DEVELOPMENT OF ANNUAL XYLEM RINGS AND SHOOT GROWTH OF YOUNG BEECH (*FAGUS SYLVATICA* L.) GROWN IN SOIL WITH VARIOUS Cd AND Zn LEVELS

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Abstract. In a pot culture experiment two-year-old beech (*Fagus sylvatica* L.) were planted in soil amended with different concentrations of Cd and Zn or combinations of both. Concentrations ranged up to ca 180 μmol Cd and 7500μmol Zn kg^{-1} soil dry weight (1 M ammonium acetate extracts). After 2 seasons of growth plants were harvested. Annual xylem growth rings in stems were significantly smaller at 50 μmol Cd kg^{-1} and 1000 μmol Zn kg^{-1} as compared to controls. Elongation of apical shoots was significantly reduced at 180 μmol Cd kg^{-1} and 1000 μmol Zn kg^{-1}. The lowest treatment of 50 μmol Zn kg^{-1} caused no significant growth depressions of stem diameter and shoot elongation. In the second year of treatment growth reductions were generally more pronounced than in the first season. Uptake and translocation of Cd and Zn into stem wood and leaves were marked and were correlated with substrate concentrations. The observed growth reductions are discussed with respect to possible adverse effects of trace elements on forest trees under field conditions.

1. Introduction

Effects of toxic trace metals on vegetation have been repeatedly documented in places with high emissions, like in mining areas (Baes and McLaughlin, 1984), around metal smelters (Buchauer, 1973; Jordan, 1975) or in industrialized regions (Glatzel and Kazda, 1985). Phytotoxic trace metals are known to interfere with various metabolic processes in plants, e.g. photosynthesis and carbon metabolism (Baszynski *et al.*, 1980; Schlegel *et al.*, 1987; Greger and Ögren, 1991; Greger and Bertell, 1992). These elements can, thus affect biomass production and growth.

Growth reductions as a result of elevated substrate trace element concentrations were observed in several investigations. Kelly *et al.* (1979) described decreased shoot elongation in some coniferous and broad-leaved tree species in culture experiments with Cd concentrations of 100 ppm after 17 weeks. Burton *et al.* (1984) found reductions in shoot yield of *Picea sitchensis* grown for 3 months with 0.1 ppm Cd added to the soil. Denny and Wilkins (1987a) examined the Zn tolerance of different genotypes of *Betula pendula* and *B. pubescens*. After 2 months of treatment stem extension growth showed an inverse correlation with Zn concentrations of the media.

Depressions in stem diameter growth of trees under trace element stress were also observed. Carlson and Bazzaz (1977) found decreases in stem diameter increments of young *Platanus occidentalis* after 3 months treatment with Pb or Cd in the substrate. In combination treatments both elements showed synergistic effects. Photosynthesis and transpiration of such plants were also reduced. In a culture

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experiment with *Acer saccharinum*, Lamoreaux and Chaney (1977) found a reduced water conducting capacity of the stems after 2 months treatment with 5 ppm Cd in the substrate. This was explained with a significant decrease of xylem tissue involved in water transport. Additionally, a partial blockage of xylem vessels by cellular debris was observed under such conditions.

Accumulations of toxic trace elements in xylem rings of deciduous broad-leaved trees, for instance *Fagus sylvatica*, have been reported in a number of field studies (Lepp, 1976; Kazmierczakowa *et al.*, 1984; Meisch *et al.*, 1986; Hagemeyer *et al.*, 1989; 1992; Glavac *et al.*, 1990). In some investigations concentration levels in stem wood were apparently related to local industrial emissions (Kazmierczakowa *et al.*, 1984; Meisch *et al.*, 1986).

Such accumulations of toxic trace elements in wood may inhibit cambial activity of trees. Jordan *et al.* (1990) observed a negative relationship between concentrations of Cd, Pb and Zn in the xylem and stem diameter increments in mature *Pinus taeda* in Alabama, USA. In northern Sweden, Symeonides (1979) found growth reductions of *Pinus sylvestris* directly related to Cu and Pb contents of the wood. Such reductions were, however, not correlated with Cd and Zn in the xylem. Obviously, differences in physical and chemical properties of the elements are meaningful in this respect.

In order to elucidate effects of different trace elements on xylem growth of *Fagus sylvatica* a pot culture experiment was conducted. Young beech were cultivated for 2 seasons in soils amended with Cd or Zn. Shoot growth and stem diameter increments were determined in plants grown with the toxic trace element Cd and the essential micronutrient Zn in elevated concentrations.

2. Materials and Methods

2.1. Experimental conditions

In spring of 1988 two-year-old saplings of *Fagus sylvatica* were planted in a soil mixture amended with Cd, Zn or combinations of both elements. The plants were cultivated for 2 growing seasons until fall of 1989, when 2 annual xylem rings had developed under conditions of trace element stress. A similar experiment was conducted with saplings of *Picea abies* (Heppel *et al.*, in prep.).

The soil mixture contained sand, peat and a forest soil rich in organic material (volume ratio 1:1.5:2). The homogenized substrate was divided into 11 portions to which solutions of $Cd(NO_3)_2 \cdot 4H_2O$ or $ZnSO_4 \cdot 7H_2O$ were added to yield extractable concentrations (1 M ammonium acetate) as given in Table I. The applied concentrations were chosen to cover the whole range from uncontaminated soil to conditions of severe trace element pollution. The prepared was substrate stored for 6 weeks to allow for a stabilization of adsorption equilibria of the added minerals between solid and liquid phases. Extractable trace element concentrations in the soil changed during the experimental period of almost 2 yr. This was assumed

TABLE I

Substrate concentrations of Cd and Zn (μmol kg^{-1} soil dw) of a culture experiment with young beech plants at the start and at the end of the treatment period of approx. 2 yr. Concentrations determined in 1 M ammonium acetate extracts. n.d. = not detectable

Treatment Group	Cd concentration μmol kg^{-1}		Zn concentration μmol kg^{-1}	
	Start	End	Start	End
Control	n.d.	n.d.	35	5
Cd10	12	11	20	2
Cd50	60	50	19	4
Cd90	89	87	14	2
Cd180	181	174	13	3
Zn50	n.d.	n.d.	46	13
Zn225	n.d.	n.d.	225	125
Zn1000	n.d.	n.d.	1060	272
Zn7500	n.d.	n.d.	7450	1550
50+150	45	47	167	144
90+1000	103	75	1040	334

to result from leaching by rain water, changes in the adsorption equilibria in the soil due to a drop in pH and plant uptake. Actual of Cd and Zn were determined with ammonium concentrations acetate extracts before and after the experiment (Table 1). Soil samples were taken before the experiment from each of the 11 portions of the prepared substrate and after the experiment from 2 randomly chosen pots per treatment group. The pH values (H_2O) of the soil before and after the experimental period were 4.8 and 3.7, respectively.

Each treatment group consisted of 20 plants, except for the control with 50 beech plants. The plants were potted individually in plastic containers with 3 L substrate. During the growing season they were kept under open conditions and watered only in periods of drought. Between December and March plants were moved to a cold greenhouse to protect them from hard frost.

2.2. Harvest and xylem growth measurements

In fall of 1989 at the end of the second growing season all plants were harvested. Radial sections were cut from basal parts of stems between soil surface and lowest branches. The cross sections were mounted on microscopic slides. The wood surface was smoothed with a sharp blade to improve the visibility of xylem growth rings. Radial widths of annual rings of both years were determined with a tree ring measuring equipment, commonly used in dendrochronological studies. It consisted of a binocular microscope, a motorized sledge for controlled sample positioning and a computer for data storage and processing (Aniol, 1983).

Plants showed some individual variability in height, stem diameter and vitality already before the treatment was started. Therefore, the measured ring widths of 1988 and 1989 were divided individually by the width of the xylem ring of 1987 of each plant, which was the last year before contamination. In this way index values of xylem ring widths were obtained. Mean values of absolute ring widths ranged between 0.32±0.40 mm (n=20, 180 μmol Cd kg^{-1}) and 0.67±0.36 mm (n=48, control) in the first year and between 0.22±0.25 mm (n=20, 180 μmol Cd kg^{-1}) and 0.57±0.39 mm (n=48, control) in the second year of treatment.

The lengths of apical shoots produced in each of the experimental seasons were separately determined for each plant.

2.3. Chemical analyses

Stem wood samples were taken adjacent to cross sections for determination of Cd and Zn incorporation. After careful removal of the bark, the wood was dried in an oven at 60 °C for 24 hr.

A sample of leaves of each plant was collected for analysis of mineral contents. In order to remove superficially adhering dust, leaves were washed for 2 min in 2% HNO_3 and rinsed for 1 min in distilled water. The leaves were dried in an oven at 60 °C for 24 hr.

Samples of leaves and stem wood were wet ashed with conc. HNO_3 in teflon pressure vessels. Concentrations of Cd and Zn were determined with atomic absorption spectrophotometry (Perkin-Elmer 380 and 5100).

Soil samples were taken before and after the treatment period and extracted with 1 M ammonium acetate (extraction at pH 7 with soil: extractant ratio 1:10 for 2 h). Concentrations of Cd and Zn were measured by atomic absorption spectrophotometry (Table I).

2.4. Statistics

Mean values of different treatment groups were compared in an analysis of variance including the Scheffe test. Correlations between different growth parameters were calculated with Pearson's coefficient of linear correlation.

3. Results

3.1. Stem diameter growth

Stem diameter increments of young beech were reduced in plants grown on substrates with elevated concentrations of Cd and Zn. This was observed in both years of treatment (Figures 1, 2).

First year: In the first year relative xylem ring widths showed an inverse relation to soil Cd concentrations. With substrate concentrations of approx. 90 μmol Cd kg^{-1} growth rings were 38% smaller than in control plants ($p<0.05$, Figure 1). At lower Cd levels (50 μmol kg^{-1}), the diameter reduction was not statistically significant.

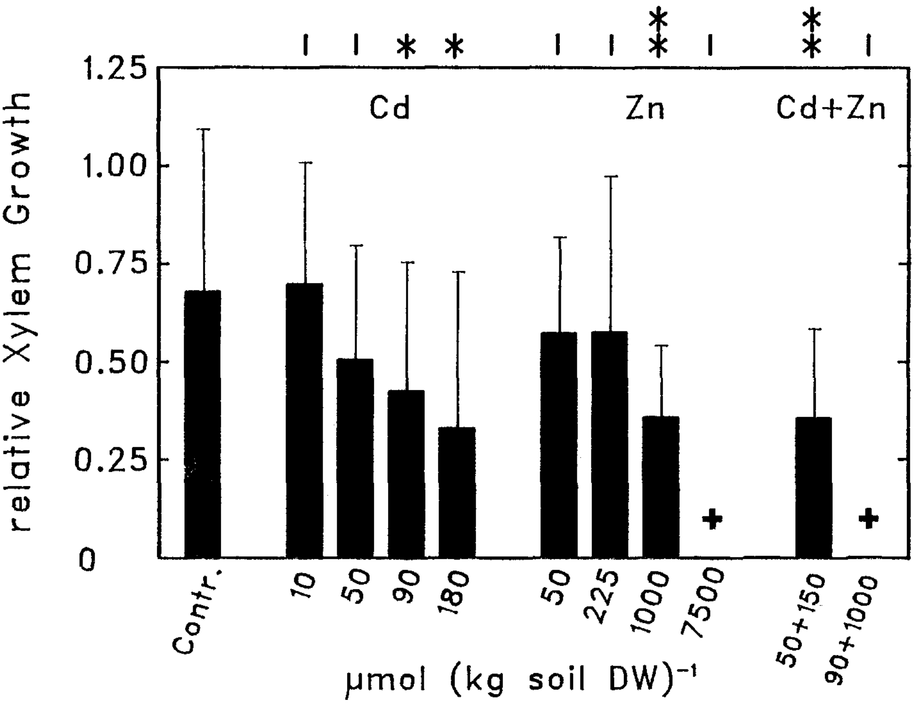

Fig. 1. Relative growth of annual xylem rings in stems of young beech in the first year of treatment with Cd and Zn in the soil. Widths of annual rings were divided by ring widths of the last year before metal treatment to reduce individual variability. Levels of significance for differences to controls: – = $p \geq 0.05$, * = $p < 0.05$, ** = $p < 0.01$. + = no plants survived. Means ± SD of 19-48 plants.

Zinc concentrations of initially 50 and 225 µmol kg^{-1} did not result in significant xylem diameter growth reductions. At higher Zn levels of initially 1000 µmol kg^{-1} the relative ring widths were 48% smaller than in control plants ($p < 0.01$). In the highest Zn concentration no plant survived until the end of the first season.

In the lower combination treatment of Cd+Zn xylem growth was reduced by 48% ($p < 0.01$). In the higher combination treatment group all plants died before the end of the first season.

Second year: In the second year of treatment the effects of Cd and Zn on xylem growth of beech were even more pronounced (Figure 2). Plants grown for 2 seasons in substrate with 50 µmol Cd kg^{-1} produced 43% smaller growth rings than the controls ($p < 0.01$). Higher Cd levels caused still stronger reductions of diameter growth.

With the lowest Zn addition of initially 50 µmol kg^{-1} annual rings of the second year were not significantly smaller than in control plants. When 1000 µmol Zn kg^{-1} were added to the soil widths of xylem rings reached only 50% of the controls ($p < 0.01$).

In the lower combination treatment stem diameter growth was reduced by 53% ($p < 0.01$).

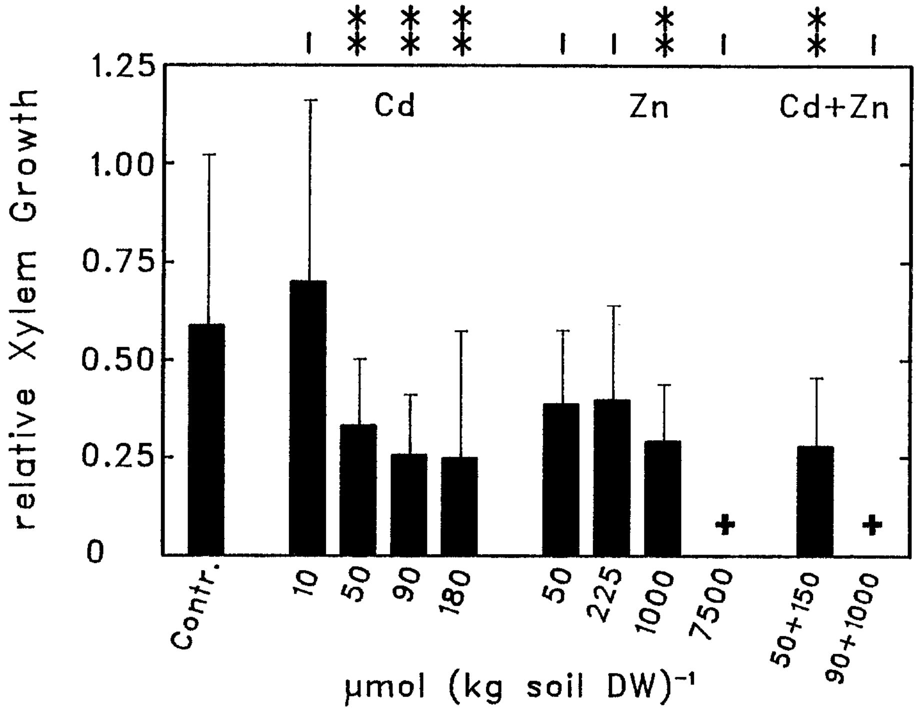

Fig. 2. Relative growth of annual xylem rings in stems of young beech in the second year of treatment with Cd and Zn in the soil. For further details see Figure 1.

3.2. Shoot elongation

The elongation of apical shoots of both years was also reduced by Cd and Zn in the soil (Figure 3). At the lowest Cd level of 10 μmol kg^{-1} height growth was slightly, though not significantly, increased in the first year. In higher Cd concentrations stem extension was inversely correlated to soil Cd levels in both years (Figure 3). At 180 μmol Cd kg^{-1} apical shoot lengths were only 49% and 39% of the control shoots of the first and second year, respectively ($p<0.001$).

With Zn concentrations of initially 1000 μmol kg^{-1}, shoot lengths were reduced by 21% in the first year and 39% in the second year ($p<0.05$).

In the lower combination treatment with Cd and Zn, height growth was slightly reduced in both years of the experiment (Figure 3). However, due to the large variations within the treatment groups these effects were not statistically significant.

Significant correlations of shoot elongation and stem diameter growth were found in both years of the experiment. Coefficients of correlation were $r=0.436$ ($p<0.01$) in the first year and $r=0.459$ ($p<0.01$) in the second year of treatment.

3.3. Concentrations of Cd and Zn in stem wood and leaves

Cadmium and Zn concentrations in stem wood samples of young beech after 2 seasons of treatment showed positive correlations with substrate levels of the elements (Figure 4). Concentrations of Cd increased with soil Cd levels up to 63 μmol kg^{-1}

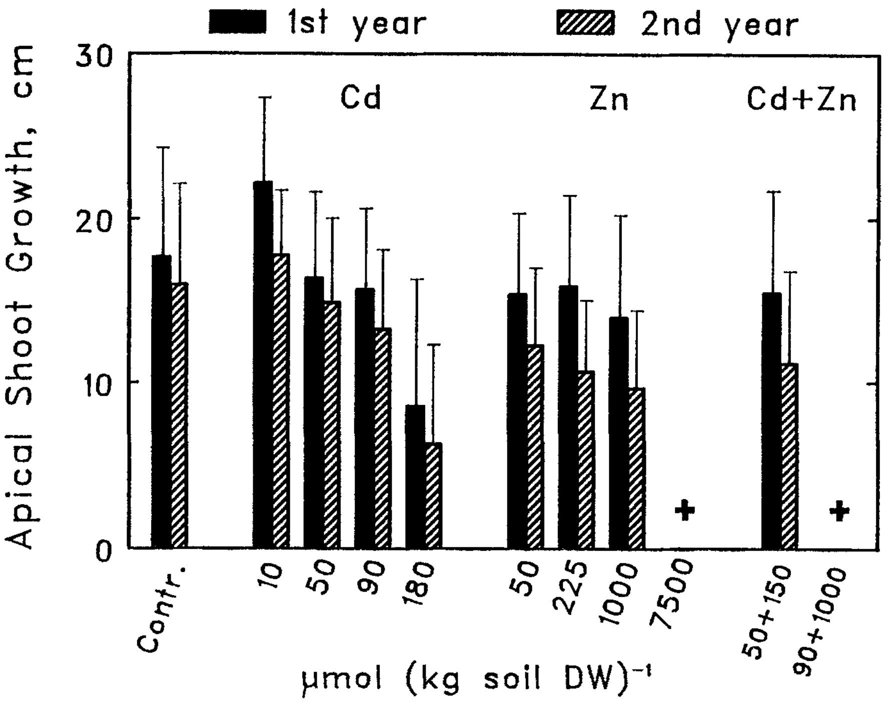

Fig. 3. Length of apical shoots of young beech grown in the first and second year of treatment with Cd and Zn in the soil. + = no plants survived. Means ± SD of 19–48 plants.

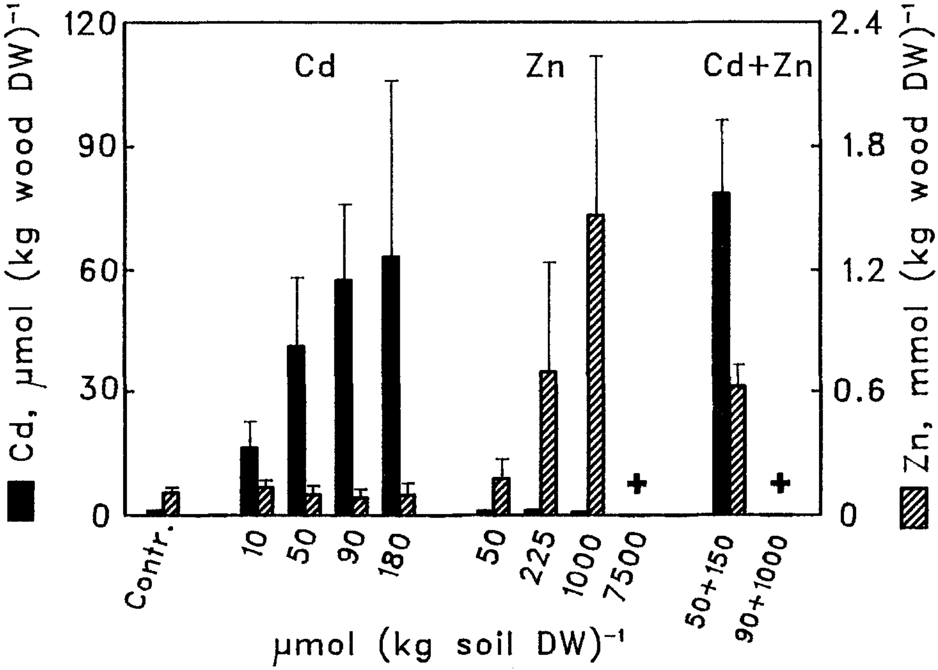

Fig. 4. Concentrations of Cd and Zn in stem wood of young beech grown for 2 years in soils of different trace metal contents. + = no plants survived. Means ± SD of 10 plants.

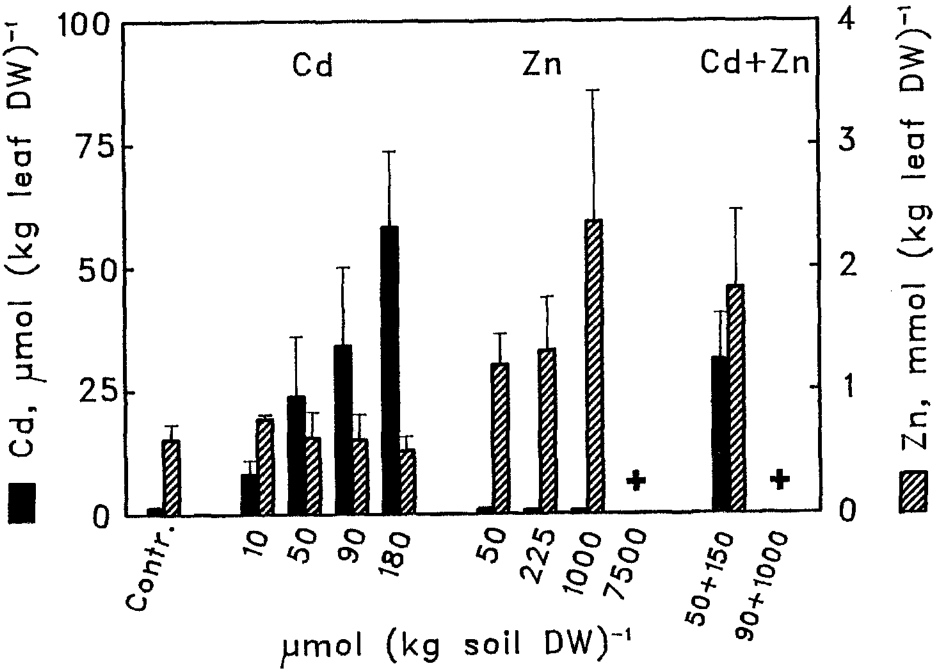

Fig. 5. Concentrations of Cd and Zn in leaves of the second year of young beech grown for 2 years in soils of different trace metal contents. + = no plants survived. Means ± SD of 10 plants.

wood dry weight. Zn concentrations reached maximum values of 1.47 mmol kg^{-1} wood dry weight in plants of the 1000 µmol Zn kg^{-1} treatment group. Plants of the lower combination treatment had significantly higher Cd concentrations than plants supplied with Cd alone ($p<0.05$). Zinc concentrations in wood of such plants did not differ from those of plants treated with Zn only (Figure 4).

Concentrations of Cd and Zn in leaves revealed a similar pattern as those in stem wood (Figure 5). However, Cd concentrations were generally lower in leaf samples than in wood, whereas Zn concentrations tended to be higher in leaves. The contents of both elements in beech leaves were correlated to soil levels. Concentrations of Cd and Zn in leaves of trees of the combination treatment did not differ significantly from concentrations of the elements in plants of the separate treatments (Figure 5).

4. Discussion

Young beech grown for 2 seasons in substrates amended with Cd or Zn showed significant reductions in stem diameter increments. Such growth depressions were observed in absolute ring widths (data not shown) as well as in the presented relative xylem growth data. In order to reduce the influence of the individual variability in growth and vitality of the plants, relative ring widths were used to assess the effects of Cd and Zn on diameter growth. Shoot elongation was also reduced by

Cd and Zn. Uptake of both metals was directly proportional to soil concentrations. Substrate levels of Cd and Zn in this investigation ranged from uncontaminated soil to levels encountered only in regions of high trace element pollution (Table I). Ernst (1972) found ammonium acetate extractable Cd concentrations up to 71 μmol kg^{-1} in soil near a zinc smelter. Zinc concentrations reached more than 37 mmol kg^{-1}. Such values are well within or even exceed the range of experimental applications and, thus underline the significance of the results for trees under certain field conditions (Heppel *et al.*, in prep.).

Another way to examine the relevance of the experimental data is to compare trace element concentrations accumulated in wood (Figure 4) with levels found in wood of forest beech. Meisch *et al.* (1986) analyzed stem wood of mature beech in western Germany and reported Zn concentrations of up to 673 μmol kg^{-1}. Similar maximum values of up to 760 μmol kg^{-1} were also found by Hagemeyer *et al.* (1992) in beech of an industrialized area. Such values are comparable with concentrations found in the beech saplings under the described experimental conditions, in which growth reductions were established (Figure 4). Apparently, in some polluted areas forest trees are exposed to similar conditions of trace element stress like the young beech in the described culture experiment. Thus, one may assume, that in such trees growth reductions as a result of trace element pollution are possible.

Trace elements can reduce shoot growth either by direct or by indirect action. Direct effects are conceivable, since Cd and Zn were readily absorbed and incorporated into stem wood and leaves (Figures 4, 5). The metals can, therefore, affect the activity of growing meristems, e.g. in the cambium or in buds. For instance, significant reductions in the size of the cambial zone were found in bush bean (*Phaseolus vulgaris*) plants under Cd stress (Barcelo *et al.*, 1988). Stem diameters of such plants were smaller than those of the controls. On the other hand, also indirect effects are possible through an impairment of organic and inorganic plant nutrition. Cadmium and Zn were shown to reduce root growth of young *Picea abies* (Heppel *et al.*, in prep). In such conditions uptake of water and nutrients are inhibited and water transport and transpiration are decreased (Hagemeyer *et al.*, 1986). In stems of *Phaseolus vulgaris* plants treated with 45 μM Cd, Barcelo *et al.* (1988) observed reductions in the number and size of xylem vessels. In older vessels deposits were found which may obstruct the water flow. The authors conclude that Cd can induce water stress in plants. Furthermore, Cd was shown to reduce photosynthesis and transpiration and increase dark respiration of leaves of *Acer saccharinum* (Lamoreaux and Chaney, 1978). It was assumed that Cd interferes with stomatal function (Bazzaz *et al.*, 1974). The same conclusion was proposed by Schlegel *et al.* (1987) in an investigation of CO_2 uptake and water relations of *Picea abies*. With increased concentrations of Cd, Zn and Hg chlorophyll concentrations were reduced. Stomatal resistance was increased by Cd and Hg, due to water stress in needles. Assimilation of carbon may be reduced, which, in turn, results in a lack of organic material for shoot growth. The observed reductions in stem diameter growth and shoot elongation may be caused by an impairment of the assimilatory metabolism of the beech plants. Similar effects were observed in a related study of the growth of young spruce under comparable conditions (Heppel *et al.*, in prep.). The spruce were, however, less sensitive to Zn and showed stem diameter growth even in the highest substrate concentration of initially 7500 μmol kg^{-1}.

In recent years forest trees are exposed to an increasing number of stress factors, e.g. gaseous pollutants and soil acidification, which affect their vitality and stress resistance. The described results of combination treatments with Cd and Zn show a significantly higher Cd incorporation in the wood as a result of combined stressors. With respect to other parameters, e.g. relative xylem growth, shoot elongation or Cd and Zn concentrations in leaves, the response of plants did not differ significantly in combined and single trace element applications. However, since the actual substrate concentrations in the described experiment changed considerably during the investigation as a result of variable exchange equilibria in the soil, it seems difficult to compare the plant responses in single and combination treatments. The effects of combined stressors need a more thorough investigation considering the importance of such relations under field conditions.

The observed growth depressions in beech plants were stronger in the second season than in the first one. Under field conditions a long-lived organism like a tree may even suffer from lower concentrations of toxic substances after a long period of exposure. This may explain why woody plants are sometimes missing in the most contaminated areas near emission sources. Trees need special strategies to survive in substrates with high trace element concentrations, e.g. the formation of mycorrhiza (Brown and Wilkins, 1985; Denny and Wilkins, 1987a, b), the ability to sustain slow growth under sub-optimal conditions or genotypic variations (Dickinson *et al.*, 1991).

Growth reductions of annual xylem rings of mature beech in Germany were examined by Eckstein *et al.* (1984). Losses in stem diameter growth had started in the 1970s. A dendroclimatological study revealed climatic extremes combined with effects of environmental pollution as a likely reason for decreased growth. According to the results of the described culture experiments soil acidification and a subsequent mobilization of toxic trace elements may result in such growth inhibitions of xylem rings.

Toxic trace elements, like Cd or Zn, can be additional stress factors in the complex process of forest decline (Godbold and Hüttermann, 1985; Jordan *et al.*, 1990). Currently, in most regions soil concentrations are still below the levels of acute toxicity. However, potentially toxic trace elements continue to accumulate in forest ecosystems (Friedland *et al.*, 1984). In the long run such substances will become harmful, especially for long-lived, immobile organisms like forest trees.

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