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SHORT COMMUNICATION

Zinc Alleviates Copper Toxicity to Lettuce and Oat in Copper-Contaminated Soils

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Abstract

Copper (Cu) mining operations have a global footprint and have led to contamination of soils and Cu toxicity to plants. Understanding the controls of plant Cu uptake, including competition with other metals, such as zinc (Zn), is essential for improving plant growth in Cu-contaminated soils. The objective of this study was to evaluate the capacity of Zn to alleviate the toxicity of Cu in crops grown in Cu-contaminated soils. Lettuce was grown in 27 soils with ranges of total Cu and Zn concentrations of 82–1295 mg Cu kg^{-1} and 86–345 mg Zn kg^{-1} for a period of 21 days. Oat was grown in 21 soils with the same total Cu and Zn concentration ranges for a period of 62 days. Regression analyses were used to evaluate the impact of total soil Zn on plant growth in the Cu-contaminated soils. We show for the first time that Zn alleviates Cu toxicity in lettuce and oat grown in soils. Specifically, we observed a negative (toxicity) effect of total soil Cu and a positive (protective) effect of total soil Zn on shoot growth response for lettuce and oat. The effective concentration 50% (EC_{50}) of Cu/Zn mass ratio was 7.0 ± 1.8 for lettuce shoot length and 5.9 ± 1.0 for oat shoot weight. These results indicate that the previously demonstrated efficacy of Zn in mitigating Cu phytotoxicity in hydroponic systems can extend to more complex soil systems. Further research should be done to evaluate specific Zn amendments for restoring vegetative growth in Cu-contaminated soils.

Keywords Bioavailability · Cu · Zn · Antagonism · Toxicity · Metal competition

1 Introduction

Contamination of agricultural soils with trace metals can occur through mining activities or the use of sewage wastewater in irrigation (Eissa and Negim 2018; Prudnikova et al. 2020). Zinc (Zn) and copper (Cu) are essential nutrients for plants but may be phytotoxic when present in high (~ 1–5 × 10^2 mg kg^{-1}) soil concentrations (Long et al. 2003; Xu et al. 2006); cadmium (Cd), on the other hand, is not a plant essential nutrient and can be phytotoxic at low (~ 1–5 × 10^1 mg kg^{-1}) soil concentrations (Liu et al. 2009), though trace metal bioavailability is also dependent on other soil physicochemical factors, such as pH (Kumpiene et al. 2017; McBride et al. 1997). It is important to study the soil-plant transfer of metals in contaminated agricultural soils, including metal competition for plant uptake in order to inform land remediation and management efforts.

Zinc and Cd have shown differential soil-plant transfer behavior for plants grown in sewage sludge amended soils, though Zn-Cd interactions were not assessed (Green et al. 2003; Green et al. 2006; Green and Tibbett 2008). The

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specific mechanisms of Zn-Cd interactions in soil-plant systems are not fully resolved and are typically studied in hydroponic systems or with unpolluted soils enriched (spiked) with metals (De Oliveira and Tibbett 2018; Sarwar et al. 2010; Zhou et al. 2019). The former approach cannot represent real soil, whereas the latter approach has been criticized due to discrepancies between metal toxicity measurements in freshly spiked soils and those in long-term contaminated soils (Neaman et al. 2020a). Nevertheless, in hydroponic studies, Zn (as Zn^{2+}) may inhibit Cd uptake within plant roots (Dong et al. 2019; Gao et al. 2020). Likewise, foliar application of Zn may be used to limit Cd transfer to important staple crops, such as rice and wheat (Javed et al. 2016; Wang et al. 2018).

The capacity of Zn to limit Cd transfer from soils to plants may extend to other divalent metals, such as Cu, though the potential of Zn to alleviate Cu toxicity to plants grown in Cu-contaminated soils is still largely unexplored. What is known about the role of Zn in alleviating Cu phytotoxicity has been limited to aquatic systems. For instance, Cu toxicity to plants may be alleviated by Zn, as shown for aquatic duckweed (Dirilgen and Inel 1994; Upadhyay and Panda 2010). Furthermore, hydroponic studies have shown that Zn may alleviate Cu toxicity to lettuce (Le et al. 2013; Liu et al. 2014; Versieren et al. 2014).

More recently, an increase in shoot biomass of common bean (*Phaseolus vulgaris* L.) was best explained by the Cu/Zn ratio of a Cu-contaminated soil in a bioassay evaluating symbiotic N_2 fixation (Stowhas et al. 2018). Symbiotic N_2 fixation increased with decreasing soil Cu/Zn ratio, suggesting that Zn alleviated Cu toxicity to N_2-fixing microbes. This finding reveals the potential importance of Zn in alleviating Cu toxicity in crops grown in Cu-contaminated soils. In the light of this new insight, the objective of the present study was to assess the impact of soil Zn on Cu toxicity to lettuce (*Lactuca sativa* L.) and oat (*Avena sativa* L.) grown in these soils. We provide the first demonstration of Zn alleviating Cu toxicity to lettuce and oat grown in field-collected soils.

2 Material and Methods

Twenty-seven agricultural topsoils (0–20 cm) located within mining impacted areas of the Aconcagua River basin and the Puchuncaví Valley in Chile were sampled to obtain a range of total concentrations of Cu and Zn based on prior sampling knowledge. The Aconcagua River basin contains diffuse pollution (Aguilar et al. 2011; Delgadillo et al. 2017; Verdejo et al. 2015), whereas the Puchuncaví Valley soils have received point source pollution from a Cu smelter (González et al. 2014; Tapia-Gatica et al. 2020). The samples were dried in an oven at 40 °C for 48 h, then sieved through a 2-mm mesh and homogenized.

The physicochemical characterization of the soils including texture, organic matter content, pH, electrical conductivity, and available macronutrient (N, P, K) concentrations (Tables 1 and 2) was conducted using standard methods (Rodríguez 1992; Sadzawka et al. 2006; Sheldrick and Wang 1993; Sparks et al. 1996). Total metal concentrations were determined by atomic absorption spectroscopy after soil digestion in boiling nitric acid followed by perchloric acid addition (Maxwell 1968). Quality was assured by similarly digesting in duplicate certified reference material (PACS-2 obtained from the National Research Council Canada). The obtained values were within 10% of the certified value. Soluble metal concentrations in 0.1 M KNO_3 extracts were measured by atomic absorption spectroscopy (Stuckey et al. 2008). Free Cu^{2+} activity was measured in a 0.1 M KNO_3 extract using a Cu^{2+} ion selective electrode (Rachou et al. 2007a).

Lettuce (*Lactuca sativa* L.) and oat (*Avena sativa* L.) are recommended as representative organisms in bioassays to evaluate soil quality (ISO 11269-2 2005; ISO 22030 2005; OECD 208 2006) and were used for evaluation of metal phytotoxicity in the present study. For the lettuce bioassay, each of the 27 topsoils was placed in its own set of four replicate containers, which were arranged according to a fully randomized design within a growth chamber with 16 h of light (at an intensity of 30,000 ± 1000 Lux and active photosynthetic

Table 1 General physicochemical properties of soils used for lettuce bioassay ($n = 27$)

Soil property	Unit	Median	Mean ± SD	Range
Electrical conductivity	dS m^{-1}	2.0	2.6±2.2	0.2–10.4
pH in KNO_3		7.2	7.0±0.5	5.7–7.6
pCu^{2+} in KNO_3		8.6	8.6±0.7	6.8–9.8
Organic matter	%	3.3	3.1±1.3	0.7–5.8
Available N	mg kg^{-1}	25	33±26	4–134
Available P	mg kg^{-1}	32	48±35	8–123
Available K	mg kg^{-1}	255	302±250	78–1143
Total Cu	mg kg^{-1}	355	418±285	82–1295
Total As	mg kg^{-1}	21	20±9	7–41
Total Zn	mg kg^{-1}	147	160±59	86–345
Total Pb	mg kg^{-1}	43	46±17	25–97
Soluble Cu	mg L^{-1}	0.14	0.22±0.17	0.04–0.71
Soluble As	mg L^{-1}	0.02	0.04±0.05	0.002–0.18
Soluble Zn	mg L^{-1}	0.015	N/A	<0.005–1.38
Soluble Pb	mg L^{-1}	<0.050	N/A	N/A
Sand	%	52	54±21	21–95
Clay	%	17	18±9	5–37
Silt	%	30	28±13	0–44

SD standard deviation, *N/A* not available due to majority of values were found under the detection limit

Table 2 Physicochemical properties of soils used for oat bioassay (n = 21)

Soil property	Unit	Median	Mean ± SD	Range
Electrical conductivity	dS m^{-1}	2.0	2.3±1.4	0.2–4.7
pH in KNO_3	–	7.3	7.1±0.5	5.7–7.6
pCu^{2+} in KNO_3	–	8.6	8.6±0.8	6.8–9.8
Organic matter	%	3.3	3.1±1.4	0.7–5.8
Available N	mg kg^{-1}	26	30±18	4–76
Available P	mg kg^{-1}	30	46±37	8–123
Available K	mg kg^{-1}	190	285±251	78–1143
Total Cu	mg kg^{-1}	426	439±287	82–1295
Total As	mg kg^{-1}	22	21±9	7–41
Total Zn	mg kg^{-1}	139	155±59	86–345
Total Pb	mg kg^{-1}	47	46±14	25–88
Soluble Cu	mg L^{-1}	0.14	0.21±0.15	0.04–0.56
Soluble As	mg L^{-1}	0.02	0.04±0.05	0.002–0.18
Soluble Zn	mg L^{-1}	0.015	N/A	<0.005–1.38
Soluble Pb	mg L^{-1}	<0.050	N/A	N/A
Sand	%	52	55±23	21–95
Clay	%	17	17±10	5–37
Silt	%	31	28±14	0–43

SD standard deviation, *N/A* not available due to majority of values were found under the detection limit

radiation of 366 ± 13 μmol m^{-2} s^{-1}). Relative humidity was 50 ± 5% (in the "Material and Methods" section, mean value ± standard deviation) during the day and 70 ± 5% during the night; diurnal temperature was 25 ± 1 °C, whereas nocturnal temperature was 20 ± 1 °C. In each container, ten seeds were planted initially. After 7 days, the planting density was thinned such that 5 plants remained in each container. The lettuce plants were harvested at 21 days from planting. Shoot length was measured from the root neck up to the distal end of the last leaf. Dry shoot mass was determined after 48 h of drying at 70 °C.

A long-term oat bioassay was performed on 21 topsoils in quadruplicate. The containers were arranged in a fully randomized design in a greenhouse under natural light at the Escuela de Agronomía of the Pontificia Universidad Católica de Valparaíso in Quillota, Chile. The average temperature was 18 ± 8 °C, and the relative humidity was 80 ± 13%. Ten seeds were planted per container. Shoot length and dry shoot mass were measured 62 days after sowing using the same methods as for the lettuce bioassay.

Simple and multiple regressions were used to assess the relationships between the plant shoot growth and the physicochemical soil properties. Statistical analyses were performed using Minitab 17 Statistical Software (2010). Effective concentrations (EC_x) were calculated using the Toxicity Relationship Analysis Program (TRAP) version 1.22 (US EPA 2013). The 3D figures were plotted using SYSTAT for Windows 13.1.

3 Results and Discussion

The lettuce shoot Cu concentrations were well explained by total soil Cu concentrations and soil organic matter, with both variables being significant ($p < 0.05$). Specifically, we found a positive effect of total soil Cu and a negative effect of soil organic matter on shoot Cu concentrations, as described by the following regression equation:

$$\text{Shoot Cu concentration} = 6.77 + 0.018 \text{ total soil Cu} \quad (1)$$
$$-1.50 \text{ soil organic matter}; (R2 = 0.56; p < 0.001),$$

where shoot Cu concentration is in mg kg^{-1}; total soil Cu is in mg kg^{-1}; soil organic matter is in %. The interaction between total soil Cu and soil organic matter was not significant ($p > 0.05$), indicating that these two variables acted as independent factors. Importantly, there was no correlation between total soil Cu and soil organic matter ($p > 0.05$); thus, there was no collinearity in Eq. (1).

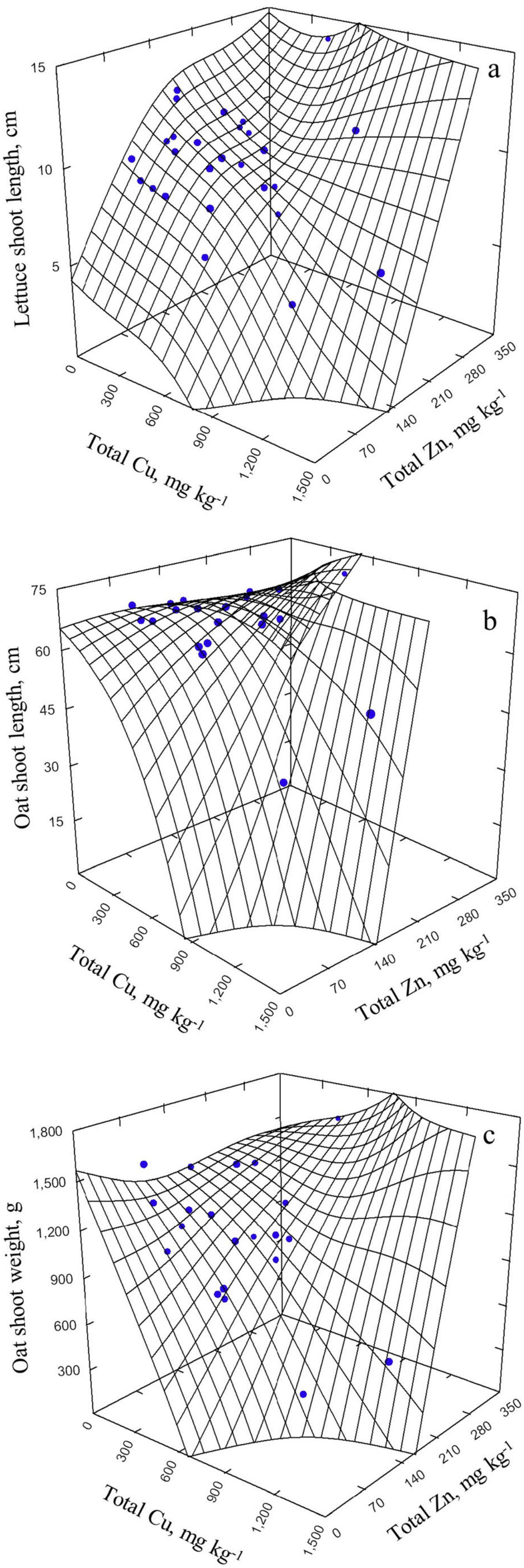
According to Rachou et al. (2007b), the kinetics of the mobilization of the elements from the solid phase to the soil solution decreases with increasing concentration of soil organic matter. Thus, the decrease in the flow of Cu from the solid phase to the soil solution can, in turn, reduce its phytoavailability for lettuce. However, oat shoot Cu concentrations were not explained by total soil Cu nor by soil physicochemical characteristics. Likewise, physicochemical characteristics such as soil pH, soluble Cu and Zn, or free Cu^{2+} did not have a significant effect on plant growth ($p > 0.05$).

On the other hand, the lettuce shoot growth response was well explained by total soil concentrations of Cu and Zn (Fig. 1a), with both variables being significant ($p < 0.05$). Specifically, we found a negative (toxicity) effect of total soil Cu and a positive (protective) effect of total soil Zn on shoot growth response, as described by the following regression equation:

$$\text{Shoot length} = 8.0 - 0.005 \text{ total soil Cu} \quad (2)$$
$$+ 0.02 \text{ total soil Zn}; \left(R^2 = 0.45; p < 0.001\right),$$

where shoot length is in cm; total soil Cu and total soil Zn are in mg kg^{-1}. The interaction between total soil Cu and total soil Zn was not significant ($p > 0.05$), indicating that Cu and Zn acted as independent factors. Importantly, there was no correlation between total soil Cu and total soil Zn ($p > 0.05$); thus, there was no collinearity in Eq. (2). Unlike shoot length, shoot weight was not a sensitive response variable for lettuce.

Similar to the lettuce bioassay, total soil Zn improved oat biomass growth and total soil Cu decreased biomass growth,



◀ **Fig. 1** Lettuce shoot length (**a**), oat shoot length (**b**), and oat shoot weight (**c**) as a function of total soil concentrations of Cu and Zn. The surface is obtained using a distance-weighted least square smoothing

though these relationships were clearer for oat shoot mass than for oat shoot length (Fig. 1b and c). Nevertheless, increasing the total soil Cu/Zn ratio significantly decreased both oat shoot length and oat shoot mass according to the following regression equations:

$$\text{Shoot length} = 70503\text{–}18180 \text{ total soil Cu} / \text{total soil Zn}; \left(R^2 = 0.56; p < 0.0002\right) \quad (3)$$

$$\text{Shoot mass} = 144852\text{–}12340 \text{ total soil Cu} / \text{total soil Zn}; \quad \left(R^2 = 0.54; p < 0.0002\right) \quad (4)$$

where shoot length is in cm; shoot mass is in g; total soil Cu and total soil Zn are in mg kg^{-1}.

Taken together, the lettuce and oat bioassays suggest that Zn alleviates Cu toxicity. Likewise, comparative toxicity studies provide strong evidence that Cu is considerably more phytotoxic than Zn under the same experimental conditions (Cheung et al. 1989; Ebbs and Kochian 1997; Kinraide et al. 2004; Pillay et al. 1994). Copper, similar to Cd, can interfere with the activities of antioxidative enzymes in plants, resulting in the overproduction of reaction oxygen species (e.g., H_2O_2) that induce oxidative damage and lipid peroxidation (Milone et al. 2003). The ability of Zn to counteract this metal-induced oxidative stress mechanism of phytotoxicity has been more clearly demonstrated in the case of Cd as the toxicant (Hassan et al. 2005; Milone et al. 2003; Venkatachalam et al. 2017; Zhao et al. 2011). Yet, in the case of Cd or Cu as the toxicant, Zn can protect lipids from oxidative degradation and may increase the biosynthesis of antioxidative enzymes (Aravind and Prasad 2003; Cakmak 2000; Upadhyay and Panda 2010). Furthermore, Zn may increase photosynthetic pigment production, promoting plant growth (Upadhyay and Panda 2010).

Table 3 Effective concentration (EC_{10}, EC_{25}, and EC_{50}) of Cu/Zn molar ratios for responses of shoot length in lettuce and shoot length and shoot growth in oat, along with the 95% confidence intervals

Plant response	Effective concentration		
	EC_{10}	EC_{25}	EC_{50}
Lettuce shoot length	3.3	5.0	7.0
	(1.8–4.8)	(3.9–6.2)	(5.2–8.8)
Oat shoot length	5.0	6.5	8.3
	(3.9–6.0)	(5.7–7.4)	(6.8–9.9)
Oat shoot weight	2.9	4.3	5.9
	(1.5–4.2)	(3.4–5.2)	(4.8–6.9)

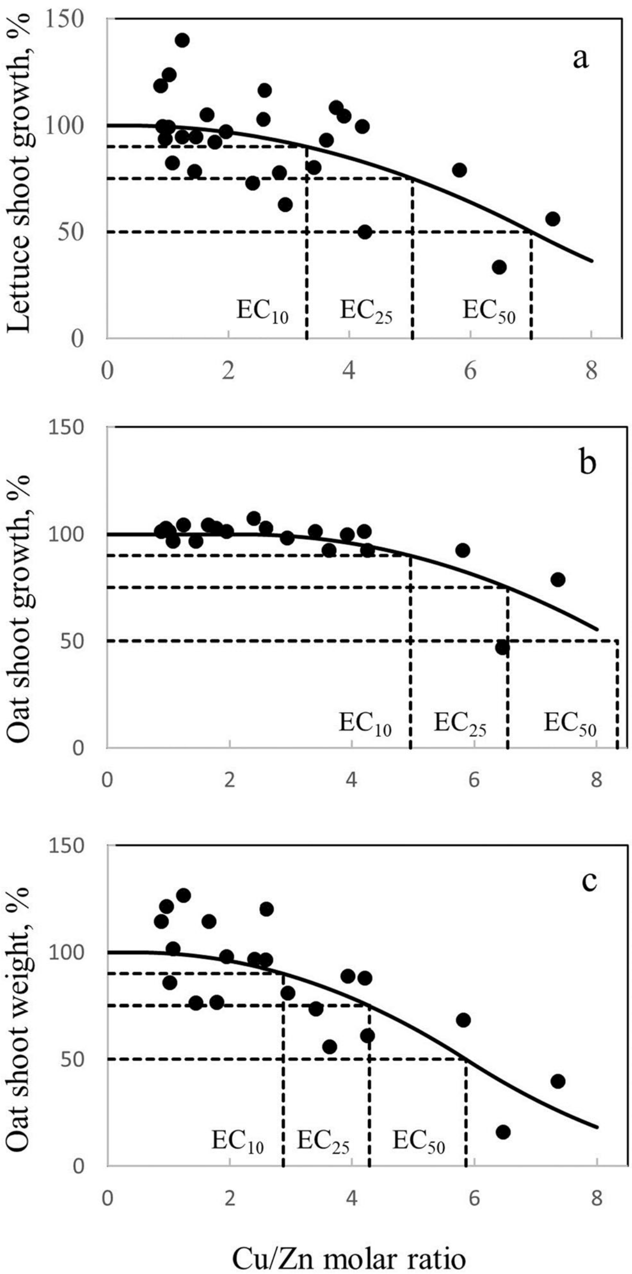


Fig. 2 Lettuce shoot length (**a**), oat shoot length (**b**), and oat shoot weight (**c**) respect to the control, as a function of the soil Cu/Zn molar ratio. The *y*-axis represents plant response in experimental pots expressed as a percentage of the response respect to the control pots. A threshold sigmoid regression analysis was used to fit the data (Toxicity Relationship Analysis Program, US Environmental Protection Agency)

At an atomic level, the efficacy of Zn^{2+} in inhibiting Cu^{2+} activity and toxicity in plants potentially may be explained by a shared classification of Lewis acidity (Lewis 1923). Metals of the same Pearson classification of Lewis acidity, such as Cu^{2+} and Zn^{2+}, may compete for the same functional groups of biological membranes or proteins (Tomasik et al. 1995). In this way, Zn^{2+} may inhibit the plant uptake of excessive Cu^{2+} (Montvydiene and Marciulioniene 2007). Versieren et al. (2014) showed that Zn^{2+} decreased Cu^{2+} toxicity to barley roots grown in resin-buffered nutrient solutions, a phenomenon explained by the Terrestrial Biotic Ligand Model that accounts for ion competition for plant root binding sites (Antunes et al. 2007; Thakali et al. 2006; Wang et al. 2012).

The shoot growth of lettuce decreased with increasing soil Cu/Zn ratio (Fig. 2a). The Cu/Zn ratios of the studied soils are equal or greater than the uncontaminated background Cu/Zn ratio of 0.7 for the Valparaiso region (Neaman et al. 2020b). The effective concentration 50% (EC_{50}) of Cu/Zn ratio for lettuce shoot growth response was 7.0 with a 95% confidence interval of 5.2–8.8 (Table 3). The mean EC_{50} values for oat shoot length and weight fell within a similar range (Table 3). Oat weight had a lower EC_{25} value than oat length did, indicating a more severe phytotoxicity response to the Cu/Zn molar ratio (Fig. 2b and c; Table 3). The EC_{50} values for the Cu/Zn ratio derived in this study were higher than the EC_{50} value of 1.0 reported by Stowhas et al. (2018) for symbiotic N_2 fixation, using soils of the same study area. Therefore, these findings suggest that lettuce and oat are more tolerant of Cu than are bacteria, consistent with previous evidence that soil microorganisms are more sensitive to metals than are plants or animals (Giller et al. 1999; Sauvé et al. 1998).

4 Conclusions

Our results showed that zinc (Zn) alleviated copper (Cu) toxicity to lettuce and oat grown in Cu-contaminated soils. These results suggest that the demonstrated capacity of Zn to alleviate phytotoxicity of other divalent metals, such as Cu and cadmium (Cd), in hydroponic systems may extend to actual soil systems. Zinc is less phytotoxic to plants and may compete with Cu through a similar uptake mechanism. Further work to demonstrate the efficacy of Zn in mitigating Cu phytotoxicity under field conditions is warranted.

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Declarations

Conflict of Interest The authors declare no competing interests.

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