CHLOROPHYLL DETERMINATION IN SILVER BIRCH AND SCOTS PINE FOLIAGE FROM HEAVY METAL POLLUTED REGIONS USING SPECTRAL REFLECTANCE DATA

Lucie Kupková[1], Markéta Potůčková[1], Kateřina Zachová[1], Zuzana Lhotáková[1], Veronika Kopačková[2], and Jana Albrechtová[1]

1. Charles University in Prague, Faculty of Science, Czech Republic; e-mail: lucie.kupkova(at)gmail.com
2. Czech Geological Survey, Prague, Czech Republic

ABSTRACT

The foliage chlorophyll content can be used as one of the indicators of tree stress caused by adverses oil conditions with heavy metal contamination being the major stress factor. Linear regression models showing the relation between spectral indexes derived from spectroscopic measurements of Norway Spruce foliage and its chlorophyll content were successfully applied in previous studies. Silver Birch (*Betulapendula* Roth), a pioneer tree species, and Scots Pine (*Pinus sylvestris* L.) used for revegetation after mining activities are typical tree species in the mine reclamation area of the Sokolov Basin (West Bohemia, Czech Republic). In August 2010 hyperspectral images with a resolution of 5 × 5 m^2 were acquired with the HyMap sensor. In four selected areas with a different level of soil contamination samples of Silver Birch leaves and Scots Pine needles were collected during the field campaign. Spectral reflectance curves of samples were measured in a laboratory with an ASD Field Spec3 spectrometer using the contact probe. The goal of this study is to prove the correlation between spectral reflectance data and the chlorophyll content determined spectrophotometrically in the laboratory using different spectral indices (*MCARI*, *TCARI* / *OSAVI*, $mNDVI_{705}$, $ANMB_{650–725}$) and to find a mathematical description of this relation. The suitability of different indices for application on coniferous foliage of pine and broad leaved birch is discussed. Although the relations between different indices and the chlorophyll content show similar trends, the $ANMB_{650–725}$ index revealed the best results regarding the statistical significance. While the sought relation between spectral indices and the chlorophyll content showed to be statistically significant in the case of Scots Pine, it was rather weak and thus not applicable in the case of Silver Birch. The present work is a part of a study aiming to create a methodology for chlorophyll determination in Silver Birch and Scots Pine from available hyperspectral data.

INTRODUCTION

Imaging spectroscopy, namely hyperspectral data have been used rather widely in the last decade for the natural environment monitoring (geology, vegetation cover and health status) and for an extraction of information and knowledge related to environmental change, pollution effects, and ecosystems degradation (1,2,3,4).

The present outputs were obtained in the framework of the project ‘Assessment of Mining Related Impacts Based on Utilization of HyMap Airborne Hyperspectral Sensor’ supported by the Czech Science Foundation (GACR). Focusing on the Sokolov lignite mining area in the Czech Republic, this project combines image spectroscopy laboratory data, high resolution hyperspectral data and laboratory biochemical determinations with the goals (5) to assess the current extent of the area affected by mining activities (tailing pounds, acid and heavy metal polluted zones, irritated vegetation, and changes in protection zones of water) (6) to find relationships between stress conditions and the consequential physiological state of vegetation (focus is given to observation of coniferous trees – Norway spruce and Scots Pine and pioneer vegetation – Silver Birch), (3) to prepare integrated data analyses based on the thematic maps resulting from the aerial and *in situ* hyperspectral data, implementing new mapping techniques providing a high level of spatial and spectral distinction among all the major environmental components, present at the open pit mine area and its closest surrounding (e.g. mineral extraction, waste extraction, contaminated water identification, chlorophyll, cellulose and lignin contents in vegetation). In this specific study we wanted to answer the questions what the relationships between the biochemically determined needles/leaves chlorophyll content and spectral/optical indices are, if there is any dependence and how significant it is. We wanted to test if there are some differences between Scots Pine as a representative of coniferous trees and Silver Birch that represents broadleaved trees.

First results of the project part concerned with chlorophyll determination in silver Birch and Scots Pine are a subject of the present study. Photosynthetic pigments and other biochemical compounds like phenolic compounds, lignin, etc. may provide information concerning the physiological state of foliage (7,4,5,8,9,10) and indicate the vegetation resistibility to various types of stress (11,12,13).

It has been proved that foliar optical properties are among others driven by variations in the biochemical composition (14,15,7,16,17). Laboratory biochemical determinations of these compounds have several disadvantages (a limited number of analysed samples, highly labour- demanding and relatively expensive method). However laboratory and especially remote spectra measurements and analyses have the potential to bring significant contributions to the understanding of dependences of the physiological state of vegetation and environmental conditions and may enable to detect and monitor the vegetation condition/health in large areas. The laboratory biochemical determinations are usually an important part of the methodology providing ground truth data, for example, for scaling measurements and result validation.

To determine foliage biochemical compounds, various spectroscopic and statistical methods employing spectral data have been used, e.g. stepwise multiple linear regression, band-depth analysis of absorption features, single reflectance ratios, spectral derivative indices, radiative transfer modelling (18,19,20,7,21). Significant attention has been paid to a methodology of chlorophyll retrieval on both leaf/needle and canopy levels (6,15,22,23,24,25,26).

Reliable methods for pigment quantification by remote sensing techniques are crucial for understanding and monitoring of photosynthetic processes and vegetation stress caused by various environmental factors (17). Despite the fact that all higher plants contain the identical photosynthetic pigments, the empirical relationships between chlorophyll content and spectral indices are species specific (7).

A lot of attention has been recently paid to the spectroscopic chlorophyll determination in needles of spruce species, e.g. Norway Spruce (24,27,26) or black spruce (28,29). On the contrary, scarce attention has been paid to other coniferous species (but see (7,25,21)). In spite of the fact that some widely used analytical methods of spectroscopic chlorophyll content determination have been developed originally for broadleaved species (6), the relation between the chlorophyll content and spectral reflectance for some common species such as birch or aspen have not been analysed yet.

The goal of the present study is therefore to analyse spectral reflectance characteristics of Silver Birch and Scots Pine foliage and to establish the correlation between foliage laboratory spectra measurements and chlorophyll content laboratory biochemical determinations. The results will be used in our further work for silver Birch and Scots Pine chlorophyll content analysis based on airborne hyperspectral data (HyMap) acquired over the study site of the Sokolov basin mining area and to prepare chlorophyll maps of the study site.

TEST AREA DESCRIPTION

The test area is a part of the lignite mining area located in the north-west part of Czechia – the Krušné mountains, in the Sokolov basin. Oligoceneto Mioceneinage, Sokolov basin extends over a total area of about 200 km^2. It consists of 60% of volcanic ejecta resulting from faults and volcanic cones and of 40% of sediments. Lignite is found in the western part of the basin and comprises of three coal seams (Josef, Anežka, Antonín). Some of them are very rich in sulphur (up to 8%) and arsenic (60-70 ppm) (30,31).

Dumps composed of overburden material originated from the mines are a typical territory of silver-Birch (pioneer species) and Scots Pine (replanted species) growth. The growths of replanted species were originally founded about 10 - 20 years ago as a part of revitalisation of former mining areas. Four study sites within the test area were selected. The first one located on the Lítov dump, the second located on the Lomnice dump, the further abandoned sand pit Erika and finally the control site Svatava.

Lítov is a locality with extreme acidity (pH from 2.3 to 2.9), heterogeneously distributed herbaceous pioneer species as *Calamagrostis epigejos and Tussilago farfara,* with sparse woody species cover – self-seeded pioneer *Betula pendula* Roth and replanted *Pinus sylvestris*. At Lomnice there is sparse heterogenously distibuted *Calamagrostis epigejos* and groups of self-seeded pioneer *Betula pendula* growing at open substrate on spoil heaps, while replanted *Pinus sylvestris* trees grow above the spoil heap on recultivated soil. Abandoned sand pit Erika is characterised by dense self-seeded *Betula pendula* with *Calamagrostis epigejos* undergrowth and sparse replanted *Pinus sylvestris* with almost no or moss undergrowth. Svatava consists of mixed pine-birch forest and is considered a control site almost free of heavy metal contamination (Figure 1).

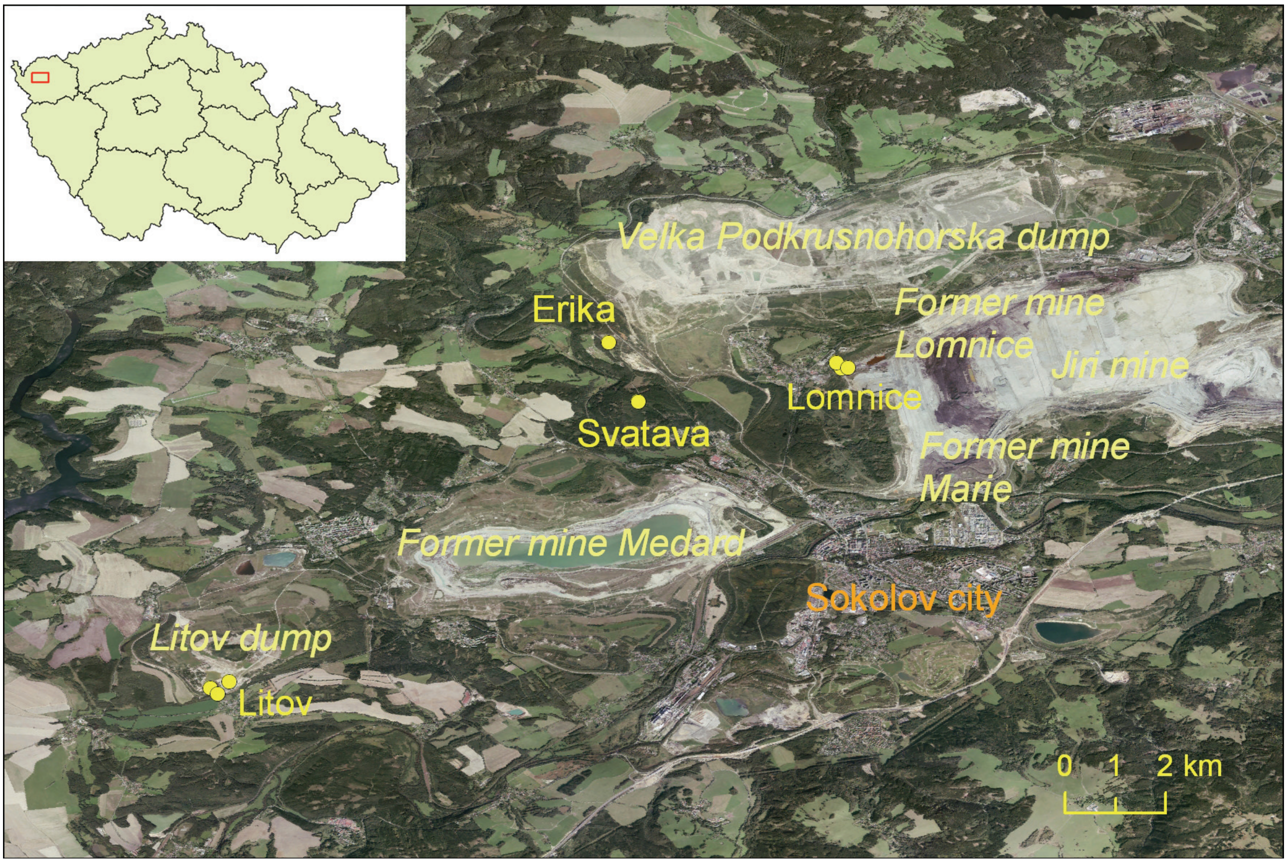

Figure 1: Ortophoto of the Sokolov lignite mining area. The study sites are in dark yellow.

MATERIAL AND METHODS

Foliage sample collection

At each study site two or three groups of birch and pine trees (up to 15 years old) were selected, each group consisting of five trees (altogether 45 trees for each species). Sunlit branches from two canopy levels were collected from each tree: upper branches (sun exposed branches from the 3rd top whorl) and lower branches (also sunlit branches from the middle part of the canopy).The upper branches were cut near the tree top; the lower branches were cut at the middle still sunlit part of the canopy. The branches were sampled using pruning poles. Samples of current year foliage were taken, altogether 170 samples: 80 for pine and 90 for birch foliage (an average value was measured for the upper and lower canopy levels).

Foliage for photosynthetic pigment assessment was immediately removed from the branch, sealed in plastic vials, transported in a cooled box into laboratory and stored at -20°C until processed.

Foliage for spectral measurements remained attached to the branch, placed into the plastic bag with wet tissue, transported to the laboratory and stored at 4°C until spectral measurements were made.

Photosynthetic pigments content determination

Photosynthetic pigments (chlorophyll a, chlorophyll b) were extracted from leaves and needles in dimethylformamide (DMF). Up to 0.2 g of foliage was chopped to small segments and left in 10 ml of DMF solvent for 7 days in the dark at 4°C according to (32). The amount of pigments was determined spectrophotometrically according to equations from (33). The concentration of pigment was expressed as weight of pigment per gram of foliage dry mass. The foliage dry mass was determined on a parallel set of samples dried at 80°C for 48 hours.

Laboratory spectra measurement at leaf level

The spectral reflectance of birch and pine foliage was measured in the range between 350 and 2,500 nm. Spectral measurements were taken using an ASD FieldSpec 3 spectrometer equipped with two sensors. The first sensor measured reflected light in wavelengths between 350 and 1,050 nm by sampling the reflected light every 1.4 nm. The second sensor measured reflected light in wavelengths between 1,000 and 2,500 nm by sampling the reflected light every 2 nm. The reflectance of foliage was expressed in % of reflectance compared to a calibrated white reference panel (100% reflectance). For spectral measurements at leaf level a fibre optic contact probe was used similarly as in (34).

The contact probe was pressed on the adaxial leaf surface in such a way that the full field of view of the contact probe was covered by the leaf and the leaf surface was only illuminated by a constant light source inside the contact probe. In the case of birch a stack of at least five leaves was piled together. In the case of pine the needles were arranged in the same direction and arranged to create a consistent layer to fill in the field of view of the contact probe. Both types of foliage were placed on spectrally black surface during the measurement to minimise the background spectral noise or radiation transmitted through the leaf.

For each foliage stack five independent measurements on different parts of the stack were taken. The scan average on the ASD spectrometer was set to 15 to avoid overheating the foliage.

Spectral Indices calculation

To stress the functional dependence of the chlorophyll content in analysed species the following optical vegetation indices were calculated using a continuum removed ASD FieldSpec 3 data:

1) *MCARI* (Modified Chlorophyll Absorption in Reflectance Index) (35)

$$MCARI = \left[\left(R_{700} - R_{670}\right) - 0.2\left(R_{700} - R_{550}\right)\right]\frac{R_{700}}{R_{670}}$$

2) *TCARI*/*OSAVI* (Transformed Chlorophyll Absorption in Reflectance Index) / (Optimised Soil-Adjusted Vegetation Index) (22)

$$TCARI = 3 \cdot \left[\left(R_{700} - R_{670}\right) - 0.2\left(R_{700} - R_{550}\right)\right]\frac{R_{700}}{R_{670}}$$

$$OSAVI = \frac{1.16\left(R_{800} - R_{670}\right)}{R_{800} + R_{670} + 0.16}$$

3) $mNDVI_{705}$ (36)

$$mNDVI_{705} = \frac{R_{750} - R_{705}}{R_{750} + R_{705} - 2R_{445}}$$

4) $ANMB_{650-725}$ (Area under curve normalized to maximal band depth between 650 and 725 nm)

$$ANMB_{650-725} = \frac{0.5 \cdot \sum(\lambda_{j+1} - \lambda_j)(R_{j+1} + R_j)}{MDB}$$

where λ_j is the wavelength, R_j is the reflectance after continuum removal at wavelength λ_j, and *MBD* is the maximum curve depth after continuum removal (37).

Statistical analysis

Vegetation indices were calculated based on ADS spectrometer laboratory measurements. Spectral reflectance values were obtained in the 350 - 2,500 nm wavelength range with a step of 1 nm. In order to minimise the influence of outliers, a median of five reflectance measurements per sample was used in further analyses. A relation between the chlorophyll content and spectral indices was derived by means of a regression analysis.

The regression curve fitting was performed in the *R* statistical environment using linear models (in some cases polynomial or power-law trends). The significance of relationships was tested also in *R* software. The models that gave the best results are presented in the following paragraph.

RESULTS

The results of the analyses are demonstrated in the graphs of selected spectral indices as a function of total leaf chlorophyll related to dry leaf / needle mass for both Scots Pine (Figures 2-4) and silver Birch (Figure 5). The values of the spectral indices represent a median of five measurements collected for each sample.

In the case of Scots Pine all used spectral indices provided rather good correlations with the needle chlorophyll content (coefficient of determination R^2 reached values above 0.8 depending on the selected index and *P* values were for all indices < 0.001. The best result (R^2 = 0.8645, P < 0.001) was achieved with the $ANMB_{650-725}$ index (Figure 2). Very good results were achieved also for the *TCARI*/OSAVI (Figure 3) and for the *MCARI* index (Figure 4).

However, the applied spectral indices for silver Birch foliar spectra failed to show any significant correlation with the chlorophyll content determined in the laboratory. The trend was uncertain and correlations were rather low (R^2 around 0.35, P < 0.001). No significant relation was proved (Figure 5).

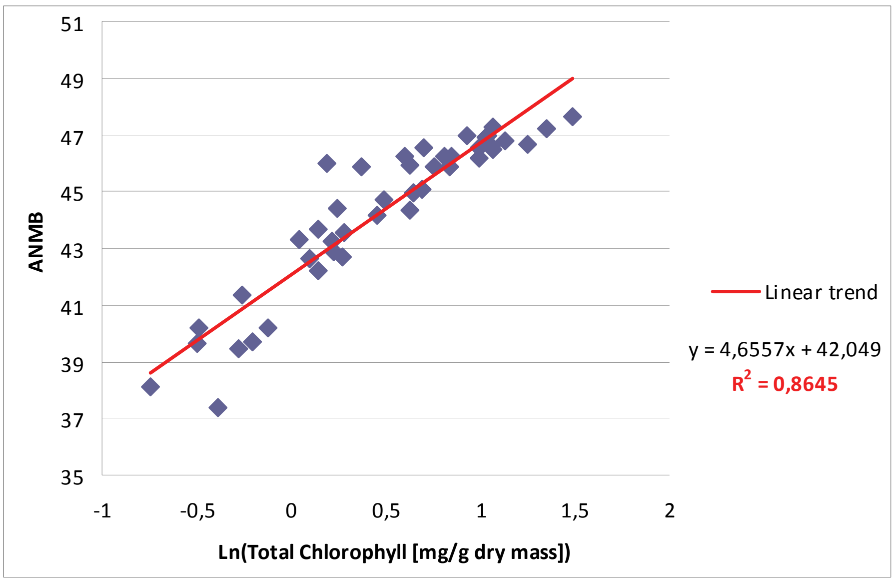

Figure 2: Scots Pine $ANMB_{650-725}$ index as a function of total needle chlorophyll in mg/g dry mass.

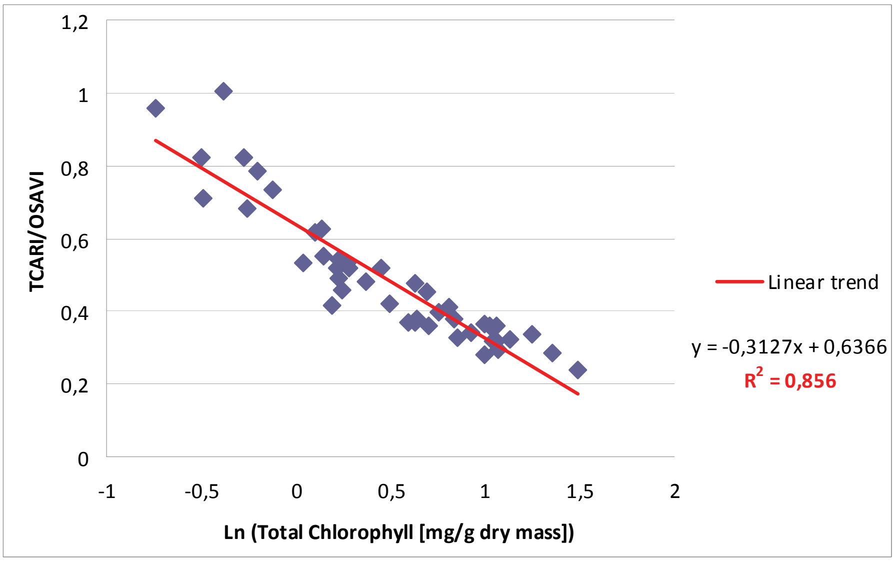

Figure 3: Scots Pine TCARI/OSAVI index as a function of total needle chlorophyll in mg/g dry mass.

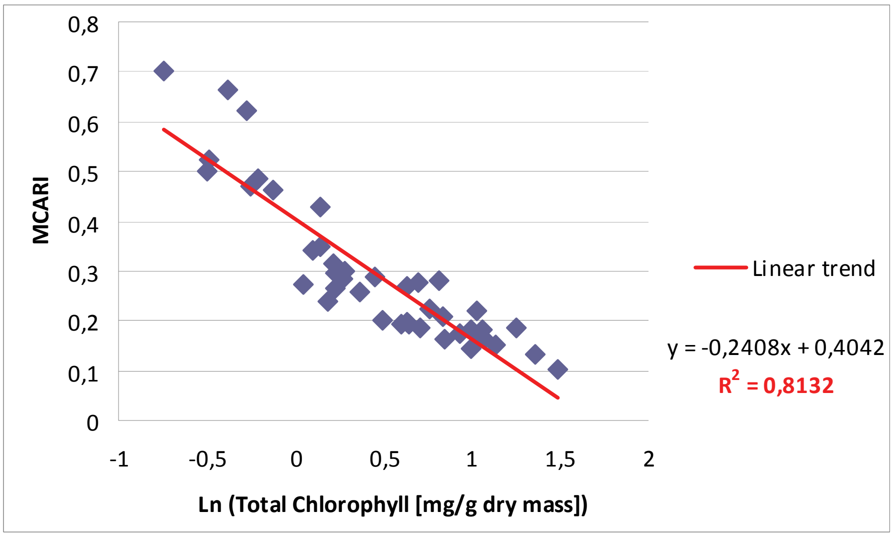

Figure 4: Scots Pine MCARI index as a function of total needle chlorophyll in mg/g dry mass.

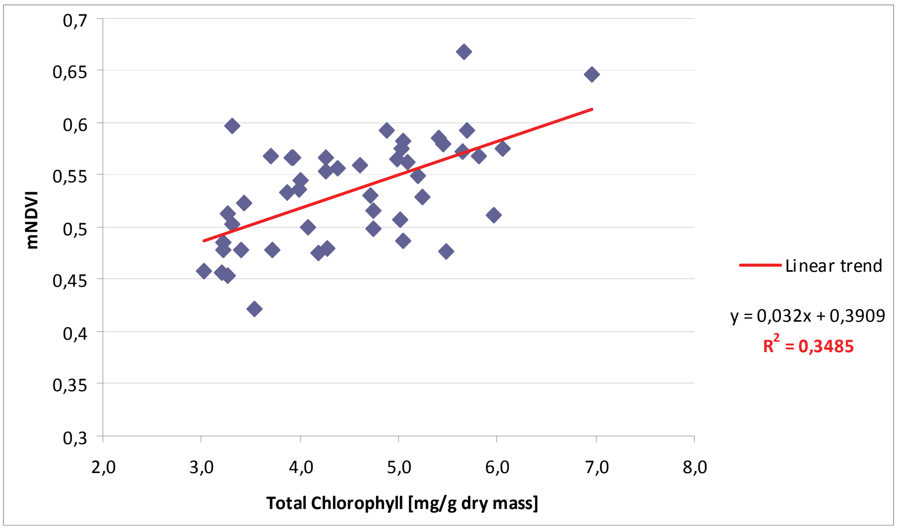

Figure 5: Silver Birch mNDVI$_{705}$ index as a function of total leaf chlorophyll in mg/g dry mass.

DISCUSSION AND CONCLUSIONS

The results of the regression analysis between the needles/leaves chlorophyll content determined biochemically and spectral/optical indices for Scots Pine proved a rather strong relationship while a very poor, non-significant relationship was observed for silver Birch.

In the case of Scots Pine, all calculated indices showed similar trends with respect to the chlorophyll content, nevertheless the *TCARI/OSAVI* index yielded the strongest correlation result. These findings allow us to conclude that *TCARI/OSAVI* and the other three tested indices have a great potential for chlorophyll retrieval from laboratory reflectance spectra of Scots Pine foliage. However, the validation of chlorophyll prediction needs to be verified on an independent data set. A strong relationship between *TCARI/OSAVI* and the needle chlorophyll content was also shown for other conifers: red spruce (R^2 = 0.89) and balsam fir (R^2 = 0.93) (5) and Norway spruce (R^2 = 0.64; (21)). Despite that, *TCARI/OSAVI* index was developed on reflectance of corn canopy (22) and being further used for chlorophyll retrieval in corn and winter wheat canopies (38), it appears to be also suitable for various conifers because it takes into account several disturbance effects, such as the reflectance of non-photosynthetic materials. The $ANMB_{650-725}$ index was originally developed for chlorophyll retrieval in Norway spruce (20) and it also proved to be strongly related to chlorophyll content in young balsam fir needles (R^2 = 0.99) (1).

Standardised and commonly used methods for laboratory chlorophyll content determination and also for laboratory spectra measurements (7,34) were applied for both silver Birch and Scots Pine. The reflectance in NIR is known to depend among others on the internal leaf structure (39). We assume that the reasons for the observed weak relationship between the chlorophyll content and optical indices tested in the case of silver Birch could not only be due to different leaf morphology and anatomy (needle vs. bifacial leaf) but also to different leaf architecture on shoots. This phenomenon may be overcome by spectral measurements at leaf level, however, it could be attenuated at canopy level when dealing with air-borne hyperspectral data. Nevertheless pine needles display more xeromorphic character (i.e., adaptation for drought) than birch leaves, and the decrease of chlorophyll may be faster due to physiological changes including faster water loss in birch leaves after the twig detachment. However, the leaves remained attached to twigs during the measurement and we do not suppose such a massive loss of chlorophyll.

These results can also indicate that the indices used are sensitive enough for the coniferous species (or for selected coniferous species) but not for broadleaved species (or just for some of them). (7) pointed out that some published spectral indices provided a relatively poor correlation with the leaf chlorophyll content when applied across a wide range of broadleaved species. Their conclusion is that the leaf surface reflectance and the leaf functional type were the most important factors in this variation. They developed a new set of indices that were less sensitive to leaf structural variations according to their experiments. We used one of their published indices $mNDVI_{705}$ for silver Birch spectra, however the results were not satisfactory and the expected relation was not found. An analysis that would compare a wider spectrum of various indices preferentially designed for broadleaved or herbaceous species would be desirable to achieve a strong relationship between chlorophyll and reflectance spectra at leaf level for silver Birch. We see the potential in derivative indices, as quite good results in chlorophyll prediction using different derivative indices were achieved e.g. for leafy vegetable species (40). Also other methods for the analysis of the broadleaves species could be applied, for example methods of radiative transfer (6).

In the next steps the analysis of airborne hyperspectral data from the HyMap sensor acquired over the study site of the Sokolov basin mining area is going to be accomplished. We assume that the regression analysis results of the HyMap spectra and biochemical laboratory determination could show similar strong dependence for Scots Pine and poor correlation results for silver Birch. Further analyses and methodology improvements should answer the question if the difference between Scots Pine and Silver Birch results is of methodological origin (if some methodological improvements are needed, for example measurements using integration sphere) or a conceptual problem (the difference between coniferous and broadleaved species).

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