
Numerical Indicators of Absorption Spectra of Leaf Extracts Obtained from Conifer Family Plants

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Abstract: The purpose of this study is to examine the ratios of absorption by photosynthetic complex substances and other components of leaf extracts obtained from conifer family plants of different genera. Such ratios were determined by absorption spectrometry method using unconventional numerical values of absorption spectra, such as wave length of the highest maximum, coefficient of relative photoabsorption, blue-red coefficient, total absorption intensity and relative share of absorption by photosynthetic complex substances in total absorption. The intensity of light energy's utilization by conifers was shown to be the highest among conifers of pine family, to be the lowest among spruce family plants and to have intermediate values among plants of fir and cypress genera. Numerical indicators of absorption spectra used in this article reflect the properties of photoreceptive system of coniferous plants and could be used in photosynthesis studies and also as criteria in environmental science and plant resources monitoring during assessment of the impact anthropogenic, climate and other factors have on coniferous forests.

Keywords: Photosynthesis, Spectrophotometry, Spruce, Fir, Pine, *Chamaecyparis Lawsoniana*

1. Introduction

Photosynthesis mechanisms [1, 2] and properties of basic pigments of the photosynthetic complex (PSC) of chlorophyll and carotenoids, which absorb in a visible section of the optical spectrum, have so far been thoroughly studied [3, 4]. Comparatively less attention is paid to other leaf components (OLC) not included into the photosynthetic system, such as anthocyanins [5], coumarins and saponins, which absorb ultraviolet light. The ratios of absorption by PSC substances and OLC are determined by absorption spectrophotometry methods using numerical indicators of absorption spectra (NIAS) [6, 7] and could be used as photoabsorption characteristics [8]. However, in case of conifer family plants such ratios are poorly studied and underrepresented in scientific literature.

Currently, it is important to use NIAS to compare the intensity of light absorption by the leaves of coniferous trees, which prefer different habitats in the forest. The purpose of this study is to examine the ratios of absorption by PSC substances and other components of leaf extracts obtained

from conifer family plants of different genera. To achieve the goal, it is advisable to use coniferous trees, preferring growth conditions with different illumination. Spruce trees usually prefer shaded areas, pines grow in lighter areas, silver fir and cypress trees grow in areas with medium light. Research can be performed on the following plan: 1) select several species of spruce, fir, pine and cypress for comparison, 2) to conduct spectrophotometry of extracts from the leaves of these trees, 3) to determine the numerical indicators of the absorption spectra of the extracts, 4) to compare the obtained numerical indicators and 5) to conclude on the priorities of absorption activity among conifers.

2. Material and Methods

10 species of 25-30-year-old coniferous plants belonging to 4 genera and growing in the environment specific to the southern part of Primorsky Krai, Russia, were used for this study. In July 4 to 6 independent samples of new needles were taken from each plant species in the afternoon under dry weather conditions, followed by immediate production of

their extracts in a shaded room. Needle bunches were grinded in a porcelain jar filled with quartz sand, magnesium carbonate and 95% ethanol. In 5 to 6 minutes the extracts were filtered through PTGE-H 0.45 μm filter (HyundaiMicro, Republic of Korea) in to dark glass flask. A digital spectrophotometer UV-2501PC (Shimadzu, Japan) was used within range of 220 to 700 nm with a 1 nm interval to register absorption spectra. The spectra were rated by the highest maximum. The results were statistically treated using small sample methods [9].

The first-order derivative of the normalized absorption spectrum (NAS) was calculated using numerical method. The coordinates of inflection points of the absorption band contour were determined by an absolute value of local maximums (Figure 1, points 1, 2 and 3). NIAS used in the study are as follows:

Wave length λ_{HM} of the highest maximum (HM);

Coefficient of relative photoabsorption (CRP), equal to the ratio of extinction A_R at the analytical wave length of chlorophyll absorption in the red section to extinction A_{HM} at the wave length of the highest maximum (Figure 1):

$$CRP = \frac{A_R}{A_{HM}} \quad (1)$$

blue-red coefficient (BRC) was calculated as the ratio of

extinction A_B at the blue wave length and extinction A_R at the red one of chlorophyll absorption:

$$BRC = \frac{A_B}{A_R} \quad (2)$$

The integral absorption intensity of the OLC corresponds to the area S_{OLC} , limited by the band contour in the ultraviolet section. The area was calculated in accordance with Simpson's rule [10] within range between λ_1 (inflection point1) near the maximum of A_{HM} and λ_2 (inflection point2) on the left slope of A_B 's "blue" maximum contour (Figure 1, diagonal hatching). The integral intensity of absorption by PSC substances, which was numerically equal to the area S_{PSC} under the contour of absorption band's visible range (Figure 1, vertical hatching), was calculated in the same way, using integration range between λ_2 (inflection point 2) on the left slope of A_B 's contour and λ_3 (inflection point 3) near the A_R 's "red" maximum. The total intensity of absorption (S_T) of a whole leaf was taken as a sum of the foregoing areas:

$$S_T = S_{OLC} + S_{PSC} \quad (3)$$

The relative share (F) of absorption by PSC substances in total absorption by a leaf was expressed in percentage points:

$$F = 100 \frac{S_{PSC}(\%)}{S_T} \quad (4)$$

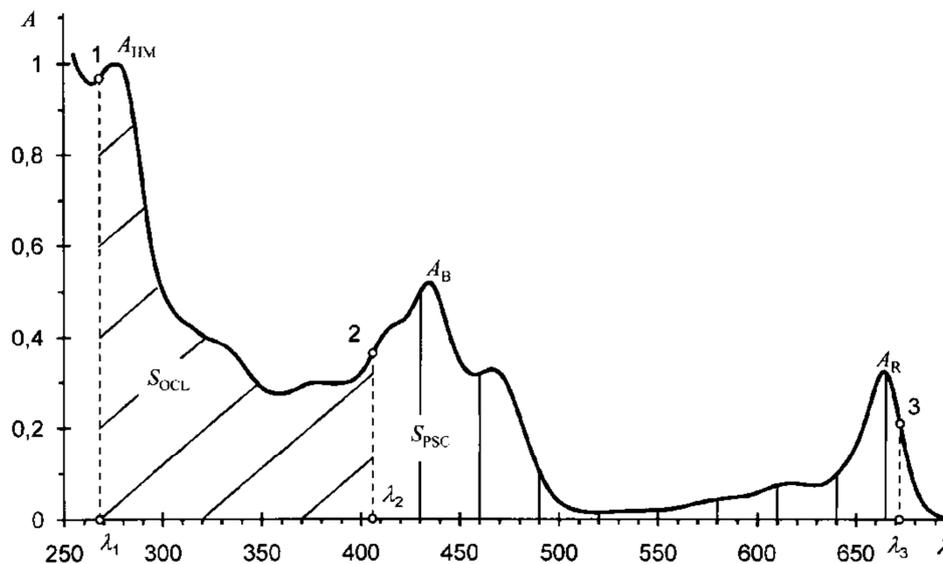


Figure 1. Normalized absorption spectrum of *Picea ajanensis* (Fisch. ExCarr.) extract. 1, 2 and 3 are inflection points of the band contour. A_{HM} , A_B and A_R are extinctions of the highest, blue and red maximums; λ_1 , λ_2 and λ_3 are x-coordinates inflection points; S_{OLC} and S_{PSC} areas corresponding to intensity of absorption by OLC and PSC substances. X-axis: wavelength λ in nm; y-axis-extinction A in relative units.

All calculations were carried out using original software [11, 12].

3. Results

Absorption spectra of the same type were obtained for needle extracts produced from all studied plants (Figure 2). The visible range of registered NAS included four maximums at the wave length of 434 ± 1 ; 466 ± 2 ; 618 ± 1 and 664 nm. At these wave length values the lowest extinctions

were obtained for spruce needles, medium ones for fir and cypress needles, and the highest ones for pine needles.

As the study results show (see Table 1), λ_{HM} in the needle extracts' NAS is within ultraviolet range between 272 and 282 nm. The lowest λ_{HM} values were reported for spruces, with their mean value of 272 ± 1.04 nm. The mean λ_{HM} values in the fir and pine extracts' NAS exceed that of spruces by 3 to 5 nm, and the mean λ_{HM} value in *Chamaecyparis lawsoniana* extract's NAS exceeds it by 10 nm.

The lowest CRP (formula (1)) values averaging

0.084±0.005 were obtained for spruce needle extracts' NAS. The mean *CRP* values of the fir and cypress extracts and the pine needle extracts exceed those of the spruce needle extracts 1.54 to 1.61 times and 2.92 times respectively.

The lowest S_T (formula (3)) values were reported for spruce needle extracts averaging 65.57±2.15, and similar mean values were also reported for the fir and pine extracts, i.e. 64.25±1.64 and 67.87±2.78 respectively.

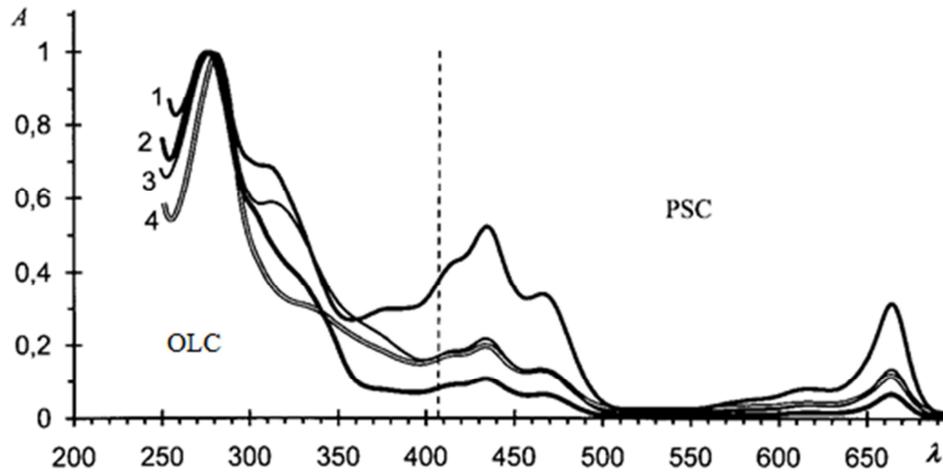


Figure 2. Examples of normalized absorption spectra of needle extracts obtained from *Pinus koraiensis* (1), *Picea smithiana* (2), *Abies koreana* (3) and *Chamaecyparis lawsoniana* (4). PSC: area of absorption by photosynthetic complex substances, OLC: area of absorption by other leaf components. X-axis: wave length λ in nm; y-axis-extinction A in relative units.

Table 1. Wave length of the highest maximum (λ_{HM}), coefficient of relative photoabsorption (*CRP*), blue-red coefficient (*BRC*), total intensity of absorption (S_T) and share (*F*) of absorption by photosynthetic complex substances in total absorption for absorption spectra of needle extracts obtained from trees of different species.

Plant		Numerical value				
Genus	Species	λ_{HM}	<i>CRP</i>	<i>BRC</i>	S_T	<i>F</i>
Picea A. Dietr.	<i>P. smithiana</i> (Wall.) Boiss.	277 ±0.32	0.084 ±0.005	1.58 ±0.015	72.18 ±1.387	15.33 ±0.733
	<i>P. pungens</i> Engelm	271 ±2.33	0.088 ±0.004	1.58 ±0.013	70.29 ±1.332	17.56 ±0.548
	<i>P. ajanensis</i> Fisch. ex Trautv. & C.A. Mey	269 ±0.60	0.065 ±0.012	1.63 ±0.016	55.82 ±1.927	16.88 ±1.512
	<i>A. nephrolepis</i> Maxim.	275 ±1.91	0.179 ±0.017	1.59 ±0.015	80.52 ±5.254	31.71 ±2.636
Abies Mill.	<i>A. koreana</i> E.H. Wilson	270 ±3.86	0.099 ±0.017	1.62 ±0.018	90.88 ±1.816	14.83 ±2.419
	<i>A. hollophylla</i> Maxim.	280 ±0.41	0.118 ±0.011	1.61 ±0.016	64.25 ±1.637	26.16 ±2.609
	<i>P. koraiensis</i> Siebold et Zucc.	280 ±0.71	0.289 ±0.028	1.62 ±0.019	122.31 ±8.219	35.07 ±2.336
Pinus L.	<i>P. densiflora</i> Siebold et Zucc.	282 ±0.41	0.177 ±0.019	1.65 ±0.007	82.97 ±2.017	30.15 ±1.517
	<i>P. sibirica</i> DuTour	280 ±0.23	0.213 ±0.017	1.58 ±0.025	111.44 ±7.432	29.32 ±2.054
	<i>C. lawsoniana</i> Parl.	282 ±0.49	0.104 ±0.011	1.65 ±0.022	67.87 ±2.781	23.02 ±1.677

Each value is expressed as mean ± square error of mean (n = 6)

The mean S_T value of the pine needle extracts exceeded that of the spruce needle ones 1.6 times ($p < 0,001$). The share of absorption by photosynthetic complex substances in total absorption for the spruce needle extracts averaged (16.47±0.65)%. The mean *F* value for the fir and cypress needle extracts and the pine needle extracts exceeded that for the fir needle ones 1.40 to 1.46 times and 1.93 times respectively.

The obtained results show that *BRC* (formula (2)) values for the needle extracts obtained from all studied plants are

within range of 1.58 to 1.64, with the mean value of 1.61±0.06, while the *BRC* variation reached 0.37% and the confidence interval boundaries were 1.598 and 1.622.

According to the obtained data, the highest mean NIAS values of the extracts obtained from pine genus trees, such *F* (formula (4)) and *CRP*, exceed those obtained from spruce genus ones by 48.2 to 65.8%, while in case of such NIAS as *BRC* and λ_{HM} the excess is only 1.2 and 3.5% respectively.

4. Discussion

As the study results show, the shapes of absorption band

contours of NAS of the needle extracts obtained from different coniferous tree species are quite similar to each other and the corresponding maximums are similar to each other in terms of wave lengths (Figure 2), while their set matches the maximums of chlorophyll absorption [13]. Such similarity is due to the qualitative identity of PSC substances in leaves of different plant species [1]. Moreover, *BRC* numerical value is virtually the same for all studied coniferous plant species, which is also confirmed by the constancy of PSC substances in their needles.

In all studied needle extracts' NAS the λ_{HM} value is within ultraviolet range, which is consistent with our previously obtained data [6, 8] for the leaf extracts of plants and broad-leaved trees. The fact that the highest maximums are within ultraviolet range is apparently a feature of absorption spectra of leaf extracts of all terrestrial green plants and is probably due to the optical properties of flavonoids, tannins, saponins etc. of the OLC, which absorb exactly in this range. Yet, in case of conifers the contribution to absorption in the extracts' NAS is probably also made by resins present in the needles, which content in, say, pine needles may be as high as 7 to 12%.

The numerical *CRP* value by default depends on the ration of extinctions at the red wave and λ_{HM} , yet since the extinction A_{HM} in the NAS is unity, the *CRP* value reflects a relevant absorption by chlorophylls, while higher *CRP* values point to increased photoreception of their needle extract and lower ones, to the contrary, point to lower photoreception. According to the obtained data, the highest and the lowest *CRP* values are specific to pines and spruces respectively, while other studied trees belong to an intermediate position in terms of this indicator.

The total intensity of absorption S_T provides an image of cumulative absorption of all leaf components within examined range. The most interesting part is undoubtedly the area of absorption carried out by photosynthetic complex substances, and this part is reflected by the *F* value expressed in percentage points as the ratio of intensities of absorption by PSC substances and total one. As the obtained data show, pines are featured by higher S_T and *F* values respectively and also stronger ability to absorb light energy by PSC substances, while spruces are featured by the lowest ones and other studied trees belong to an intermediate position in terms of these indicators.

Thus, in terms of aggregated NIAS increase and, accordingly, in ascending order of the light utilization level of their needle extracts the studied trees could be lined up as follows:

[*Picea spruces*] < [*Abies firs* and *Chamaecyparis cypress*] < [*Pinus pines*].

Since the NIAS used in this study have not been previously used for the spectrophotometric analysis of needle extracts, direct comparisons with literature data are excluded. However, there may be correlations with published data on biochemistry and physiology of coniferous plants. For example, a number of studies show lower levels of photochemical processes in the needles of different spruce

species [14-18] comparing with firs [19, 20] and pines [21] under normal conditions. At the same time, it was found out that pine needles [22] were featured by higher photosynthesis intensity that that of cypress needles [23, 24]. In other words, pines and spruces are featured by higher and the lowest levels of photosynthetic processes respectively, while other studied coniferous plants belong to an intermediate position in terms of photosynthetic process level, which is quite consistent with the presented results, which were obtained in this study.

The used NIAS reflect the properties of plants' photoreceptive system. The most valid ones among the used NIAS are *F* and *CRP* values, which are appropriate to be used in further studies.

It should be noted that, comparing to, say, liquid chromatography [25], the used method of absorption spectrophotometry is less time-consuming and featured by quite high accuracy and also does not require additional reagents (bar extraction agents), which is quite important for the field practice of plant studies.

5. Conclusions

Using unconventional numerical indicators for the analysis of light absorption by plant leaves extends the field of applications of molecular spectrophotometry, which is important for increasing the amount of useful data extracted with a spectrophotometer and for further development of methodology of absorption spectrophotometry.

The experimental material presented in this study demonstrates a new approach to a spectrophotometric assessment of light absorption by plant leaves, which could be dubbed "method of numerical indicators of absorption spectra" or, in short, "NIAS method". The results obtained using the NIAS method is not at odds with modern perceptions about biochemistry of coniferous plants.

The obtained results point to the prospects of using unconventional numerical indicators, especially *CRP* and *F* ones, as criteria in plant resources monitoring during assessment of the impact anthropogenic, climate and other factors have on coniferous trees.

Confirmation

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Contribution of Authors

All the authors have equally contributed to this study.

Ethics

This is an original article that contains unpublished materials. The authors hereby confirm that there are no any ethical issues.

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