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# APPLICATION OF METHODOLOGY OF TERPENES AND TERPENOIDS ANALYSIS IN NORWAY SPRUCE ASSIMILATION ORGANS *(Picea abies* (L.) K a r s t.) WITHIN MONITORING HEALTH CONDITIONS OF FOREST TREE SPECIES

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Abstract

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In spite of the imission reduction in middle Spiš region, the health conditions of spruce sites are still deteriorating. The most severe changes in the health conditions of spruce sites have been caused by climatic factors, biotic factors (particularly the presence of bark beetles), abiotic factors as well as the impact of a long-term imission load. The aim of the present study was to justify the appropriateness of methodology for terpenes and terpenoids analysis in spruce assimilation organs when evaluating the health conditions of forest tree species within sustainable monitored areas of 2$^{nd}$ level. The differences of selected components content at individual degrees of Norway spruce assimilation organs damage were processed by multivariable analysis in the years 1996, 1999 and 2000. From the principal component analyses results, it may be said that based on the method of terpenes and terpenoids analysis, the differences between samples of various degree of spruce needles damage may be found out. From the results of cluster analysis, where the component content in individual years represented variables within a given year and degree of damage the content of analysed samples was found to be dependent mainly on the age of forest tree species and then on the degree of damage. The methodology of terpenes and terpenoids analysis in common spruce assimilation organs is recommended as appropriate for observing the health conditions of trees within sustainable monitored areas of 2$^{nd}$ level, where obtained results represent selective data corresponding to the crest – assimilation organs type of damage.

*Key words:* spruce sites, monitoring, terpenes and terpenoids analysis, gas chromatography, mass spectrometry, principal component analysis, cluster analysis

## Introduction

If the environmental conditions are changed, one can observe, from the eco-physiology point of view, changes in the level of metabolites, changes in diameter and level increments, appearance of diseases and pests, as well as early root kill. The cell membrane properties in conifer needles are changed by atom-gene photo oxidants through lipids per oxidation. Changed membrane permeability leads towards the mono-terpene release from needles, which as endogenous factors either stop or slow down the electron photosynthetic and respiratory transport. During illumination more radicals are created in the damaged electron photosynthetic transport than detoxification mechanisms are capable to cope with, and plastid pigmentation is destroyed (Wagner et al., 1987).

The assimilation area of by air pollutants damage trees, seeking many needle year classes, is rather small. There is obvious inequality between photosynthetic active assimilation area and nourished tree biomass (trunk, root). Consequently, there is a lack of tree and shoot growth, and hardly cognisable rings. If the photosynthesis runs continuously in the rest of needles, the labile balance is created and the saccharine photosynthesis production and its consumption by breathing are kept balanced within an annual period. This may survive for many years within any notable increment or root kill. If such a labile situation is accompanied with other photosynthesis organs damage, e.g. by photo oxidants (direct damage) or by the lack of water in contexture caused by long-lasting draughts (indirect damage), then the minimum rest photosynthesis is inefficient to nourish the whole biomass. Those parts of trees that cannot be supplied die off. More often it is the tree top that most suffers the lack of water, and is influenced by high temperatures and air pollutants (Kmeť, 1996). Short lasting hairpin roots die in the soil without any reproduction. Mycorise mushrooms withdraw or completely disappear; harmful soil mushrooms interfere to the trunk through roots i. e. dieing from wood parenchyma cells inside trunks. Biotic pests accelerate this process of such weak forest tree species. Terpenes and terpenoides determined components content in sound and damaged trees of Norway spruces sways and is dependent on wood age, too. The damage degree may be stated also as an important impact on the determined component content (Jűttner, 1988), as this trend changing the determined component content in samples is equal, it represents some reference level in every year.

## Material and methods

Assimilation organ samples of Norway spruce were taken from the trees in accordance with UN/ECE, ICP Forests (1998) serving as the visually assessment of the forest health condition. The samples were taken from the research area aimed to appraise the Norway spruce health condition close to Kovohuty Krompachy air pollution source as well as from the research area with no direct damage by air pollution in Nižný Komárnik (damaged only by air pollution distance transmission). The assimilation organ samples from these Norway spruces were taken in accordance with the damage degree (0, 1, 2, 3) originating with Krompachy research area in August 1996, 1999 and 2000. Due to the comparison reason the control samples were taken from Nižný Komárnik research area in the year 2000. Norway spruce wood ages from Krompachy and Nižný Komárnik amount to 80 years, their height in Krompachy area 30.5 ms, in Nižný Komárnik one 32 ms. Both research area exposition in North-western. In agreement with the mentioned methodology the second year of Norway spruce assimilation organ needles was taken from the seventh tree vertical. The aim of this research was to determine the terpen and terpenoid amount in the samples damaged by the different degree by means of gas chromatography and to search the important parameter preponderance being common for the Norway spruce damage individual degrees.

Prior to actual analysis, taken 2-year old needles were flour to small pieces of 1 mm, and then they underwent water vapour distillation for 12-hour period. Prior to distillation, known amount of internal standard – cyclohexane was added to samples, being used for quantitative evaluation of samples. Distillate was pre-extracted by diethyl ether and dried by $Na_2SO_4$. Gas chromatography and mass spectrometry (GS/MS) were applied to analyse terpene and terpenoids samples of Norway spruce assimilation organs. The analysis were run on HP 5970B mass spectrometer / HP 5890A gas chromatograph equipped with HP ULTRA-1 capillary column (25x0.2 mm I.D., 0.33 μm film). Helium (25 cm/s) was used as a carrier gas. Injection temperature was 250 °C, temperature of the detector was 275 °C, temperature of the GC column was 40 °C (1 min), and consequently programmed at 7.5 °C/min to 260 °C, and kept for a period of 10 minutes. The mass spectrometer operated in scan mode, the mass scale of analysis was from 33 to 300 amu, the energy used was 70 eV, solvent delay: 2.5 min, EMV: 2200V, the threshold: 1500. The identification of separated components was performed automatically using probability based matching, and mass spectra were interpreted with the help of Wiley mass spectra library database.

## Results

Data obtained from gas chromatography and mass spectrometry (GS/MS analysis) were used as an entry data at present study, marked as: S1, S2, S3, S4 for the years 1996, 1999 and 2000 from an area of middle Spiš, as well as control samples S1K, S2K, S3K, S4K for the year 2000 from an area of Nižný Komárnik.

The quantitative and qualitative characterization of analysed samples chemical composition in this study was determined by GS/MS analysis. The list of the selected identified compounds from the complete data sets (year 1996, 1999 and 2000) used in the statistical evaluation only for the year 2000 is cited in the Table 1.

T a b l e 1. Identified samples components used in statistical evaluation

| Sample | labels | S1 | S1K | S2 | S2K | S3 | S3K | S4 | S4K |
|---|---|---|---|---|---|---|---|---|---|
| CAS Numbers | Figure N. Compound Name \ Time | 1 min | 2 min | 3 min | 4 min | 5 min | 6 min | 7 min | 8 min |
| 108-94-1 | internal standard | 3.17 | 3.15 | 3.17 | 3.21 | 3.15 | 3.15 | 3.17 | 3.15 |
| 80-56-8 | .alpha.-pinene, (-) | – | – | – | 3.40 | 3.45 | 3.45 | - | 3.45 |
| 79-92-5 | Camphene | 3.63 | 3.61 | 3.61 | 3.66 | 3.61 | 3.61 | 3.63 | 3.62 |
| 76-22-2 | Camphor | 6.09 | 6.07 | 6.09 | 6.17 | 6.08 | 6.12 | 6.16 | 6.05 |
| 98-55-5 | l-.alpha.-Terpineol | 6.75 | 6.71 | 6.73 | 6.84 | 6.71 | 6.71 | 6.75 | 6.71 |
| 5655-61-8 | Bicyclo[2.2.1]heptan-2- | 8.34 | 8.32 | 8.30 | 8.44 | 8.31 | 8.31 | 8.35 | 8.30 |
| 80-26-2 | 1-P-Menthen-8-yl acetate | 9.38 | – | 9.35 | 9.37 | 9.34 | 9.34 | 9.38 | 9.34 |
| 644-30-4 | Benzene, 1-1,5-dimethyl- | – | 12.08 | – | 11.80 | 11.67 | – | 11.70 | 11.70 |
| 10208-80-7 | .alpha.-Muurolene | – | 12.33 | – | 12.18 | 12.06 | – | 12.08 | • |
| 483-76-1 | Delta-Cadinene | – | – | – | 12.46 | 12.44 | 12.58 | 12.36 | 12.43 |
| 39029-41-9 | .gamma.-Cadinene | 14.49 | 14.46 | 14.47 | 14.48 | – | – | – | 14.45 |
| 3856-25-5 | epi-bicyclosesquiphellan | 14.71 | 14.68 | 14.69 | 14.71 | 14.46 | 14.45 | 14.48 | 14.67 |
| 1438-62-6 | 13-Epimanool | 20.64 | 20.61 | – | 20.63 | 20.62 | 20.61 | 20.63 | 20.60 |

Note 1. Identified compounds in the Figs 1–8 are marked by the retention time. Records of GS/MS analysis from the year 2000 are given in Figs 1–8.

Input data matrixes represent no homogeneously matrixes; therefore auto scaling was performed to put data on a common scale. The result is a variable with zero mean and a unit standard deviation. Standardized input data matrix of following dimensions 95x4 (year 1996), 34x4 (year 1999) and 52x8 (year 2000) were obtained by the above-mentioned procedure. Due to large extent of variables measured it is difficult to see patterns and relationships so multivariate statistic methods were used to evaluate the input matrixes by principal component analysis (PCA) and by cluster analysis (CA).

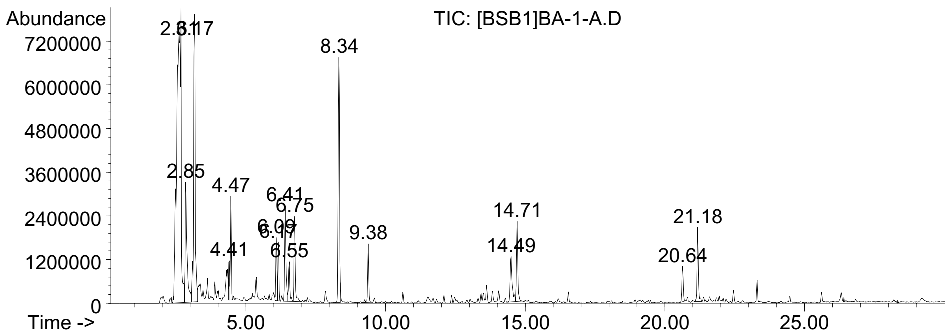


Fig. 1. Record of GS/MS analysis of Norway spruce assimilation organs samples S1 – degree of damage 0 from an area of middle Spiš in the year 2000.

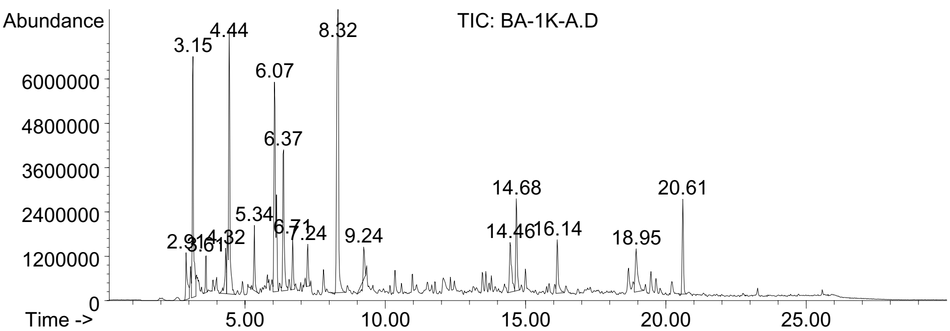


Fig. 2. Record of GS/MS analysis of Norway spruce assimilation organs samples S1K– degree of damage 0 from an area of Nižný Komárnik in the year 2000.

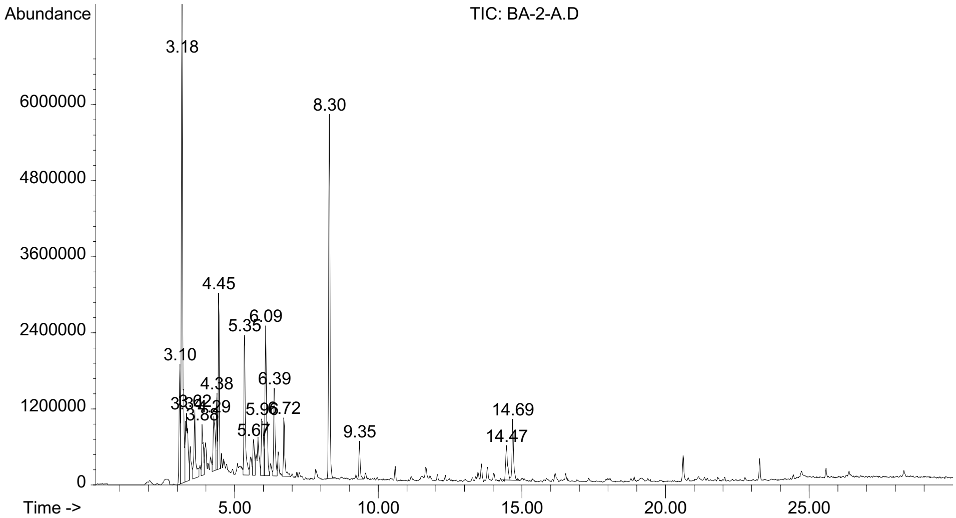


Fig. 3. Record of GS/MS analysis of Norway spruce assimilation organs samples S2 – degree of damage 1 from an area of middle Spiš in the year 2000.

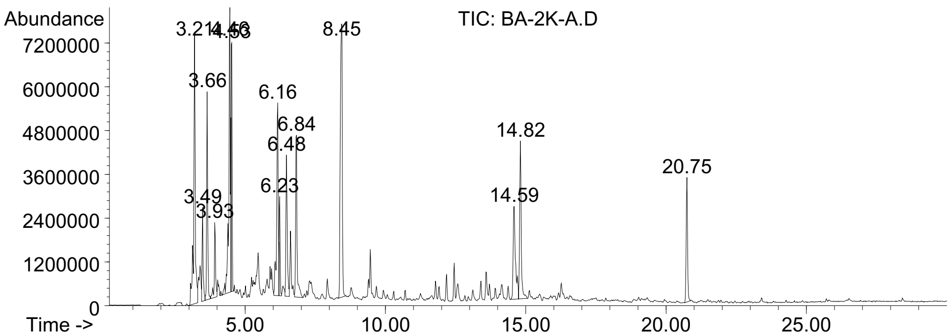


Fig. 4. Record of GS/MS analysis of Norway spruce assimilation organs samples S2K – degree of damage 1 from an area of Nižný Komárnik in the year 2000.

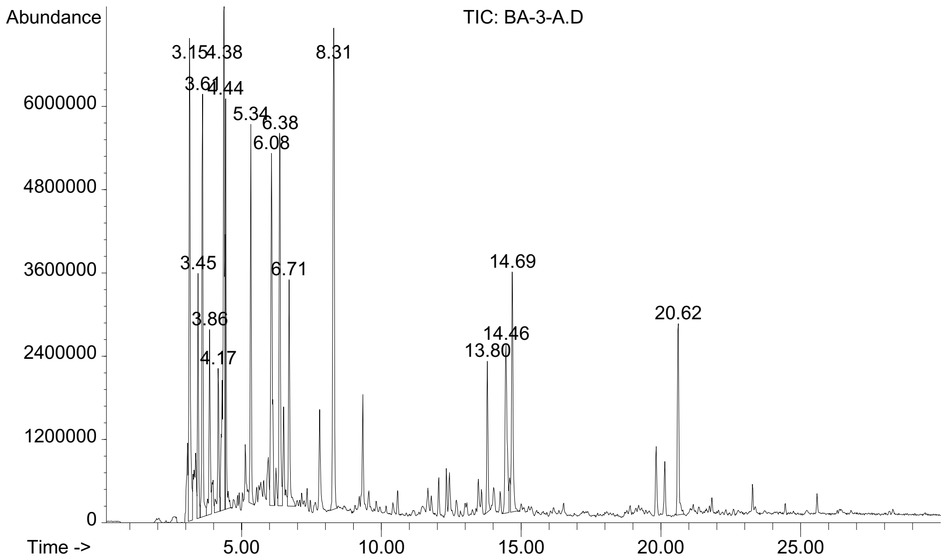


Fig. 5. Record of GS/MS analysis of Norway spruce assimilation organs samples S3 – degree of damage 2 from an area of middle Spiš in the year 2000.

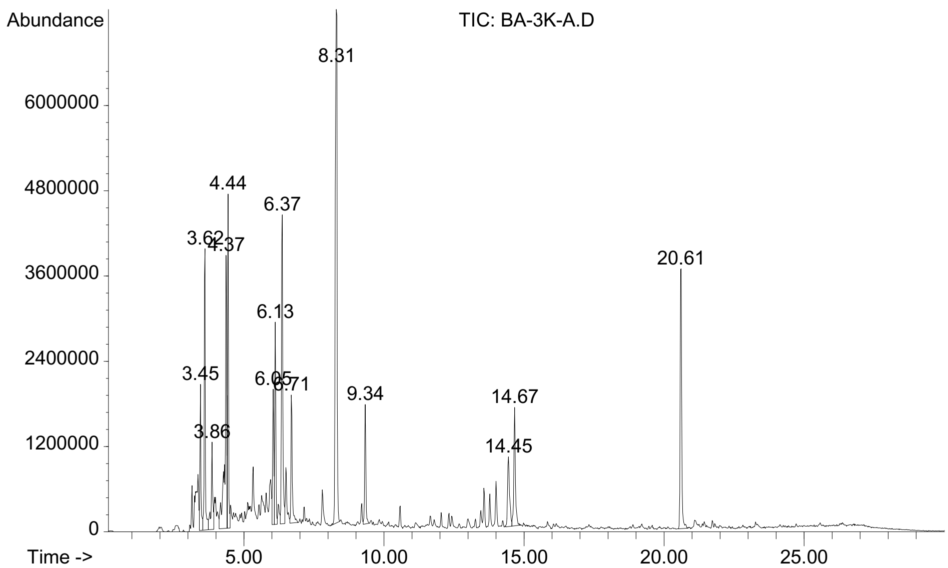


Fig. 6. Record of GS/MS analysis of Norway spruce assimilation organs samples S3K – degree of damage 2 from an area of Nižný Komárnik in the year 2000.

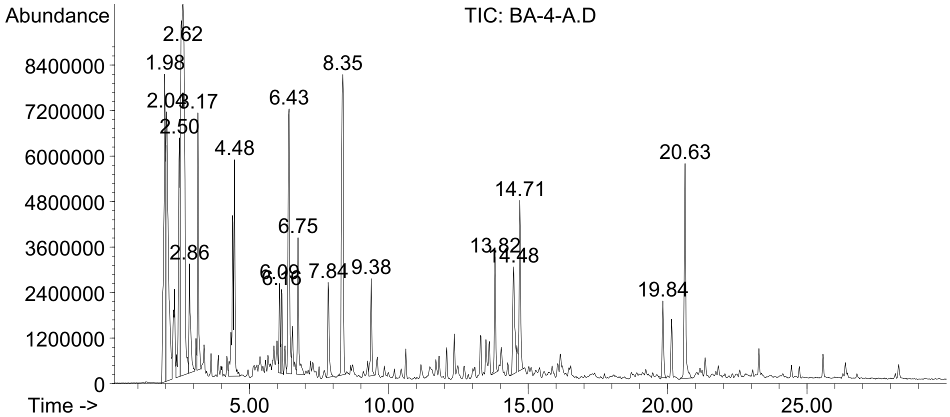


Fig. 7. Record of GS/MS analysis of Norway spruce assimilation organs samples S4 – degree of damage 3 from an area of middle Spiš in the year 2000.

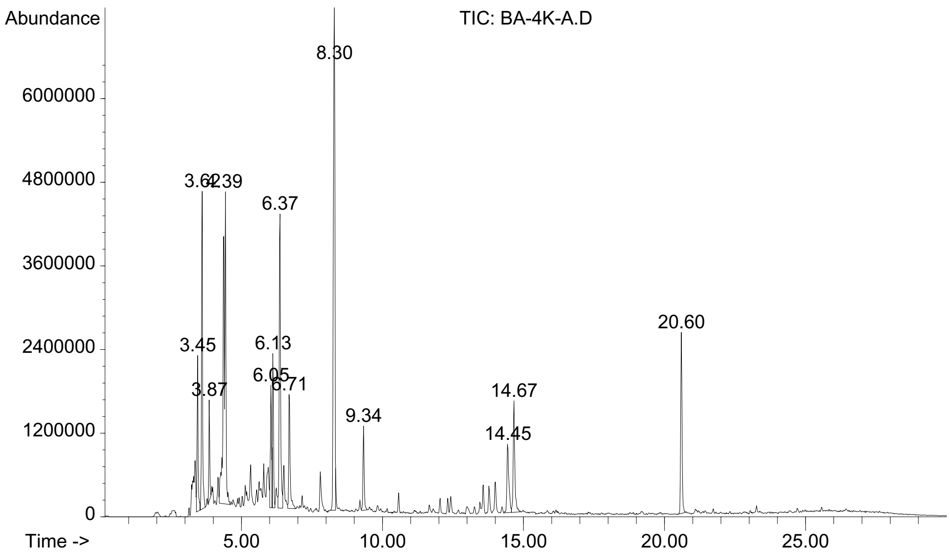


Fig. 8. Record of GS/MS analysis of Norway spruce assimilation organs samples S4K – degree of damage 3 from an area of Nižný Komárnik in the year 2000.

*Principal component analysis (PCA)*

In order to justify the appropriateness of proposed methodology, mainly the principal component analysis was used for individual years, and check samples (for the year 2000 also cluster analysis) in order to find out:

a) important difference within the investigated years between individual samples S1-S4, as well as S1K-S4K
b) whether the proposed methodology is able to by recognized, within a given year (only 2000) difference between individual samples S1-S4 and S1K-S4K, as well as individual trends according to the degree of damage
c) whether the content of selected and analysed components in the individual years is dependent both on the degree of damage as well as on the duration of a observation (age of forest tree species).

Usually, such a number of principal components (latent variables) is selected which covers cumulative value of total variance 80–95% of total variability. The orthogonal rotation (varimax) was used for the decomposition of data matrixes to transform (by calculation of a rotation matrix) the abstract factors (from principal components) into interpretable factors with physical-chemical importance to new variables. In this paper the individual latent variables in the years 1996, 1999, 2000 after rotation and following decomposition are given on Tables 2–6. To improve the orientation in tables the coefficients $c_{ij} > 0.7$ of linear combination for the single factors are given in bold type.

It is evident, from the matrixes of the linear combinations, which form latent variables (years 1996, 1999, 2000 and 2000K – Tables 2–6), any reduction of the variables is impossible and for the description of properties 4 latent variables are needed. The significant difference between individual samples S1-S4 as well as S1K-S4K was proved within the given years.

*Cluster analysis (CA)*

In order to prove the results gained by PCA analysis, that is to find out certain similarity or difference between samples, the other method of investigation – cluster analysis was used. The input data for the statistical evaluation by cluster analysis were the same (data processing and matrix labels) and the hierarchical agglomerative cluster analysis was performed. The Manhattan distance was selected as a measure of similarity because of its robustness in p-dimensional space and Ward method for aggregation of individual classes to clusters is very efficient, nevertheless it prefers clusters with of small size. The following dendrogram is a graphical result of mentioned chemometric procedure – see Fig. 9 (dendrogram of hierarchical cluster analysis of Norway spruce assimilation organs the individual samples as variables. Similarity measure: Manhattan distance. Cluster linkage: Ward method).

The samples S1 and S2 are similar (involve similar information) as seen in results of cluster analysis by the use of above-mentioned matrixes, and they are closest to each other within n-dimensional area. Second group is represented by S1K and S2K samples of prob-

T a b l e 2. Factor loadings for individual latent variables in the year 1996

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|---|---|---|---|---|
| S1 | 0.344 | **0.862** | 0.361 | 0.086 |
| S2 | **0.752** | 0.403 | 0.385 | 0.350 |
| S3 | **0.907** | 0.294 | 0.298 | 0.004 |
| S4 | 0.421 | 0.466 | **0.772** | 0.092 |
| Expl.Var | 1.686 | 1.211 | 0.964 | 0.138 |
| Prp. Totl | 0.421 | 0.302 | 0.241 | 0.034 |

T a b l e 3. Factor loadings for individual latent variables in the year 1999

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|---|---|---|---|---|
| S1 | **0.806** | 0.129 | 0.339 | 0.465 |
| S2 | 0.394 | 0.279 | **0.758** | 0.435 |
| S3 | 0.091 | **0.983** | 0.148 | 0.056 |
| S4 | 0.391 | 0.054 | 0.321 | **0.860** |
| Expl.Var | 0.968 | 1.064 | 0.816 | 1.150 |
| Prp. Totl | 0.242 | 0.266 | 0.204 | 0.287 |

T a b l e 4. Factor loadings for individual latent variables in the year 2000 – from an air pollution area of middle Spiš (S1–S4)

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|---|---|---|---|---|
| S1 | 0.614 | 0.447 | 0.107 | 0.641 |
| S2 | **0.949** | 0.050 | 0.260 | 0.168 |
| S3 | 0.231 | 0.205 | **0.948** | 0.063 |
| S4 | 0.082 | **0.960** | 0.208 | 0.164 |
| Expl.Var | 1.339 | 1.167 | 1.022 | 0.470 |
| Prp.Totl | 0.334 | 0.291 | 0.255 | 0.117 |

T a b l e 5. Factor loadings for individual latent variables in the year 2000 – check sample from an area of Nižný Komárnik (S1K–S4K)

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|---|---|---|---|---|
| S1 | **0.879** | 0.267 | 0.347 | 0.182 |
| S2 | 0.555 | 0.301 | **0.745** | 0.212 |
| S3 | 0.422 | 0.591 | 0.420 | 0.543 |
| S4 | 0.226 | **0.940** | 0.203 | 0.150 |
| Expl.Var | 1.311 | 1.397 | 0.893 | 0.396 |
| Prp.Totl | 0.328 | 0.349 | 0.223 | 0.099 |

T a b l e 6. Factor loadings for individual latent variables in the year 2000 (S1–S4) and (S1K–S4K)

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 | Factor 7 | Factor 8 |
|---|---|---|---|---|---|---|---|---|
| S1 | 0.079 | **0.818** | 0.468 | 0.140 | 0.065 | 0.041 | 0.275 | –0.044 |
| S1K | 0.259 | 0.495 | 0.202 | 0.700 | 0.123 | 0.377 | 0.015 | 0.011 |
| S2 | 0.174 | **0.896** | –0.031 | 0.348 | 0.109 | 0.016 | –0.170 | 0.047 |
| S2K | 0.294 | 0.350 | 0.174 | **0.829** | 0.251 | –0.075 | 0.019 | –0.050 |
| S3 | 0.537 | 0.187 | 0.153 | 0.451 | 0.669 | 0.035 | 0.002 | 0.016 |
| S3K | 0.615 | 0.147 | 0.309 | 0.640 | 0.154 | 0.028 | –0.061 | 0.254 |
| S4 | 0.214 | 0.141 | **0.944** | 0.184 | 0.079 | 0.025 | 0.003 | 0.014 |
| S4K | **0.926** | 0.146 | 0.184 | 0.235 | 0.167 | 0.035 | 0.012 | –0.028 |
| Expl.Var | 1.762 | 1.940 | 1.338 | 2.022 | 0.600 | 0.154 | 0.109 | 0.072 |
| Prp.Totl | 0.220 | 0.242 | 0.167 | 0.252 | 0.075 | 0.019 | 0.013 | 0.009 |

ably similar properties. Last cluster contains S3K, S4K and S3, S4 samples. This cluster is divided to sub clusters of S3K and S4K samples of similar properties, indicate S3 and S4 being very close to probably moderately different properties within a given cluster.

The results of PCA as well as of CA indicate proposed methodology is able to find a difference between samples S1-S4 and S1K-S4K within a given year as well as individual trends according to the degree of derogation.

To test the appropriateness and correctness of the proposed methodology, the comparison of several selected analysed components representing selected properties in all analysed samples from an area of Spiš as well as check samples from an area of Nižný Komárnik

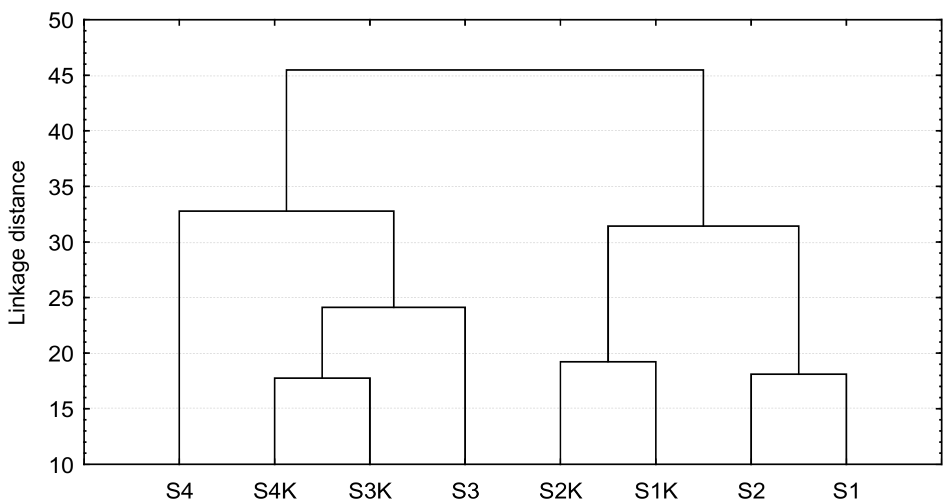


Fig. 9. Dendrogram of hierarchical cluster analysis.

T a b l e 7. Factor loadings for individual latent variables

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 | Factor 7 | Factor 8 |
|---|---|---|---|---|---|---|---|---|
| S1_96 | 0.005 | **0.958** | 0.018 | 0.124 | –0.098 | –0.119 | 0.040 | –0.045 |
| S2_96 | –0.104 | **0.968** | –0.020 | 0.059 | –0.038 | –0.051 | –0.075 | 0.011 |
| S3_96 | 0.327 | **0.854** | 0.188 | 0.036 | 0.104 | 0.193 | –0.168 | 0.095 |
| S4_96 | 0.064 | **0.953** | 0.110 | 0.082 | –0.032 | 0.017 | 0.111 | –0.001 |
| S1_99 | 0.192 | 0.261 | **0.866** | 0.008 | 0.033 | –0.345 | –0.046 | 0.045 |
| S2_99 | 0.415 | 0.017 | **0.829** | –0.053 | 0.184 | 0.166 | –0.163 | –0.069 |
| S3_99 | –0.028 | –0.194 | 0.042 | **–0.977** | –0.039 | 0.003 | 0.001 | 0.002 |
| S4_99 | 0.176 | –0.052 | **0.952** | –0.015 | –0.063 | 0.128 | 0.092 | 0.003 |
| S1_00 | **0.852** | 0.138 | 0.288 | –0.068 | 0.198 | 0.242 | 0.046 | –0.092 |
| S2_00 | **0.774** | –0.173 | 0.102 | 0.081 | 0.540 | 0.140 | –0.045 | –0.009 |
| S3_00 | **0.939** | 0.027 | 0.132 | 0.033 | –0.056 | –0.068 | 0.027 | 0.234 |
| S4_00 | **0.570** | 0.429 | 0.571 | –0.091 | 0.137 | –0.046 | 0.000 | 0.318 |
| S1K_00 | **0.730** | –0.111 | 0.090 | 0.092 | 0.639 | –0.066 | 0.007 | 0.024 |
| S2K_00 | **0.941** | 0.074 | 0.162 | –0.010 | 0.040 | 0.012 | 0.194 | –0.136 |
| S3K_00 | **0.840** | 0.066 | 0.369 | 0.026 | 0.137 | –0.143 | –0.247 | 0.105 |
| S4K_00 | **0.838** | 0.188 | 0.329 | 0.009 | 0.005 | –0.013 | –0.342 | –0.050 |
| Expl.Var | 5.728 | 3.897 | 3.113 | 1.016 | 0.846 | 0.329 | 0.306 | 0.215 |
| Prp.Totl | 0.358 | 0.243 | 0.194 | 0.063 | 0.052 | 0.020 | 0.019 | 0.013 |

was done. Such comparison may indicate whether the content of selected analysed components in individual years is dependent on the degree of derogation, respectively on the age of forest tree species, or on both mentioned factors.

The factor loadings of linear combination are given for individual latent variables in Table 7. After the orthogonal transformation with varimax rotation, new variables may be assigned to the following properties:

- the first factor represents, respectively constitutes properties of the year 2000, as samples (S1-S4) and (S1K-S4K)
- the second factor is formed by linear combination, respectively includes properties of the year 1996, as samples (S1-S4))
- the third factor represents, respectively includes properties of the year 1999, as samples (S1-S4).

In 2D or 3D plot for principal components (PC1 vs. PC2 or vs. PC3) covering more than 80% of total variance, it individual clusters indicate the creation by samples, representing individual years disregard the degree of damage, and physical sense was assign to the first three factors, where individual factors represent investigated years – see Fig. 10.

The results of cluster analysis, where the component content in individual years represented variables within a given year and degree of damage, obtained the similar image as by PCA by the use of above mentioned methodology (Similarity measure: Manhattan dis-

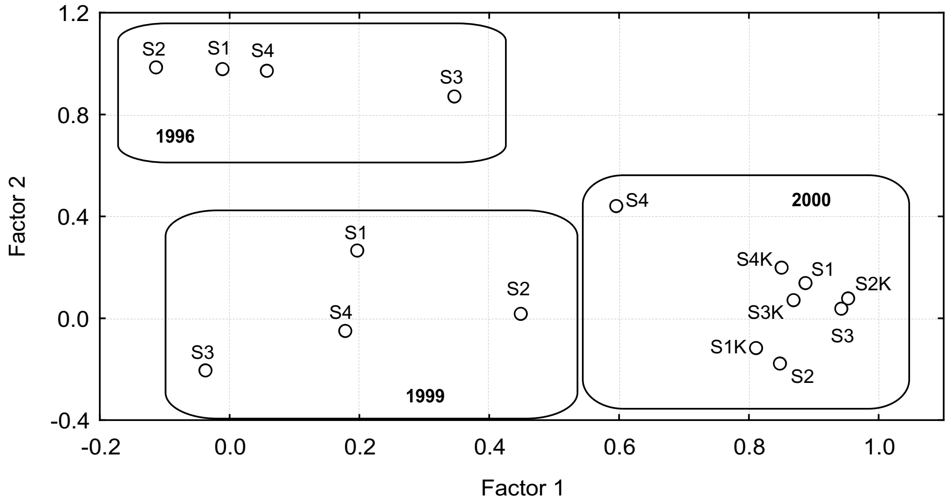


Fig. 10. PCA – dependence PC1-PC2 evaluating the content of analysed components within individual years.

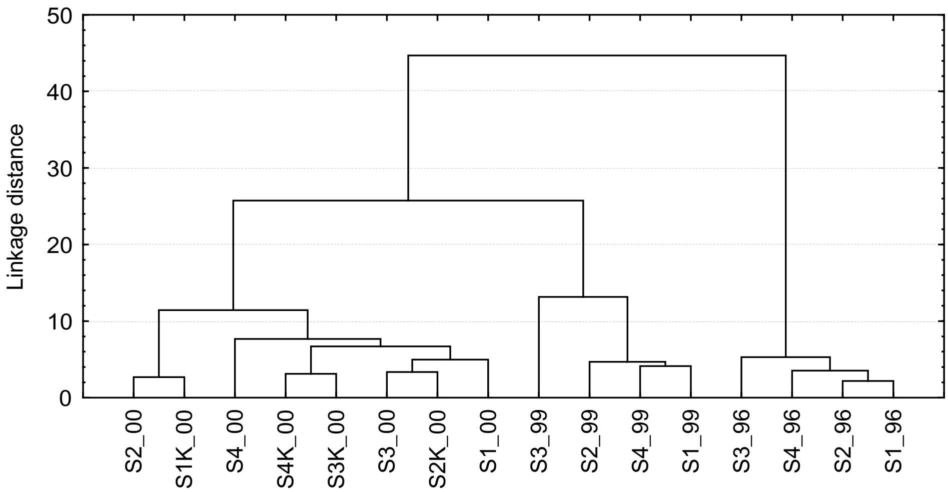


Fig. 11. Dendrogram of the complete data sets.

tance. Cluster linkage: Ward method). Analysed samples are divided to three clusters relatively far from each other, and representing S1-S4 samples for individual years. It also results to the fact the content of analysed ingredients is dependent on the age of forest tree species and then on the degree of damage. Dendrogram of hierarchical cluster analysis is given in Fig. 11.

## Discussion

Changed environmental conditions influence biotic component of ecosystems in a negative way, where they stimulate different degrees and forms of damage, from cell to organ, sort to prize-logical level (Supuka, 1996). The composition of tree needles is investigated from various points of view. Several studies concentrate on chemotaxonomy works, others justify geographical and ecotype difference in population, famous are studies justifying relation between terpenes production and forest tree species attacks by insect pests. Berta (1990, 1993) analysed volatile substances of terpenoid character from 67 branches of needle trees belonging to 7-plant family. These substances are important from the point of resistance potential against biotic harmful factors. The composition of terpenes allowed the chemotaxonomy differentiation between European Black pine (*Pinus nigra* A r n o l d.) and Scots pine (*Pinus sylvestris* L., K a r s t.) with significant content difference like camphene, beta-pinene, delta-3-carene, spathulenol and several sesquiterpenes (Jurášek et al., 1993). The change in the composition of European black pine needle terpenes was found in changed conditions of urbanised environment (Supuka et al., 1997). The influence of various environmental factors was studied from the point of quantitative composition of monoterpenes in spruce needles (Merk et al., 1988). The content of mono-terpenes in 3-month, 1-year, 2-year, 3-year and 4-year old spruce needles was analysed within the work of abovementioned authors. The significant differences of content between 1-year and 2-year spruce needles were found out within several terpenes. The increased concentration of terpenes in spruce trees, which were influenced by the air pollution was found out by Jűttner (1988). The comparison of terpenes inside sound needles and inside damaged needles of yellow colour was carried out by this author, and the different contents of terpenes concentration between compared groups, mainly at alfa-pinine, beta-pinine, camphene, borneol and delta-cardinene were found out.

The results of terpenes and terpenoids gained within the analysis of spruce needles in an area of middle Spiš and Nižný Komárnik prove the difference between sound and damaged needles, as described by Jüttner (1988).

The multivariate analysis was used to evaluate the contents of selected components in the years 1996, 1999 and 2000. To justify the appropriateness of proposed methodology for individual years, the principal component analysis and for the year 2000 also the method of cluster analysis were applied. Our aim was to find the significant differences between individual samples within the investigated period, to find out whether proposed methodology is capable to recognize within a given year the differences between samples and trends according to the degree of damage, as well as to find out whether the content of selected analysed components in the individual years is dependent on the degree of damage and the age of forest tree species.

The results of the method of main components indicate that based on the method of terpenes and terpenoids analysis, the differences between samples of various degree of Norway spruce needles damage may be found out.

The results of cluster analysis, where the component content in individual years represented variables within a given year and degree of damage, indicate that the content of analysed samples is dependent mainly on the age of the forest tree species and secondary on the degree of damage.

## Conclusions

In an area of middle Spiš, a long-term unfavourable air pollution influence can be documented. Despite its reduction, the unfair condition of forest prevails. Decrease in ecological stability of forest ecosystems has been caused by the fact that naturally slightly buffer and poor soils underwent a long-term influence by acid air pollution load, and also by heavy metal air pollution accumulated in the soil for a long time. According present knowledge base on stress issue, on influence of individual stressors, on complex stress situations and air pollution, it is obvious that stress bio indications are possible at various levels of biological systems and by different physical-biochemical reactions, respectively parameters. The research based on eco-physiology of either selected process or group of processes is becoming more important during these days. This approach results from the fact that any influence of external environment on forest tree species has its own final realisation in a form of certain physiology process. The present paper aimed to justify the appropriateness of methodology for terpenes and terpenoids analysis in Norway spruce assimilation organs aimed at finding differences of selected components content. Within individual degrees of damage, the multivariable analysis in the years 1996, 1999 and 2000 were used.

The results of the principal component analysis indicate that based on the method of terpenes and terpenoids analysis, the differences between samples of various degree of spruce needle damage may be found out.

The results of cluster analysis, where the component content during individual years represented variables within a given year and degree of damage, indicate that the content of analysed samples is dependent mainly on the age of the forest tree species and then on the degree of damage.

The methodology of terpenes and terpenoids analysis in Norway spruce assimilation organs and its multivariate analysis confirm the differences between degrees of damage.

This methodology is recommended as appropriate to observe the health conditions of trees within sustainable monitored areas of 2$^{nd}$ level, where obtained results represent selective data corresponding with the crest – assimilation organs type of damage.

*Translated by the authors*

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Bilá A., Májek P., Šurina I.: **Využitie metodiky analýz terpénov a terpenoidov v asimilačných orgánoch smreka obyčajného (***Picea abies* (L.) K a r s t.) **v rámci monitorovania zdravotného stavu lesných porastov.**

Napriek zníženiu imisného zaťaženia v oblasti stredného Spiša, naďalej pretrváva zhoršenie zdravotného stavu smrekových porastov. Príčinou najväčších zmien v zdravotnom stave smrekových porastov v jednotlivých rokoch sú klimatické faktory, biotické faktory (najmä prítomnosť podkôrneho hmyzu), abiotické faktory, ako aj dopad dlhodobého imisného zaťaženia. Predmetom výskumu bolo posúdiť vhodnosť metodiky pre analýzy terpénov a terpenoidov v asimilačných orgánoch smreka obyčajného pri hodnotení zdravotného stavu lesných porastov na trvalo monitorovacích plochách II. úrovne. Rozdiely obsahov vybratých komponentov pri jednotlivých stupňoch poškodenia asimilačných orgánov smreka obyčajného sme spracovali pomocou multivariantných analýz vzoriek z rokov 1996, 1999 a 2000. Z výsledkov metódy hlavných komponentov môžeme konštatovať, že na základe metódy terpénov a terpenoidov sa dajú získať diferencie medzi vzorkami s rôznym stupňom poškodenia ihličia smreka. Z výsledkov metódy zhlukovej analýzy, kde obsah komponentov v jednotlivých rokoch predstavoval závislé premenné v rámci daného roku a stupňa poškodenia, sme zistili, že obsah analyzovaných vzoriek závisí najmä od veku dreviny a až potom od stupňa poškodenia. Metodiku analýz terpénov a terpenoidov v asimilačných orgánov smreka obyčajného navrhujeme ako vhodnú na sledovanie zdravotného stavu lesných porastov na trvalo monitorovacích plochách II. úrovne, kde získané výsledky predstavujú voliteľné údaje vzťahujúce sa na korunu – typ poškodenia asimilačných orgánov.