Bull. Environ. Contam. Toxicol. (1994) 52:444–451

Environmental Contamination and Toxicology

Effect of Acidification on Metal Uptake of *Picea abies* Seedlings

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Received: 8 March 1993/Accepted: 30 July 1993

The effects of air pollutants on forest ecosystems are based on complex interactions where no single dominating factor has been found. The reasons for forest die-back are extremely difficult to determine because of the lack of long-term data and the difficulties in determining the "normal" state of the forest ecosystems (Krause *et al.* 1986). The forest die-back in central Europe has been interpreted on the basis of many different theories, including so called stress hypothesis. According to this hypothesis all stress factors are summed together so that the limits of biological stress tolerance of different organisms can be exceeded (Schulze 1989). One potential stress factor is soil acidification (Godbold and Hüttermann 1986, Bergkvist 1987). Anthropogenic pollutants increase soil acidification, which is known to increase the solubility of many metals. This has raised the question whether metals could be one potential stress factor to forest organisms. Root growth and the uptake of nutrients and water are in some cases sensible parameters of metal toxicity (Godbold and Hüttermann 1986). Although it is evident that metals are not the main factor in forest decline, it is important to understand the role of metals as a stress factor on forest ecosystems.

Metal uptake by different plant species, especially trees, is unclear (Martin and Coughtrey 1982, Smith and Brennan 1984, Kabata-Pendias 1992). For example the synergistic and antagonistic effects of different metals in plant tissues and the availability of organic metal complexes are still unknown. Plant roots also play an important active role in mobilizing metals from soil particles by producing organic compounds that are effective in releasing substances bound to soil particles (Kabata-Pendias 1992). It is important to study the quantities of metals taken up by forest plants because plants are one way for soil metals to enter forest food chains. It is also important to know whether metals can accumulate into plants in concentrations harmful to the plant itself. This study was designed to determine whether metal uptake by Norway spruce (*Picea abies* Karst.) is dependent on acidification or humus content of the soil.

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MATERIAL AND METHODS

Two different soils were used in the experiment: "sand" and "humic". They were prepared by mixing sieved (2-mm mesh) sand and homogenized, unlimed peat. The first soil had a sand:peat proportion of 8:1 (vol:vol) and the other soil 2:1. The final densities of the soils were 1.58 and 1.36 g/ml, respectively. The sand and peat were mixed thoroughly and separately for each seedling. The soil quality in the beginning of the experiment is shown in Table 1.

Table 1. Soil quality before treatments; the concentrations of soluble elements were measured after digestion in NH_4COOH-EDTA (pH 4.65).

	"Sand"	"Humic"
Conductivity, μS / cm	0.18	0.38
Organic matter, % of dry weight	1.1	2.9
Soluble P, mg/l	1.6	2.4
Soluble N, mg/l	17	33
Soluble K, mg/l	26	26
Soluble Ca, mg/l	140	160
Soluble Mg, mg/l	16	29

Three-year-old seedlings of Norway Spruce (*Picea abies*) were planted in pots (4.5 l of soil in a 5 l pot) in a greenhouse (glass roof, no walls) in spring 1988. During the growth periods in 1988 and 1989, the climate was near outdoor conditions. During the winter they were kept in a dark laboratory in the cold (+0°C, humidity 70%).

The spruce seedlings were subjected to three different treatments:
- artificial acidification, pH approx. 6 (distilled water + nutrients = D)
- artificial acidification, pH 5.4 (simulating North European rain = N)
- artificial acidification, pH 3.6 (simulating Central European rain = C)

Laboratory prepared water was added directly to the soil once a week in amounts corresponding to an annual precipitation of 650 mm. This artificial precipitation was prepared by adding KCl (25 mg/l), NaCl (38 mg/l), $CaSO_4$ (99 mg/l), $MgNO_3$ (18 mg/l) and NH_4SO_4 (29 mg/l) to distilled water. The pH was adjusted by adding H_2SO_4 and HNO_3 (Lodenius and Autio 1989). Six individual seedlings were subjected to the same treatment. Samples were taken in September 1989 from soil, roots (ø < 0.1 mm), needles 1989 and needles 1988. The root samples were washed in distilled water (400 ml in a shaker bath at 300 rpm for 5 min). All samples were dried and stored in paper bags.

The samples were homogenized and dried over night at +105°C. They were digested in HNO_3 (2.5 hours at +50°C, 4 hours at +105°C and 4 hours at +180°C). After filtration and dilution the samples were analyzed by graphite furnace AAS (Cd) or flame AAS (Fe, Mn, Zn, Cu, Al; Varian SpectrAA 40 and Perkin Elmer 360). The acid treatment used here does not dilute silicates, which is refelected as a lower recovery for Al in the standard plant samples (Table 2). The amounts of plant-available elements were analyzed from soil samples after digestion by NH_4COOH - EDTA at pH 4.65.

Table 2. Certified (mean + 95% confidence interval) and own (means ± std deviation) results (μg /g dry weight) from two standard samples: A = NBS, Pine needles, B = BCR, Olive leaves.

	Fe	Mn	Cu	Zn	Cd	Al
A cert.	200 ± 10	-	3.0 ± 0.3	-	< 0.5	545 ± 30
own	180 ± 4.3	680 ± 8.1	3.9 ± 0.24		0.20 ± 0.009	440 ± 18
B cert.	-	57.0 ± 2.4	46.6 ± 1.8	16.0 ±0.7	0.10 ± 0.02	450 ± 20
own		57 ± 2.0	44 ± 2.3	18 ± 1.8	0.07 ± 0.01	310 ± 36

RESULTS AND DISCUSSION

The soil pH-values were affected as follows:

	"SAND"	"HUMIC"
Beginning	4.6	4.2
Distilled water	4.6	4.1
pH 4.4	4.7	4.2
pH 3.6	4.4	4.0

The strongest acid treatment lowered the pH value only 0.2 pH units. It is obvious that the buffering capacity of the soil was sufficient to eliminate the effect of acid addition to the pH value. Neither treatment had significant effects on the Fe levels of the plant tissues (Fig. 1).

Acidification had no significant effect on plant Mn-levels, but Mn-levels were significantly higher in plants growing in more humus-containing soils (Fig. 2). In general the seedlings seemed to concentrate Mn in the needles rather than in roots.

Copper concentrated in the roots (Fig 3). The copper concentrations in plant tissues were not affected by the acid treatment or the soil organic matter content.

Zinc concentrated in the plant needles rather than roots (Fig. 4). This is not surprising because Zn is a component in at least 20-30 enzymes situated especially in plant leaves (Hewitt 1983).

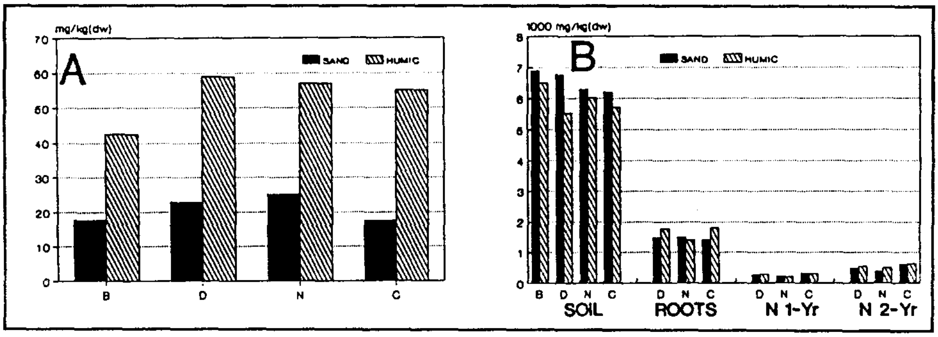

Figure 1. A: Plant-available iron in soil (B= beginning, D= distilled water + nutrients, N= north-European rain, C= central European rain). **B**: Fe concentrations in soil (tot Fe) and in plant roots and needles of one year and two years of age.

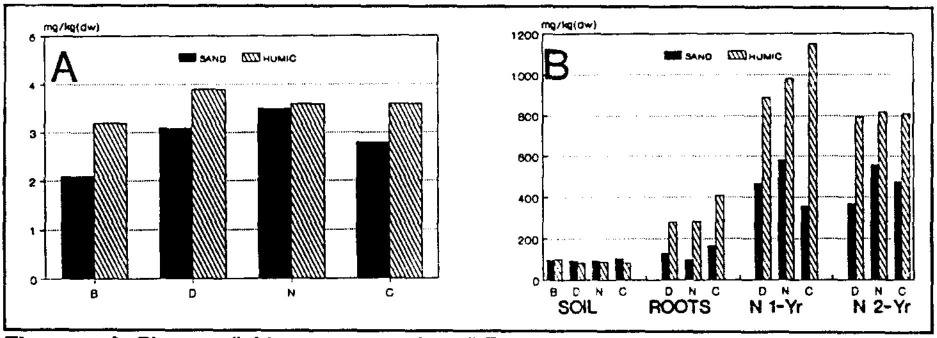

Figure 2. A: Plant-available manganese in soil **B**: Mn concentrations in soil (tot Mn) and in plant roots and needles of one year and two years of age. (Legends as in Fig. 1).

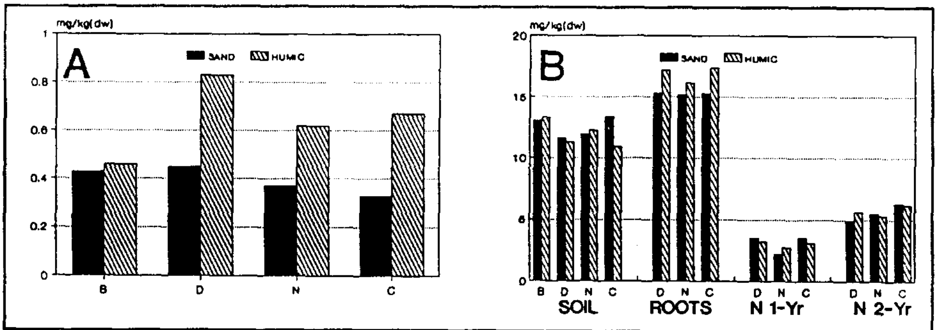

Figure 3. A: Plant-available copper in soil **B**: Cu concentrations in soil (tot Cu) and in plant roots and needles of one year and two years of age. (Legends as in Fig. 1).

Aluminium concentrated in plant roots (Fig. 5). It is difficult to say if the Al levels in roots were toxic, because the critical Al levels are extremely difficult to define (Hutchinson *et al.* 1986). Also, Al tolerance changes with many variables including plant age (Joslin *et al.* 1988). The Al concentrations were, however, not affected by the acid treatments or the soil organic matter content.

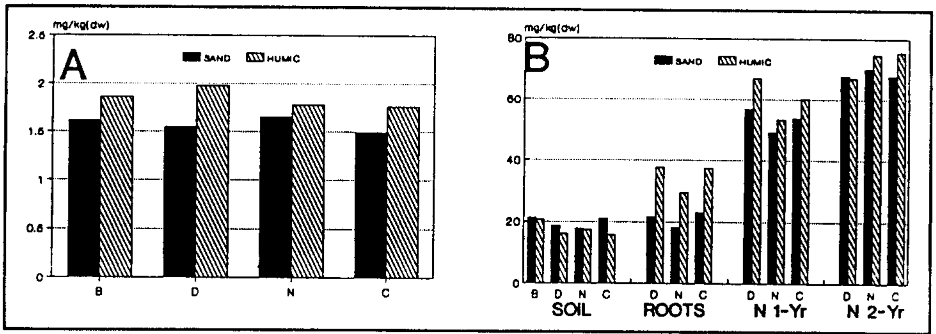

Figure 4. **A**: Plant-available zinc in soil **B**: Zn concentrations in soil (tot Zn) and in plant roots and needles of one year and two years of age. (Legends as in Fig. 1).

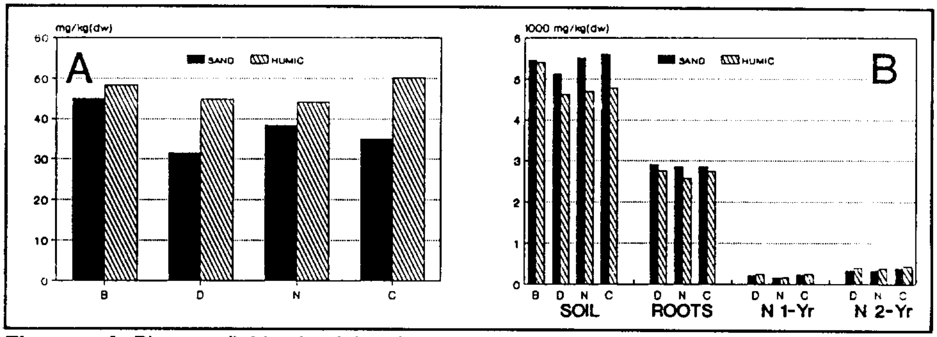

Figure 5. **A**: Plant-available aluminium in soil **B**: Al concentrations in soil (tot Al) and in plant roots and needles of one year and two years of age. (Legends as in Fig. 1).

It has been claimed that Al is not transferred from roots to leaves in Al-sensitive species (Foy 1974). Norway spruce has been classified as an Al-sensitive plant, but exact critical levels have not been presented (Godbold and Kettner 1991). Also the role of mycorrhizae in plant uptake of Al is incompletely known.

The plants accumulated cadmium mainly in the roots (Fig. 6). Cd is usually easily mobilized at decreasing soil pH. In our study soil pH was not affected by the treatments.

Artificial acidification had no significant effect on metal levels of *Picea abies*. Organic matter in the soil had no clear effect on metal levels except on Mn levels in roots and needles. The results of corresponding studies are contradictory: some obtained similar results (Lodenius and Malm 1990, Carlson and Ragsdale 1988) while others obtained the opposite (Wyttenbach *et al.* 1991). This would support the idea that metal absorption into plants is dependent on so many soil variables that equivalent results are not possible unless the conditions are absolutely equal. Some studies indicate that metal mobilization and absorption into plants would be clearly pH-dependent, but pH would be decisive only at very low pH values (Carlson and Ragsdale 1988).

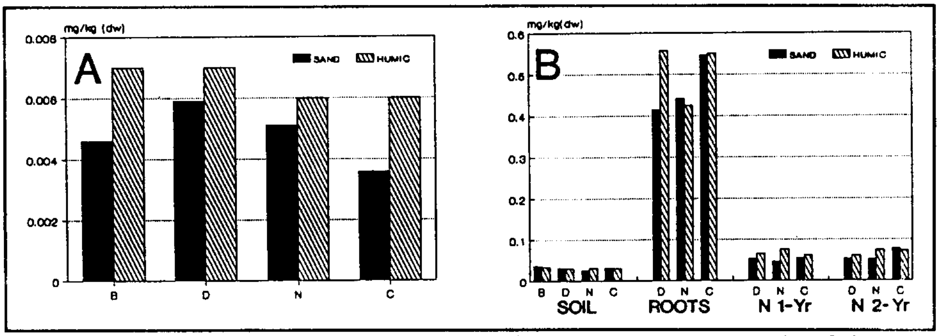

Figure 6. A: Plant-available cadmium in soil B: Cd concentrations in soil (tot Cd) and in plant roots and needles of one year and two years of age. (Legends as in Fig. 1).

The metal levels of plants are usually related to plant-available metal concentrations in soil (Kabata-Pendias 1992). In our study the plant-available metal concentrations did not increase significantly during the test. It was obvious that the buffering capacity of the soil was high enough to prevent the changes in pH value. Norway spruce also grows in naturally acid soils and must thus be adapted to acidic soils.

Although no clear trend in metal accumulation by seedlings was detected, it would be an oversimplification to claim that metals do not play any role as a stress factor in forest ecosystems. It is obvious that acidification as a process is so slow that short time tests cannot show any changes in plant metal levels. Metal problems in terrestrial ecosystems probably depend on how long the buffering capacity in soils is able to bind the increasing amounts of mobilized metals.

The exact role of metals in forest ecosystems is relatively unclear. The difficulties in interpreting the role of different metal concentrations in plant tissues are well known (Verklej and Schat 1989, Kabata-Pendias 1992). Determining the exact critical levels for certain plants is extremely difficult and would require studies at the cellular or even molecular level (MacNicol and Beckett 1985). Many metal toxicity tests are performed using a liquid medium and their applications in the natural environment is thus problematic.

Also, tolerance mechanisms complicate the assessment of metal stress to the organisms. In assessing the metal toxicity to plants, metal sensitivity is an essential factor. No known tolerance mechanism, however, explains completely the metal tolerance of higher plants (Verklej and Schat 1990). The role of mycorrhizae as a tolerance mechanism against metals is still unclear. It seems that some mycorrhizal species increase the hosts' metal absorption and some even seem to prevent it (Dixon and Buschena 1988). Mycorrhizae can also suffer from gaseous air pollutants. The final effect depends on the combined effects of all stress factors.

Chemical reactions of metals and humic substances in soils should be studied in more detail. For example, the chemical and physical properties of humic substances have been studied for more than 200 years, but their nature as a group is still poorly understood (Livens 1991). Metal-humus complexes are, however, one essential factor determining the amount of plant-available metals in the soil. It seems unlikely that metal toxicity would be the dominant causative factor in forest decline. However, over long time periods they cannot be excluded as a potential contributing stress factor on forest ecosystems.

Acknowledgments. This work was supported by The Academy of Finland. We are also indebted to Jukka Malm, Esa Tulisalo, Anna-Liisa Lodenius and Marjo-Riitta Kojo for technical assistance.

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