MICROANALYSIS OF RELATIVE WEIGHT ELEMENTS PERCENTAGE IN NEEDLES OF DAMAGED SILVER FIR TREES (ABIES ALBA MILL.) AT TWO SITES

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Concentration of carbon (C), oxygen (O), nitrogen (N), magnesium (Mg), aluminium (Al), silicon (Si), phosphorus (P), mercury (Hg), sulphur (S), lead (Pb), chlorine (Cl), potassium (K), cadmium (Cd), calcium (Ca), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu) and zinc (Zn), in the form of weight percentage, in the needles of different age classes, current and previous year one, of two sites, Risnjak National Park and Donja Dobra (control), were measured by X-ray microanalysis (EDAX) in the Scanning Electron Microscope in silver fir trees (Abies alba Mill.). The analysis suggests that accumulation of elements, in both years, particularly that of heavy metals, appeared in polluted site, like Risnjak, in the higher amount, whereas in non polluted, like Donja Dobra, in the lower amount. Moreover, it seems likely that elements at the first sites were accumulated and contaminated in very young current year needles, while in non polluted in somewhat older, the previous year ones.

Key words: Abies alba, concentration, EDAX, elements, needles, X-ray microanalysis

INTRODUCTION

High levels of extremely harmful toxic emissions into the atmosphere bring inadvertent changes in the environment. Their deleterious effects manifest themselves in contamination of soils and water, and lead to the destruction of vegetation. The forest ecosystems, particularly coniferous ones, have been always susceptible to the adverse effect of emissions. Even if the level of emissions remains stable, the effects of some harmful factors have tendency to be accumulated (Smith 1981, Zelinski 1984). In recent years, these particularly endangered coniferous forests have attracted investigations in Europe and United States of America (Persson 1980, Smith 1981, Grodzinski et al. 1984).

In Croatia, like many European countries (Huttunen 1976a, b, Bernadzki 1983, Schulze et al. 1989, Tikvić et al. 1995), the coniferous forests are declining and this process is specially pronounced in Gorski Kotar where the Risnjak National Park is situated. Gorski Kotar is the region between the continental and Mediterranean area, and this part is exposed to strong emissions of sul-
phur dioxide and other pollutants from the Rijeka region, the northern Italy and the north and middle Europe as a consequence of wind directions.

The data published by Prpić (1990), on high sulphur dioxide level in the Risnjak area, and by Komlenović and Rastovski (1992) on increased content of sulphur and numerous plastoglobules within chloroplasts stroma in the needles of damaged trees, indicate to an increased pollution in this part of country. Kušan et al. (1993) estimated that the average damage in this region amounted about 50%, and Durbešić and Kerovec (1990) that the air pollution might be a possible and primary cause of silver fir forests damage. The last conclusion has been confirmed by the most recent research (Bačić et al. 2003) about the considerable lower content of chloroplasts pigments and higher concentrations, mostly upper limits, of heavy metals in the soil, as well as the reduced and swelling chloroplasts into roundish or irregular shape, reduction of their thylakoides, specially those of grana, presence of many plastoglobuli, particularly the light ones in the stroma, etc., in the needles of damaged trees of the Risnjak site (Bačić et al. 2004).

Since yellowing of needles and nutrient deficiency, have received much attention and since this process has greatly increased in recent decades, particularly in the needles at the Risnjak site, in comparison with the control site, where green needles prevailed, we have examined element compositions by HAX in order to see, not only the difference between the sites, but the difference between the two needles of different age classes.

MATERIALS AND METHODS

Two stands were chosen as distinguishing in the percentage of damaged trees level. The first was the Risnjak National Park, and second Donja Dobra, about 30 km away to the southeast. In the Risnjak National Park, which is to be consider the most polluted one, three trees were selected with the level of damage of 20%, 55% and 85%, and in Donja Dobra, which is consider to be nearly the clean one (control site), also three trees were chosen, but two with a 5% of damage and the other with a 10% level of damage. The estimation of damage has been done by Roša (2001). The needles of different age classes, of the current and of the previous year, were collected from both stands, per trees, at regular intervals during 1999 and 2000 season, in May, July and September. Segments from their middle region and abaxial surface were subsequently examined on the stomatal rims of stomata at three separate places by SEM. In total, 72 needles, 36 of the current and 36 of the previous year (1999 and 2000), from both stands, were involved in the experiment. The measuring of elements was done at 216 places of each needle, respectively.
The segments of middle region were air dried, fixed to the stubs using double-sided adhesive tape, and then sputter-coated with gold. The investigation was done by identification of piques in High Automatic X-Ray Analysis System using Scanning Philips XL 30 Electron Microscope with the energy-dispersive system EDAX DX 4. The accelerating voltage was 120 kV, the take-off angle 25° and the counting time 60–100 live seconds. In this way the weight percentage of elements in needles was obtained.

The results obtained were statistically analysed with Student’s t-test (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

The weight percentage of twenty elements was analysed: carbon (C), nitrogen (N), oxygen (O), magnesium (Mg), aluminium (Al), silicon (Si), phosphorus (P), mercury (Hg), sulphur (S), lead (Pb), chlorine (Cl), cadmium (Cd), potassium (K), calcium (Ca), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu) and zinc (Zn). They included in both the current and the previous year needles, of silver fir trees (Abies alba Mill.), at both stands, the Risnjak National Park and the Donja Dobra site (control), during the 1999 and 2000.

*Fig. 1. EDAX of a current year needle, the fifth tree, the first place, July 1999, from the Risnjak National Park*
As seen in Figures 1 and 2 various differences exist between the two sites. The mean element concentration for 1999 and 2000, respectively, was generally higher at the Risnjak than in the Donja Dobra. The current year needles there were able to take up effectively nearly more than twice the amount of elements in comparison with the previous year ones, particularly the amount of heavy metals, such as aluminium (Al), magnesium (Mg), mercury (Hg), potassium (K), calcium (Ca), cadmium (Cd), chromium (Cr), manganese (Mn), cobalt (Co), iron (Fe), copper (Cu) and zinc (Zn). At the Donja Dobra site the situation was just the opposite. The previous year needles there, although less pronounced but still clearly evident, were able to take up effectively nearly more than twice the amount of elements, as compared with the current year needles. Such finding seems to suggest that the accumulations of elements in a polluted area appear in very young current year needles, and in a non-polluted area, however, they appear somewhat late in the previous year ones. This supports the opinion of Tuovinen et al. (1993), Tamm (1968), Raitio et al. (1995), etc. of an easier accumulation in younger needles. No significant differences among elements were found during 1999 and 2000, respectively, except of iron (Fe) in 1999 in current year needles at the Donja Dobra site.

The mean element concentrations for both 1999 and 2000 were as seen in Table 1. The highest values of weight percentage went for the first group of elements: carbon (63%), oxygen (12%) and nitrogen (11%). In total 86 weight percentage goes to this elements. No significant differences were detected among

![Fig. 2. EDAX of a current year needle, the fifth tree, the third place, July 1999, from the Donja Dobra site (control)](http://example.com)

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them. Mercury holds the fourth position (9.2%) and had even between seven and ninth weight percentage. Potassium (about 3.8%) and calcium (about 3.4%) with over the one weight percentage, were behind mercury.

The remaining elements go to the second group. These are: magnesium (0.3%), aluminium (0.4%), silicon (0.2%), phosphorus (0.8%), sulphur (0.08%), lead (0.5%), chlorine (0.5%), cadmium (0.4%), chromium (0.4%), manganese (0.7%), iron (0.5%), cobalt (0.5%), copper (0.9%) and zinc (0.6%). At the site of Risnjak significant values in the current year needles of chromium (Cr) and zinc (Zn) were found, whereas those of potassium (K), manganese (Mn), cobalt (Co) and copper (Cu) were in the previous year ones. The increasing tendency predominantly of most of other heavy metals in the current year needles was also observed at the Risnjak National Park, while the opposite trend was found in the previous year needles at the Donja Dobra.

Table 1

Weight percentage (Wt) of elements, in current and previous year needles at sites Risnjak and Donja Dobra

<table>
<thead>
<tr>
<th>Elements</th>
<th>Risnjak Current year</th>
<th>Risnjak Previous year</th>
<th>Donja Dobra Current year</th>
<th>Donja Dobra Previous year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium (Al)</td>
<td>0.42±0.3</td>
<td>0.21±0.1</td>
<td>0.27±0.1</td>
<td>0.40±0.3</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.42±0.2</td>
<td>0.35±0.1</td>
<td>0.41±0.1</td>
<td>0.43±0.1</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>2.47±2.1</td>
<td>1.9±4.0</td>
<td>1.8±1.3</td>
<td>3.4±2.9</td>
</tr>
<tr>
<td>Carbon (C)</td>
<td>57.7±7.7</td>
<td>62.6±1.93</td>
<td>62.3±3.8</td>
<td>60.8±3.4</td>
</tr>
<tr>
<td>Chlorine (Cl)</td>
<td>0.57±0.4</td>
<td>0.32±0.07</td>
<td>0.41±0.06</td>
<td>0.45±0.1*</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>0.39±0.2</td>
<td>0.2±0.05</td>
<td>0.2±0.03*</td>
<td>0.27±0.04*</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>0.46±0.2</td>
<td>0.28±0.05</td>
<td>0.29±0.07</td>
<td>0.32±0.05*</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.88±0.5</td>
<td>0.43±0.06</td>
<td>0.49±0.1</td>
<td>0.54±0.06*</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>0.5±0.2</td>
<td>0.33±0.05</td>
<td>0.30±0.08</td>
<td>0.34±0.08</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>0.5±0.7</td>
<td>0.23±0.1</td>
<td>0.32±0.2</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>0.22±0.1</td>
<td>0.26±0.1</td>
<td>0.21±0.1</td>
<td>0.25±0.1</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>0.43±0.1</td>
<td>0.26±0.04</td>
<td>0.41±0.1</td>
<td>0.7±0.2*</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>9.25±3.0</td>
<td>8.57±1.7</td>
<td>7.79±1.1</td>
<td>8.39±0.7</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>9.1±2.6</td>
<td>10.7±1.8</td>
<td>10.5±2.0</td>
<td>9.5±0.9</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>11.8±1.9</td>
<td>11.7±1.6</td>
<td>10.6±1.7</td>
<td>11.5±1.9</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>1.02±0.68</td>
<td>0.62±0.1</td>
<td>0.76±0.27</td>
<td>0.68±0.1</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>3.83±4.8</td>
<td>0.9±0.3</td>
<td>2.9±1.35</td>
<td>1.61±0.5*</td>
</tr>
<tr>
<td>Silicon (Si)</td>
<td>0.17±0.1</td>
<td>0.17±0.1</td>
<td>0.15±0.1</td>
<td>0.15±0.1</td>
</tr>
<tr>
<td>Sulphur (S)</td>
<td>0.01±0.1</td>
<td>0.02±0.03</td>
<td>0.06±0.1</td>
<td>0.08±0.08</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.65±0.2</td>
<td>0.4±0.05</td>
<td>0.36±0.07*</td>
<td>0.49±0.1</td>
</tr>
</tbody>
</table>

Mean value±SD, * = significant differences at p < 0.05, N = 6
Concentration of elements in coniferous needles and standards for quantification X-ray microanalysis, as well as of heavy metals stress and localisation of different elements, have been reported by numerous authors (Huttunen, 1976a, b, Fritz 1989, 1991a, b, Fritz and Jentschke 1994, Fritz et al. 1994, Wytenbach et al. 1995, Wytenbach and Tobler 2000, Wienhaus et al. 2001, Bäucker et al. 2003).

As it seen from the results presented this is the only one method of elemental composition pattern for silver fir needles investigations. This method could be usefully used in most case, but first of all as the complementary research.

Taking into account the facts, such as the level of pollution, the percentage of stages of damaged trees and the wind direction at the Risnjak place (Bačić et al. 2003), it seems logical and not excluded, that the concentrations presented are probably the consequence of accumulation and contamination by heavy metals (Hg) or lack by non metals (S), especially in very young needles. The most important symptom of the first consequence is the poisoning whereas of the second one is the lack of synthesis proteins. In comparison with the Risnjak site, the needles of the “clean” Donja Dobra area, retain their green colour longer (Bačić et al. 2003). In this site there is less amount of elements, with lower intensity, spread over the already formed previous year needles. Such occurrence it looks like is in accordance with our assumption of the Risnjak National Park as a more damaged part than is the Donja Dobra or control site.

Generally, it is known that several elements investigated have been accumulated more extensively in younger than in older needles, and these statements have also been confirmed by this research. Further investigations are needed to show the validity of this method.

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