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RESEARCH PAPERS

Rising Copper Exposure Effects on Nutrient Uptake in Two Species with Distinct Copper Tolerance

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Abstract—Excessive copper concentrations affect nutrient uptake in sensible species. However, the effects of copper on nutrient uptake in tolerant species have not been studied equally well. Thus, the main objective of this study was to determine the effect of rising Cu exposure on nutrient uptake rates and root/shoot nutrient contents in two species with distinct copper tolerance: *Acacia caven* (Mol.) Mol. and *Helianthus annuus* L. To this end, a hydroponic experiment was conducted. Copper treatments were applied at concentrations of 0, 2.0, 3.9, 7.9, and 15.7 μM. Relative root elongation was chosen as the endpoint for Cu toxicity assessment. The results of our study demonstrate that *A. caven* and *H. annuus* differ greatly in their tolerance to Cu. Copper concentration in the solution associated with the calculated EC_{25} was found to be six times higher in *A. caven* (15 μM) than in *H. annuus* (2.3 μM). When effective concentrations were instead based on the measured root Cu concentrations, the difference observed was ten times greater (1044 and 98 mg/kg for *A. caven* and *H. annuus*, respectively). Both species showed equal Cu uptake kinetics, with root absorbing power (α) of $7.5 \pm 0.7 \times 10^{-6}$ and $7.8 \pm 0.5 \times 10^{-6}$ cm s^{-1} for *A. caven* and *H. annuus*, respectively. Rising Cu concentrations in the exposure solution progressively diminished the influx of Ca into the roots of *H. annuus*, whereas no significant effect was noted for *A. caven*.

Keywords: *Acacia caven*, *Vachellia caven*, *Helianthus annuus*, ion competition, ion influx

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INTRODUCTION

Copper (Cu) is an essential plant micronutrient, but it can be phytotoxic in concentrations even slightly above the level of sufficiency [1]. Plant species and genotypes differ greatly in their ability to withstand damage induced by Cu exposure. The values of the effective Cu concentrations that lead to 25 to 50% (EC_{25} or EC_{50}, respectively) degradation in biological performance of sensitive versus tolerant species and genotypes [2] may differ by up to two orders of magnitude.

Copper damage is associated with reactive oxygen species, where it may manifest as unspecific injuries to cellular membrane [3, 4]. Enzyme problems and metallic cation replacement in metalloproteins have also been reported [1, 5]. Furthermore, excessive copper concentrations affect nutrient uptake in sensible species [3, 4]. However, the effects of copper on nutrient uptake in tolerant species have not been studied equally well.

Acacia caven (Mol.) Mol. (Fabaceae) is a typical native species in the central part of Chile that has Mediterranean-type climate [6]. This species is also known as *Vachellia caven* (Molina) Seigler & Ebinger. In particular, it can be found in abandoned mine tailings in north-central Chile [7]. It has been shown to have constitutive tolerance to Cu stress [6]. On the other hand, *Helianthus annuus* L. (Asteraceae), the common sunflower, exhibits high Cu sensitivity [8]. Both species have large germplasm, making them suitable for hydroponic experiments.

The main objective of the study was to determine the effect of rising Cu exposure on nutrient uptake rates and

root/shoot nutrient contents in two species with distinct copper tolerance: *A. caven* and *H. annuus*.

MATERIALS AND METHODS

Hydroponic experiment. A hydroponic experiment was conducted in order to evaluate plant Cu and nutrient uptake. The seeds of certain Chilean native woody species need to be pretreated with acids to achieve germination [9]. Hence, *Acacia caven* (Mol.) Mol. seeds (Til Til ecotype) were pretreated in concentrated sulfuric acid (90%) for 2 hours and then rinsed with deionized water. A commercial *Helianthus annuus* L. hybrid variety was chosen for the experiment. Sunflower seeds were disinfected with 70% ethanol for 30 seconds and then rinsed with deionized water. Seeds of both species were germinated on A-6 grade perlite until the radicle reached 10 to 15 mm in length, which occurred at 4 and 3 days for *A. caven* and *H. annuus*, respectively.

After germination, one hundred uniform seedlings were harvested as initial growing seedlings (hence forth referred to as the first harvest) and another thirty were transplanted onto polystyrene sheets and then placed over 2.5 L containers with 1/5 strength Hoagland solution. Preference was given to the diluted Hoagland solution because of the plants' tendency for a stronger Cu response in lower ionic strength solutions, and also to avoid Cu-phosphate precipitation [10]. The composition of the nutrient solution was as follows: 0.5 mM KNO_3, 0.5 mM $Ca(NO_3)_2$, 0.2 mM $MgSO_4$, 0.1 mM K_2HPO_4, 0.02 mM Fe-EDDHA 6%, 10 μM H_3BO_3, 2 μM $MnCl_2$, 0.2 μM $ZnSO_4$ and 0.1 μM MoO_3. No buffer was added to the nutrient solution.

Copper treatments were applied upon transplantation at concentrations of 0, 2.0, 3.9, 7.9, and 15.7 μM. Copper was applied from a concentrated Cu stock solution of 18.7 mM $CuSO_4$, stirred and left to equilibrate for 1 hour. Solution pH was in the range of 6.6–6.7, with no statistical differences between the treatments. Copper treatments continued until plant harvest (henceforth referred to as the second harvest). Nutrient solutions were continuously aerated and replaced on a daily basis. Each treatment was replicated three times. All experiments were performed in a controlled climate chamber (light/dark regime: 12/12 h at 22 ± 3°C, relative humidity 46% and light intensity of 27 μmol/(m^2 s).

In pre-test, secondary roots appeared on 5th and 4th day after transplant in *A. caven* and *H. annuus*, respectively. In order to obtain the maximum principal root elongation, the seedlings were harvested one day before the emergence of secondary roots, which occurred 4 and 3 days after transplant for *A. caven* and *H. annuus*, respectively. Seedlings were rinsed with deionized water, following the protocol of other studies on the sample preparation of plant material for the quantitative analysis of metals [11–13]. Subsequently, root length and fresh weight of shoots were recorded. Biomass was dried in a forced-air oven at 45°C for seven days, and then the dry matter was weighed. Dried plant tissues were ground to powder and digested with HNO_3–HF–H_2O_2 in a microwave oven (Milestone 1200; Milestone Microwave System, United States). Cu, Ca, Mg, Fe, and Zn values were established using ICP-MS (Perkin Elmer ELAN 6100).

Data analysis. Relative root elongation was chosen as the endpoint for Cu toxicity assessment due to the simplicity and accuracy of this method [14]. RRE was calculated using the following formula: $RRE_i = 100\ \Re_i/\Re_{\max}$, $RRE_i = 100\ \Re_i/\Re_{\max}$, where RRE_i is relative root elongation in treatment i, RE_i is root elongation in treatment i and $RE_{\max}$ is the maximum root elongation in all treatments. Since root elongation was not normally distributed ($P = 0.0001$), we used the 75% percentile of the relative root elongation values in our assessment.

Subsequently, we calculated the effective concentrations of Cu in the nutrient solution that inhibited root elongation by 25% (EC_{25}), along with EC_{25} for foliar Cu concentrations. In order to determine EC_{25}, we used the Hill equation of Excel^{MR} macro REGTOX version 7.0.6.

The net influx (I_n) is the net amount of nutrient taken up per unit of root surface area per unit of time. To calculate I_n, dry-mater nutrient concentration was measured, and root surface was calculated in each harvest. Given that young plants exhibit exponential root growth rates, the average nutrient influx (I_n) was calculated using the Williams' formula [15]:

$$I_n = \frac{(U_2 - U_1)}{(AR_2 - AR_1)}\ln,$$

where I_n is the net influx in pmol/cm^2 s, U is the entire plant nutrient content per plant in pmol, RA is root surface area per plant in cm^2, t is the time between two harvests in seconds, and where subscripts 1 and 2 signify the first and second harvest, respectively.

The specific root length (RL_S) is the ratio between root length (RL) and root fresh weight (R_{fw}). It was established for each species by weighing three samples of approximately 0.2 g of fresh root and directly measuring the length (in cm) of the sample. Root surface was calculated on the basis of root fresh weight assuming a cylindrical shape [16], using the following formula:

$$RA = 2\pi\sqrt{\frac{1}{\pi\rho RL_s}}RL_S R_{fw},$$

where RA is root surface in cm^2, R_{fw} is root fresh weight in g, RL_S is the specific root length in cm/g and ρ is root density in g/cm, which was assumed at 1 [17]. Partition coefficient (PC) was calculated as the ratio

Table 1. Fresh and dry weight (g per seedling) and 75th percentile of root length (mm) of *Acacia caven* and *Helianthus annuus* cultivated in Hoagland solution spiked with raising copper concentrations.

Endpoint	Cu treatment, μM	*Acacia caven*		*Helianthus annuus*	
Root fresh weight	0.0	0.068 ± 0.005	A	0.064 ± 0.018	A
	2.0	0.066 ± 0.006	A	0.059 ± 0.005	A
	3.9	0.066 ± 0.010	A	0.040 ± 0.005	AB
	7.9	0.063 ± 0.003	A	0.028 ± 0.005	B
	15.7	0.053 ± 0.004	A	0.022 ± 0.006	B
	ANOVA F:	3.0		11.3**	
Shoot fresh weight	0.0	0.192 ± 0.012	A	0.225 ± 0.042	AB
	2.0	0.195 ± 0.002	A	0.249 ± 0.061	A
	3.9	0.182 ± 0.013	A	0.175 ± 0.036	AB
	7.9	0.204 ± 0.010	A	0.161 ± 0.019	AB
	15.7	0.187 ± 0.006	A	0.128 ± 0.025	B
	ANOVA F:	2.3		4.7	
Root dry weight	0.0	0.007 ± 0.001	A	0.003 ± 0.001	A
	2.0	0.007 ± 0.001	A	0.003 ± 0.001	A
	3.9	0.007 ± 0.001	A	0.002 ± 0.001	A
	7.9	0.006 ± 0.001	A	0.002 ± 0.000	A
	15.7	0.006 ± 0.001	A	0.002 ± 0.001	A
	ANOVA F:	0.2		2.5	
Shoot dry weight	0.0	0.037 ± 0.002	AB	0.046 ± 0.002	A
	2.0	0.037 ± 0.002	AB	0.047 ± 0.004	A
	3.9	0.037 ± 0.001	AB	0.043 ± 0.003	A
	7.9	0.039 ± 0.001	B	0.043 ± 0.001	A
	15.7	0.035 ± 0.001	A	0.045 ± 0.002	A
	ANOVA F:	4.1		1.3	
Root length	0.0	72 ± 7.4	A	111 ± 13.8	A
	2.0	69 ± 8.5	A	103 ± 5.2	A
	3.9	67 ± 5.5	AB	37 ± 3.6	B
	7.9	64 ± 6.1	AB	19 ± 1.0	BC
	15.7	52 ± 0.6	B	13 ± 2.3	C
	ANOVA F:	4.5		139.9**	

Values are mean ± S.E. ($n = 3$). Mean values followed by the same letter are not statistically different according to the Tukey test ($P < 0.05$). Statistically significant differences at: $^{**}P < 0.001$. $^{*}P < 0.01$ (one-way ANOVA).

between cation concentration in shoots and cation concentration in roots. In the discussion that follows, the results are presented as the element concentrations in the shoots or roots (expressed in mg/kg) or the element content in the shoots or roots (expressed in μg/plant). In both cases, dry plant weight was used for calculations.

Results were analyzed statistically using the Infostat program with one-way ANOVA. The mean values of the treatments were compared using the Tukey test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Among all plant responses, the root length was found to be the most sensitive endpoint (Table 1). Based on this endpoint, *A. caven* and *H. annuus* differ greatly in their tolerance to Cu (Table 2). Cu concentration in the solution associated with the calculated EC_{25} was found to be six times higher in *A. caven* (15 μM) than in *H. annuus* (2.3 μM). When effective concentrations were instead based on the measured root Cu concentrations, the difference observed was found to be ten times greater (1044 and 98 mg/kg for *A. caven* and *H. annuus*, respectively). The calculated EC_{25} in this study places *A. caven* in the first quartile of the most tolerant species and *H. annuus* in the second quartile, very close to the median [2]. The EC_{25} value of 7.85 μM of Cu is normally used as the limit between tolerant and non-tolerant species [14], which places *A. caven* among tolerant species and *H. annuus* among non-tolerant ones.

Table 2. Effective concentration for 25% reduction in root elongation (EC_{25})

Species	Cu in solution, μM	Cu in roots, mg/kg
Acacia caven	15 (11–22)	1044 (180–1498)
Helianthus annuus	2.3 (1.9–3.0)	98 (62–142)

Numbers in parenthesis indicate lower and upper limits at 95% confidence level.

The increase of copper in the tissues of both species occurred mainly in the roots (Fig. 1). This phenomenon is known as soil-root barrier [18]. Given its high EC_{25} value and Cu accumulation in roots, *A. caven* can be classified as a non-hyperaccumulating tolerant species.

Copper uptake kinetics in both species adjust to a straight line as described by Nye [19]. Such line is given by $I_n = \left(C|L - C_{L\min}\right) I_n = \left(C|L - C_{L\min}\right)$, where I_n is net influx (pmol/cm^2 s), α is root absorbing power (cm/s), C_L is Cu concentration in solution (pmol/cm^3) and $C_{L\min}$ is C_L at $I_n = 0$. The difference in α between the species was not statistically significant. Likewise, the difference in $C_{L\ \min}$ between the species was not statistically significant (Table 3). Such behavior was unexpected because species [14] or even genotypes of the same species [20] usually exhibit some differences.

In this study, rising Cu concentrations in the exposure solution resulted in a reduction in the Ca influx rate in *H. annuus*, whereas no significant effect was noted for *A. caven* (Table 4). The same Cu exposure concentrations did not have any impact on the influx rates calculated for Fe, Mg, or Zn.

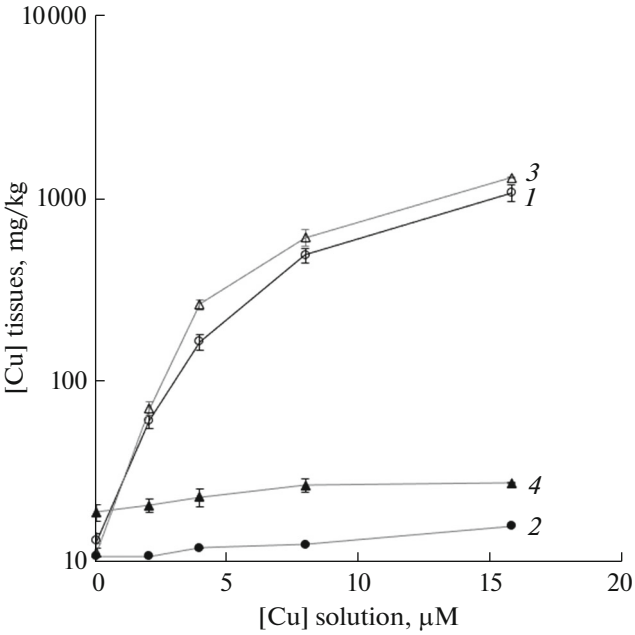

Fig. 1. Copper concentration (mg/kg) in shoots and roots of *Acacia caven* and *Helianthus annuus* cultivated in modified Hoagland solution spiked with rising copper concentrations. Bars indicate standard deviation. 1—*Acacia caven* (roots), 2—*Acacia caven* (shoots), 3—*Helianthus annuus* (roots), 4—*Helianthus annuus* (shoots).

Table 3. Calculated influx values for *Acacia caven* and *Helianthus annuus* cultivated in Hoagland solution spiked with rising copper concentrations

Value	*Acacia caven*			*Helianthus annuus*		
	estimated	lower limit	upper limit	estimated	lower limit	upper limit
Root absorbing power (α)	7.5E-06	6.8E-06	8.3E-06	7.8E-06	7.3E-06	8.3E-06
$C_{L\min}$	727	182	1349	1274	441	2286
r^2 adj	0.97			0.99		
Significance level (*p*-value)	<0.0001			<0.0001		

Root absorbing power is in pmol/cm^2 s, $C_{L\min}$is in pmol/cm^3. Lower and upper limits are at 95% confidence levels.

Table 4. Net influx (pmol/cm^2 s) of Ca, Fe, Mg and Zn in seedlings of *Acacia caven* and *Helianthus annuus* cultivated in Hoagland solution spiked with rising copper concentrations

Species	Cu treatment, μM	Ca		Fe		Mg		Zn	
Acacia caven	0.0	0.84 ± 0.10	A	0.0028 ± 0.0018	A	0.22 ± 0.19	A	–0.0016 ± 0.0011	A
	2.0	0.70 ± 0.02	A	–0.0006 ± 0.0008	A	0.04 ± 0.24	A	–0.0027 ± 0.0006	A
	3.9	0.87 ± 0.06	A	–0.0006 ± 0.0013	A	–0.59 ± 0.16	A	–0.0027 ± 0.0007	A
	7.9	0.87 ± 0.02	A	–0.0009 ± 0.0016	A	–0.53 ± 0.09	A	–0.0023 ± 0.0005	A
	15.7	0.77 ± 0.05	A	–0.0009 ± 0.0006	A	–0.43 ± 0.14	A	–0.0019 ± 0.0010	A
	ANOVA F:	1.80		1.53		4.46		0.36	
Helianthus annuus	0.0	2.3 ± 0.03	A	0.0042 ± 0.0020	A	1.72 ± 0.55	A	0.0120 ± 0.0026	A
	2.0	2.9 ± 0.22	B	0.0059 ± 0.0033	A	1.70 ± 0.71	A	0.0148 ± 0.0070	A
	3.9	1.7 ± 0.14	C	0.0013 ± 0.0067	A	1.12 ± 0.27	A	0.0107 ± 0.0082	A
	7.9	1.0 ± 0.03	D	0.0496 ± 0.0280	A	0.61 ± 1.37	A	0.0114 ± 0.0025	A
	15.7	0.91 ± 0.01	D	–0.0006 ± 0.0028	A	1.73 ± 1.33	A	0.0065 ± 0.0033	A
	ANOVA F:	54.16**		2.62		0.28			0.32

Mean values followed by the same letter are not statistically different according to the Tukey test ($P < 0.05$). Statistically significant differences at: $^{**}P < 0.001$, $^{*}P < 0.01$ (one-way ANOVA). Values are mean ± S.E. ($n = 3$).

Table 5. Calcium concentrations (expressed in mg/kg) and calcium contents (expressed in μg/plant) in roots and shoots of *Acacia caven* and *Helianthus annuus* seedlings exposed to rising copper concentrations in the nutrient solution

Variable	Cu treatment, μM	*Acacia caven*		*Helianthus annuus*	
Root concentration	Germinated	897 ± 126	A	1607 ± 80	A
	0.0	2590 ± 20	B	3959 ± 370	B
	2.0	2638 ± 21	B	4093 ± 72	BC
	3.9	2754 ± 193	BC	4267 ± 666	BC
	7.9	2796 ± 82	BC	4731 ± 599	BC
	15.7	2973 ± 83	C	5226 ± 105	C
	ANOVA F:	157.3**		29.4**	
Shoot concentration	Germinated	1596 ± 285	A	810 ± 41	A
	0.0	2655 ± 175	B	2222 ± 316	C
	2.0	2443 ± 83	B	2512 ± 220	C
	3.9	2631 ± 93	B	1591 ± 71	B
	7.9	2543 ± 56	B	1061 ± 93	A
	15.7	2525 ± 169	B	906 ± 38	A
	ANOVA F:	18.2**		56.1**	
Root content	Germinated	4.1 ± 0.9	A	1.3 ± 0.1	A
	0.0	18 ± 1.2	B	12 ± 2.4	B
	2.0	18 ± 2.0	B	13 ± 1.7	B
	3.9	18 ± 1.4	B	10 ± 1.8	B
	7.9	17 ± 0.6	B	10 ± 1.1	B
	15.7	18 ± 1.2	B	11 ± 2.5	B
	ANOVA F:	60.7**		17.8**	
Shoot content	Germinated	67 ± 12.3	A	34 ± 1.7	A
	0.0	98 ± 10.3	B	102 ± 12	C
	2.0	89 ± 2.1	B	118 ± 9.3	C
	3.9	97 ± 4.8	B	69 ± 8.2	B
	7.9	97 ± 0.8	B	46 ± 4.0	A
	15.7	87 ± 4.7	B	40 ± 0.6	A
	ANOVA F:	9.7**		67.8**	
Partition coefficient	Germinated	0.063 ± 0.009	A	0.038 ± 0.004	A
	0.0	0.185 ± 0.018	B	0.125 ± 0.009	AB
	2.0	0.202 ± 0.025	B	0.113 ± 0.025	AB
	3.9	0.193 ± 0.013	B	0.160 ± 0.038	BC
	7.9	0.180 ± 0.008	B	0.234 ± 0.046	CD
	15.7	0.215 ± 0.017	B	0.291 ± 0.061	D
	ANOVA F:	36.3**		18.6**	

Calcium concentrations and calcium contents in germinated seedlings before treatments are also shown, along with the partition coefficients (ratio of root/shoot Ca content). Values are mean ± S.E. ($n = 3$). Mean values followed by the same letter are not statistically different according to the Tukey test ($P < 0.05$). Statistically significant differences at: ** $P < 0.001$, * $P < 0.01$ (one-way ANOVA).

In *A. caven*, no differences were found between Cu treatments in terms of Ca contents or Ca concentrations in shoots and roots (Table 5). Likewise, in *H. annuus*, Ca concentrations varied slightly in the roots between Cu treatments, whereas Ca contents remained unchanged in the roots between Cu treatments. On the other hand, there was a considerable reduction in Ca concentrations and Ca contents in the shoots of *H. annuus* (Table 5). The observed behavior cannot be related to plant tolerance to Cu. In *Minuartia hirsuta*, Ca concentrations in roots and shoots decreased in tolerant population and increased in sen-

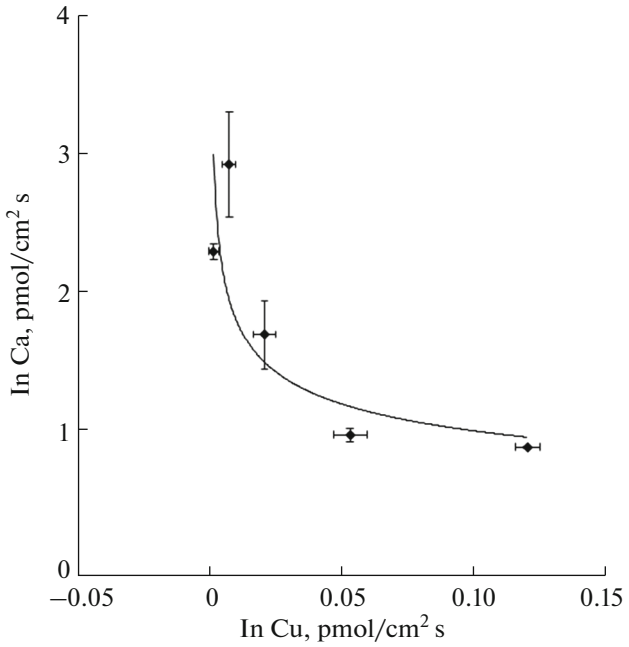

Fig. 2. Relation between In Cu and In Ca of *Helianthus annuus* cultivated in modified Hoagland solution spiked with rising copper concentrations. Bars indicate standard deviation.

sitive one as a response to the increase of Cu concentration in the nutrient solution [21]. However, in *Elsholtzia argyi,* Ca concentrations maintained in roots and decreased in shoots in tolerant population, but decreased in both roots and shoots in sensitive population [22]. Thus, more studies should be carried out with other species in order to explain the observed patterns.

In other words, *H. annuus* was unable to maintain root-to-shoot Ca translocation due to rising concentrations of Cu in the nutrient solution. In the toxic range of Cu in *H. annuus* (>2.3 μM), the Ca influx dropped with rising concentrations of Cu in the nutrient solution (Fig. 2), indicating that both cations compete for the same binding sites. This behavior is consistent with cations competition described in the biotic ligand model [23–25].

Basic divalent cations in general and Ca in particular have the potential to ameliorate metal toxicity through ion competition and the decreased electrical potential of the cell membrane [26–28]. In addition, Ca uptake can further protect the plants due to its role in the synthesis and activation of antioxidants that are essential for cell membrane integrity [29, 30].

To conclude, *Acacia caven* and *Helianthus annuus* displayed great differences in their tolerance to Cu, the former being a tolerant and the latter being a non-tolerant species. However, rising Cu concentrations in the nutrient solution cause an increase in Cu concentrations mainly in the roots in both species. Furthermore, both species exhibited equal Cu uptake kinetics parameters indicating that density and affinity of Cu binding sites in roots are similar in both species. Thus, our findings suggest that distinct species exhibit different toxicity responses at the same level of binding site saturation. Since we are not aware of any other study discussing this tendency, this adds an aspect of novelty to the present study.

Moreover, Cu had a greater affinity for Ca binding sites on the roots of *H. annuus* in comparison to *A. caven*. Further studies are necessary to uncover the tolerance mechanisms of *A. caven* that protect it from Cu toxicity. Nevertheless, the ability of *A. caven* to maintain Ca influx in view of rising Cu may have a role in the high tolerance of this species.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

REFERENCES

1. Nagajyoti, P.C., Lee, K.D., and Sreekanth, T.V.M., Heavy metals, occurrence and toxicity for plants: a review, *Environ. Chem. Lett.*, 2010, vol. 8, p. 199.
2. Kopittke, P.M., Blamey, F.P.C., Asher, C.J., and Menzies, N.W., Trace metal phytotoxicity in solution culture: a review, *J. Exp. Bot.*, 2010, vol. 61, p. 945.
3. Kholodova, V.P., Vasil'ev, S.V., Efimova, M.V., Voronin, P.Yu., Rakhmankulova, Z.F., Danilova, E.Yu., and Kuznetsov, V.V., Exogenous melatonin protects canola plants from toxicity of excessive copper, *Russ. J. Plant Physiol.*, 2018, vol. 65, p. 882.
4. Ivanova, E.M., Kholodova, V.P., and Kuznetsov, V.V., Biological effects of high copper and zinc concentrations and their interaction in rapeseed plants, *Russ. J. Plant Physiol.*, 2010, vol. 57, p. 806.
5. Yruela, I., Copper in plants, *Braz. J. Plant Physiol.*, 2005, vol. 17, p. 145.
6. *Uso de Recursos Fitogenéticos Nativos para la Fitoestabilización de Relaves Mineros en la Región de Coquimbo*, Coquimbo: Inst. Invest. Agropecuarias INIA Intihuasi, 2008.
7. Orchard, C., León-Lobos, P., and Ginocchio, R., Phytostabilization of massive mine wastes with native phy-

togenetic resources: potential for sustainable use and conservation of the native flora in north-central Chile, *Cienc. Investig. Agrar.*, 2009, vol. 36, p. 329.

8. Pillay, S.V., Rao, V.S., and Rao, K.V.N., Comparative effects of copper and zinc toxicity and tolerance of *Hyptis suaveolens* (L.) Poit. and *Helianthus annuus* (L.), *Int. J. Environ. Stud.*, 1994, vol. 46, p. 173.
9. Figueroa, J.A. and Jaksic, M., Seed bank and dormancy in plants of the Mediterranean region of central Chile, *Rev. Chil. Hist. Nat.*, 2004, vol. 77, p. 201.
10. Xiong, Z.-T., Li, Y.-H., and Xu, B., Nutrition influence on copper accumulation by *Brassica pekinensis* Rupr, *Ecotoxicol. Environ. Saf.*, 2002, vol. 53, p. 200.
11. Wu, L., Thurman, D.A., and Bradshaw, A.D., The uptake of copper and its effect upon respiratory processes of roots of copper-tolerant and non-tolerant clones of *Agrostis stolonifera, New Phytol.*, 1975, vol. 75, p. 225.
12. Chen, B.-C., Ho, P.-C., and Juang, K.-W., Alleviation effects of magnesium on copper toxicity and accumulation in grapevine roots evaluated with biotic ligand models, *Ecotoxicology*, 2013, vol. 22, p. 174.
13. Antunes, P.M.C., Hale, B.A., and Ryan, A.C., Toxicity versus accumulation for barley plants exposed to copper in the presence of metal buffers: Progress towards development of a terrestrial biotic ligand model, *Environ. Toxicol. Chem.*, 2007, vol. 26, p. 2282.
14. Ait Ali, N., Bernal, M.P., and Ater, M., Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays, Plant Soil*, 2002, vol. 239, p. 103.
15. Williams, R., The effects of phosphorus supply on the rates of intake of phosphorus and nitrogen and upon certain aspects of phosphorus metabolism in gramineous plants, *Aust. J. Biol. Sci.*, 1948, vol. 1, p. 333.
16. Claassen, N., Syring, K., and Jungk, A., Verification of a mathematical model by simulating potassium uptake from soil, *Plant Soil*, 1986, vol. 95, p. 209.
17. Barber, S.A., *Soil Nutrient Bioavailability: A Mechanistic Approach*, New York: Wiley, 1995.
18. Meier, S., Alvear, M., Borie, F., Aguilera, P., Ginocchio, R., and Cornejo, P., Influence of copper on root exudate patterns in some metallophytes and agricultural plants, *Ecotoxicol. Environ. Saf.*, 2012, vol. 75, p. 8.
19. Nye, P.H., The relation between the radius of a root and its nutrient-absorbing power [α]: Some theoretical considerations, *J. Exp. Bot.*, 1973, vol. 24, p. 783.
20. Hartley-Whitaker, J., Ainsworth, G., and Meharg, A.A., Copper- and arsenate-induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity, *Plant Cell Environ.*, 2001, vol. 24, p. 713.
21. Ouzounidou, G., Symeonidis, L., Babalonas, D., and Karataglis, S., Comparative responses of a copper-tolerant and a copper-sensitive population of *Minuartia hirsute* to copper toxicity, *J. Plant Physiol.*, 1994, vol. 144, p. 109.
22. Jiang, L.Y., Yang, X.E., Shi, W.Y., Ye, Z.Q., and He, Z.L., Copper uptake and tolerance in two contrasting ecotypes of *Elsholtzia argyi, J. Plant Nutr.*, 2004, vol. 27, p. 2067.
23. Li, B., Zhang, X., Wang, X., and Ma, Y., Refining a biotic ligand model for nickel toxicity to barley root elongation in solution culture, *Ecotoxicol. Environ. Saf.*, 2009, vol. 72, p. 1760.
24. Wang, X., Ma, Y., Hua, L., and McLaughlin, M.J., Identification of hydroxyl copper toxicity to barley (*Hordeum vulgare*) root elongation in solution culture, *Environ. Toxicol. Chem.*, 2009, vol. 28, p. 662.
25. Lin, Y., Allen, H.E., and Di Toro, D.M., Validation of Cu toxicity to barley root elongation in soil with a Terrestrial Biotic Ligand Model developed from sand culture, *Ecotoxicol. Environ. Saf.*, 2018, vol. 148, p. 336.
26. Wang, P., Zhou, D., Kinraide, T.B., Luo, X., Li, L., Li, D., and Zhang, H., Cell membrane surface potential (ψ0) plays a dominant role in the phytotoxicity of copper and arsenate, *Plant Physiol.*, 2008, vol. 148, p. 2134.
27. Wang, P., Kinraide, T.B., Zhou, D., Kopittke, P.M., and Peijnenburg, W.J.G.M., Plasma membrane surface potential: Dual effects upon ion uptake and toxicity, *Plant Physiol.*, 2011, vol. 155, p. 808.
28. Wang, P., Menzies, N., Wang, Y.-M., Zhou, D.-M., Zhao, F.-J., and Kopittke, P., Identifying the species of copper that are toxic to plant roots in alkaline nutrient solutions, *Plant Soil*, 2012, vol. 361, p. 317.
29. Tian, S., Lu, L., Zhang, J., Wang, K., Brown, P., He, Z., Liang, J., and Yang, X., Calcium protects roots of *Sedum alfredii* H. against cadmium-induced oxidative stress, *Chemosphere*, 2011, vol. 84, p. 63.
30. Rahman, A., Mostofa, M.G., Nahar, K., Hasanuzzaman, M., and Fujita, M., Exogenous calcium alleviates cadmium-induced oxidative stress in rice (*Oryza sativa* L.) seedlings by regulating the antioxidant defense and glyoxalase systems, *Braz. J. Bot.*, 2016, vol. 39, p. 393.