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MORPHOLOGICAL AND ANATOMICAL CHANGES OF NORWAY SPRUCE NEEDLES *(Picea abies (L.) Karst.)* IN THE ŠOŠTANJ STEAM POWER PLANT INFLUENCE AREA

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Abstract

Morphological and anatomical changes of Norway spruce needles from 16 sampling plots in the vicinity of the Steam Power Plant in Šoštanj (TEŠ) were examined. The visible needle injuries were analysed and compared to the sulphur contents in the same samples. On the basis of this comparison and analysis of needle infections by microorganisms a conclusion was made on the possible causes of needle injuries. A new method of evaluation of needle injuries was tested. The method was based on autofluorescence of chlorophyll in spruce needles. After DCMU treatment of needle samples, the fluorescence intensity rose significantly, yet not proportionally to the autofluorescence intensity. The difference between the autofluorescence and the secondary fluorescence intensity (after DCMU treatment) showed, to which degree was damaged the photosynthetic apparatus of the Norway spruce-needles.

Key words: Norway spruce, needles, injuries, autofluorescence of chlorophyll, DCMU, secondary fluorescence of chlorophyll

MORFOLOŠKE IN ANATOMSKE SPREMEMBE SMREKOVIH IGLIC *Picea abies (L.) Karsten* V VPLIVNEM OBMOČJU TERMOELEKTRARNE ŠOŠTANJ

Izvleček

Ugotavljali smo morfološke in anatomske spremembe smrekovih iglic s 16 vzorčnih mest v okolici TEŠ. Poškodovanost iglic smo primerjali z vsebnostjo žvepla v istih vzorcih. Na podlagi omenjene primerjave in analize okuženosti iglic z mikroorganizmi smo sklepali o izvoru njihovih poškodb. Preizkusili smo novo metodo vrednotenja poškodovanosti iglic na osnovi avtofluorescence klorofila v njih. Po dodatku DCMU se je fluorescenca močno povečala, vendar ne sorazmerno z avtoflorescenco. Razlika v intenzivnosti avtofluorescence in sekundarne fluorescence pokaže, v kolikšni meri je poškodovan fotosintetski aparat pri rastlini.

Ključne besede: Picea abies, iglice, poškodovanost, avtofluorescenca klorofila, DCMU (3 3,4 diklorfenil - 1,1 dimetil urea), sekundarna fluorescenca klorofila

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CONTENTS

1 INTRODUCTION

Forest decline has recently been considered one of the major ecological problems of the developed countries. Slovenia had to face this problem similarly as almost every other European country - due to its geographical position and developmental level. The first systematic inventory of forest decline in Slovenia (in 1985) showed, that the situation was alarming. The greatest area of declining forest was found to be the valley of Šaleška dolina and its surroundings.

The steam power plant in Šoštanj (TEŠ) is the greatest source of air pollution in Slovenia, and the relief of the valley of Šaleška dolina contributes greatly to the bad pollution circumstances. The local inventorization of 10140ha of forest showed, that 98% of the forest had already been damaged (HRČEK et all. 1988).

Because of the worrying results of the forest decline inventory, numerous investigations of air pollution and damage to forests in Šaleška dolina were undertaken. Our investigation was a part of this wide research project, and contributed some data and results to it. Beside this, a new method of evaluation of Norway spruce needle injuries was tested. The method was based on the difference of autofluorescence and secondary fluorescence of chlorophyll in differently injured needles, and was expected to show indirectly the quantity of chlorophyl in the Norway spruce needles.

It has been possible to test "our" methods of evaluation of chlorophyl quantity, because at the same time a biochemical investigation of the chlorophyl contents has been carried out and we could compare the results of the latter investigation (courtesy of Missis RIBARIČ - LASNIK - see Appendix 3) with the results of ours.

In this article, much less attention is dedicated to the other two parts of investigation - to the morphological analysis of the needles and to the analysis of needle infections by microorganisms as this was routine work and has been exactly described elsewhere (ŠLIBAR 1990).

2 MATERIAL

Norway spruce needles for the investigation were sampled at the same time and from the same trees as the needles, used for the analyses of chlorophyll and sulphur contents. Therefore the comparison of results of all the three analyses could give an answer about the influence of air pollution on Norway spruce trees.

The goal of this study was to analyse the injuries of needles from the following 16 localities:

Sampling localities	Height above sea level (m)	Distance from TEŠ (m)	Exposition
Vrh Smrekovca	1577	12.750	NW
Koča na Smrekovcu	1400	12.700	NW
Kramarice	1200	12.750	NW
Slanica	950	9.500	NW
Zavodnje	770	8.250	NW
Pod Zavodnjami	690	6.000	NW
Lajše	400	2.000	NW
Lokovica	505	500	SW
Veliki vrh	560	2.750	SE
Andraž	410	6.000	SE
Graška gora	770	7.200	NE
Šmiklavž	485	10.500	NE
Podgorica	460	11.000	NE
Kope	1542	20.200	NE
Komisija	1300	21.750	NE
Paški Kozjak	1108	10.500	NE

The Šoštanj Steam Power Plant (TEŠ) is situated at height of 360m above sea level, at the bottom of the valley of Šaleška dolina.

The sampling plots were located at various distances from TEŠ, at different altitudes and in variously damaged regions of forest. On each sampling plot, the most vital two Norway spruce trees of the age of 60-80 years were chosen for the investigation. From these trees, the current year needles and the one year old needles were sampled from twigs of the seventh spruce whirl, counted from the tree crown top downwards. The sampling took place five times: in spring, summer and autumn 1989 and in winter and spring 1990.

3 MORPHOLOGICAL ANALYSIS

3.1 Methods

From each tree, 60 needles of the mentioned two age classes were analysed microscopically (stereomicroscope ZEISS, JENA). The injured surface of every needle was evaluated and the data were registered. Later, the total damaged surface was summed for each group of 60 needles. Among injuries, only the necroses and chloroses were taken into account. The mechanical injuries were not taken into consideration, because they did not result from the influence of gas pollutants. However, the mechanical injuries occured only seldom.

After assessment of injuries, the needles were dried until no further loss of weight could be noticed and then weighed. The data on the dry weight of needles were used for comparison of needle injuries, originating from different sampling plots.

3.2 Results

The results are presented in figures 1 and 2.

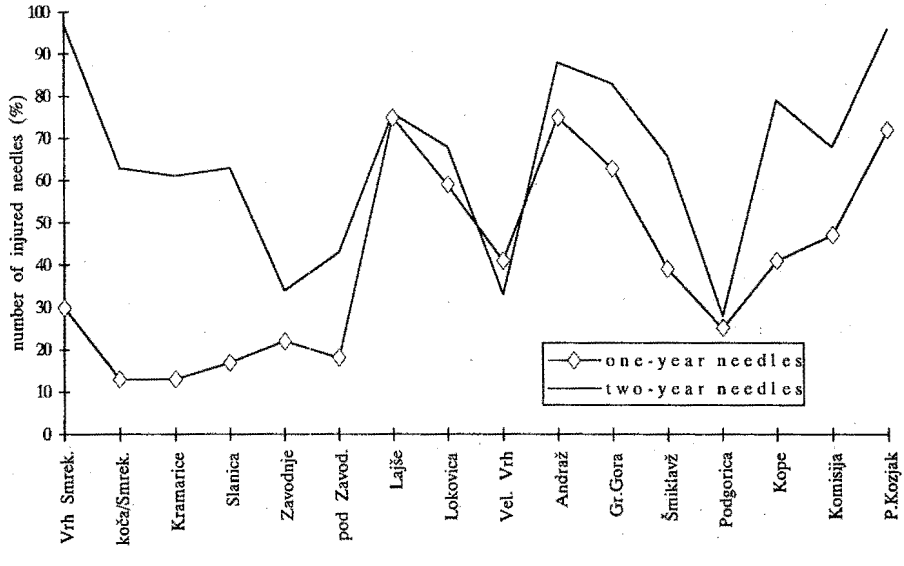


Figure 1: Comparison of injuries of current-year and one-year old needles (autumn, 1989)

The comparison of injuries of current-year and one-year old needles is shown, according to the number of injured needles to a needle-group (the 60 needles of the same age). It could be seen from the diagram, that in the majority of cases the current-year needles are less damaged than the one-year needles. The difference in damage among sampling plots varies significantly. The highest degree of injury occurs by needles from the localities of higher altitude, although the sulphur contents in these same needles is the lowest (courtesy of Mr. Kalan - see Appendix 1).

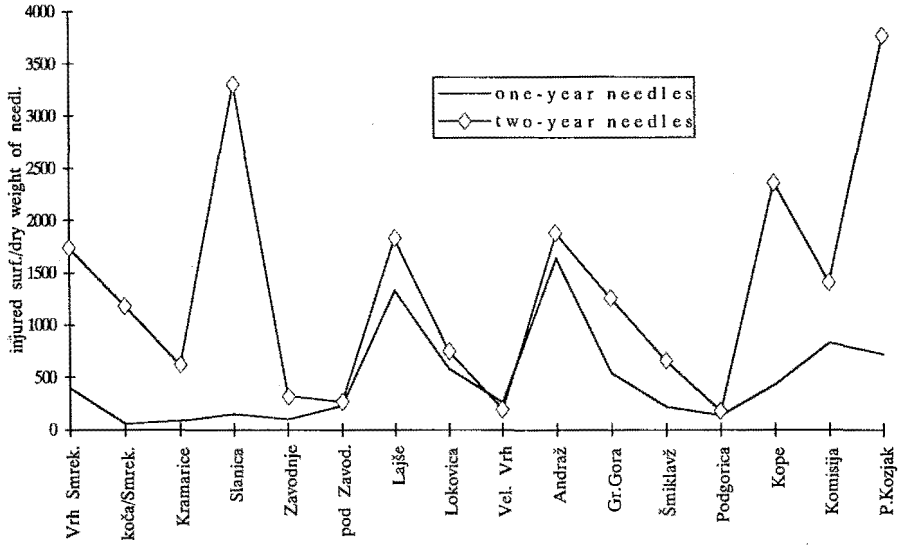


Figure 2: Comparison of injuries of current-year needles and one-year old needles (autumn, 1989)

The comparison of injuries of current-year and one-year old needles from different sampling plots is shown as a quotient: injured surface/dry weight. Except for one case, the current-year needles are less injured than the one-year old needles, and again the extent of injuries varies significantly from one locality to another.

The course of the curves on both diagrams is surprisingly similar. It had been expected that after dry weight of needles was taken into consideration, the relations between sampling plots would change significantly - yet this had not happened. Neither in Figure 2, the connection between external damage to needles and the contents of sulphur could be noticed.

It is interesting to compare our results with the results of the analysis of sulphur contents in Norway spruce needles (KALAN). For this reason, the diagram of sulphur contents has also been appended to the article (Appendix 1).

The analysis of external injuries of the Norway spruce needles of different age confirmed, that the current year needles were less damaged than the older ones. This had been expected, because the injuries increase with the longer time of exposure to damaging substances.

In comparison of injuries to needles from various sampling plots it was expected, that the visible injuries would mainly depend on the sulphur contents in the needles. This proved not to be true. Far the most injured were the needles from higher elevations, in which the sulphur contents was rather low. These injuries could therefore not be a result of damaging effects of sulphur, and the cause should be sought elsewhere. As high concentrations of ozone have often been measured in the surroundings of TEŠ it is possible for this gas to be the major pollutant in this case. Its concentrations increase with the growing altitude - probably as a consequence of greater number of days without fog, stronger UV radiation and possibly also the vicinity of stratosphere (which makes passing of the ozone to the lower air layers easier). Therefore the trees' injuries on these elevations are most possibly caused by ozone.

4 ANALYSIS OF NEEDLE INFECTIONS BY MICROORGANISMS

4.1 Methods

Parts of fresh needles with different types of injuries were sterilized and inoculated on nutrient medium. Some days later the colonies were sterilly transfered to fresh media. After four weeks of incubation, the species of fungi were determined (ŠLIBAR 1990).

4.2 Results

10 types of micelia grew from the needles with various types of injuries. The micelia were not characteristical for different types of injuries - no connection was discovered between the type of injury and the fungi species. All the determined species of fungi were saprofitic, with an exception of the Sirococcus strobilinus Preuss (a parasitic species). Therefore it could be infered, that needle injuries did not occur as a consequence of infection. Most probably the microorganisms infected the already feeble needles. No hiphae or sporangies were noticed in the needle tissue, so that it could also be possible, that the presence of fungi on the needle surface was merely casual. It has been anticipated, that the only parasitic fungus, Sirococcus strobilinus Preuss, had not led to extensive needle injuries, for it only appeared once among the 75 inoculated samples (ŠLIBAR 1990).

5 ANALYSIS OF CHLOROPLAST FLUORESCENCE

5.1 Methods

If chlorophyll is exposed to UV light, strong red fluorescence appears. As a consequence of chlorophyll degradation, the red colour changes to orange-yellow. The healthy needles fluorescred, the chlorotic needles orange-yellow and the necrotic needles have no fluorescence at all. The fluorescence intensity depends on the quantity of chlorophyll in the needles.

By intensive illumination with UV light, the autofluorescence fades away very quickly. The discolouration can be delayed, if a drop of 3 (3,4dichloro-phenil)-1,1 dimethyl urea (DCMU) solution is added to the sample instead of water. The fluorescent colour is blood-red at the beginning and soon changes to lighter red, which is more persistent.

DCMU is a herbicide that specifically blocks the photosystem II (DENFFER, ZIEGLER 1982). Due to the blockade, the resonance transfer (the transfer of energy from one molecule to another) is made impossible. The energetically rich chlorophyll molecules can not transfer their energy surplus to the acceptor molecules, but loose this surplus in the form of photones. This is reflected in great increasment of fluorescence intensity.

My goal was to evaluate the efficiency of photosynthetic apparatus in the needles on the basis of intensity of primary and secondary fluorescence of chlorophyll. Fresh needles without visible external injuries were used for this part of investigation. One current-year and one one-year old needle from each tree were choosen for the analysis. The needles were always taken at the distance of 1cm below the twig top or below the limit between the current and the one-year old needles, always from the upper side of a twig. Cross-sections of the middle part of needles (40 x 10^{-6}m thick) were done with a criomicrothome (HANOVIA). Autofluorescence of objects in water and their secondary fluorescence after addition of DCMU solution was observed and photographed. The DCMU solution was prepared by dissolving 0.02g DCMU (M=233.1) in 100ml of ethyl alcohol.

The microscop OLYMPUS - BHS and the photographic equipment of the same company were used for the work. The majority of photographs were taken by the 10x10 magnification, only some details were photographed by the magnification of 10x20. For the fluorescent microscopy, the exciter filter U was applied (transmits the light of the wavelenghts of 334nm and 365nm), the dichroic mirror U (DM-400 + L-420) and the supplement barrier filter L-435 and up. The film AGFA CHROME, CT 100, DIA was used for all the photographs. The values on the automatic photographic equipment were by all photographs adjusted to: ASA/400, RECIPROCITY/4, EXPOSURE ADJUSTMENT/1. 30% of the eye-field was highlighted for each photography. The efficiency of photosynthetic apparatus was evaluated on the basis of luminosity of objects. That was also a measure of chloro-

plast damage caused by air pollution or other external factors.

The object on dark background fluoresces red. Its luminosity depends on the size of the object (the fluorescing surface) and on its fluorescence intensity. None of the two parameters could have been directly measured.

Size of the object - Each of the objects was photographed. The objects' contours were than copied from the photographs to paper sheets of invariable quality, the shapes were cut out and weighed. The size of a whole photograph represented the value of 100% and the contours of the objects represented corresponding shares of the surface. So the relative values of size of the objects were obtained.

Fluorescence intensity - Because there was no light sensitive cell (photocell) available, with which the fluorescence could have been accurately measured, the fluorescence was measured with help of the automatic photographic equipment. We assumed, that the camera needed equal quantity of light for each photograph. If light was faint, the time of photography had to be longer. The time (t), which was automatically adjusted by the photographic equipment, is therefore inversely proportional to the object's luminosity (S):

$$t = 1/S$$

Luminosity is a product of fluorescing surface (P) and fluorescence intensity (IF). According to this, the following expression is gotten:

$$t = 1/(P \times IF)$$

For each photograph, the time value was registered. The fluorescence intensity was then calculated with the following equation:

$$IF = 1/(P \times t)$$

Besides the photographs needed for the analysis of fluorescence intensity in healthy needles, some photographs of chlorotic and necrotic needles were also taken.

5.2 Results

The results are presented in figures 3 and 4 (the diagrams have been made after the values presented in the table - Appendix 2).

The comparison of autofluorescence intensity and secondary fluorescence intensity (after DCMU treatment) in current year needles from different sampling plots is presented in the diagram (Figure 3). The course of both curves is contrasting, with the assumption, that the extreme values are much more emphasized by the secondary fluorescence curve. The greatest difference between the curves occurs for the

localities, where the chloroplasts in needles are the least damaged.

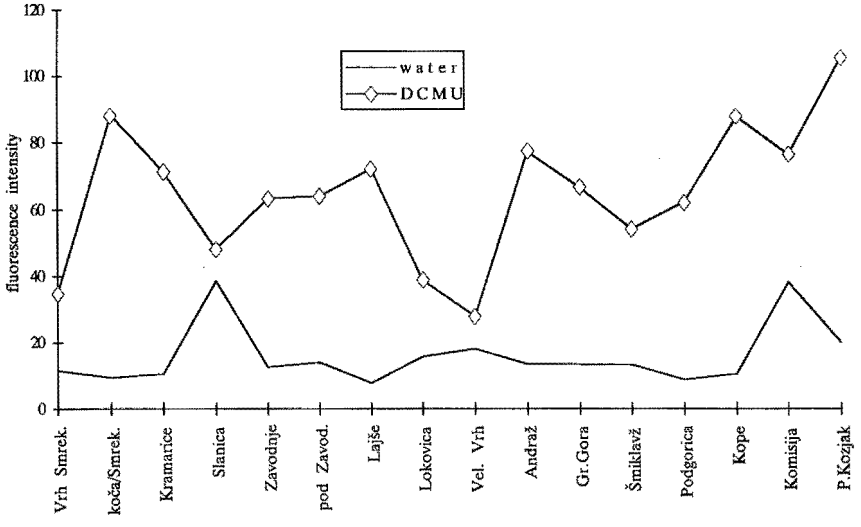


Figure 3: Comparison of autoflorescence intensity and secondary fluorescence intensity in current-year needles (spring 1990)

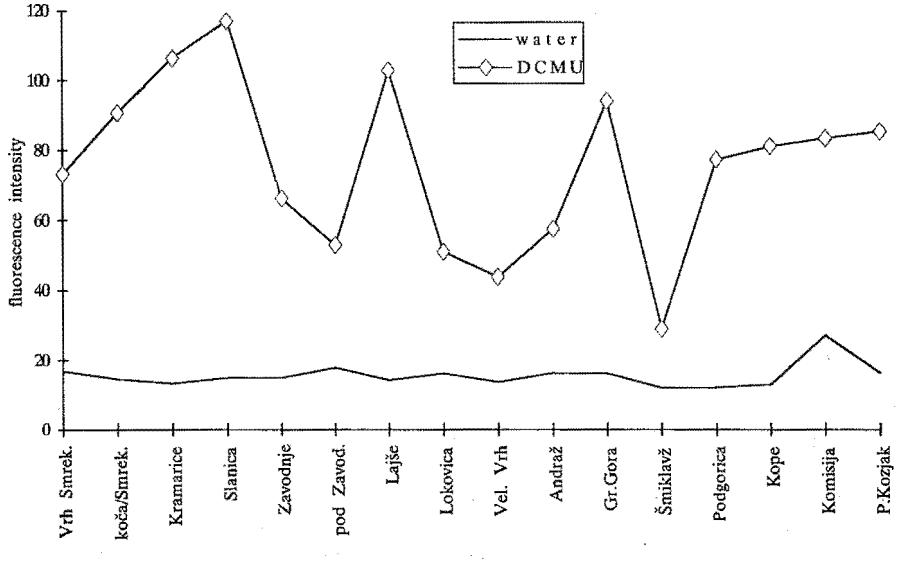


Figure 4: Comparison of autoflorescence intensity and secondary fluorescence intensity in one-year old needles (spring 1990)

The comparison of autofluorescence intensity and secondary fluorescence intensity (after DCMU treatment) in one year old needles from different sampling plots is presented in the diagram (Figure 4). The course of both curves is similar as in Figure 3, with identical assumption. In this case as well the greatest difference occurs, where the chloroplasts are the least injured and chlorophyll contents the highest.

When comparing the curves of autofluorescence of the current-year and one-year old needles it can be seen that their courses are similar, but the curve representing the current-year needles lies lower than the curve of one-year old needles. That indicates, that the chlorophyll quantity in one-year needles is lower than in two-year needles. This is normal, and corresponds to our earlier expectations.

6 DISCUSSION

Interesting results were obtained with the analysis of chloroplast injuries on the basis of chlorophyll fluorescence. It had been expected, that the fluorescence intensity depended on the quantity of chlorophyll and integrity of chloroplasts in needles. The fluorescence intensity in current-year needles with lower chlorophyll contents (Figure 3), should therefore be weaker from the fluorescence intensity of one-year old needles (Figure 4).

Despite some deviations and relatively small difference between the fluorescence intensities of current-year and one-year old needles it could be said, that the course of curves presented in the figures 3 and 4 corresponds to our expectations. Yet unusual it is, that our results do not agree with the results of the analysis of chlorophyll contents (courtey of Missis Ribarič - Lasnik; Appendix 3). It seems, that the mechanism of electron transport has to be taken into consideration. As already mentioned, the DCMU is a herbicide, blocking the photosystem II of photosynthesis. That means, that it causes the electron transport breakdown, and this is reflected as strong fluorescence increasment. This increasement is the greatest by the objects, by which the electron transport before DCMU addition was the least damaged. By the objects, where the electron transport had been damaged already before the DCMU treatment, the fluorescence increasement could be almost negligible (a typical example of this kind is the sampling site Slanica; see Figure. 3).

With the method tested it is not possible to evaluate the quantity of chlorophyll in the needles. The most interesting result, obtained from the curves of autofluorescence and secondary fluorescence (after DCMU addition) would certainly be their difference. Let us suppose, that DCMU leads to complete (100%) electron transport breakdown. If this was true, the secondary fluorescence would show the extent of undamaged electron transport (100% electron transport). Autofluorescence represents the actual level of damage to electron transport mechanism. The difference of both values shows, to what degree the mechanism of electrone transport is damaged by the examined tree. As the electron transport is of a vital importance for photosynthesis, the results actually directly show the level of

disturbance in plant's photosynthetic apparatus. Most probably this is one of the best presentations of vital force of a plant which shows, to what degree the plant was able to adapt to the environmental changes.

The tested method should be worked out more precisely. One of the greatest deficiencies was, that it was not possible to measure the fluorescence intensity directly. For this purpose, automatic photographic equipment was applied, while for completely reliable results highly accurate measuring equipment (photocell) would be necessary. Another deficiency could have been, that the needle cuts were too thick (40×10^{-6}m). Such thickness was necessary, because entire (not torn or otherwise damaged) needle cuts were needed for the calculations, but the needles tear very easily if the cut is not thick enough. Tearing could probably be prevented by pre-cooling of the criomicrothome blade.

7 LITERATURE

DENFFER, ZIEGLER 1982: Botanika - morfologija i fiziologija (Botany - Morphology and Physiology). Školska knjiga, Zagreb.

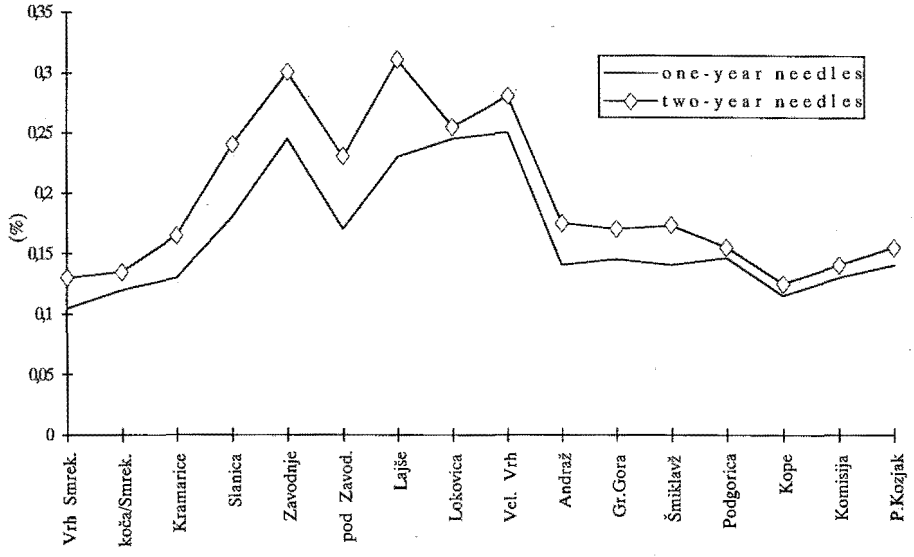
HRČEK D., CIGLAR R., GREGORČIČ B., LEŠNJAK M., PEČENKO A., PLANINŠEK T., VEDENIK - NOVAK M., 1988: Proučitev mezoklimatskih razmer v občini Velenje (A study of mesoclimatic conditions in Velenje). Hidrometeorološki zavod SRS, Ljubljana.

KALAN, J., in preparation: Vpliv TEŠ na tla in vegetacijo (The influence of Šoštanj steam power plant on the soil and vegetation). 2nd part. Inštitut za gozdno in lesno gospodarstvo, Ljubljana.

RIBARIČ - LASNIK, C., in print: Ekofiziološke lastnosti smreke /Picea abies (L) Karsten/ na vplivnem območju Termoelektrarne Šoštanj (Ecophysiological characteristics of Norway spruce /Picea abies (L) Karsten/ in the influence area of the Šoštanj Steam Power Plant). Magistrsko delo. Ljubljana.

ŠLIBAR, A., 1990: Morfološke in anatomske spremembe smrekovih iglic (Picea abies /L/ Karsten) v vplivnem območju Termoelektrarne Šoštanj (Morphological and anatomiceal changes of Norway spruce needles (Picea abies /L/ Karsten) in the Šoštanj steam power plant influence area). Diplomska naloga. Ljubljana.

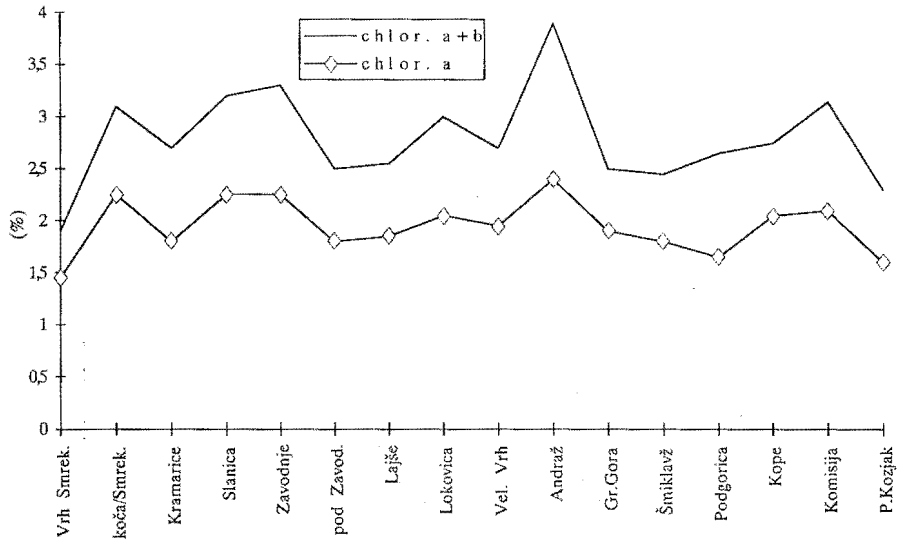
Appendix 1: Sulphur contents (%); Kalan, 1990



Appendix 2: Fluorescence intensity in current year and one year old needles; IF = 1/(P x t).

Sampling plot	Fluorescence intensity			
	One year needles		Two year needles	
	water	DCMU	water	DCMU
Vrh Smrekovca	11,53	34,87	16,79	73,12
Koča na Smrekovcu	9,42	88,21	14,44	90,72
Kramarice	10,62	71,38	13,26	106,44
Slanica	38,63	48,18	14,87	117,02
Zavodnje	12,54	63,31	14,91	66,22
Pod Zavodnjami	13,98	64,06	17,87	53,03
Lajše	7,87	72,22	14,20	102,80
Lokovica	15,76	38,99	16,22	51,10
Veliki vrh	18,17	27,94	13,63	43,79
Andraž	13,52	77,20	16,23	57,55
Graška gora	13,55	66,61	16,20	94,07
Šmiklavž	13,34	54,28	12,02	29,13
Podgorica	8,81	62,29	12,18	77,42
Kope	10,54	87,90	12,99	81,02
Komisija	38,39	76,49	27,19	83,41
Paški Kozjak	20,16	106,12	16,23	85,32

Appendix 3: Chlorophyll contents - spring, 1989 (Ribarič-Lasnik, 1990)



Explanation to the photographs 1 and 2

Both photographs show a cross-section of a Norway spruce needles by fluorescence microscopy.

On Photograph 1, the autofluorescence of chlorophyll in needle cells can be seen and on Photograph 2 the secondary fluorescence of the same object after addition of fluorochrome DCMU (dichloro-phenyl dimetil urea).

The difference between fluorescence intensities shows to which level is damaged the photosynthetis system of the needle under examination. The lowest the difference, the more chlorophyll has been damaged, or in other words, the smaller is photosynthetis activity of the needle - and of the whole tree.

This method of evaluation shows, to which degree is damaged the photosynthetic mechanism of a tree and thus directly illustrates the level of tree's vitality.

The method is used on camparative basis. The photographs were taken on OLYMPUS BHS microscope, with photographyc equipment of the same producer (10 x 10 magnification, fluorescence michroscopy).

Photographs 1 and 2: