MULTIELEMENTAL ANALYSIS OF TREATED PINE SEEDLINGS

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A comparison study concerning the effects of acid rain on pine seedlings has been performed. Two different X-ray fluorescence methods, PIXE and IXRF were employed to produce multielemental analyses of the samples.

Seedlings were treated for 3 months with watering of pH=7 or pH=3 liquids on the needles and on the roots. One year and two years old needles of the seedlings were inspected for changes in photosynthetic rate as well as for changes in elemental concentration.

Twelve elements from Si to Zn were compared in the samples. The PIXE results show that the amounts of most of these elements in the needles of the seedlings grown in sand increase, when treated with acid water. This growth is more clear, when acid treatment is effected on the roots.

The elemental concentrations of the needles grown in soil on the other hand decrease slightly.

INTRODUCTION

The use of fossil fuels around the globe has increasingly added to the burden of pollutants in the atmosphere. In considering the effects of these pollutants sulfur dioxide is frequently recognized as the most serious in the northern hemisphere. The total effects of man-made pollutants have, however, not yet been the subject of systematic scientific studies. It is on the other hand, known that sulfur dioxide interacts with other constituents in the atmosphere, thus also e.g. with fine particles emitted or otherwise present in air. After transport over long distances complex sulfur containing aerosols and chemical species are deposited on the ground and on the vegetation. The physical state and variations in the reactions under different meteorological conditions should affect the true influence of these pollutants.

The transformation of sulfur dioxide in the atmosphere produces compounds with different oxidation states. It is difficult to specify the transformation exactly. The SO₃ in air is probably adsorbed on solid particles or absorbed in liquid droplets, but may also be released continuously during evaporation process. Sulfites and sulfates as well as sulfuric acid are found in air (Proceedings ... 1978). The sulfuric acid occurs first in the form of ultrafine particles, but after transformation by condensation to sulfates is present also in droplets of size diameter less than 2 μm. The sulfite is thought to occur in all particle sizes (HANSEN et al. 1978).

The presence of gaseous and solid sulfur compounds simultaneously in the atmosphere, and the dynamics involved with their transport and transitions, reflect the need for diversified analysis. Most of the effects of sulfur compounds must be coupled also with changes in nutrient mobility in the soil. All these effects are almost impossible to trace with sufficient precision. Some main aspects can, however, be revealed by simple field experiments.

In the following a simulation experiment on young pine seedlings treated with acid water is described. The analyses of the seedlings were performed multielementally. Care should, however, be taken in drawing direct conclusions from the results achieved herein. As such, the results reflect only the suitability of multielemental X-ray fluorescence methods in delicate ecological research.

SAMPLES

In the experiments, altogether 40 seedlings of tree year old pines (Pinus sylvestris L.) were subjected to controlled treatment. Half of the plants were growing in sand, the other half in mould soil. In each group of seedlings a reference subgroup was watered with neutralized water, pH=7, (group A), part of the seedlings were treated with an acid water, pH=3, sprayed on the shoots (group B), and the remainder watered with an acid water, pH=3, on the soil (group C). See fig. 1.

The treatment was carried out continuously during three months in the summer of 1978 at the Hyytiälä Forestry Station of the University of Helsinki. During the study period the seedlings were under the influence of daily meteorological conditions. The photosynthetic rates, respiration rates and damage on the needles were measured continuously. At the end of the treatment period, growth of the seedlings and radiative reflection characteristics of the groups A, B and C, at 410 to 950 nm wavelengths (JAAKKOLA et al. 1980), were measured. The present experiments contributed towards a research project concentrating more fully on the effects of air pollution on the Finnish forests (Contract ... 1979).

Representative samples consisting of one to two years old needles of the seedlings were processed for analysis. The needles were dried in 105°C for 24 hours, then ground by an ultracentrifuge into fine powder of particle size less than 50 μm. From this powder—1 mm thick pellet with a diameter of 10 mm was compressed into a polyacrylate support. Use of plastic materials is necessary to maintain a low background in the analysis (RAUNEMAA et al. 1980).

The sample compositions were scanned through two separate methods by radio-isotope excited X-ray fluorescence, IXRF, and by particle induced X-ray fluorescence, PIXE. As both these methods are non-destructive, the samples can be stored after analysis for reuse in any way.

THE EXPERIMENTAL METHODS

X-ray fluorescence effect

In X-ray fluorescence electromagnetic interaction is employed to produce momentary radiation which is characteristic to each element. The yield of the X-ray production can be calculated using refined theoretical formulations (MASSEY and BURHOP 1936). However, when high analytical applicability is required, different semiempirical methods must be applied. In practice, the X-ray fluorescence method determines the elemental composition of any sample in a measurement which is non-destructive and takes only a few minutes. However, the data processing requires that large computer programs (KAUFMANN et al. 1980) must be utilized to obtain well analyzed final results.
IXRF

When using the isotope excited fluorescence method (IXRF) X-rays were produced by the MnK-radiation from an Fe-55 radioactive source. This radiation induces fluorescence up to the element vanadium. In the facility used here other sources enclosed in the equipment can be used to study the elements up to lead semiquantitatively (RAUNEMAA et al. 1978). A careful IXRF measurement takes 15 to 30 minutes to execute.

PIXE

In the PIXE method 2.0 MeV protons from the Van de Graaff accelerator of Helsinki University were used (fig. 2). The elements from aluminum up to lead can be studied in this method with varying sensitivities.

In the present experiments a proton beam with 1.5 mm diameter and an average 2 nA current was employed. This implies that \(\sim 10^{10}\) protons/s strike the sample, which means about \(10^{11}\) protons in an average experiment lasting 10 minutes. A detailed description of the PIXE facility is given elsewhere (RAUNEMAA et al. 1980).

4. RESULTS

The fluorescence spectra arising from the IXRF and PIXE method are presented in Fig. 3. In PIXE analyses the elements Si, P, Cl, Ti, V, Cr, Mn, Fe, Cu, and Zn were detected in the samples in addition to the elements K and Ca obtained in the IXRF analyses.

In Table 1 the average concentrations in ppm of the needles as determined by the PIXE method are tabulated. In addition to the statistical errors ranging from 1 for element K) to 30 (element Cr) percent of the values shown in Table 1, a 10 percent systematic error should be included.

As no calibration standards were measured with the IXRF method, only element intensity changes between the different seedling groups can be compared with those due to PIXE determinations. The K/Ca ratios (Fig. 4 and Table 2) show that a rather good agreement between the IXRF and PIXE results was achieved.

Some general conclusions can be drawn from the PIXE results. Firstly, the amounts of different elements in the two year old needles do not change much, only by +5 percent, when considering the seedlings grown in sand and sprinkled on the shoots, but increase considerably, by a factor of 3.0, when acid treatment is effected on the roots. The concentration of sulfur behaves differently, increasing by 50 percent when the shoots are sprinkled. The most intense change in sand seeded pines occurs with the element zinc, the concentration of which increases by a factor of 20 when the seedlings are watered with acid water on the roots.

Secondly, the amounts of the elements in the needles slightly decrease when we consider the seedlings grown in soil and subjected to acid treatment. When the shoots are sprinkled, all element intensities except sulfur decrease by 24 percent, and when the roots are watered, the decrease is 12 percent, as compared to the reference groups. The sulfur behaves again differently; its concentration increases somewhat (\(\sim 7\) percent)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Si</th>
<th>P</th>
<th>S</th>
<th>Cl</th>
<th>K</th>
<th>Ca</th>
<th>Ti</th>
<th>V</th>
<th>Cr</th>
<th>Mn</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference A Sand</td>
<td>115</td>
<td>197</td>
<td>185</td>
<td>116</td>
<td>1400</td>
<td>414</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>63</td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>259</td>
<td>407</td>
<td>686</td>
<td>327</td>
<td>8130</td>
<td>1120</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>14</td>
<td>280</td>
<td>98</td>
<td>65</td>
</tr>
<tr>
<td>Acid sprinkling B</td>
<td>118</td>
<td>164</td>
<td>281</td>
<td>119</td>
<td>1540</td>
<td>460</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>45</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Sand Soil</td>
<td>197</td>
<td>345</td>
<td>785</td>
<td>202</td>
<td>2580</td>
<td>709</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>200</td>
<td>75</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Acid watering C</td>
<td>255</td>
<td>424</td>
<td>601</td>
<td>383</td>
<td>4080</td>
<td>863</td>
<td>4</td>
<td>12</td>
<td>2</td>
<td>298</td>
<td>101</td>
<td>63</td>
<td>29</td>
</tr>
<tr>
<td>Sand Soil</td>
<td>244</td>
<td>550</td>
<td>629</td>
<td>262</td>
<td>2710</td>
<td>995</td>
<td>19</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>55</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The average concentrations in ppm of the needles determined by PIXE analysis using 2.0 MeV protons.

490
when seedlings are sprinkled with acid water, but decreases slightly (~8 percent), when watered on the roots. Also a few other elements show differences in acid watering; e.g., zinc, the intensity of which increases slightly.

In each sample, intensity ratios of each element to the element Ca were also calculated to obtain a common norm and also to reveal possible changes as between different elements (see Table 5). Averaged over each group of seedlings these ratios show that the elements Cl, K, V, and Mn change similarly to the element S both in sand grown and in soil grown groups. In acid sprinkling, an average of 20 percent increase was obtained in both groups. In acid watering no change in soil grown, but an average of 48 percent increase in sand grown seedlings was found as compared to the reference group.

The concentration ratios S/Ca, Cl/Ca and K/Ca, as measured by PIXE method, are presented in Fig. 5. The ratios of the elements Si, P and Ti to Ca have almost constant values in each group; and in each treatment. The ratios of the elements Fe and Cr to Ca increase in sand seedling groups, but decrease in soil seedling groups when subjected to acid treatment.

**DISCUSSION**

The elemental concentrations of pine needles show that when the seedlings are sprinkled with acid water the sulfur concentration increases independently of the growth environment. When the acidiying is effected through watering on the roots, the growth environment may affect the needle composition; in sand the mineral content is effectively increased, in moulded soil no large change occurs. This may be due to differences in the mineral leaching.

The decrease in photosynthesis must be coupled with the apparent increase in concentration of all elements in sand grown and the moderate decrease in soil grown seedlings. This coupling may be deduced through the observed growth changes, which showed decrease in sand, and respectively, increase in soil grown seedlings.

An optimal equilibrium between the mineral content and growth of the needle biomass may be possible. Verification of the existence of this equilibrium requires further careful examination. The results achieved so far by this study indicate that multielemental PIXE analyses can be beneficially put to use for this purpose.
REFERENCES


THE EFFECT OF AIR POLLUTION ON TRANSPLOANTED MOSSES

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The use of forest mosses as bioindicators was tested with transplantation experiments. One transplantation experiment was made to study effects of air pollutants on two forest moss species, *Hylocomium splendens* and *Pleurozium schreberi*. Another transplantation was used to study nitrogen fixation capacity of blue-green algae in the *Hylocomium* and *Pleurozium* moss layers. The surface structure of the moss species was studied by scanning electron microscopy. The air pollution induced changes in the surface structure of moss cells were observable soon after the transplantation. In polluted industrial areas the fertilizing effect of air-borne nitrogen compounds increased the photosynthetic activity of mosses before their destruction. Stress respiration was also observable in polluted areas. The nitrogen fixing capacity decreased or was almost inhibited in all the air-polluted environments.

INTRODUCTION

Two common moss species in Finnish forests, *Hylocomium splendens* (Hedw.) B. S. G. and *Pleurozium schreberi* (Brid.) Mitt., were used as bioindicators in transplantation experiments in air polluted areas. The levels of CO\(_2\) fixation and N\(_2\) fixation were studied in the transplanted mosses in polluted and clean environments.

Several studies have proved mosses to be very sensitive to air pollutants (i.e. GILBERT 1968, 1969, LeBLANC 1969, LeBLANC et al. 1971, WINKLER 1976, WINNER and BEWLEY 1978a and b, FERGUSON and LEE 1978). Air pollutants affect the growth and reproduction of mosses. Several species have been shown to disappear from industrial and urban environments, though suitable stands for moss growth can still be found (RAO and LeBLANC 1967, GILBERT 1968, BARKMAN 1969). The suitability of mosses to bioindication is mainly due to their primitive morphological and anatomical structure. Mosses can absorb and accumulate nutrients and pollutants directly from the atmosphere.

The use of mosses as indicators of soil, water and air pollution and the importance of mosses as accumulators of metal ions have recently been reviewed by HISATSUGU 1980, LeBLANC and RAO 1974.

The nitrogenase activities in moss epiphytic blue-green algae were used as one of the indicators of air-pollution induced effects on mosses. BASILIER et al. (1978) found the nitrogenase activity of mosses to correlate with the number of blue-green algae in natural clean air areas. This is in accordance with our findings obtained during several years of study (KALLIO, unpubl.). Only few studies of pollution effects have been made earlier using nitrogenase activity measurements by asetylene reduction (KALLIO and VARHEENMAA 1974, HÅLLGREEN and HUSS 1975, KALLIO 1976).

The morphology of *Hylocomium splendens* with groove-like surface structure of leaves and *Pleurozium schreberi* provides a good shelter for algae against drying. *Pleurozium schreberi* is a moss of somewhat dryer forests, and its