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Effect of Zinc, Cadmium and Mercury on Root Elongation of *Picea abies* (Karst.) Seedlings, and the Significance of These Metals to Forest Die-Back

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ABSTRACT

Inhibition of root elongation was used to assess the toxicity of Zn. Cd and Hg to Picea abies *seedlings. The order of toxicity was found to be Hg > Cd > Zn. A comparison of the levels of these metals, shown to inhibit root elongation in nutrient solution, with those in forest soils under stands showing symptoms of die-back, suggests that metal levels in such soils are sufficiently high to influence root growth.*

INTRODUCTION

The current die-back occurring in forests in the Federal Republic of Germany has been attributed mainly to the effect of acid deposition produced by atmospheric pollution. Associated with trees showing the symptoms of die-back are high heavy metal contents in both roots and aerial parts (Mayer & Heinrichs, 1981; Mayer, 1983).

Similarly, Mayer (1981) has shown that the metals Cr, Fe, Co, Ni, Zn, Cu, Cd and Pb accumulate in the humus layer of soils under tree stands showing die-back. This soil layer has been shown to be an important area for root growth of spruce *Picea abies* (Ulrich, 1983). However, it is not known whether the levels of heavy metals found in this soil layer and the trees themselves are sufficiently high to contribute to the decline of the trees. Inhibition of root elongation has been shown to be a sensitive parameter for assessing the toxicity of heavy metals (Hassett *et al.*, 1976:

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Godbold *et al.*, 1984), and was used in this work to assess the relative toxicity of Zn, Cd and Hg to spruce seedlings and to estimate whether these metals could be a contributing factor to forest die-back.

MATERIALS AND METHODS

Culture of plants

All plant material was grown from seed under sterile conditions. Seeds were surface sterilised in 1% w/v $Ca(OCl)_2$ and germinated on 1% w/v water agar. After 3 weeks, seedlings were transferred to sterile nutrient solutions based on Ingestad solution (Ingestad, 1959) of the following composition: (μM) $Ca(NO_3)_2$, 450; $MgSO_4$, 315; KH_2PO_4, 250; NH_4NO_3, 225; $FeCl_3$, 5; H_3BO_3, 5; $MnSO_4$, 1; Na_2MoO_4, 0·1; $CoSO_4$, 0·02; $ZnSO_4$, 0·1; $CuSO_4$, 0·1; pH 4·3, and grown for a further 7 days. Nutrient solutions were constantly aerated with sterile air. All work was carried out at 23/21°C day/night temperatures, 44·7 W m^{-2} light intensity (Osram L 18 W/25 lamps) and 16 h daylength.

Estimation of root elongation rate

Seedlings were transferred from the sterile culture to non-sterile nutrient solutions and allowed to equilibrate for 2 days. The seedlings were then transferred to nutrient solutions containing a range of Zn (0·1–60 μM), Cd (0–60 μM) or Hg (0–15 μM) concentrations for a further 7 days. Zn and Cd were added as $ZnSO_4$ and $CdSO_4$, respectively, Hg as $HgCl_2$. Plants were withdrawn from nutrient solutions and root lengths were measured daily, from a reference point marked with indian ink 10 mm behind the initial root tip, using a binocular microscope fitted with a calibrated eyepiece.

RESULTS AND DISCUSSION

The effect of metals on the rate of root elongation was used as a screening method to estimate their toxicity to *Picea abies* seedlings. Root elongation was greatly inhibited by 30 and 60 μM Zn (Fig. 1) and inhibition was evident after only 24 h of Zn treatment. At 5 and 15 μM Zn a significant inhibition of root elongation could not be detected (data not

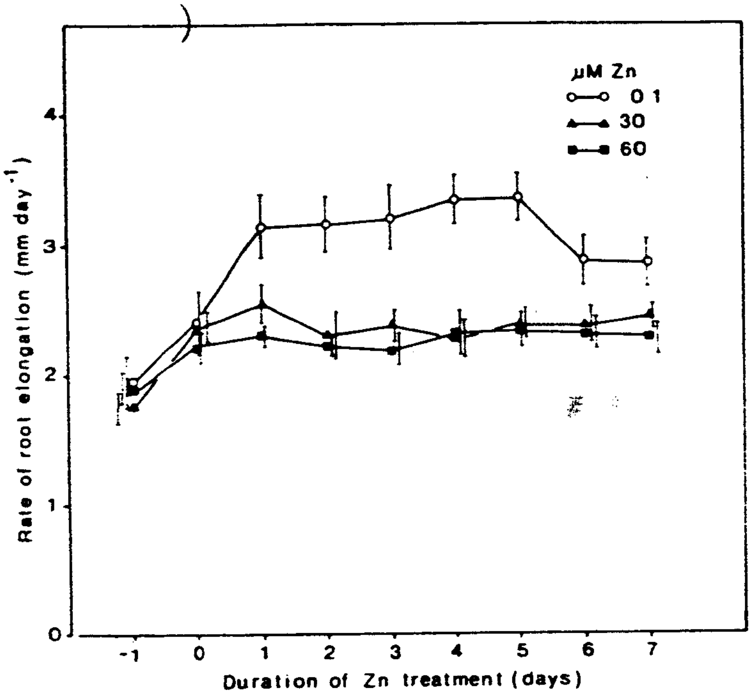

Fig. 1. Root elongation rate of 4-weeks-old seedlings of *Picea abies* grown for 7 days in a range of Zn concentrations (0·1–60 μM). Bars indicate the standard error. Each point represents the mean of thirty replicates.

presented). Cadmium was found to inhibit root elongation at all levels tested (Fig. 2), the degree of inhibition being dependent upon the concentration of Cd in the nutrient solution. Similar to Zn, Cd inhibited root elongation within 24 h. At the levels 30 and 60 μM Cd, root elongation almost completely ceased after 5 days. Mercury was found to be considerably more toxic than both Zn and Cd. Root elongation was severely inhibited by 0·1 and 0·5 μM Hg after 24 h (Fig. 3). At 5 and 15 μM Hg root elongation ceased completely within 24 h, and a radial shrinkage was observed in 1–5 mm behind the root tip.

The data show that the elements Zn, Cd and Hg differ in toxicity to *Picea abies* seedlings, although they are chemically similar. The order of toxicity was Hg > Cd > Zn, with Hg being over a 100 times more toxic than Zn. The same order of toxicity for Hg, Cd and Zn has been described for inhibition of respiration in *Saccharomyces cerevisiae* (Grafl & Schwantes, 1983), and may be a reflection of the higher stability constants of Hg than Cd, and Cd than Zn, with negatively charged organic groups (Aylett, 1979).

Many factors in soil have been shown to influence the uptake of heavy

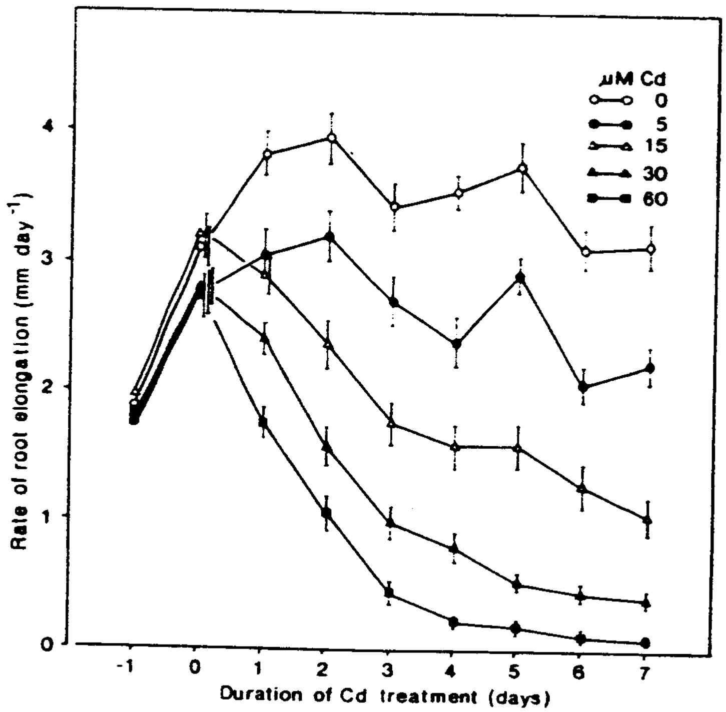

Fig. 2. Root elongation rate of 4-weeks-old seedlings of *Picea abies* grown for 7 days in a range of Cd concentrations (0·1–60 µM). Bars indicate the standard error. Each point represents the mean of thirty replicates.

metals into plants. Cadmium uptake increases with decreasing soil pH (Lagerwerff, 1971; Miller *et al.*, 1976) and decreases with increasing soil cation exchange capacity (Haghiri, 1974). Competition from other ions has been shown to both decrease (John, 1976) and increase (Haghiri, 1973) Cd uptake into plants. This effect tends to vary with plant species and conditions of growth (see review in Jastrow & Koeppe, 1980). Combinations of high levels of heavy metals have also been shown to be more toxic to plants than single metals (Hassett *et al.*, 1976), and particularly to micro-organisms (Hutchinson, 1973; Baldry *et al.*, 1977; Babich & Stotzky, 1983). Thus, under conditions of lower nutrient availability and low pH, and in combination with other heavy metals, heavy metals in the soil solution may be more toxic than in nutrient solution. When the levels of metals shown to inhibit root elongation in nutrient solution are compared with those in the humus layer under stands of *Picea abies* showing die-back symptoms (Table 1), it can be seen that the Zn and Hg contents of the humus layer are high enough to influence root growth. Direct comparison of concentrations of metals between nutrient and soil solution is difficult, as the speciation and activity of the metals in the soil

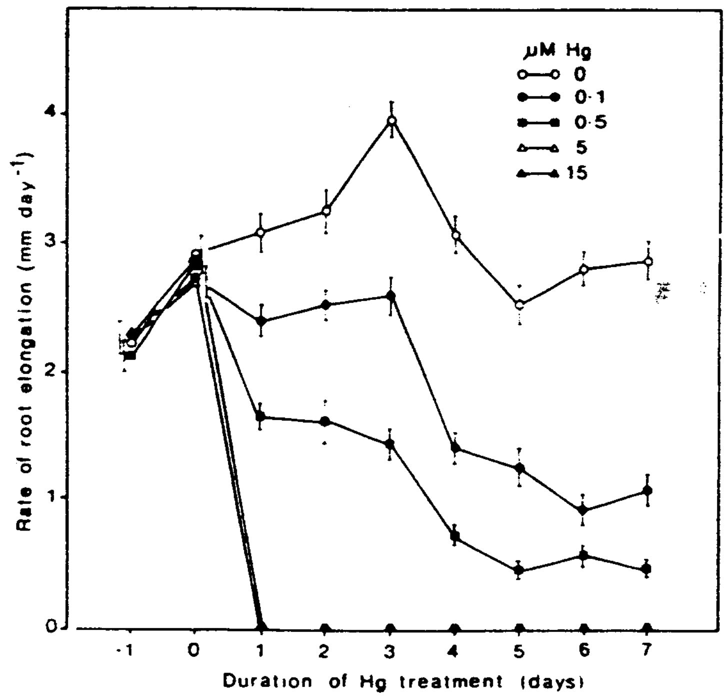

Fig. 3. Root elongation rate of 4-weeks-old seedlings of *Picea abies* grown for 7 days in a range of Hg concentrations (0–15 μM). Bars indicate the standard error. Each point represents the mean of thirty replicates.

solution are not known, although, on some heavily polluted soils, Cd in the soil solution was found to be mainly present as $CdCl_2$ (Tills & Alloway, 1983). It is probable that Zn and Hg also exist as inorganic complexes in the soil solution. For Hg, the concentration in the soil solution is not known; however, if only a small fraction (2 %) of the total Hg in the humus layer were plant available, this would be sufficient to influence root growth.

TABLE 1

Zinc and Cadmium Concentrations in Soil Solution (μM) and Total Soil Mercury Contents (μM kg^{-1}) of the Humus Layer of a Forest Soil at Solling, FRG[a]

	Zn *Soil solution*	*Cd* *Soil solution*	*Hg* *Total soil content*
Mean	5·1	0·01	4·5
Minimum	2·2	0·01	4·1
Maximum	11·4	0·02	4·8

[a] Source: E. Matzner (pers. comm.).

Due to the problems of comparing data obtained from nutrient solution with data obtained in the field, it cannot be conclusively shown that heavy metal toxicity is a causative factor in forest die-back. However, the data show that the levels of metals are sufficiently high in the humus layer that they can no longer be excluded as a possible contributing factor to forest die-back. Further work is necessary to investigate the effects of heavy metals on tree physiology before this problem can be conclusively answered.

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