Radial distributions of Pb in stems of young Norway spruce trees grown in Pb-contaminated soil

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Summary Concentrations of Pb in individual stem xylem rings of 5-year-old Norway spruce trees (Picea abies (L.) Karst.) were determined after one growing season in soil containing a low, medium or high concentration of Pb. In trees in the control and low-Pb soil treatments, Pb concentrations increased from the outer annual rings toward the stem center, whereas in trees grown in the soil treatment containing a medium Pb concentration, all of the four tree rings analyzed contained similar concentrations of Pb. Although trees grown in the high-Pb soil treatment had higher concentrations of Pb in the outer annual rings than both control trees and trees in the low-Pb soil treatment, the highest concentrations of Pb were never observed in the outermost rings, which were formed during the period of exposure to increased soil Pb. We conclude that radial distribution patterns of Pb in Norway spruce stems do not directly reflect changes in soil Pb concentration but depend on several internal, physiological factors and therefore, do not provide reliable information about past variations in Pb contamination of soil.

Keywords: Pb, Picea abies, tree rings, xylem.

Introduction

Trace element concentrations of tree rings have been used for retrospective biomonitoring of past environmental pollution (Ferretti et al. 1993, Momoshima and Bondietti 1994, Eklund 1995). The method, called dendroanalysis (Gilboy et al. 1976), is based on the assumption that element concentrations in tree rings are closely related to the environmental abundance of an element at the time the growth rings are formed. Thus, changing element concentrations in the environment should be reflected in changing concentrations of the same elements in tree rings. It is assumed that trees incorporate and store absorbed elements mainly in currently growing xylem rings. However, this assumption has not been validated, although dendroanalytical techniques have been used in many investigations (Burton 1985, Cutter and Guyette 1993, Hagemeyer 1993). Donnelly et al. (1990) conducted an experiment in which 2-year-old Picea rubens Sarg. trees were grown hydroponically and supplied with Pb either in the first or second year of growth and found that stem xylem formed in the absence of Pb contained significant amounts of Pb.

Hagemeyer and Lohrie (1995) studied uptake and incorporation of Cd and Zn in annual xylem rings of young Picea abies (L.) Karst. trees and found that growth rings formed during treatment with high concentrations of Cd and Zn contained lower concentrations of these metals than older growth rings. However, the total amount of Cd and Zn incorporated into the annual xylem rings increased from the stem center toward the outer xylem rings. It was suggested that the lower metal concentrations in outer rings than in inner rings could be explained by the greater wood dry matter of the outer rings compared with the rings at the stem center. In the present study, we examined the accumulation of the relatively less mobile element Pb in individual annual xylem rings and bark of 5-year-old Picea abies trees grown for one year in Pb-contaminated soil.

Materials and methods

Experimental conditions

Four-year-old Norway spruce trees (Picea abies (L.) Karst.) were obtained from a tree nursery and planted in pots containing 5 liters of a mix of composted garden soil, peat and sand (23/17/10, v/v), to which fixed concentrations of Pb were added to provide four soil treatments (control (no Pb added), low-Pb, medium-Pb and high-Pb). Before planting, Pb was added as Pb(NO$_3$)$_2$ in aqueous solution to the soil treatments to adjust the Pb concentration of the soil to the extractable Pb concentrations (in 1 M ammonium acetate) given in Table 1. After adding Pb, the soils were stored for 2 months and frequently mixed to stabilize ion exchange equilibria. Both at planting and at the time of harvest, soil samples were taken from each pot in the four treatment groups and analyzed for extractable Pb (Table 1). Variations in extractable Pb concentrations of the substrate during the experimental period are presumed to be caused by shifts in the exchange equilibrium between mobile and immobile Pb, as a result of pH changes, microbial degradation of organic matter, leaching of soluble substances from the containers, and plant uptake.
Table 1. Soil concentrations of Pb (1 M ammonium acetate extracts) and pH \((\text{H}_2\text{O})\) at the beginning and at the end of a pot culture experiment with young Norway spruce trees. Values are means \(\pm\) SD of 11--12 containers, otherwise values are means of two samples.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pb ((\mu\text{mol kg}_\text{dw}^{-1}))</th>
<th>Soil pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>4.7 (\pm) 1.7</td>
</tr>
<tr>
<td>Low-Pb</td>
<td>74</td>
<td>74.9 (\pm) 4.9</td>
</tr>
<tr>
<td>Medium-Pb</td>
<td>127</td>
<td>147 (\pm) 41.2</td>
</tr>
<tr>
<td>High-Pb</td>
<td>646</td>
<td>575 (\pm) 106</td>
</tr>
</tbody>
</table>

At the end of January 1993, the Norway spruce trees were planted in pots containing the Pb-treated soils. Each treatment group included more than 30 plants. The potted trees were kept in an unheated greenhouse until mid March when they were moved outdoors until the time of harvest in November 1993. During dry weather the trees were irrigated with tap water.

**Harvest procedure**

After the end of the growing season, November 1993, 12 trees per treatment were randomly chosen for harvest. A 5-cm-long section was cut from the basal part of the stem directly above the soil surface. Adhering dust and epiphytes were removed from the bark with a brush. The surfaces at both ends of each stem section were cut with a microtome to improve the visibility of growth rings. The stem section was then clamped upright in a holder and the bark was removed with a scalpel. For analysis, the bark was divided into the outer dead part and the inner live part. The wood was then dissected with a scalpel and divided into single annual growth increments, starting from the outermost ring. Thin layers near ring boundaries were discarded in order to obtain a clear separation of tissue of adjacent rings. Because the innermost 1989 ring was usually very narrow and did not yield sufficient material for a separate analysis, it was combined with the 1990 growth ring. To minimize sample contamination, scalpel blades were cleaned with isopropanol between different harvest steps. Bark and wood samples were collected in paper bags and dried at 105 °C to constant weight.

**Analytical procedures**

Samples of wood and bark were wet ashed in Teflon pressure vessels with concentrated \(\text{HNO}_3\). Whenever sufficient sample material was available, analyses were carried out in replicates. Concentrations of Pb in wood and bark were determined by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer 5100). For all samples, the method of standard additions calibration was applied including a Pd-Mg(NO\(_3\))\(_2\) matrix modifier.

To determine exchangeable Pb concentrations in soil samples, 5 g of air-dried soil was extracted with 50 ml of 1 M ammonium acetate. The extracts were stirred for 2 h and then filtered. The filtrates were analyzed by flame atomic absorption spectrophotometry (Perkin-Elmer 380). Soil pH \((\text{H}_2\text{O})\) was measured in 10 g of air-dried soil in 25 ml of demineralized water after stirring for 15 min.

The quality of the analytical results was checked with the certified reference material Tea (GBW 08505) of the National Research Centre for Certified Reference Material, People’s Republic of China. Based on 43 determinations, a mean Pb concentration of \(1.03 \pm 0.15\) ppm was found which was in good agreement with the certified value of \(1.06 \pm 0.1\) ppm.

**Statistical analysis**

Lead concentrations of different annual rings within a treatment group were compared with the Wilcoxon matched pairs signed rank test (Sachs 1992). An analysis of variance including the Scheffé test was conducted to compare data between treatment groups.

**Results**

Concentrations of Pb in stem wood increased with increasing Pb concentration in the soil (Figure 1). Radial distribution patterns of Pb varied among the soil treatments. In control trees, Pb concentrations increased from the outer rings toward the stem center. This pattern was also observed in the low-Pb treatment, although at a higher range of concentrations. In trees in the medium-Pb soil treatment, Pb concentrations increased relative to that in control trees in all annual rings, but particularly in the outermost xylem ring \((P < 0.01)\). As a consequence, these trees had similar Pb concentrations in all four of the xylem rings analyzed. For trees in the high-Pb soil treatment, highest Pb concentrations were found in the 1992 xylem ring, which was formed before the experiment began. Lead concentrations in the outermost ring, which developed during the period of exposure to Pb-contaminated soil, were significantly lower \((P < 0.01)\) than in the 1992 xylem ring.

Concentrations of Pb in bark were higher than in stem wood (Figure 2) and higher in outer bark than in inner bark. In both outer and inner bark, Pb concentrations increased with increas-
Values are means ± SD of 8–11 plants.

Discussion

Although the Pb concentration of the soil was increased sharply in the 1993 growing season, the highest Pb concentrations were not found in the growth ring formed during that period. Under conditions of increased Pb availability in the soil, the overall Pb uptake of the Norway spruce trees was enhanced. However, the additional quantities of absorbed Pb were deposited in both currently growing wood and in older growth rings. Therefore, Pb concentrations in annual xylem rings do not represent a chronological record of variations in substrate Pb concentrations.

Deposition of Pb in the inner xylem rings can be explained by the pattern of sap flow in the stem. In coniferous trees the xylem stream passes through several annual rings at the same time. Ėrmák et al. (1992) found that, in *Picea abies* trees with 18 xylem rings, between 10 and 12 rings conduct the xylem flow. Thus, there is a direct supply of absorbed metals from the root system via the xylem sap stream to several xylem rings at the same time. It can reasonably be assumed that, in the five-year-old Norway spruce trees studied, all xylem rings were part of the conducting system and were supplied with Pb.

Control trees and trees in the low-Pb soil treatment had similar radial distribution patterns of Pb, though Pb concentrations in all of the annual growth rings were higher in trees in the low-Pb soil treatment than in control trees. Lead concentrations increased from the outermost growth rings toward the stem center. Similar distribution patterns have also been found for Cd and Zn (Hagemeyer and Lohrie 1995). Such patterns may be related to spatial differences in the cation binding capacity of the wood tissue. In mature red spruce trees (*Picea rubens*), the cation binding capacity of xylem tissue increased from the outer growth rings to the stem center (Momoshima and Bondietti 1990). Because the Ca concentration of the xylem tissue was highest at the stem center, Momoshima and Bondietti (1990) concluded that xylem Ca concentration increased with increasing cation binding capacity of the tissue. A similar explanation may account for the distribution of Pb in xylem tissue of the Norway spruce trees used in the present investigation.

Concentrations of Pb were higher in bark than in stem wood. These tissues are connected by rays crossing the cambial zone (Van Bel 1990), which suggests that there may be radial transport of elements, including Pb, between them. In support of this hypothesis, we found close correlations between Pb concentrations in xylem and bark. The transport of toxic substances, such as Pb, to the outer bark, where they will become incorporated in tissues that die and are eventually shed by the tree, may constitute a detoxification mechanism.

Because elements can move through rays in the radial direction (Ziegler 1964), radial transport processes could affect Pb distribution patterns within the xylem. Furthermore, an exchange of minerals between tissues of adjacent annual rings is possible through the pits, which connect tracheids of different rings (Bosshard 1976). Radial transport of mineral elements has been described for mature trees (Ziegler 1968, Bamber 1976, Daube 1883). The radial transport of toxic substances to be deposited in inner stem parts was suggested as part of a detoxification mechanism (Stewart 1966). However, the extent of such radial movements of Pb in stems is not known.

Our study provided no indication of the stability of the observed Pb distribution patterns. However, because an appreciable part of Pb in wood of coniferous and of ring-porous trees appears to be mobile (Balk and Hagemeyer 1994, Hagemeyer and Shin 1995), it seems possible that the radial distribution pattern of Pb will change, perhaps in response to variations in xylem sap composition. Seasonal changes in pH (Glavac et al. 1990) and in the concentrations of cation-complexing organic molecules (e.g., amino acids or citrate) in xylem sap have been described in various tree species (Sauter 1981, Rennenberg et al. 1994, Schneider et al. 1994). Such changes could give rise to mobilization of exchangeably bound Pb in the xylem. Hagemeyer and Shin (1995) investigated the potential mobility and binding strength of metal cations in wood of mature *Pinus sylvestris* L. trees and found that, depending on the radial position in the stem, only about 15–40% of the total Pb was immobilized. The major portion of Pb was exchangeably bound. Thus, the possibility of changes in the radial distribution of an element should be considered when radial distributions are evaluated in terms of pollution records.

We conclude that the radial distribution of Pb in Norway spruce stems depends more on internal physiological factors than on variations in the concentration of the external Pb supply and, therefore, Pb concentrations in annual xylem rings do not represent a chronological record of variations in substrate Pb concentrations (cf. Donnelly et al. 1990). Some of the factors affecting Pb distributions in xylem are: (1) retention of Pb in the root system, which can result in a delayed transport to the stem (Donnelly et al. 1990); (2) the amount of xylem sap flow through individual rings that determines Pb input; (3) the cation binding capacity of growth ring tissue; and (4) the
potential for remobilization of deposited Pb caused by variations in xylem sap composition. Thus, the observed distribution patterns of Pb represent the integrated results of effects of several factors, which can vary with time, making it difficult to interpret the physiological significance of element concentrations in tree rings.

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References


