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Interactions between calcium and copper or cadmium in Norway spruce

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Abstract

The accumulation of calcium (Ca), copper (Cu) and cadmium (Cd) in roots and stem of Norway spruce (*Picea abies* [L.] Karst) was examined. Two-year-old Norway spruce seedlings were treated with elevated concentrations of Ca, Cd or Cu, or as combinations of Ca with Cu or Cd in nutrient solutions for three months. The stem was divided into bark, wood formed during the treatment period (new wood), and wood formed before the treatment period (old wood). The accumulation of the metals in stem and roots increased with addition of the respective metal into nutrient solution. Addition of Cu decreased the accumulation of Ca in roots and wood, and Ca addition decreased the accumulation of Cu in the new wood. By adding Ca in combination with Cu the accumulation of Cu in the stem was decreased even more by Ca and the negative effect of Cu on the Ca content in the stem was diminished. Addition of Cd decreased the accumulation of Ca in wood, especially the old wood, and Ca addition decreased the accumulation of Cd in roots, bark and new wood. By adding Ca in combination with Cd the Ca content was reduced in the bark, instead of in the old wood.

Additional key words: accumulation of metals, bark, *Picea abies*, roots, stem, wood.

Introduction

Wood ash, a waste product from the forest industry, has been suggested to be used as a liming and fertilising agent in forest soils, to counteract nutrient depletion caused by whole tree harvesting and acidification (Eriksson 1998, Demeyer *et al.* 2001). Spreading of wood ash in the forest can increase the bioavailability of calcium (Ca) as well as heavy metals in the soil (Bramryd and Fransman 1995, Rumpf *et al.* 2001, Arvidsson and Lundkvist 2003). Even small changes in the metal availability in forest soils are thought to be reflected in the metal content of forest trees (Arduini *et al.* 1998, Österås and Greger 2003). It is therefore of interest to study the effect of elevated contents of Ca and heavy metals, such as copper (Cu) and cadmium (Cd), in order to understand the effect of wood ash on the metal content in forest trees.

Elevated concentrations of Ca in forest soils may decrease the uptake and accumulation of toxic heavy metals in trees due to competition between the ions. In the same way, elevated concentrations and bioavailability of heavy metals in forest soils can decrease the uptake and accumulation of calcium, an essential plant nutrient element in trees. Calcium is known to have a positive influence on the growth of plants and also to ameliorate the toxicity of heavy metals (Marschner 1995, Hagemeyer 1999). Further, Ca may also decrease the uptake, translocation and accumulation of Cd and Cu in plants (Jarvis *et al.* 1976, Wallace *et al.* 1980, Tyler and McBride 1982, Kawasaki and Moritsugu 1987, Saleh *et al.* 1999, Österås and Greger 2003). Cadmium and Cu are known to be toxic to plants in low concentrations (Hagemeyer 1999, Liu *et al.* 2003) and may reduce the Ca content of roots, shoots, leaves, wood and/or bark of trees (Burton *et al.* 1986, Gussarsson 1994, Arduini *et al.* 1998, Kim *et al.* 2003, Österås and Greger 2003) and other plants (Ouzounidou 1994, Dražić *et al.* 2004). A reduced Ca content of trees may adversely affect the growth and wood formation, since Ca is involved in several functions in trees, such as cell division, cell extension, synthesis and function of cell walls and membranes (Jones and Lunt 1967, Demarty *et al.* 1984, McLaughlin and Wimmer 1999).

This study was undertaken to prove or deny the hypothesis that elevated Ca contents would decrease the accumulation of Cu or Cd in roots, wood and bark of young Norway spruce, and *vice versa*, due to interactions.

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Materials and methods

Plants, growth conditions and metal treatments: Two-year-old plug-plants of Norway spruce (*Picea abies* [L.] Karst.) raised from seeds in a tree nursery were used in this experiment. The seeds originated from Belorussia (54°38'). The plants were adapted in a greenhouse for two weeks in slowly raised temperatures, first in a day/night temperature of 10/5 °C for one week and then at 15/10 °C during the next week, before they were put into a climate chamber where the day/night temperature was 19/15 °C. The climate chamber was equipped with halogen lamps (*Powerstar HQI-E*, *Osram*, Munich, Germany) giving a photon flux density of 200 µmol m^{-2} s^{-1} at the top of the plants for 16-h a day. The relative humidity was 70 %.

After the adaptation to temperature in the greenhouse, the roots were cleaned of peat as thoroughly as possible using distilled water. Then the plants were placed in vessels filled with 2.3 dm^3 nutrient solution, with one plant in each vessel placed on a single styrofoam plate. The nutrient solution according to Ingestad (1979) was modified and contained [µM]: 122 KNO_3, 1497 NH_4NO_3, 122 K_2SO_4, 248 KH_2PO_4, 99 $Mg(NO_3)_2 . 6\,H_2O$, 3.5 $MnSO_4 . H_2O$, 8.9 H_3BO_3, 0.23 $CuCl_2 . 2\,H_2O$, 0.22 $ZnSO_4 . 7\,H_2O$, 0.033 $Na_2MoO_4 . 2\,H_2O$, 55 HNO_3, 3.0 $Fe(SO_4)_3 . 5\,H_2O$, 6.2 EDTA, 60 $Ca(NO_3)_2 . 4\,H_2O$. The solution was constantly aerated and changed every week. The pH of the medium during the experiment varied between 3.8 and 4.0 and was not regulated.

The experiment was started one week after the plants had been placed in the vessels. At the start of the treatment, the total fresh mass and stem diameter of all plants were measured in order to enable calculations of the fresh mass and stem diameter increase during the treatment. The stem diameter was measured in four directions with a micrometer one cm above the styrofoam plate, which enabled a mean stem diameter value of each plant to be calculated. The plants were treated with elevated concentrations of Ca (0.30 mM), Cd (0.5 µM) or Cu (1.5 µM), or as combinations of Ca (0.30 mM) with Cd (0.5 µM) or Cu (1.5 µM) for 3 months, except the control, which only contained the diluted nutrient solution. The additional metals of the different treatments were added as chlorides and were changed once per week together with the nutrient solution. Metal concentrations were selected to try to avoid negative effects on growth.

Analyses of metal content: After the 3-month treatment, the stem diameter and total fresh mass were measured in the same manner as at the start of the treatment. Then, the roots were washed in redistilled water for 2 × 30 s, followed by rinsing 1 × 30 s in 20 mM EDTA, and again in redistilled water for 2 × 30 s, in order to remove metals adsorbed to the roots surface. Thereafter, the shoots were separated from the roots and the fresh mass of roots and shoots were registered. The roots were then dried for 24 h at 105 °C to measure dry mass. The lowest part of the stem (1 cm) was separated from the shoot since it had been exposed to the nutrient solution and then, above this part, about 1cm of the stem part was cut off and frozen at -20 °C until sample preparation.

The one-cm stem parts of all replicates were then prepared for metal analysis by separating the bark from the wood with a stainless razor blade and the wood was cut into two smaller sections. The plants were brought in from the nursery during the winter season when they were dormant and therefore the wood formed during the treatment period (new wood) could be cut from the rest of the wood (old wood) at the late wood border of the previous growth ring. This border had been studied under the light microscope in stems kept from both before and after the treatment. The new and old wood was separated with a stainless razor blade as accurately as possible from the small wood sections. The razor blade was cleaned with ethanol between each stem section in order to remove any metal contamination remaining from the previous stem section. Bark and wood were dried for 24 h at 105 °C and then wet digested in $HClO_4$:HNO_3 (3:7) and analysed for Ca, Cd and Cu using atomic absorption spectrophotometry, flame atomiser and graphite furnace (*Spectra AA-100* and *GTA100*, *Varian*, Springvale, Australia). Standards were added to the samples to eliminate the interaction of the sample matrix.

Calculations and statistics: The metal content of the stem parts was calculated on a dry mass basis. The percentage change in content of metals in wood and bark compared with the control was calculated as the ratio of metal content in treated and control plants × 100.

There were 9 replicates of each treatment, but due to abnormal growth of some of the plants, probably due to some bacterial infection, they had to be excluded from the analyses, which resulted in 6 to 9 replicates, depending on which treatment. A two-sided t-test was used for comparison of mean values. The least significant difference in *t*-tests was calculated at the $P \leq 0.05$.

Results

Growth responses: All treatments had a positive influence on growth, except the Cd treatment, which gave no effect on the measured growth parameters (Table 1). In the presence of elevated Ca, all the measured growth parameters were significantly higher compared with the control. At elevated Cu, the stem diameter increase was significantly larger compared with the control. Addition of Cu and Ca in combination did not affect any of the growth parameters differently than when adding Ca alone. Addition of Cd and Ca in combination did not

affect the stem diameter differently than when adding Ca alone, however the fresh mass increase and root dry mass were significantly lower than when only adding Ca.

Table 1**.** Total fresh mass (FM) and stem diameter increase and root dry mass (DM) of two-year-old Norway spruce after three months' treatment without or with Ca (0.3 mM), Cu (1.5 µM) and Cd (0.5 µM) in indicated combinations. The values represent the means ± SE (n = 6 - 9). Significant differences ($P \leq 0.05$) between the different treatments are marked with different letters.

Treatment	Total FM increase [g]	Stem diameter increase [mm]	Root DM [g]
Control	41.1 ± 3.7a	4.0 ± 0.1a	3.9 ± 0.3a
Ca	56.5 ± 3.3b	4.6 ± 0.1b	5.2 ± 0.3b
Cu	47.8 ± 4.9ab	4.6 ± 0.2b	4.9 ± 0.5ab
Cu+Ca	58.4 ± 3.4b	4.7 ± 0.2b	5.5 ± 0.2b
Cd	35.4 ± 4.0a	3.7 ± 0.3a	3.6 ± 0.3a
Cd+Ca	42.9 ± 2.2a	4.4 ± 0.1b	3.8 ± 0.3a

Calcium: The Ca content in the different plant parts increased in the order old wood < new wood ≤ roots < bark (Table 2). The Ca treatment increased the Ca content in roots, bark and wood significantly compared with the control. The Ca content of the wood of the Ca treated plants tended ($P < 0.10$) to be higher in both new and old wood, compared with the control (Table 2). On the other hand, the Cu treatment decreased the content of Ca in roots and wood significantly compared with the control. The Ca content of the wood of the Cu treated plants was significantly lower both in new wood and old wood, compared with the control (Table 2). The highest decrease in accumulation of Ca of the Cu treated plants tended to be in the old wood, which was 37 % lower compared with the control. In the roots and new wood, the accumulation of Ca was about 20 % lower, compared with the control. In the presence of enhanced Cu and Ca addition in combination, the Ca content of roots and bark, but not wood, was increased, compared with the control. However, the Ca content of roots and new wood was significantly lower compared with only adding Ca. The accumulation of Ca in roots and new wood of the plants treated with Cu and Ca in combination was reduced with 21 and 11 %, respectively, compared with the plants treated with Ca alone.

The Cd treatment decreased the content of Ca in the wood significantly compared with the control (Table 2). The Ca content of the wood for the Cd treated plants was significantly lower in the old wood, but not in the new wood, compared with the control. In the presence of enhanced Cd and Ca addition in combination, the Ca content of the bark was increased significantly compared with the control. However, when adding both Ca and Cd, the enhanced accumulation of Ca in bark was significantly lower compared with only adding Ca.

Table 2**.** Calcium content in roots, bark, new wood and old wood of two-year-old Norway spruce after three months' treatment without or with Ca (0.3 mM), Cu (1.5 µM) and Cd (0.5 µM) in indicated combinations. The values represent the mean ± SE (n = 6 - 9). Significant differences ($P \leq 0.05$) between the different treatments are marked with different letters and significant differences compared with the control are highlighted with a star.

Treat-ment	Ca [mg g^{-1}(d.m.)] roots	bark	new wood	old wood
Control	0.80 ± 0.02a	2.6 ± 0.1a	0.76 ± 0.05ab	0.36 ± 0.02a
Ca	1.18 ± 0.10b*	3.7 ± 0.4b*	0.88 ± 0.04b	0.43 ± 0.03a
Cu	0.65 ± 0.06c*	2.2 ± 0.2a	0.59 ± 0.05c*	0.23 ± 0.03c*
Cu+Ca	0.93 ± 0.05d*	3.5 ± 0.2b*	0.78 ± 0.04a	0.39 ± 0.02a
Cd	0.66 ± 0.07ac	2.4 ± 0.1a	0.64 ± 0.03ac	0.28 ± 0.04c*
Cd+Ca	1.09 ± 0.07b*	2.8 ± 0.2a	0.82 ± 0.05b	0.41 ± 0.03a

Copper: The Cu content of the different plant parts of the control plants was significantly higher in the bark than in roots and wood (Table 3). The Ca treatment significantly decreased the content of Cu in new wood by 28 %, compared with the control (Table 3). The Cu treatment increased the content of Cu significantly in all examined parts compared with the control. The enhanced accumulation of Cu increased in the order old wood < new wood and bark < roots. The highest accumulation of Cu was found in the roots, which was 331 % higher than the control. In the bark and new wood, the enhanced accumulation of Cu was about 80 % and in the old wood 38 %. In the presence of enhanced Cu and Ca addition in combination, the Cu content of roots and wood was increased significantly and it also tended to increase the Cu content in bark, compared with the control. However, addition of Cu and Ca in combination gave a significantly lower Cu content in bark and wood, compared with only enhanced Cu addition. The Cu content in wood for the combined Cu and Ca treated plants was significantly lower in new wood, but not in old wood, compared with

Table 3**.** Copper content in roots, bark, new wood and old wood of two-year-old Norway spruce after three months' treatment without or with Ca (0.3 mM), Cu (1.5 µM) or Cu+Ca. The values represent the mean ± SE (n = 6 - 9). Significant differences ($P \leq 0.05$) between the different treatments are marked with different letters and significant differences compared with the control are highlighted with a star.

Treat-ment	Cu [µg g^{-1}(d.m.)] roots	bark	new wood	old wood
Control	12.6 ± 2.1a	29.2 ± 2.3a	9.9 ± 1.2a	7.5 ± 0.5a
Ca	9.6 ± 0.5a	25.8 ± 3.3a	7.1 ± 0.6b*	7.8 ± 0.8a
Cu	54.4 ± 5.5b*	53.7 ± 4.4b*	17.9 ± 1.8c*	10.3 ± 0.8b*
Cu+Ca	53.4 ± 7.4b*	37.5 ± 3.4a	12.8 ± 0.6a	9.1 ± 0.6ab

the Cu treated plants. The Cu content of bark and new wood was about 30 % lower in the Cu+Ca treated plants compared with the Cu treated plants.

Cadmium: The Cd content in the different plant parts of the control plants increased in the order roots and old wood < new wood < bark (Table 4). The Ca treatment significantly decreased the content of Cd in roots, bark and wood, compared with the control (Table 4). Within the wood the Cd content was only significantly lower in new wood, compared with the control. The Ca treatment decreased the Cd content of roots, bark and new wood, by 20, 30 and 55 %, respectively, compared with the control. The Cd and Cd+Ca treatments increased the content of Cd significantly in all examined parts, compared with the control. The highest accumulation of Cd was in the roots and it was about 30 times higher than in the bark. For the different plant parts examined the enhanced accumulation of Cd increased in the order bark < new wood < old wood < roots. In the presence of enhanced Cd and Ca addition in combination, the Cd content of roots, bark and new wood was significantly lower than with only enhanced Cd addition. The Cd content and accumulation in roots, bark, and new wood was 23, 20 and 50 %, respectively, lower for the Cd+Ca treated plants than for the Cd treated plants.

Table 4. Cadmium content in roots, bark, new wood and old wood of two-year-old Norway spruce after three months' treatment without or with elevated concentrations of Ca (0.3 mM) and Cd (0.5 µM) in indicated combinations. The values represent the mean ± SE (n = 6 - 9). Significant differences ($P \leq 0.05$) between the different treatments are marked with different letters and significant differences compared with the control are highlighted with a star.

Treat-ment	Cd [mg g^{-1}(d.m.)] roots	bark	new wood	old wood
Control	0.33 ± 0.02a	6.1 ± 0.7a	1.0 ± 0.2a	0.5 ± 0.1a
Ca	0.26 ± 0.02b*	4.2 ± 0.3b*	0.5 ± 0.1b*	0.5 ± 0.1a
Cd	111.3 ± 9.4 c*	72.4 ± 4.3c*	28.6 ± 1.8c*	23.6 ± 2.9b*
Cd+Ca	85.4 ± 3.5 d*	58.1 ± 2.0d*	14.4 ± 2.8d*	22.5 ± 2.3b*

Discussion

As was expected, the content of the metals (Ca, Cd, Cu) in roots and stem (bark and wood) of two-year-old Norway spruce increased with the elevated addition of the metal in question (Tables 2 - 4). The results found suggests that an enhanced bioavailability of Ca, Cu or Cd in the forest soil will enhance the accumulation of the metal in question in mainly the roots for Cu and Cd and in the roots and bark for Ca. A restricted translocation of Cu and Cd to the shoot has been shown in several plant species and it may be a way of decreasing the risk of negatively affecting photosynthesis of the shoot (Coughtrey and Martin 1978, Fernandes and Henriques 1991, Greger *et al.* 1991, Landberg and Greger 1996, Österås *et al.* 2000).

In the stem, the highest increase in accumulation of the metals was in the bark for Ca, in the bark and new wood for Cu, and in the old wood for Cd (Tables 2 - 4). Similar results were found in young Norway spruce treated with high concentrations of Ca, Cd or Cu (Österås and Greger 2003). The Ca and Cu treatment in the present study had a positive influence on growth, increasing the stem diameter (Table 1). Thus, the high accumulation of Ca and Cu in bark and/or new wood may reflect a higher need of these ions in the cambial region, where new cells are formed. Similar suggestions have been made earlier for Ca (Kuhn *et al.* 1997, Österås and Greger 2003).

All treatments, except the Cd treatment, gave a positive growth response of the plant, increasing the stem diameter, compared with the control (Table 1). The effect of Ca on growth seemed independent of Cu, since treatment with Cu and Ca in combination did not enhance the growth of the spruce plants more than in treatments with only Ca. Cadmium acted as an antagonist to Ca, since treatment with Cd and Ca in combination gave a lower fresh mass increase and roots dry mass compared with only Ca treatment. This antagonistic effect of Cd on Ca may be explained by a reduced translocation of Ca to the shoot, which is further discussed below (Tables 1, 2). Thus, enhanced bioavailability of Ca and Cu, but not Cd, may give a positive influence on the growth and wood formation of young Norway spruce if their availability is limited. Further, enhanced Cd bioavailability can decrease the positive growth influence of Ca, probably partly due to a reduced translocation of Ca to the shoot.

As was hypothesised, Ca and Cu interacted with each other, influencing each other's accumulation in roots and/or stem of young Norway spruce (Tables 2, 3). Addition of calcium did not affect the accumulation of Cu in roots. Enhanced Cu, on the other hand, reduced the accumulation of Ca in roots. The root apoplast is thought to be of great importance for uptake of nutrients like Cu and Ca because of its negative charges that can bind and accumulate cations (Thornton and Macklon 1989, Sattelmacher 2001). Copper ions are selectively adsorbed onto plant cell walls compared with Ca (Van Cutsem and Gillet 1982) and have a higher affinity to poly-galacturonic acids in cell walls than Ca (Ernst *et al.* 1992). Thus, the main explanation why, in the present study, Cu interferes with the accumulation of Ca in roots, but not *vice versa*, may be that Cu can reduce the amount of Ca bound in the apoplast, but not *vice versa*. Once Cu has passed the root apoplast it can probably also block the uptake of Ca or compete with Ca for the same uptake sites in the plasmalemma (Clarkson and Lüttge 1989, Piñeros and Tester 1997, White 1998).

Enhanced Ca addition is suggested to reduce the

translocation of Cu, since the Ca treatment decreased the Cu content in new wood, but not in roots (Table 3). Similarly, Cu also seemed to reduce the translocation of Ca, since the Ca content in new and old wood was decreased by elevated Cu (Table 2). However, the decreased Ca content in the wood resulting from the Cu treatment is probably not only an effect on the translocation but also an effect of the reduced uptake of Ca at elevated Cu (see above). Copper has earlier been suggested to interfere with the internal transport of Ca in trees (Arduini *et al.* 1998). Enhanced Cu has also been shown to decrease the accumulation of Ca in wood and bark of Norway spruce (Österås and Greger 2003). Many cations are transported in the xylem *via* ion-exchange and divalent ions are known to exchange each other in the xylem and to affect each other's translocation (Bell and Biddulph 1963, Shear and Faust 1970, Fergusson and Bollard 1976, Wolterbeek 1987). Copper and Ca might compete with each other for the exchangeable sites in the cell walls of the xylem, as in the case of the root apoplast mentioned above, thereby affecting each other's translocation, binding and accumulation in the stem.

The inhibitive effect of Ca on the Cu accumulation in the stem seemed to be larger when both Cu and Ca were added. This was apparent since treatment with Cu and Ca in combination not only decreased the accumulation of Cu in new wood, such as treatment with only Ca, but also in bark (Table 3). Moreover, and perhaps an effect of the results above, the reducing effect of Cu on the accumulation of Ca in the wood seemed to decrease when also enhancing the Ca, since treatment with Cu and Ca in combination gave a lower decrease in the accumulation of Ca in wood compared with only treatment with Cu (Table 2).

Calcium and Cd interacted with each other, influencing each other's accumulation in roots and/or stem of Norway spruce, as was hypothesised (Tables 2, 4). However, Cd does not seem to affect accumulation of Ca in roots, since addition of Cd did not decrease the accumulation of Ca in roots in either the Cd or Cd+Ca treatment. Similarly, Arduini *et al*. (1998) found no decreased Ca content of roots of three Cd treated tree species. Thus, Cd does not seem to compete for, or is less attracted to, the exchangeable sites in the root apoplast compared with Ca. Retainment of Ca in fine roots of *Betula pendula* after treatment with Cd has been suggested to be a possible mechanism of the plant to reduce the toxic effects of Cd (Gussarsson 1994).

Calcium, on the other hand, seems to reduce accumulation of Cd in roots of Norway spruce, since enhanced Ca addition decreased the accumulation of Cd in roots both for the Ca and Cd+Ca treatments (Table 4). This implies that Ca is a stronger competitor than Cd for exchangeable sites in the cell wall and, thereby, can reduce the amount of Cd bound in the apoplast. A similar explanation was suggested by Österås and Greger (2003) for the reduced Cd content in bark of young Norway spruce after treatment with Ca. As in the case of the Cu-Ca interactions, the competition between Cd and Ca for binding sites in the apoplast of root cells ought to have a large influence on accumulation of Cd in roots. The amount of Cd bound in the cell wall in roots of Norway spruce seedlings treated with the same Cd concentration (0.5 µm) for 2 weeks was shown to be nearly 95 % of the total amount taken up in the roots (unpublished). Further, addition of Ca has been shown to reduce absorption of Cd in excised barley roots (Kawasaki and Moritsugu 1987).

Calcium seems to decrease the translocation of Cd, since the accumulation of Cd in new wood and/or bark was decreased more than in the roots by the Ca and Cd+Ca treatment (Table 4). In addition to the reducing effect of Ca on the translocation of Cd, there also ought to be an ion exchange directly in the tissue where Cd was bound, since no Cd was supplied to the control or in the Ca treatment. Cadmium also seems to affect the translocation of Ca, since elevated Cd addition decreased the Ca content in old wood, but not in roots (Table 2). However, by adding more Ca the effect of Cd on the Ca content in the wood disappears and instead the Ca content of the bark was decreased. Arduini *et al*. (1998) suggested that Cd interfered with the internal transport of Ca, since the Ca content of the shoot but not of the roots of three tree species was reduced by Cd treatment. Cadmium, as in the case of Cu, ought to compete with Ca for the exchangeable sites in the xylem, affecting each other's translocation, binding and accumulation in the stem, since they have a similar ionic form.

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