Effect of copper on germination and seedling growth of *Minuartia*, *Silene*, *Alyssum* and *Thlaspi*

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Abstract

The influence of increasing copper concentrations on seed germination, seedling survival and radicle length of *Minuartia hirsuta*, *Silene compacta*, *Alyssum montanum* and *Thlaspi ochroleucum* was studied. Seed germination was highly affected by the higher Cu^{2+} concentrations (80 and 160 μM), while lower Cu^{2+} concentrations seemed to be necessary for seed germination, even for the plants originated from non-Cu^{2+}-rich soils (*i.e.* *A. montanum*). Nevertheless, plants originated from Cu^{2+}-rich soils (*M. hirsuta*, *S. compacta*) showed a higher demand of Cu^{2+} for rapid seed germination. Cu^{2+} at higher concentrations severely reduced growth rate of radicle, especially in *A. montanum* and *T. ochroleucum*. These data clearly indicate the reduced suitability of the above mentioned plant species for reclamation on Cu^{2+} soils. Lower Cu^{2+}-concentrations had no influence on seedling survival in *M. hirsuta* and *S. compacta*, but a progressive reduction of a number of survived seedlings with increasing Cu^{2+} concentration was found, that was more pronounced in *A. montanum* and *T. ochroleucum*.

Key words: Cu^{2+}-rich soils, radicle length, reclamation, seedling survival

Introduction

Soils with elevated total concentrations of heavy metals generally owe this property to contamination resulting from close proximity to natural metalliferous ore outcrops, or as a result of man's metalliferous mining, smelting or other industrial activities. Early observations of plants growing on metal-contaminated soils have posed intriguing questions about the nature, scale and mechanisms of adaptation involved (Antonovics *et al.* 1971, Ernst 1976, Woolhouse 1983). Recent study has focused on the development of metal-tolerant cultivars for use in the revegetation of metalliferous wastes and refinement of geobotanical and biogeochemical techniques of mineral exploration (Baker 1987). Our work here is confined to Cu as it is probably among the most common contamintants of metalliferous soils in Northern Greece. Moreover, Cu is also among the essential micronutrients for plant growth.

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However, in excessive quantities it becomes toxic as it interferes in photosynthetic and respiratory processes, in enzyme synthesis and in ultrastructural development of the plants (Wainwright and Woolhouse 1975, Karataglis *et al.* 1988, Ouzounidou *et al.* 1992, Ouzounidou *et al.* 1993).

According to Reeves *et al.* (1986) and Baker (1987) some species of the metallophytes genera *Minuartia* and *Silene* (*Caryophyllaceae*) are widespread on shallow, coarser, nutrient-poor and Cu-rich soils and they could be characterized as "excluders". Studies concerning the species of the genus *Alyssum* and *Thlaspi* (*Cruciferae*) classified many of them to "accumulators" of Ni, Zn and Pb which are restricted to a Ni, Zn and Pb outcrop (Reeves *et al.* 1983, Baker and Brooks 1989).

The purpose of the present study was to quantify the effects of Cu on seed germination, seedling survival and primary root growth for *Minuartia hirsuta*, *Silene compacta*, *Alyssum montanum* and *Thlaspi ochroleucum*, over a Cu-concentration range of 0 to 160 μM. In addition, an effort has been made to determine the respective tolerances to Cu of species originated from Cu-rich and Zn-Pb-rich soils.

Materials and methods

Seed of *Minuartia hirsuta* Hand.-Mazz was obtained from mine-contaminated soils with Cu^{2+} (Gerakario, Kilkis, N. Greece), of *Silene compacta* Fischer from mine-contaminated soils with Cu^{2+} (Kokkinolakkos, Chalkidiki, N. Greece), of *Alyssum montanum* L. from mine-contaminated soils with Zn^{2+} and Pb^{2+} (Sotiras, Thassos, N. Greece) and of *Thlaspi ochroleucum* Boiss. & Heldr. from mine-contaminated soils with Zn^{2+} and Pb^{2+} (Limenaria, Thassos, N. Greece).

Six $CuSO_4$ treatments (0, 8, 16, 32, 80 and 160 μM) were used to test the effects of Cu^{2+} on seed germination, seedling survival and primary root length. For each treatment, 30 seeds were placed on filter paper in Petri dishes (3 replications per treatment) soaked with 6 cm^3 of $CuSO_4$ solutions and the pH was adjusted to 5.5 ± 0.1. The dishes were then placed in clear plastic bags to maintain constant high humidity and were subjected to a 16 h photoperiod, irradiance of 15 W m^{-2} and a constant temperature of 25 ± 1 °C (Ouzounidou *et al.* 1993). The number of germinated seeds were checked daily and were considered to be fully germinated when the radicle was about 1.5 mm in length, until no more germination occurred (10 d). At 3-d intervals after the onset of germination, primary root length was measured for 15 randomly chosen survived seedlings from each treatment. At 13-d the survival of the seedlings was also determined.

All data were analysed with Student's t-test, to assess the statistical significance of differences between Cu^{2+}-treatments.

Results

Seeds of *M. hirsuta* of all the Cu^{2+}-treatments started to germinate 3 d after the experiment began (Fig. 1a). The time necessary for maximum seed germination was

10 d for all Cu^{2+}-treatments. Exposure to 8 and 16 µM of Cu^{2+} resulted in an appreciable increase in seed germination (by 10 and 3 % compared with the control), while 160 µM of Cu^{2+} caused a significant reduction (by 25 % of the control; $P < 0.05$) in germination (Fig. 1a).

After 3 d the initial seed germination of *S. compacta* occurred at all Cu^{2+} concentrations (Fig. 1b). The maximum germination for control and 8 µM of Cu^{2+} was after 10 d, whereas for the higher Cu^{2+} concentrations after 7 d. 8 µM of Cu^{2+} stimulated germination by 10 % compared with the control, while 160 µM of Cu^{2+} inhibited it by 16 % ($P < 0.05$, Fig. 1b).

All Cu^{2+}-treatments and control for *A. montanum* seeds started germination 3 d after the experimental beginning (Fig. 1c). The maximum seed germination occurred under 8, 16 and 32 µM of Cu^{2+} (95 %), while the corresponding value of control was 90 %. Higher Cu^{2+}-treatments (80 and 160 µM) induced a significant delay in germination and a small percentage of germinated seeds that was 25 and 30 % lower than that of the control ($P < 0.05$, Fig. 1c). The maximum seed germination for *A. montanum* at 80 and 160 µM of Cu^{2+} was observed after 6 d, whereupon no further germination occurred.

Simultaneous seed germination for control and Cu^{2+}-treatments of *T. ochroleucum* was observed (4 d; Fig. 1d). At control and 8 µM of Cu^{2+} progressive increase in seed germination during the 10 d occurred, whereas at the higher Cu^{2+} concentrations maximum germination was achieved after 8 d. The maximum value appeared in

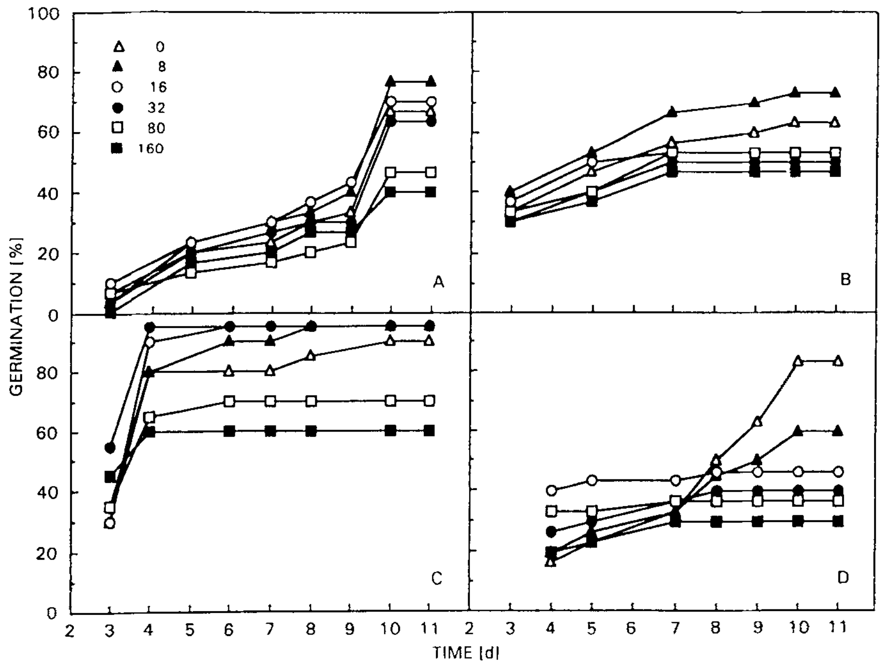

Fig. 1. *M. hirsuta* (*A*) *S. compacta* (*B*) *A. montanum* (*C*) *T. ochroleucum* (*D*) seed germination on solutions with varying Cu^{2+} concentrations.

control solution (84 %) and was depressed by 64 % at 160 μM of Cu^{2+} compared with the control ($P < 0.05$, Fig. 1d).

Cu^{2+} influenced primary root growth in a different manner in the four plant species (Table 1). Lower Cu^{2+}-treatments (8 and 16 μM) had positive effect on radicle elongation in the two species of *Caryophyllaceae* (*M. hirsuta* and *S. compacta*). So, *M. hirsuta* seedlings treated with 8 and 16 μM of Cu^{2+} showed higher root length by 44 and 25 %, respectively, than control seedlings ($P < 0.05$), while at 8 μM of Cu^{2+} *S. compacta* roots revealed a significant increase by 38 % of the control ($P < 0.05$, Table 1). An inhibition of radicle elongation at 160 μM of Cu^{2+} was observed by about 30 and 46 % for *M. hirsuta* and *S. compacta*, respectively. In contrast, for the species of *Cruciferae* (*A. montanum* and *T. ochroleucum*) the increase of Cu^{2+} concentration showed increasing inhibition of root growth (Table 1). An important root growth inhibition even in lower Cu^{2+} treatment by 13 and 16 % of the control for *A. montanum* and *T. ochroleucum* was observed. The seedling root growth displayed more severe reduction under 160 μM of Cu^{2+} equal to 61 and 68 % of the control for *A. montanum* and *T. ochroleucum*, respectively ($P < 0.05$, Table 1).

Table 1. Primary root lenght of 13-d-old seedlings, germinated under various Cu^{2+} concentrations. Values are means ± S.E. of 15 measurements.

Cu^{2+}-treatment [μM]	Primary root length [mm]			
	M. hirsuta	*S. compacta*	*A. montanum*	*T. ochroleucum*
0	8.0 ± 0.7	6.5 ± 0.8	14.0 ± 0.6	13.0 ± 0.7
8	11.5 ± 1.0	9.0 ± 1.1	12.2 ± 0.7	10.9 ± 0.8
16	10.0 ± 0.9	6.0 ± 0.5	11.8 ± 1.0	9.7 ± 0.5
32	7.0 ± 0.3	5.3 ± 0.6	8.3 ± 1.1	7.0 ± 0.6
80	6.0 ± 0.4	4.3 ± 0.3	6.0 ± 0.5	5.3 ± 0.2
160	5.5 ± 0.2	3.5 ± 0.2	5.5 ± 0.3	4.1 ± 0.3

No necrotic symptoms under 8, 16, 32 μM of Cu^{2+} in *M. hirsuta* and *S. compacta* were observed, while at 160 μM of Cu^{2+} the seedling survival decreased by 25 and 68 % of the control, respectively (Fig. 2). A progressive reduction of a number of survived seedlings of *A. montanum* and *T. ochroleucum* with increasing Cu^{2+} in solution was found. 160 μM Cu^{2+}-treatments resulted in seedling mortality by 52 and 64 % of control, respectively (Fig. 2).

Discussion

The degree of seed germination in the presence of toxic metals is to some extent a measure of tolerance of that particular ecotype to the element concerned (Baker and Brooks 1989). Seed germination was highly affected by the higher Cu^{2+} concentrations (80 and 160 μM), while lower Cu^{2+} concentration seemed to be necessary for seed germination, even for *A. montanum*. Nevertheless, plants originated from Cu^{2+}-rich soils (*M. hirsuta, S. compacta*) showed a higher demand of Cu^{2+} for rapid seed germination. This fact could be explained by adaptation of plants to Cu^{2+}. Similar observations concerning germination experiments of tolerant and non-tolerant ecotypes have been reported *e.g.* by Baker *et al.* (1983), Symeonidis *et al.* (1985), Turner *et al.* (1988).

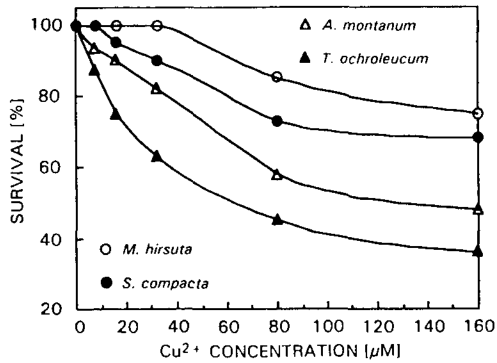

Fig. 2. Seedling survival for *M. hirsuta, S. compacta, A. montanum* and *T. ochroleucum* germinated on solutions with varying Cu^{2+} concentrations.

Excess of Cu^{2+} affected negatively enzyme substrate affinity (Stiborová *et al.* 1986, Agarwal *et al.* 1987), the chlorophyll content (Gadallah 1994), the nucleic acid content in the embryo and amylase, RNAase and protease activity in the endosperm (Das Gupta and Mukherji 1977).

Higher Cu^{2+} concentrations severely reduced extension growth rates of radicle, especially in *A. montanum, T. ochroleucum*. These data clearly indicate the reduced suitability of these plant species for reclamation on Cu^{2+} rich soils. Moreover, even at lower Cu^{2+} levels these plant species suffered damage at root and leaf ultrastructure and photosynthesis (Ouzounidou *et al.* 1992, Ouzounidou 1994b). In contrast, ecotypes originated from Cu^{2+}-rich soils, as *M. hirsuta* and *S. compacta,* showed a more vigorous growth at elevated Cu^{2+} levels (Wu and Lin 1990, Ouzounidou 1994a, 1995) and a greater survival. The suite of adverse effects associated with germination, seedling survival and growth in *A. montanum* and *T. ochroleucum* exposed to high Cu^{2+} concentrations demonstrates that these species are not cuprophytes as *M. hirsuta* and *S. compacta* seem to be. We can assume that the successful establishment of the first two species, if it exists, in such environments may be due to the selective occupation of less cupriferous microsites.

In conclusion, it is obvious that germination process was less influenced by excess of Cu^{2+} than the seedling survival and growth. Besides, low Cu^{2+} concentrations seemed to enhance seed germination in the four plant species we used. The most detrimental effect of Cu^{2+} concerned the seedlings survival and the primary root length.

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