71°26’35.15”	32°43’41.69”
40	71°25’36.63”	32°43’34.94”
41	71°24’59.15”	32°43’26.77”
42	71°24’41.18”	32°43’12.41”
43	71°24’43.06”	32°42’46.66”
44	71°24’21.61”	32°42’17.24”
45	71°24’35.32”	32°42’18.83”
46	71°25’55.64”	32°41’30.50”
47	71°28’59.75”	32°43’49.00”
48	71°29’21.75”	32°44’14.96”

tion (Maxwell, 1968). To prevent volatilization of As during the digestion process, a Teflon stopper with a 30-cm-long glass reflux was used (adapted from Verlinden, 1982). Quality was assured by similarly digesting in duplicate the following certified reference samples: PACS-2 obtained from the National Research Council Canada, and GRX-2 obtained from the United States Geological Survey (USGS). The obtained values were within 10% of the certified value. The soluble concentration of metals was determined using a solution of 0.1 M KNO_3 as an extractant (Stuckey *et al.*, 2008). Total and soluble concentrations of metals were measured by atomic absorption spectroscopy. Spikes of Cu, Zn, Cd, Pb, and As were performed on every 10th sample and recovery was 100% ± 7%. The Cd, Pb, and As data are not addressed in this study, as Cu is the foremost contaminant of concern for phytotoxicity in these soils (Verdejo *et al.*, 2016; Mondaca *et al.*, 2017).

Kd-Cu was calculated as the ratio between dissolved Cu (Cu_{dis}) and the total Cu concentration in the soil (Cu_T) (Sauvé *et al.*, 2000). The activity of Cu^{2+} was determined in the 0.1 M KNO_3 extract with an ion selective electrode (Rachou *et al.*, 2007). The results were expressed as Cu^{2+}, which is the negative logarithm of the activity of the free Cu^{2+} ion.

Phytotoxicity Bioassay

Each soil was assessed through a bioassay with ryegrass (*Lolium perenne* L.). Ryegrass is recommended by the ISO and OECD methods (ISO 11269-2, 2005, OECD-208, 2006) and has been used often as a bioindicator for Cu toxicity in soils contaminated by mining activities (Stuckey *et al.*, 2009; Gandarillas *et al.*, 2019).

Plants were grown in a growth chamber with 16 h of light (photosynthetic active radiation of 366 ± 13 μmol m^{-2} s^{-1}). Relative humidity was 50 ± 10% and temperature was 23 ± 3 °C. Plastic conic pots of 1.2 L were used holding 800 g of soil. Biotesting was carried out with four replicates. The study included 168 pots (23 x 4 + 19 x 4). The pots 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. Soils were kept wet by a cotton wick (based on ISO 11269-2, 2012).

After 21 days of growth on each soil, shoot length of ryegrass was measured from the root neck to the distal ends of the last leaf. Shoots were thoroughly washed in the following sequence: tap water, 0.1 M HCl, distilled water, 0.05 M EDTA, distilled water, and distilled water again (Steubing, 1982). Samples were dried in an oven at 70 °C for 48 h. Later, the samples were ground, sieved (2 mm), and homogenized.

The concentrations of metals were measured using standard methods (Kalra, 1997). Quality was assured by similarly digesting IPE 951 reference sample (Wageningen University), with the experimental values being within 10% of the certified values.

Statistical Analyses

Effective concentrations (EC_x) were determined by the Toxicity Relationship Analysis Program (TRAP) version 1.22 (US EPA, 2016) (Figure 1). For instance, EC_{50} is the effective concentration of total soil Cu producing a 50% decline in shoot length. Simple and multiple regressions were carried out between the ryegrass shoot length and the physicochemical characteristics of the

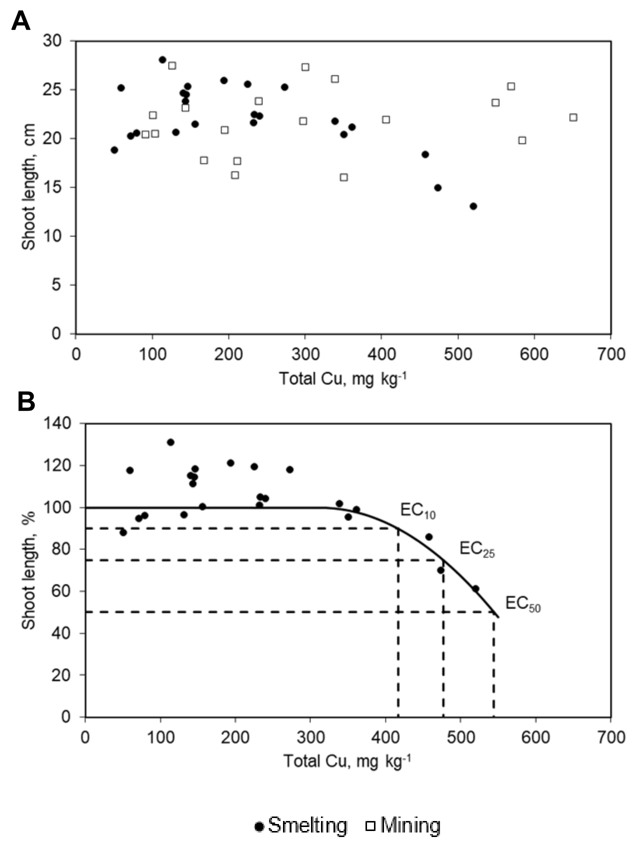

Figure 1. (A) Scatterplot between ryegrass (*Lolium perenne* L.) shoot length and total soil Cu concentration, and (B) the effect of total soil Cu on shoot length of ryegrass grown in soils of the smelter area. For the determination of the ECx values, a control response (100%) was established as the average of the responses obtained in the soils with total Cu concentration <155 mg kg^{-1}.

Figura 1. (A) Diagrama de dispersión entre la longitud del vástago de ballica (*Lolium perenne* L.) y la concentración total de Cu en el suelo, y (B) efecto del Cu total del suelo sobre la longitud del vástago de ballica cultivada en suelos del área de la fundición. Para la determinación de los valores de ECx, se estableció una respuesta control (100%) como el promedio de las respuestas obtenidas en los suelos con concentración de Cu total <155 mg kg^{-1}.

soils (Figure 2 and Table 2). Differences in physicochemical characteristics of the soils between the two study areas were tested by the Mann-Whitney test (Table 3). Statistical analyses were carried out using Minitab 17 Statistical Software.

RESULTS AND DISCUSSION

Our results show distinct Cu phytotoxicity responses for perennial ryegrass grown in soils contaminated by different Cu mining sources despite decades of contaminant aging. Shoot length was independent of total soil Cu concentration in the mining area (Figure 1A; Table 2). In contrast, shoot length decreased markedly for the soils with total Cu concentrations greater than ~350 mg kg^{-1} in the smelting area (Figure 1A and 1B). The EC_{10}, EC_{25} and EC_{50} were 416 mg kg^{-1}, 477 mg kg^{-1} and 544 mg kg^{-1}, respectively. These results suggest that copper contaminant source may continue to impact Cu phytotoxicity responses after decades of contaminant aging, despite our previous results showing no discernable impact of contaminant source on Cu solubility in soils after decades of aging (Mondaca *et al.*, 2015).

The contrasting responses of ryegrass shoot length to increasing total soil Cu concentration in the smelting-contaminated versus mine tailing-contaminated soils may be explained, at least in part, by the role of Zn. The median total soil Cu concentration of the smelting area and mining area was not significantly different, whereas the median total soil Zn concentration was greater in the mine tailing area than in the smelting area ($p < 0.05$) (Table 3). Increasing total soil Cu and total soil Zn correlated with an increase in foliar Cu and foliar Zn, respectively, in the mine tailing area (Figure 2A and 2B). In contrast, total soil metal concentration and foliar metal uptake were not significantly correlated in the smelting area (Figure 2A and 2B). Foliar Cu uptake had no impact on shoot length for either field area (Figure 2C; Table 2). However, increasing foliar Zn uptake coincided with increasing shoot length in the mine tailing area, whereas foliar Zn concentration showed no impact on shoot length in the smelting area (Figure 2D; Table 2). Indeed, foliar Zn was the strongest predictor of ryegrass shoot length in the mine tailing area ($R^2 = 0.52$; Table 2), whereas total soil Cu was the best explanatory variable at predicting ryegrass shoot

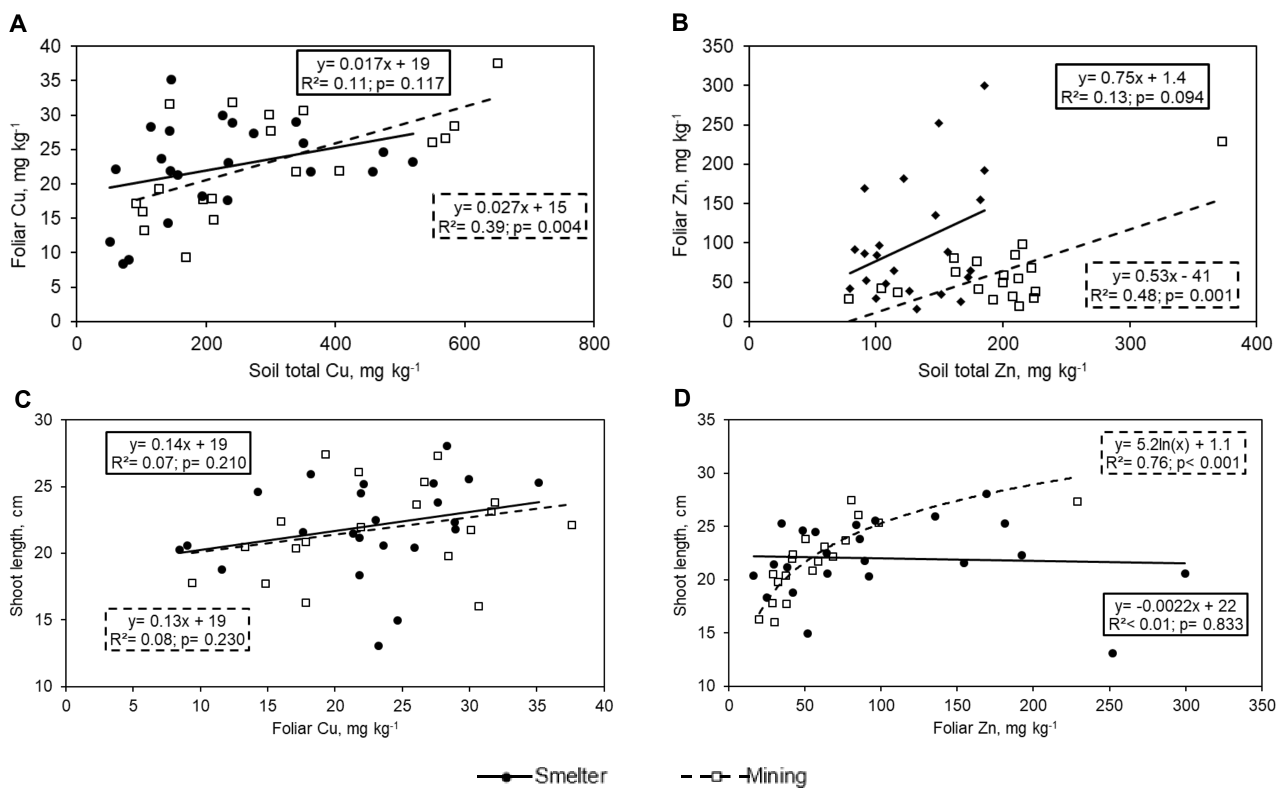

Figure 2. Foliar copper concentration as a function of total soil concentrations of Cu (A) and Zn (B) for the two study areas. Ryegrass (*Lolium perenne* L.) shoot length as a function of the foliar concentrations of Cu (C) and Zn (D) for the two study areas.

Figura 2. Concentración foliar de cobre en función de las concentraciones totales de Cu (A) y Zn (B) en el suelo para las dos áreas de estudio. Longitud del vástago de ballica (*Lolium perenne* L.) en función de las concentraciones foliares de Cu (C) y Zn (D) para las dos áreas de estudio.

Table 2. Determination coefficients and P-values (in parentheses) of the regression models for ryegrass shoot length and soil physicochemical properties in the two studied areas.

Cuadro 2. Coeficientes de determinación y valores P (entre paréntesis) de los modelos de regresión para la longitud del vástago de ballica y las propiedades físico-químicas de suelos en las dos áreas estudiadas.

Row	Response variable	Continuous predictor	Surrounding activity			
			Smelter (n=23)		Mining (n=19)	
			Regression model	R^2	Regression model	R^2
A)	SL	Me_T, pH, OM, Sand	25 – 0.016 Cu_T (0.003)	0.36	49 – 4.5 pH (0.018) + 0.11 SOM (0.007)	0.43
B)	SL	Cu_T	25 – 0.016 Cu_T (0.003)	0.36	ns	–
C)	SL	Cu_{dis}	24 – 3.5 Cu_{dis} (0.005)	0.32	ns	–
D)	SL	pCu^{2+}	ns	–	ns	–
E)	SL	Cu_T / Zn_T	25 – 1.9 Cu_T / Zn_T (0.003)	0.31	ns	–
F)	SL	Cu_F	ns	–	ns	–
G)	SL	Zn_F	ns	–	19 + 0.053 Zn_F (<0.001)	0.52
H)	SL	Cu_F / Zn_F	ns	–	27 – 9.8 (Cu_F / Zn_F) (0.001)	0.44
I)	Cu_F	Cu_T, pH, SOM, Sand	ns	–	15 + 0.027 Cu_T (0.004)	0.39
J)	Zn_F	Zn_T, pH, SOM, Sand	95 + 1.2 Zn_T (<0.001) – 53 pH (0.004) + 22 SOM (0.007) + 2.0 Sand (0.004)	0.77	- 41 + 0.53 Zn_T (0.001)	0.48

All the variables were significant in the regression model ($p < 0.05$).
SL= shoot length; Me_T= soil total metal concentration; SOM= soil organic matter content; Sand= sand content; Cu_{dis}= dissolved Cu; Me_F= foliar metal content; ns= not significant ($p > 0.05$).

Table 3. Comparison of the soil physicochemical characteristics between smelter and mining area. Range values and medians (in parentheses) are shown.

Cuadro 3. Comparación de las características fisicoquímicas del suelo entre el área de la fundición y el área minera. Se muestran los rangos y las medianas (entre paréntesis).

Variable	Unit	Mann-Whitney test difference	Smelter	Mining
pH	-		5.1 - 7.8 (7.1)	5.9 - 7.4 (7.0)
EC	dS m^{-1}		0.37 - 6.2 (0.74)	0.49 - 4.5 (0.81)
Sand	%	*	31 – 100 (82)	33 – 88 (46)
SOM	%	*	1.1 - 7.7 (3.0)	1.8 -10 (4.0)
DOC	mg L^{-1}	*	2.4 - 28 (13)	2.2 - 48 (6.2)
K_d-SOM	L kg^{-1}	*	1.1 – 15 (2.2)	2.2 – 29 (6.2)
Total Cu	mg kg^{-1}		51 - 520 (194)	92 - 651 (240)
Total Zn	mg kg^{-1}	*	79 - 185 (126)	79 - 373 (201)
Total Cu / Total Zn	-		0.60 – 5.1 (1.3)	0.78 - 3.1 (1.2)
Total Cd	mg kg^{-1}		0.43 - 5.1 (1.3)	1.0 - 2.1 (1.5)
Total Pb	mg kg^{-1}		19 - 71 (37)	24 - 50 (33)
Total As	mg kg^{-1}	*	11 - 60 (21)	2 - 43 (12)
Dissolved Cu	mg L^{-1}	*	0.15 - 3.1 (0.46)	0.048 - 1.1 (0.27)
pCu^{2+}	-		7.2 - 10 (9.3)	8.1 - 9.8 (9.0)
K_d-Cu	L kg^{-1}	*	153 - 1153 (379)	325 - 2190 (1023)

EC= Electrical conductivity; SOM= soil organic matter; DOC= dissolved organic carbon; pCu^{2+}= negative logarithm of the activity of free Cu^{2+}.
Asterisks indicate statistically significant difference between smelter and mining areas according to the Mann-Whitney test ($p < 0.05$).

length in the smelting area (R^2 = 0.36; Table 2). Overall, it appears that Zn uptake may have prevented Cu phytotoxicity in ryegrass grown in the mine tailing area soils, but not in the smelting area soils.

This is among the first studies to demonstrate the alleviating effect of Zn on Cu toxicity in plants grown in field-collected soils. Zinc alleviates Cu phytotoxicity under hydroponic conditions (Le *et al.*, 2013; Versieren *et al.*, 2014). Proposed mechanisms include competition for cell binding and uptake (Le *et al.*, 2013; Versieren *et al.*, 2014) and physiological protection at the cell level (Upadhyay and Panda, 2010). Both Cu^{2+} and Zn^{2+} possess parallel physicochemical properties (Weast, 1976) that explain their competitive interactions.

CONCLUSION

Overall, sites with differing Cu contaminant source may exhibit different thresholds of Cu phytotoxicity that may depend, at least in part, on the extent of Zn competition with Cu for plant uptake.

REFERENCES

Aguilar, R., Hormazábal, C., Gaete, H., Neaman, A., 2011. Spatial distribution of copper, organic matter and pH in agricultural soils affected by mining activities. Journal of Soil Science and Plant Nutrition 11, 125–144. https://doi.org/10.4067/S0718-95162011000300010

Gandarillas, M., España, H., Gardeweg, R., Bas, F., Arellano, E.C., Brown, S., Ginocchio, R., 2019. Integrated management of pig residues and copper mine tailings for aided phytostabilization. Journal of Environment Quality 48, 430. https://doi.org/10.2134/jeq2017.11.0431.

Ginocchio, R., Sanchez, P., de la Fuente, L.M., Camus, I., Bustamante, E., Silva, Y., Urrestarazu, P., Torres, J.C., Rodriguez, P.H., 2006. Agricultural soils spiked with copper mine wastes and copper concentrate: Implications for copper bioavailability and bioaccumulation. Environmental Toxicology and Chemistry 25, 712–718. https://doi.org/10.1897/05-105R.1.

González, I., Neaman, A., Rubio, P., Cortés, A., 2014. Spatial distribution of copper and pH in soils affected by intensive industrial activities in Puchuncaví and Quintero, central Chile. Journal of Soil Science and Plant Nutrition 14, 943–953.

Hamels, F., Malevé, J., Sonnet, P., Kleja, D.B., Smolders, E., 2014. Phytotoxicity of trace metals in spiked and field-contaminated soils: Linking soil-extractable metals with toxicity. Environmental Toxicology and Chemistry 33, 2479–2487. https://doi.org/10.1002/etc.2693.

ISO 11269-2, 2005. Soil quality — Determination of the effects of pollutants on soil flora. Part 2: Effects of chemicals on the emergence and growth of higher plants. International Organization for Standardization, Genève, Switzerland.

ISO 11269-2, 2012. Soil quality — Determination of the effects of pollutants on soil flora. Part 2: Effects of contaminated soil on the emergence and early growth of higher plants. Geneva, Switzerland.

Kalra, Y., 1997. Handbook of Reference Methods for Plant Analysis CRC Press, Boca Raton, FL, USA.

Le, T.T.Y., Vijver, M.G., Kinraide, T.B., Peijnenburg, W.J.G.M., Hendriks, A.J., 2013. Modelling metal–metal interactions and metal toxicity to lettuce Lactuca sativa following mixture exposure (Cu2+–Zn2+ and Cu2+–Ag+). Environmental Pollution 176, 185–192. https://doi.org/https://doi.org/10.1016/j.envpol.2013.01.017.

Lillo-Robles, F., Tapia-Gatica, J., Díaz-Siefer, P., Moya, H., Celis-Diez, J.L., Santa Cruz, J., Ginocchio, R., Sauvé, S., Brykov, V.A., Neaman, A., 2020. Which soil Cu pool governs phytotoxicity in field-collected soils contaminated by copper smelting activities in central Chile?. Chemosphere 242, 125176. https://doi.org/10.1016/j.chemosphere.2019.125176.

Maxwell, J.A., 1968. Rock and mineral analysis. Interscience Publishers, New York.

Mondaca, P., Catrin, J., Verdejo, J., Sauvé, S., Neaman, A., 2017. Advances on the determination of thresholds of Cu phytotoxicity in field-contaminated soils in central Chile. Environmental Pollution 223, 146–152. https://doi.org/10.1016/j.envpol.2016.12.076.

Mondaca, P., Neaman, A., Sauvé, S., Salgado, E., Bravo, M., 2015. Solubility, partitioning and activity of copper in contaminated soils in a semiarid zone. Journal of Plant Nutrition and Soil Science 178, 452–459. https://doi.org/10.1002/jpln.201400349.

Neaman, A., Selles, I., Martínez, C.E., Dovletyarova, E.A., 2020. Analyzing soil metal toxicity: Spiked or field-contaminated soils?. Environmental Toxicology and Chemistry 39, 513–514. https://doi.org/10.1002/etc.4654.

OECD-208, 2006. Guidelines for the testing of chemicals. Terrestrial plant test: Seedling emergence and seedling growth test. Organisation for Economic Cooperation and Development, Paris, France.

Rachou, J., Gagnon, C., Sauvé, S., 2007. Use of an ion-selective electrode for free copper measurements in low salinity and low ionic strength matrices. Environmental Chemistry 4, 90. https://doi.org/10.1071/en06036.

Sauvé, S., Hendershot, W., Allen, H.E., 2000. Solid-solution partitioning of metals in contaminated soils: Dependence on pH, total metal burden, and organic matter. Environmental Science & Technology 34, 1125–1131. https://doi.org/10.1021/es9907764.

Sheldrick, B.H., Wang, C., 1993. Particle size distribution, in: Carter, M. (Ed.) editor Soil Sampling and Methods of Analysis. Canadian Society of Soil Science. Lewis Publishers., Boca Raton, FL, USA, pp. 499–511.

Soil Survey Staff, 2003. Keys to Soil Taxonomy, 9th ed. United States Department of Agriculture. National Resources Conservation Service, Washington, DC, USA.

Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnson, C.T., Sumner, M.E., 1996. Methods of Soil Analysis. Part III: Chemical Methods. Soil Science Society of America. American Society of Agronomy, Madison, WI, USA.

Steubing, L., 1982. Problems of bioindication and the necessity of standarization, in: Steubing, L., Jäger, H. (Eds.), Monitoring Air Pollutants by Plants. Dr. Junk Publishers, The Netherlands, pp. 19–27.

Stuckey, J.W., Neaman, A., Ravella, R., Komarneni, S., Martinez, C.E., 2009. Highly charged swelling mica reduces Cu bioavailability in Cu-contaminated soils. Environmental pollution 157, 12–16. https://doi.org/10.1016/j.envpol.2008.09.009.

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 & Technology 42, 9197–9202. https://doi.org/10.1021/es801799s.

Tapia-Gatica, J., González-Miranda, I., Salgado, E., Bravo, M.A., Tessini, C., Dovletyarova, E.A., Paltseva, A.A., Neaman, A., 2020. Advanced determination of the spatial gradient of human health risk and ecological risk from exposure to As, Cu, Pb, and Zn in soils near the Ventanas Industrial Complex (Puchuncaví, Chile). Environmental Pollution 258, 113488. https://doi.org/10.1016/j.envpol.2019.113488.

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. Journal of Hazardous Materials 175, 1081–1084. https://doi.org/https://doi.org/10.1016/j.jhazmat.2009.10.016.

US EPA, 2016. Toxicity Relationship Analysis Program (TRAP) version 1.3 United States Environmental Protection Agency, Mid-Continent Ecology Division, https://archive.epa.gov/med/med_archive_03/web/html/trap.html.

Verdejo, J., Ginocchio, R., Sauvé, S., Mondaca, P., Neaman, A., 2016. Thresholds of copper toxicity to lettuce in field-collected agricultural soils exposed to copper mining activities in Chile. Journal of Soil Science and Plant Nutrition 16, 154–158. https://doi.org/10.4067/S0718-95162016005000011.

Verlinden, M., 1982. On the acid decomposition of human blood and plasma for the determination of selenium. Talanta 29, 875–882. https://doi.org/http://dx.doi.org/10.1016/0039-9140(82)80260-9.

Versieren, L., Smets, E., De Schamphelaere, K., Blust, R., Smolders, E., 2014. Mixture toxicity of copper and zinc to barley at low level effects can be described by the Biotic Ligand Model. Plant and Soil 381, 131–142. https://doi.org/10.1007/s11104-014-2117-6.

Villavicencio, G., Espinace, R., Palma, J., Fourie, A., Valenzuela, P., 2014. Failures of sand tailings dams in a highly seismic country. Canadian Geotechnical Journal 51, 449–464. https://doi.org/10.1139/cgj-2013-0142.

Weast, R., 1976. CRC Handbook of Chemistry and Physics. CRC Press, Cleveland, USA.