EARLY DETECTION OF INDUSTRIAL EMISSION IMPACT ON SCOTS PINE NEEDLES BY COMPOSITION OF PHENOLIC COMPOUNDS

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Abstract. In this work the influence of emissions from heat power station on the phenolic composition in Scots pine needles was investigated. There was no difference neither in qualitative composition nor in quantitative content. Trees state changes associated with multiple stress factors are reflected in the structure of correlation matrix. The number of significance of correlation coefficients was increased in testing group and structure of correlation bonds between individual compounds was changed too. This phenomenon is independent of nature of stress and arises only due to common regulator factor.

The problem of large-scale pollution of the air and its ruinous impact on the plants has acquired special ecological significance. Condition of the forests in highly industrialized countries is a subject of great attention. Certain progress had been achieved in developing the methods to record visually detected damage of the forest vegetation [1]. At present it is commonly recognized, that the damage is, first of all, revealed at the biochemical to spread over the ultrastructural and cell levels. The visible symptoms of the damage develop after it. In this connection it is of prime importance to develop the methods for early diagnosing of the plant condition on the basis of biochemical indices.

There is voluminous literature, devoted to the studies of the biochemical composition of plants, affected by air pollution [2, 3, 4]. Nevertheless, there are no however acceptable biochemical methods for early diagnosis yet. This seems to be due to several reasons. One of them is the fact, that most works, as a rule, compare absolute values of the concentrations of the substances or

activity of the enzymes. In such an approach the ranges of the test values often overlap the control ones. The alternative approach can be based on studying the correlations of the indices under study. The problem of their variability and the choice of the control is getting acute. This method, called correlation adaptometry, was developed in studying the degree of correlation or the lipid exchange parameters in human populations [5]. The revealed effect of growing correlations between the physiological parameters was accounted for the monofactoriality of the regulation, "apparition" in the adaptation process of a limiting factor, whose function decreases and becomes compatible with the other factors in the adapted condition. It is obvious, that the slight effect of industrial emissions, not entailing changes in the morphological indices, can be revealed at the biochemical level, and consider the plants to adapt to the adverse conditions of the environment.

Most works dealing with the early diagnosis methods study the products of primary metabolism. Meanwhile, biochemically they are very active, their daily dynamics is often very complex, they are actively transported from the leaves which makes difficult to use them as indicators of the plant condition.

To diagnose we used in our work secondary metabolites of phenolic nature. The plants are capable of synthesizing a very broad range of such substances. All of them form from the common precursor -phenilalanine, which is one of the final products of the shikimate pathway. The phenolic compounds are subject to catabolism in much less degree, that the primary exchange products, consequently, they hold the information about the impacts on the plant organism for a long time. Another important advantage of the phenolic compounds is that the modern methods of chromatographic analysis allow to separate complex mixture of these substances by minimal manipulations with the plant samples.

The aim of this work is to obtain quantitative data on the composition of the phenolic compounds of the Scotch pine needles and to process them by correlation analysis method with the purpose of early detection of thermal electric station emission impact.

Objects and Methods

The object under investigation was Scotch pine (Pinus sylvestris L). Examination was conducted in a clean 40 years old stand of the II class of quality with the stocking density 1.0. The tree stand is in the emission tongue 10 km from the thermal electric station, operating on brown coal for 45 years. The control was a stand of the same age and forest type, growing outside the industrial emission area. Ten trees without visible damage and weakening were chosen in each sample plot. The needles for analyses were one year old from the shoots in the middle part of the crown. The samples were taken in spring in bud swelling period.

Samples of needles (2 g of wet mass) were fixed in 96% ethyl alcohol and held in a refrigerator at 2^0 - 4^0C. After it the needles were ground with liquid nitrogen and extracted by 80% ethyl alcohol (15 ml 3 times) at room temperature. Unsolvable substances were separated by centrifugation for 15 min. at 5000g. The alcohol extract was evaporated dry and the remainder was dissolved in hot (60^0C) distilled water. Resins, pigments, lipids and other substances, unsolved by water, were sedimented by centrifugation. The thus purified extract was used for quantitative analysis of individual phenolic compounds, present in the pine needles, and the fraction of free proantocyanidines. In the remainder the bound proantocyanidines were determined.

Individual composition of phenolic compounds were examined by high efficiency liquid chromatography (HELC) with "Milichrome". The column 2*64 mm was filled with Silasorb C 18 sorbent (CSFR). The eluent was the linear gradient of ethyl alcohol (0 - 30%) in 5% acetic acid. The flow rate - 50 mcl/min. Detection was performed at 260 nm. The area under the chromato-graphic peaks in proportion with the concentration of the respective individual substances, were measured automatically by electronic integrator CI-100A (CSFR). The proantocyanidine content was measured by a routine procedure [6].

Results and Discussion

The morphological parameters of the needles and tree seedlings, compared earlier [7] on the sample test and control plots, showed no deviation in the growth processes in the pine crowns. Nevertheless, the sulphur content in the needles, examined by the same authors was found to be higher in the trees growing in the emission areas, and represented, mainly, by SO_2.

Table 1. Proantocyanidine Content (mg/g dry weight) in One Year Old Needles of Scots Pine

Proantocyanidines	Control	Test
Free	8.1±0.7	8.0±1.0
Bound	29.3±3.2	28.7±1.9
Total	37.4±3.2	36.8±2.0

Table 1 shows the determination of proantocyanidine content the largest, with respect to the quantity, group of phenolic compounds. No index showed sound difference between the samples. However, we should note, that in spite of the unreliability of the difference of the mean values between the two samples, within the samples we obtained reliable difference for both indices both in the test and in the control. So, there exists considerable individual variability in the content of free and bound proantocyanidines in the needles from different trees. It is this situation, that we meant, speaking about the vicissitudes of using absolute parameter values to determine the "norm - pathology" boundary.

Fig. 1 shows a typical chromatograph of phenolic compounds of the alcohol extract of the needles. All chromatographs were found to have 26 individual compounds. Thus, the qualitative composition of the phenolic compounds of the needles on the test and control plots was identical. Next we determined the content of 19 compounds, corresponding to the peaks most pronounced in the chromatographs. Standard error values show, that individual phenolic compounds are individually very variable, too. Therefore

with close mean values the difference between the samples at the 5% level of significance are unreliable. An exception is only the compound, corresponding to the second peak, revealing marked difference between the samples.

However, we think, that comparing a set of signs it is hardly possible to make some sound conclusions about the coincidence of the sets on the whole, guided by the results of comparisons of individual signs. Indeed, even if there are reliable differences between certain pairs it is not clear what quantity of such pairs makes two sets differ on the whole. Therefore, either it is necessary to prove the possibility to use the distinct signs

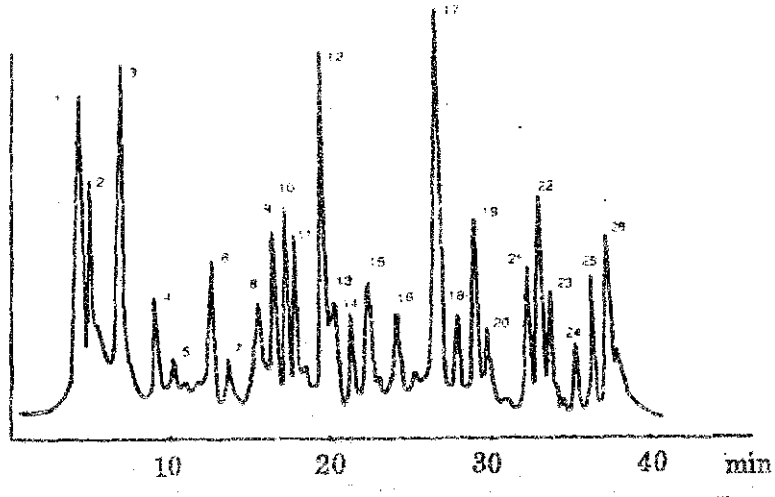

Fig. 1. Typical chromatograph of phenolic compounds of alcohol extract of one-year old needles of Scots pine.

without taking into account the rest or to study the structure of the sets of the signs and relations between them. With the absence of the indicator signs the latter direction remains the only one. It was this reason, that made us construct correlation matrices of the relations of the individual phenolic compounds' concentrations

for each of the samples. Table 2 shows their fragments, containing meaningful correlation coefficients.

Table 2. Fragments of Matrices of Correlation Bonds Between the Content of Individual Phenolic Compounds: A - test, B - control

A.

No. of compounds (peaks)	2	9	10	11	13	16	22	26
1	0.68	0	0	0	0	0	0	0
3	0	0	0	0.67	0	0	0	0
4	0	0	0	0	-0.74	0	0	0
7	0	0.78	0.72	0	0	0	0	0
8	0	0.66	0	0	0	0	0	0
9	0	1	0.65	0.74	0	0	0	0
10	0	0.65	1	0	-0.67	0	0	0
15	0	0	0	0	0	-0.69	0	0
17	0	0	0	0	0	0	0	0.64
18	0	0	-0.66	0	0.75	0	0	-0.67
21	0	0	0	0	0	0	0.67	0

B.

No. of compounds (peaks)	13	15	21	23
1	0	-0.79	0	-0.80
9	0.81	0	0	0
15	0	1	0	0.72
17	0	0	0.67	0

The number of meaningful correlation coefficients for the population in the industrial emissions' area turned out to be three times of that for the control one. The absolute values are

15 and 5, respectively. The structure of the correlation bonds was also different. The test population was found to have increased number of negative correlations, which seems to prove the engagement of the compensation mechanism with the respective changes in the concentrations of the substances under study. By the correlation adaptometry method greater correlation of the investigated parameters is accounted for the apparition of a common limiting factor, which, in our case it is but logical to identify as the air pollution.

The mechanism of the growing correlation of the biochemical indices may be accounted for the fact, that the air contaminants, penetrating the plant cell, are able to interact with different components: membranes, metabolites, enzymes and so on. At this, adaptive reactions, aimed to reduce the negative effect of the air contamination, are started and the metabolism of all trees changes identically. It is common knowledge, that any disturbance in the basic exchange affect the nature and intensity of the secondary metabolism of the plants [8]. So, the air contamination can be as factor, determining similar changes in the synthesis of the phenolic compounds.

In conclusion we should note, that the effect of higher correlation of the signs in the population, subject to the common for all trees regulating (limiting) factor, was obtained, as in [5], within the framework of the normal correlation theory. On this path there are two traditional difficulties, connected with the assumption of the normality of distributions at the initial general totalities and with the linearity of the relationships of the signs. In these cases it is more promising to apply non-parametric statistics, as the assumption about the normality is a convenient abstraction only, while the non-correlatedness condition is less rigorous, than the independence of the signs. Non-parametric methods are based on weaker assumptions about the properties of the general totalities. The preliminary results, obtained in these directions show, that the Spearman's rank correlation coefficient is reliable for the larger number of pairs in the test population, compared to the control : 11 and 8, respectively. So, even with less burdensome assumptions about the

statistical properties of the totalities the correlation of the phenolic compounds' content is higher in the trees, growing in the area of the thermal electric station impact.

The results obtained can be considered important, stimulating arguments to create a method for early diagnosis of tree stands without visual signs of damage and/or weakening if the trees.

REFERENCES

1. Lacasse, N. L. & Treshow, M. Diagnosing Vegetation Injury Caused by Air Pollution. Environmental Protection Agency; Washington D. C., USA, 1976

2. Gaseous Air Pollutants and Plant Metabolism. (M. J. Koziol and F. R. Whatley eds.); London etc., Butterworths, 1984.

3. Air Pollution and Plant Metabolism. (S. Schulte-Hostede, N. M. Darrall, L. W. Blank and A. R. Wellburn eds.). London and New-York; Elsevier Applied Science, 1987.

4. Environmental Stress in Plants. Biochemical and physiological mechanisms. (Ed. by J. H. Cherry). Berlin etc.; Springer, 1989.

5. Gorban A. N., Manchouk V. T., Petushkova Ye. V. Dynamics of Correlations between Physiological Parameters in Adaptation and Ecologo-Evolutional Principle of Polyfactoriality. In: "Problems of Ecological Monitoring and Ecosystem Modeling", 1987, vol. 10, pp. 187-198.

6. Stafford H. A and Cheng T.-Y. The Procyaninidines of Douglas Fir Seedlings , Callus and Cell Suspension Cultures Derived from Cotyledons. Phytochem., 1980, v. 19, N. 1, pp. 131-135.

7. Girs G. I., Zubareva O. N. Tolerance of Scotch Pine in the Area Affected by Coal-Operated Thermal Electric Station Emissions. Izv. AN SSSR. Ser. biol. nauk. 1990, vyp. 1, pp. 105-112.

8. Margna U. Control at the Level of Substratum Supply - an Alternative in the Regulation of Phenylpropanoid Accumulation in Plant Cell. Phytochem., 1977, v. 16, N. 4, pp. 419-426.