

# Environmental Monitoring

Cite this: *J. Environ. Monit.*, 2011, **13**, 591

[www.rsc.org/jem](http://www.rsc.org/jem)

PAPER

## Effects of silicon on copper toxicity in *Erica andevalensis* Cabezudo and Rivera: a potential species to remediate contaminated soils

Sabina Rossini Oliva,<sup>\*a</sup> Maria Dolores Mingorance<sup>b</sup> and Eduardo O. Leidi<sup>c</sup>

Received 6th October 2010, Accepted 3rd December 2010

DOI: 10.1039/c0em00549e

The influence of silicon on responses to copper excess was studied in plants of *Erica andevalensis*. Plantlets were grown in nutrient solutions containing two Cu (1 and 500  $\mu\text{M}$ ) and three Si concentrations (0, 0.5 and 1 mM). Plant growth, water content, and mineral nutrient concentration were determined. Plants grown with 500  $\mu\text{M}$  Cu showed differences in growth and shoot water content depending on Si supply. The addition of 1 mM Si in high-Cu nutrient solutions significantly improved plant growth and reduced water loss preventing plant death related to Cu-excess. Silicon supply reduced significantly leaf Cu concentration (up to 32%) and increased Cu concentration in roots. Phytoliths isolated from leaves were analysed by scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy. Such phytoliths consisted in silica deposits associated with Cu and other elements (K, Ca, P). Improvement by Si of Cu tolerance in *E. andevalensis* was clearly related to the inhibition of Cu upward transport. The leaf phytoliths formed in Si-treated plants might have some contribution to tolerance by Cu immobilisation and inactivation.

### 1. Introduction

Silicon is the second most abundant element in the earth's crust and is still unclear if it has an essential role in higher plants since its essentiality has been demonstrated only in a few plant species.<sup>1</sup> Liang *et al.*<sup>2</sup> proposed that according to the new definition of essentiality of elements,<sup>3</sup> silicon would be considered an essential element. Silicon is never found in a free form and is always combined with other elements. Plants take up Si by roots in the form of silicic acid ( $\text{Si}(\text{OH})_4$ ), which is translocated to the shoots, and with transpirational loss of water it is concentrated and polymerized to colloidal silicic acid and finally to silica gel (or

phytoliths in higher plants) on the surface of leaves and stems.<sup>4,5</sup> The term phytoliths is commonly employed to describe silicified cells, either isolated or in tissues. Silicon forms complex with metals such as Al, Cd and Zn contributing to its detoxification.<sup>6–8</sup> Higher plants differ in their capacity to take up silicon, and can be classified into three groups based on measurements of Si and the Si–Ca ratio in plant tissues: accumulators, intermediate and non-Si accumulators.<sup>4,5,9</sup> In general graminaceous plants and monocots tend to uptake much more Si than other species.<sup>2,5</sup> These species possess Si active uptake systems,<sup>10</sup> whilst the majority of species accumulate Si *via* passive diffusion following the water flux driven by transpiration.<sup>8</sup> Silicon accumulation and physiology have been extensively studied in grasses.<sup>11–15</sup> There is evidence that Si has a protecting effect against insects, parasites and pathogens<sup>16,17</sup> and also improves plant performance under abiotic stresses.<sup>5,18,19</sup> It is the only element that does not cause serious injury when present in excess.<sup>4,5</sup> Silicon plays a significant role in minimizing the toxic effects of excess of metals like Al,<sup>7,12,14,20,21</sup> Cd,<sup>15,22,23</sup> Mn,<sup>24–27</sup> and Zn.<sup>6,8</sup> In these investigations,

<sup>a</sup>Department of Plant Biology and Ecology, University of Seville, Avda. Reina Mercedes s/n, Apto de C orreo 1095, 41012 Seville, Spain. E-mail: [sabina@us.es](mailto:sabina@us.es); Fax: +34954557059

<sup>b</sup>Instituto Andaluz de Ciencias de la Tierra, (UGR-CSIC), Prof. Albareda 1, 18008 Granada, Spain

<sup>c</sup>Department of Plant Biotechnology, IRNAS-CSIC, Avda. Reina Mercedes 10, 41012 Seville, Spain

### Environmental impact

Silicon has been considered as an essential nutrient for many plant species. *Erica andevalensis* is a metallophyte species that grows in a mining area of SW of Spain and that was proposed to be used in phytoremediation of mine environments. Under hydroponic conditions *E. andevalensis* does not tolerate 500  $\mu\text{M}$  of Cu whilst when treated with 1 mM Si is able to tolerate it. *E. andevalensis* forms phytoliths in the leaves when treated with Si. The leaf phytoliths might have some contribution to enhance the Cu tolerance. The results should help to understand how to improve the Cu resistance in this species that can be useful to promote the phytostabilization and the development of a self-sustaining vegetative cover in contaminated soils.

different mechanisms were proposed to explain the Si-associated alleviation of metal toxicity. For example, in high Mn, Si would reduce Mn availability by a stronger binding of Mn to cell walls resulting in lower concentration in the symplast.<sup>25</sup> Most recently, Shi *et al.*<sup>26</sup> reported that Si decreased lipid peroxidation caused by Mn. Alleviation of toxicity by Si in plants under abiotic stresses might be a consequence of both internal and external mechanisms.<sup>2</sup> In Cd stressed plants, silicon inhibits Cd transport from roots to shoots, resulting in reduction of Cd content in cell organelle fractions of leaves and the stimulation of antioxidative enzymes.<sup>23</sup> No studies are available on the effect of Si in the response of heathers like *E. andevalensis* to Cu toxicity. Nowakowski and Nowakowska<sup>28</sup> reported that Si reduced Cu toxicity in wheat by decreasing Cu uptake. Most recently, Li and Leisner<sup>29</sup> found that Si improved tolerance to Cu stress in *Arabidopsis thaliana* at multiple levels from alteration in gene expression to physiological changes.

The endemic heath *E. andevalensis* colonizes in some areas of the Iberian Pyrite Belt, growing on mine tailings and bank sediments of Tinto and Odiel Rivers (SW Spain). It is able to survive in polluted soils and the proximity of acidic waters heavily contaminated with metals (mostly Fe, Cu and Zn). In a previous study to determine its sensitivity to metal excess in solution,<sup>30</sup> high Cu concentration (500  $\mu\text{M}$ ) significantly led to 75% mortality and significant growth reduction in *E. andevalensis*. Beneficial effects have been found for this species under Al toxicity,<sup>31</sup> in which Al and Si were concentrated in leaf upper epidermis and top of multicellular hairs.

The present work aimed to study the effect of silicon in *E. andevalensis* under Cu excess as this heath has potential for re-vegetation in the acidic and poor soil sometimes heavily contaminated with metals like Cu.

## 2. Materials and methods

The plants for studying the effects of Si treatments were obtained by germinating seeds collected in the Riotinto mining area (Andalusia, SW Spain) during 2007. Plantlets were cultivated in a full nutrient solution in which the pH was adjusted and maintained at 4 by adding  $\text{H}_2\text{SO}_4$  to simulate real growing conditions. The nutrient solution (pH 4.0) contained (in mM):  $\text{NO}_3^-$ , 4;  $\text{H}_2\text{PO}_4^-$ , 1;  $\text{SO}_4^{2-}$ , 2.5;  $\text{K}^+$ , 4;  $\text{Ca}^{2+}$ , 2;  $\text{Mg}^{2+}$ , 1. Micronutrient concentration was (in  $\mu\text{M}$ ): B, 50; Fe, 100; Mn, 10; Cu, 1; Zn, 1; Mo, 05; and Fe was provided as  $4 \text{ mg l}^{-1}$  Fe-EDDHA.<sup>30</sup> When seedlings reached about 3 g fresh weight were transferred into 8 L plastic trays (8 plants for tray) containing the nutrient solutions. The Si treatments consisted in three different Si concentrations (0, 0.5 and 1 mM added as  $\text{Na}_2\text{SiO}_3$ ) added into nutrient solutions containing either with normal Cu supply (1  $\mu\text{M}$  Cu) (control) or toxic Cu (500  $\mu\text{M}$  Cu).<sup>30</sup> Copper was added in the form of  $\text{CuSO}_4$ . Solutions were continuously aerated with an aquarium air pump, completely renewed every 10 days and its pH was checked. Plants were grown in a growth chamber with day/night cycles 16 h light–8 h darkness at 26–22 °C respectively. All treatments had four replicates with two plants per replicate. Thirty days after treatment, the plants were harvested, washed with distilled water, separated into roots, stems and leaves and weighed, except plants treated with 500  $\mu\text{M}$

of Cu + 0 Si and 0.5 mM of Si, which had to be harvested after 20 day treatments because almost all plants were wilting.

For the analysis of elements, samples were dried at 70 °C, ground in a stainless steel mill and digested in a microwave oven-assisted procedure following the protocol recommended by the manufactured microwave, which involves digestion in a mixture of  $\text{HNO}_3 + \text{H}_2\text{O}_2 + \text{HF}$  (2 + 1.5 + 0.5 mL) followed by 5%  $\text{H}_3\text{BO}_3$  treatment in PTFE vessels under pressure to complex any remaining HF. Then Ca, Cu, Fe, K, Mg, P, S, Si and Zn contents were determined by ICP-OES (Horiba Jobin Yvon). The accuracy of digestion and analytical procedure was checked by routine determination of element concentrations in two reference materials: NIST SRM-2710 (certified value,  $30.44 \pm 0.19\%$  Si) and BCR CRM 61 (reference value,  $0.075\%$  Si). The Si recoveries were  $30 \pm 3\%$  and  $0.070 \pm 0.07\%$  respectively, in good agreement with the corresponding reported values. The recovery range for the other elements was from 96 to 105%.

The translocation factor (TF) was calculated as  $\text{Cu}_{\text{shoot}}/\text{Cu}_{\text{root}} \times 100$ . Growth was estimated by determining plant biomass at the beginning and end of the experiment. Growth values result from the difference between the final fresh weight and the initial fresh weight (measured at transplantation).

Leaf phytoliths were obtained from plants grown in the different Si–Cu treatments. For extracting the phytoliths, a dry ashing technique was used as reported by Morikawa and Saigusa.<sup>32</sup> Plant leaves were dried at 70 °C overnight and then ashed at 500 °C for 4 h approximately. A diluted HCl solution was added to the ashes and the mixture was centrifuged at 15 000 rpm for 30 min and decanted. The residue was rinsed with distilled water and centrifuged at 15 000 rpm for 30 min. Then 5 mL of  $\text{H}_2\text{O}_2$  (30%, v/v) was added, rinsed with distilled water and centrifuged at 15 000 rpm for 30 min. Part of the material was resuspended with distilled water and mounted for examination by optical microscopy. A fraction of the same particles was fixed on aluminium stubs with double-sided adhesive tape, coated with vacuum-evaporated carbon. The samples were examined in a JEOL 6460LV scanning electron microscope (SEM) coupled with EDX analysis operating at 25 kV and at a working distance of 15 mm. Semi-quantitative analysis was carried out by ZAF method. All data are means from three replications per treatment. In addition, leaf samples from field-grown plants were collected to study similarly by SEM-EDX. Leaves were collected from plants growing in a contaminated site of Riotinto mining area (Nerva, UTM 29S 4175471/715131, SW Spain), with mining and smelting waste piles.

## 3. Statistical analysis

Normality of datasets was checked by Shapiro–Wilk test. When normality was fulfilled, influence of Si and Cu treatment on the different variables analyzed was evaluated by ANOVA. Multiple comparisons among treatments were made by *post hoc* Turkey's test and comparison between the Si treatments was carried out by Student's *t*-test. Otherwise, the Kruskal–Wallis non-parametric test was used. The statistical significance was set/established at  $p < 0.05$ . The statistical analyses were performed by Statistica (StatSoft Inc., USA) software program. All data presented are the mean values of at least four replicates.

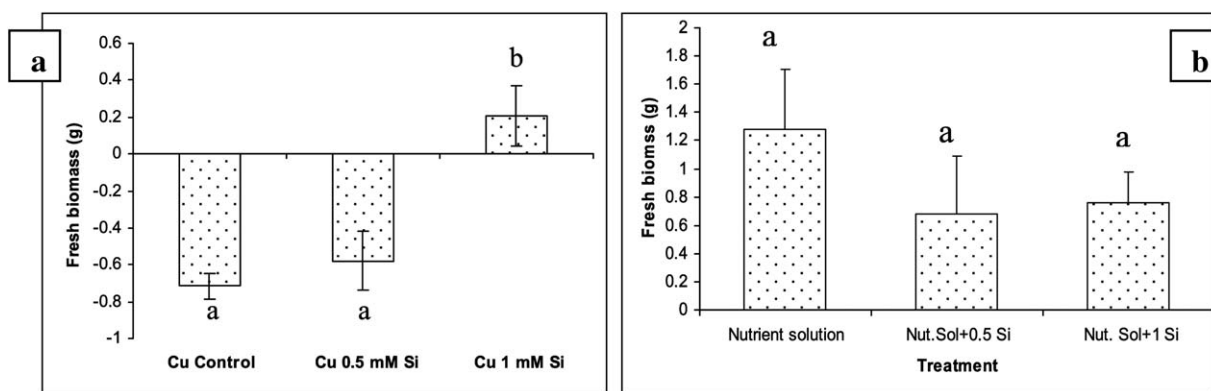
## 4. Results

### 4.1. Growth and mineral content in plant parts

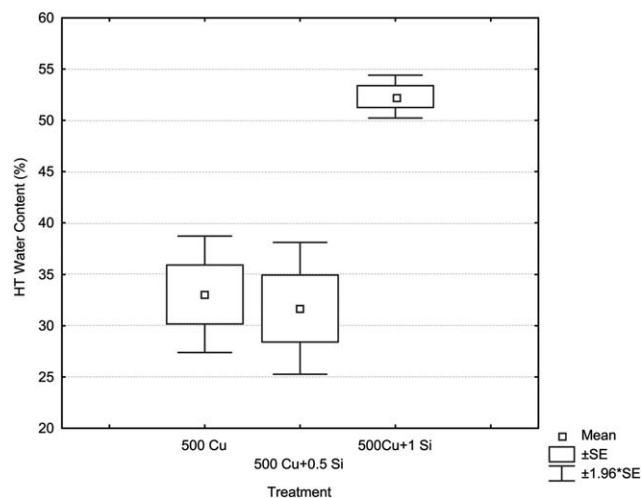
After 20 days, plants grown with 500  $\mu\text{M}$  Cu showed visible symptoms of Cu toxicity consisting in delayed growth and wilting leaves when Si was absent in the medium or when it only reached 0.5 mM Si. The addition of 0.5 mM Si only reduced the formation of brownish necrotic roots but not leaf wilting and the further leaf death induced by high Cu. However, increasing the Si concentration in the solution to 1 mM had a clear protective effect in plants grown at high Cu promoting growth and avoiding the appearance of leaf wilting. The excess of Cu produced a significant biomass reduction in plants at 0 or 0.5 mM Si but not at 1 mM Si (Fig. 1a). The presence of Si also improved shoot water contents ( $p < 0.05$ , Fig. 2). At low Cu concentration, the addition of Si in the nutrient solution had no effects on plant growth ( $p = 0.08$ , Fig. 1b).

Silicon supply had a significant effect on the plant contents of Cu at low (normal) and high Cu concentration (Tables 1 and 2). The distribution of Si between plant organs was not uniform: when Cu was present in excess, the distribution pattern was roots > leaves > stems in both Si treatments, and the mean contents were 4122 and 5422  $\text{mg g}^{-1}$  for roots and 913 and 1206  $\text{mg g}^{-1}$  for leaves from plants in 0.5 and 1 mM Si respectively. In plants growing in nutrient solutions containing normal concentration of Cu, the accumulation pattern for Si was roots > stems = leaves in all Si treatments. Regarding Cu, its distribution changed at normal or high Cu ( $p < 0.05$ ). At high Cu, the accumulation pattern was root > stems > leaves for all Si treatments. However, the application of 1 mM Si significantly reduced the Cu concentration in leaves and stems ( $p < 0.05$ ) but it increased the Cu concentration in the roots (Table 1). For both silicon treatments, no significant differences were observed in Si content of stems and roots. The supply of Si also changed the concentration of other nutrients in stems (for Ca, K, Mg, S and Zn) and roots (Ca, Fe, Mg, P and Zn) (Table 1).

When 1 mM Si was present in the nutrient solution, it promoted a significant reduction in the translocation factor (TF) for Cu in plants grown under Cu excess: the TF value reached 15.8% in the control plants (0 mM Si) and 10.4% at 0.5 mM Si but only was 2.7% in the presence of 1 mM Si.



**Fig. 1** Effect of Si supply on biomass (calculated as final-initial fresh weights) in *E. andevalensis* plants grown under high (a) and normal (b) Cu concentrations (500  $\mu\text{M}$  and 1  $\mu\text{M}$  respectively). Data are means  $\pm$  SD of four replicates. Different letters indicate statistically significant differences ( $p < 0.05$ ). Cu control, 500  $\mu\text{M}$  Cu; nutrient solution, 1  $\mu\text{M}$  Cu.



**Fig. 2** Shoot water content variation among Si treatments in *E. andevalensis* grown at high Cu (500  $\mu\text{M}$  Cu).

At low Cu in the medium (Table 2), Si also significantly reduced the concentration of Cu in leaves ( $p < 0.05$ ). A similar pattern was observed in stems but significant differences were only observed for plants in 0.5 mM Si ( $p = 0.02$ ). Silicon supply significantly changed the Cu content in roots (ANOVA,  $p = 0.03$ ). Silicon concentration in leaves, stems and roots was similar in both Si treatments at 1  $\mu\text{M}$  Cu ( $p > 0.05$ ).

### 4.2. Copper and Si distribution in phytoliths

SEM and optical observations of phytoliths from leaves of field grown plants showed bodies of various shapes (Fig. 3a). The semi-quantitative elemental microanalysis by EDX revealed that such bodies contained mostly Si (72.20 weight%) while others were constituted by Si associated with Al, K, Mn, Fe and Cu (Fig. 3b), which indicates that these formations are phytoliths. The microanalysis showed that in some phytoliths Cu reached values of 6.62 weight%. Similar deposits were also observed in the ash of plant leaves treated with 500  $\mu\text{M}$  Cu and 0.5 or 1 mM Si. The microanalysis revealed that almost all phytoliths contained a high amount of Si associated with Cu (Fig. 4), indicating

**Table 1** Mineral composition (mg g<sup>-1</sup>) of plant part of *E. andevalensis* grown in a media with 500 μM of Cu and with different Si supply (0, 0.5 and 1 mM) (average of four replicates). \* indicates significant mean differences in data group by Krushal–Wallis test and means followed by different letters indicate significant differences between treatments by Tukey or *t*-test

Si/mM	Cu	Si	Fe	Ca	K	Mg	P	S	Zn
Leaves									
0.0	86.3 <sup>a</sup>	—	113 <sup>a</sup>	4642 <sup>a</sup>	13 456 <sup>a</sup>	2964 <sup>a</sup>	1599 <sup>a</sup>	3653 <sup>ns</sup>	12.2 <sup>a</sup>
0.5	109 <sup>a</sup>	913 <sup>a</sup>	111 <sup>a</sup>	4473 <sup>a</sup>	12 612 <sup>a</sup>	2930 <sup>a</sup>	2727 <sup>a</sup>	3262 <sup>ns</sup>	32.0 <sup>b</sup>
1.0	32.8 <sup>b</sup>	1206 <sup>b</sup>	90.1 <sup>a</sup>	3386 <sup>a</sup>	11 183 <sup>a</sup>	2165 <sup>a</sup>	2173 <sup>a</sup>	2688 <sup>ns</sup>	33.1 <sup>b</sup>
Stems									
0.0	936 <sup>a</sup>	—	48.5 <sup>a</sup>	2735 <sup>a</sup>	18 022 <sup>a</sup>	1723 <sup>a</sup>	2327 <sup>a</sup>	2368 <sup>a</sup>	11.9 <sup>a</sup>
0.5	769 <sup>a</sup>	604 <sup>a</sup>	79.1 <sup>a</sup>	1867 <sup>ab</sup>	10 404 <sup>b</sup>	1261 <sup>ab</sup>	2665 <sup>a</sup>	1725 <sup>ab</sup>	35.8 <sup>ab</sup>
1.0	183 <sup>b</sup>	526 <sup>a</sup>	85.5 <sup>a</sup>	1146 <sup>b</sup>	7926 <sup>b</sup>	1009 <sup>b</sup>	2225 <sup>a</sup>	1055 <sup>b</sup>	40.4 <sup>b</sup>
Roots									
0.0	6461 <sup>a</sup>	—	19 767 <sup>a</sup>	2648 <sup>*</sup>	4745 <sup>a</sup>	721 <sup>*</sup>	10 633 <sup>a</sup>	2049 <sup>a</sup>	65.8 <sup>a</sup>
0.5	8469 <sup>b</sup>	4122 <sup>a</sup>	29 381 <sup>b</sup>	2403 <sup>*</sup>	3851 <sup>a</sup>	910 <sup>*</sup>	13 907 <sup>b</sup>	1887 <sup>a</sup>	91.7 <sup>a</sup>
1.0	7795 <sup>b</sup>	5422 <sup>a</sup>	22 046 <sup>a</sup>	4719 <sup>*</sup>	4692 <sup>a</sup>	1635 <sup>*</sup>	11 562 <sup>ab</sup>	2292 <sup>a</sup>	125 <sup>b</sup>

**Table 2** Effects of Si supply (0, 0.5 and 1 mM) on Cu and Si concentration (mg g<sup>-1</sup>) leaves, stems and roots of *E. andevalensis* grown in nutrient solution containing normal Cu content (1 μM Cu) (average of four replicates). L, leaves, S, stems, R, roots. \* indicates significant mean differences in data group by Kruskal–Wallis test and different letters indicate significant mean differences by Tukey or *t*-test

Si treatment/mM	Cu	Si
Leaves		
0.0	11.1 <sup>*</sup>	—
0.5	3.15 <sup>*</sup>	565 <sup>a</sup>
1.0	3.47 <sup>*</sup>	619 <sup>a</sup>
Stems		
0.0	14.7 <sup>a</sup>	—
0.5	4.36 <sup>b</sup>	501 <sup>a</sup>
1.0	9.85 <sup>ab</sup>	394 <sup>a</sup>
Roots		
0.0	56.5 <sup>a</sup>	—
0.5	92.2 <sup>b</sup>	3258 <sup>a</sup>
1.0	85.5 <sup>ab</sup>	4710 <sup>a</sup>

a significant Cu concentration in the leaves was associated with Si deposits.

## 5. Discussion

The excess of copper produced significantly changes in *E. andevalensis* at biochemical and physiological levels as it was reported elsewhere.<sup>30</sup> The most evident symptoms of Cu toxicity include reduction in plant growth, appearance of necrotic roots, and shoot dehydration that leads to plant death.<sup>30</sup> As shown by the present results, Si supply alleviated Cu-induced growth inhibition and toxicity symptoms. In the presence of 1 mM Si, *Erica* plants showed a healthy appearance without symptoms of metal stress and a significant increase in shoot water content. Similar effects of Si in plant growth were observed in other species treated with excess of Al, Cu, Cd, Mn and Zn.<sup>8,12,22,23,27,28,33</sup> Because of the beneficial effects of Si on growth reported in a wide variety of crops, Si is applied as fertilizer both as soil amendment and in foliar treatments.<sup>4,5</sup>

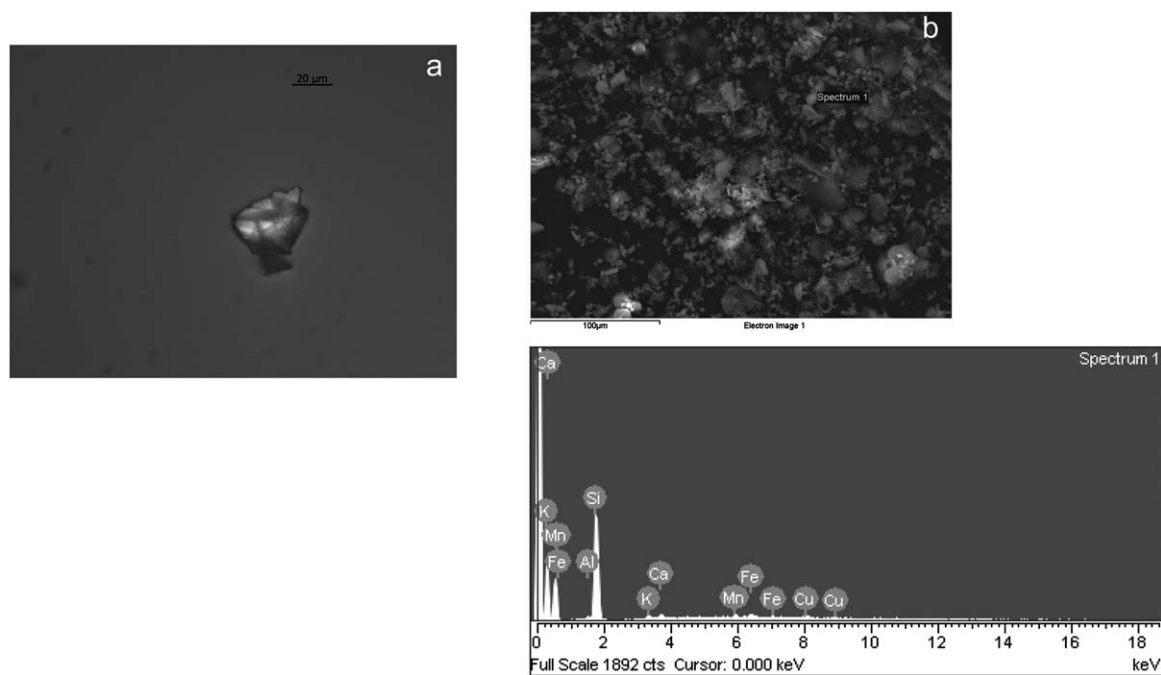
When grown under optimal Cu concentrations, Si supply did not improve the growth of *Erica* plants, results are in agreement with previous reports in other species,<sup>4,5</sup> which had shown Si-derived benefits only under stress conditions. Our results suggest

that under Cu stress, Si-associated growth stimulation may be the result of the reduction in shoot Cu concentration. In fact, the reduction in Cu TF values by 1 mM Si-treated plants implies that somehow Si inhibited translocation of Cu from roots to shoots and thereby reduced shoot Cu toxicity. A previous study in *Arabidopsis* has suggested that Si enhances the tolerance to Cu excess by affecting the metal distribution or bioavailability and not by reducing the Cu uptake or translocation.<sup>29</sup> However, silicon in *E. andevalensis* significantly reduced the Cu concentration in shoots, thereby affecting the Cu uptake and translocation, which is in agreement with previous results in wheat.<sup>28</sup> At low Cu (1 μM), Si also induced a significant reduction of Cu concentration in leaves, which reach a concentration in the range of deficiency.<sup>1</sup>

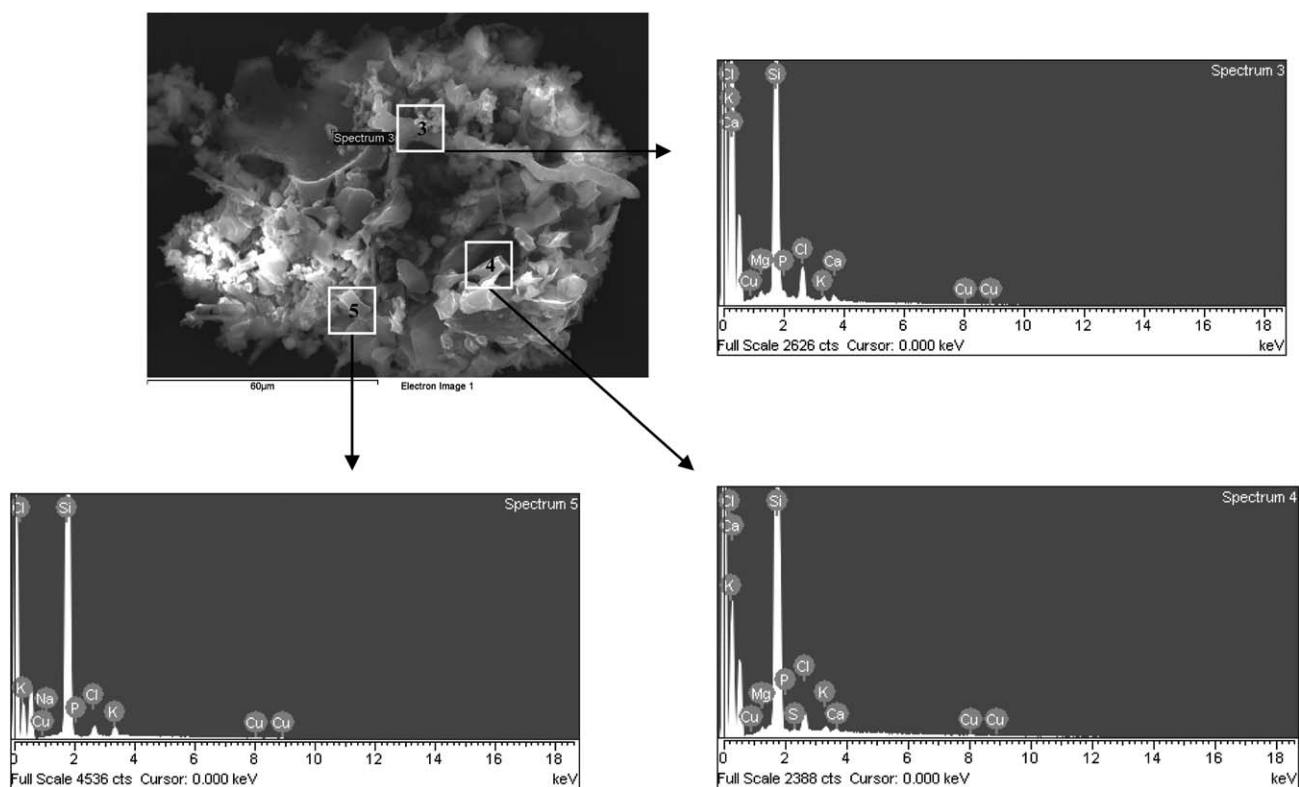
The correction of nutrient imbalances is a different proposed role of Si for explaining its reduction of metal toxicity symptoms.<sup>4</sup> Copper–Zn interactions are common as these metals are absorbed by the same mechanism and therefore Cu may competitively inhibit Zn absorption.<sup>1</sup> Our results show that Si treatment facilitated the root absorption of Zn and its transport into leaves and stems (Table 1) in agreement with other reports.<sup>1,3</sup> High Si concentration may also enhance the root concentration of Ca, an element with an antagonistic effect on Cu uptake<sup>1</sup> and therefore it might contribute to reduce the Cu content in shoots. In plants of *E. andevalensis* grown in nutrient solution, the addition of 1 mM Si significantly increased the Ca concentration in the roots. The reduction in Cu<sup>2+</sup> activity at the root cell plasma membrane induced by high Ca may also have contributed to the inhibition of Cu uptake.<sup>34</sup>

Distribution of Si between plant organs was different at low or high Cu concentration. Roots were the main sites for Si accumulation, while leaves and stems contained similar Si concentrations. In plants collected in metal contaminated soils, Si was found in both intra- and extracellular *E. andevalensis* compartments,<sup>35</sup> being greater in mesophyll cells and leaf multicellular hairs. Turnau *et al.*<sup>31</sup> found a high Si accumulation in leaf multicellular hairs and the leaf upper epidermis in the same species reporting that Si was often associated with Al and Fe.

The phytoliths from woody *Ericaceae* always contain Al<sup>36</sup> and a synchronous accumulation of Si and Cd in phytoliths was observed in shoots of rice.<sup>22</sup> The co-deposition of Si–Zn in *Cardaminopsis halleri* epidermal cell walls has been suggested as



**Fig. 3** Phytoliths recovered from ashed leaves of *E. andevalensis* collected in the field observed by optical (a) and scanning electron microscopy (b) and diagram of EDX analysis spectra.



**Fig. 4** SEM image and spectra obtained after EDX-microanalysis in phytoliths from ashed leaves of *E. andevalensis* plants grown in nutrient solutions containing 500 µM Cu and 1 mM Si.

part of the Zn tolerance mechanism in this species.<sup>6</sup> Considering those previous reports on Si-metal interactions seemed to be interesting to make a preliminary study on the composition of phytoliths in *E. andevalensis*. The leaf phytoliths obtained in the

present work presented Si-Cu associations probably forming Cu-silicates, but if such Cu sequestration has any role in reducing the Cu toxicity requires a detailed quantitative study. Copper signals appeared in both phytoliths obtained from leaves of

soil-grown plants and those from Si-added nutrient solutions. As most of Si and Cu remained in the roots, phytoliths from this organ should be quantified and analyzed in future studies to determine to which extent these structures may contribute to Cu tolerance by fixing the metal in the roots.

Silicon affected Cu uptake/translocation processes reducing significantly the Cu concentration in leaves. This might be an important effect of Si when improving the Cu tolerance in *E. andevalensis*. However, fixation and inactivation of Cu in roots would be a main process for maintaining favourable conditions for root water and selective ion uptake. The role of phytoliths formation in increasing Cu sequestration needs further research.

## 6. Conclusion

Silicon plays an important role diminishing Cu toxicity symptoms in *E. andevalensis* mostly by decreasing Cu translocation to the shoots apparently increasing Cu fixation (immobilization) in root tissues. The formation of Si bodies (phytoliths) including Cu might also sequester the metal and provide additional Cu tolerance in leaves but more studies are required to determine such a role. The present results suggest that Si might be added to soils presenting low Si availability to enhance Cu tolerance in *E. andevalensis*, and this amendment might be importance for successful re-vegetation of Cu contaminated soils.

## Acknowledgements

This work was supported by the MICINN (CGL2006/02860) and by Fundación Ramón Areces. We are also grateful to the technical staff at the Greenhouse Facilities, University of Seville for the assistance in plant cultivation.

## References

- 1 H. Marschner, *Mineral Nutrition of Higher Plants*, Academic Press, London, 1995.
- 2 Y. C. Liang, W. Sun, Y. G. Zhu and P. Christie, A review, *Environ. Pollut.*, 2007, **147**, 422–428.
- 3 E. Epstein and A. J. Bloom, *Mineral Nutrition of Plants: Principles and Perspectives*, Sinauer, Sunderland, MA, 2nd edn, 2005.
- 4 J. F. Ma, Y. Miyake and E. Takahashi, in *Silicon in Agriculture*, ed. L. E. Datnoff, G. H. Snyder and G. H. Korndörfer, Elsevier Press, Amsterdam, The Netherlands, 2001, pp. 17–34.
- 5 K. Richmond and M. Sussman, *Curr. Opin. Plant Biol.*, 2003, **6**, 266–272.

- 6 D. Neumann and U. zur Nieden, *Phytochemistry*, 2001, **56**, 685–692.
- 7 M. J. Hodson and A. G. Sangster, *J. Inorg. Biochem.*, 1999, **76**, 89–98.
- 8 K. P. V. da Cunha and C. W. A. do Nascimento, *Water, Air, Soil Pollut.*, 2009, **197**, 323–330.
- 9 E. Takahashi and Y. Miyake, Silica and plant growth, *Proc. Int. Semin. Soil Environ. Fertil. Manage. Intensive Agric.*, 1977, 603–611.
- 10 H. A. Currie and C. Perry, *Ann. Bot. (Oxford, U. K.)*, 2007, **100**, 1383–1389.
- 11 D. M. Bennett, *Ann. Bot. (Oxford, U. K.)*, 1982, **48**, 645–654.
- 12 J. Barcelo, P. Guevara and C. Poschenrieder, *Plant Soil*, 1993, **154**, 249–255.
- 13 C. W. Deren, B. Glaz and G. N. Syder, *J. Plant Nutr.*, 1993, **16**, 2273–2280.
- 14 Y. C. Liang, C. G. Yang and H. H. Shi, *J. Plant Nutr.*, 2001, **24**, 229–243.
- 15 Y. C. Liang, J. W. C. Wong and L. Wei, *Chemosphere*, 2005, **58**, 475–483.
- 16 R. K. Maiti, K. V. Ramaiah, S. S. Bisen and V. L. Chidley, *Ann. Bot. (Oxford, U. K.)*, 1984, **54**, 447–457.
- 17 E. A. H. Pilon-Smits, C. F. Quinn, W. Tapken, M. Malagoli and M. Schiavon, *Curr. Opin. Plant Biol.*, 2009, **12**, 267–274.
- 18 J. F. Ma, *Soil Sci. Plant Nutr. (Richmond, Aust.)*, 2004, **50**, 11–18.
- 19 L. E. Datnoff, W. H. Elmer and D. M. Huber, *Mineral Nutrition and Plant Disease*, APS Press, St Paul, MN, 2007.
- 20 K. M. Cocker, D. E. Evans and M. J. Hodson, *Planta*, 1998, **204**, 318–323.
- 21 R. M. Britez, T. Watanabe, S. Jansen, C. B. Reissmann and M. Osaki, *New Phytol.*, 2002, **156**, 445–456.
- 22 C. Zhang, L. Wang, Q. Nie, W. Zhang and F. Zhang, *Environ. Exp. Bot.*, 2008, **62**, 300–307.
- 23 G. Shi, Q. Cai, C. Liu and L. Wu, *Plant Growth Regul.*, 2010, **61**, 45–52.
- 24 T. Horiguchi, *Soil Sci. Plant Nutr. (Richmond, Aust.)*, 1988, **34**, 65–73.
- 25 H. Rogalla and V. Römheld, *Plant, Cell Environ.*, 2002, **254**, 549–555.
- 26 Q. Shi, Z. Bao, Z. Zhu, Q. Qian and J. Yu, *Phytochemistry*, 2005, **66**, 1551–1559.
- 27 J. P. Feng, Q. H. Shi and Z. F. Waing, *Agric. Sci. China*, 2009, **8**, 40–50.
- 28 W. Nowakowski and J. Nowakowska, *Biol. Plant.*, 1997, **39**, 463–466.
- 29 J. Li and S. M. Leisner, *J. Am. Soc. Hortic. Sci.*, 2008, **133**, 670–677.
- 30 S. Rossini Oliva, M. D. Mongorance, B. Valdés and E. O. Leidi, *Plant Soil*, 2010, **328**, 411–420.
- 31 K. Turnau, F. S. Henriques, T. Anielska, C. Renker and F. Buscot, *Environ. Exp. Bot.*, 2007, **61**, 117–123.
- 32 C. K. Morikawa and M. Saigusa, *Plant Soil*, 2004, **258**, 1–8.
- 33 J. Feng, Q. Shi, X. Wang, M. Wei, F. K. Yang and H. Xu, *Sci. Hortic. (Amsterdam, Neth.)*, 2010, **23**, 521–530.
- 34 P. M. Kopittke, F. P. C. Blamey, C. J. Asher and N. W. Menzies, *J. Exp. Bot.*, 2010, **61**, 945–954.
- 35 S. Rossini Oliva, B. Valdés and E. O. Leidi, *Sci. Total Environ.*, 2009, **407**, 1929–1936.
- 36 A. L. Carnelli, M. Madella, J. P. Theurillat and B. Ammann, *Am. J. Bot.*, 2002, **89**, 346–351.