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# THE VARIATION OF PHOTOSYNTHETIC PIGMENTS IN LINDEN LEAVES IN RELATION TO THE POSITION OF THE LEAF ON THE SHOOT

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***ABSTRACT***

*The study determined the content of chlorophyll (Chl), carotenoids (Car), and the ratios Chl/Car, Car/Chl in linden leaves, and analyzed the results in relation to the position of the leaves on the shoot. The samples of linden shoots were taken from the "Padurea Cenad" Protected Area, Timis County, Romania. 15 leaves were considered for the analysis, with consecutive position on the annual shoots growth (Lps1 to Lps15). The chlorophyll content varied between 12.87±10.92 SPAD units in the case of sample Lps15 and 46.13±10.92 SPAD units in the case of sample Lps5. The content of carotenoids (Car) varied between 2.72±1.36 units in the case of the Lps15 sample and 6.56±1.36 units in the case of the Lps6 sample. The variation of Chl and Car in relation to Lps was described by polynomial equations of the 3rd degree, under statistical safety conditions ($R^2$=0.991, p<0.001, F=426.3 for Chl, and $R^2$=0.982, p<0.001, F=207.26 for Car). The variation of Car in relation to Chl was described by a polynomial equation of degree 4, under conditions of $R^2$=0.998, p<0.001, F=1230.5. The chlorophyll content (Chl) showed very strong, positive correlations with the carotenoid content (Car), r=0.987 (p<0.001) and strong, negative correlations with the position of the leaves on the shoot (Lps), r=-0.805 (p<0.001). The content of carotenoids (Car) showed very strong correlations with chlorophyll (Car) and moderate, negative correlations with the position of the leaves on the shoot (Lps), r=-0.796 (p<0.001). Graphical models of 3D type and in the form of isoquants were obtained and described the variation of Chl in relation to Lps and Car ($R^2$=0.999, p<0.001). PCA led to the distribution diagram of Lps trials, in relation to the association with Chl, Car, Chl/Car, Car/Chl as biplot. PC1 explained 87.905% of variance, and PC2 explained 11.927% of variance. Cluster analysis facilitated obtaining the association dendrogram of Lps trials, based on similarity in relation to Chl and Car values (Coph.corr=0.857).*

**KEY WORDS:** *carotenoids, chlorophyll, Chl/Car, Car/Chl, leaves, linden*

## INTRODUCTION

Leaves are specialized organs of plants in relation to capturing solar energy and photosynthetic processes (Terashima *et al*., 2011; Gu *et al*., 2017; Joshi *et al*., 2022).

The leaves of plants show different typologies in relation to genetic factors (species, variety, cultivar), but also in relation to environmental factors (Runions and Tsiantis, 2017; Sala *et al*., 2017; Kierzkowski *et al*., 2019).

Within the same plant, the leaves show high variability in relation to internal and external factors of the plant organism, such as age, position on the plant, habitat conditions, stress factors etc. (Dkhar and Pareek, 2014; Alcántara-Ayala *et al*., 2020; Liu *et al*., 2019a; Liu *et al*., 2020; Navarro and Hidalgo-Triana, 2021). The degree of variability in leaves was studied in relation to genetic, anatomical, morphological, physiological parameters and indices (Tsukaya, 2002; Guet *et al*., 2015; Liang *et al*., 2016; Qiao *et al*., 2022).

Based on some foliar parameters and indices, the plants were studied in relation to certain habitat conditions for their evaluation (Del Vecchio *et al*., 2016; Datcu *et al*., 2017). At the level of plant leaves, quantitative and qualitative variations, including leaf health, associated with specific relationships within anthropogenic ecosystems were also quantified (Guo and Gan, 2014; Drienovsky *et al*., 2017; Joshi *et al*., 2019; Javaid *et al*., 2022). Photosynthetic pigments (chlorophyll, carotenoids), as physiological indices of plant leaves, vary in relation to plant intrinsic factors, but also to environmental or technological factors, in the case of crop plants (Constantinescu *et al*., 2018; Liu *et al*., 2019b).

Different studies evaluated the chlorophyll content both by classical methods and by non-destructive determinations with portable chlorophyll meters, or imaging by techniques based on aerial or satellite images (Pîrvulescu *et al*., 2015; Constantinescu *et al*., 2018; Kandel, 2020; Binh *et al*., 2022). The ratio between chlorophyll pigments was determined and interpreted in relation to plant species, latitudinal gradient, plant nutrition, stress factors, etc. (You *et al*., 2017; Zhou *et al*., 2019; Sonobe *et al*., 2020; Zhang *et al*., 2020; Sala, 2021).

The aim of the study was to evaluate the content of photosynthetic pigments in the leaves of linden, *Tilia tomentosa* Moench., and to describe the interdependence relationships between chlorophyll (Chl), carotenoids (Car) and the position of the leaves on the shoot, as well as with other parameters.

**MATERIAL AND METHODS**

The study analyzed the content of photosynthetic pigments in linden leaves, *Tilia tomentosa* Moench., in relation to the leaves position on the annual growth shoot.

The samples of linden shoots came from the "Padurea Cenad" Protected Area, Timis County, Romania. 15 leaves were taken into account, with consecutive position on the annual shoots of linden growth (Lps1 to Lps15), figure 1.

Photosynthetic pigments were determined by non-destructive methods. Chlorophyll was determined with the SPAD-502Plus device (KONICA MINOLTA), and carotenoids with the ACM-200 Plus device (OPTI-SCIENCES).

**FIGURE 1. The set of analyzed leaves on the linden shoot (the small, albino leaf, is on Lps 15, at the top of the shoot)**

The data series regarding Chl and Car were recorded and based on them, for each leaf category (Lps1 to Lps15) the Chl/Car ratio and the Car/Chl ratio were calculated.

The recorded data were analyzed by appropriate mathematical and statistical methods, in order to evaluate the safety of the data, the presence of variance, the level of correlation, interdependence and variation relationships in relation to the leaves position on the shoot. PAST software (Hammer *et al*., 2001), Wolfram Alpha (2020) software, and JASP software (2022) were used.

**RESULTS AND DISCUSSION**

From the determination of the content of photosynthetic pigments (chlorophyll - Chl, carotenoids - Car) in linden leaves (*Tilia tomentosa* Moench.), the results presented in table 1 were obtained, with variable values in relation to the position of the leaves on the shoot. The chlorophyll content varied between 12.87±10.92 SPAD units in the case of sample Lps15 and 46.13±10.92 SPAD units in the case of sample Lps5. Similarly, the variation of the carotenoid content (Car) was recorded with values between 2.72±1.36 units in the case of the Lps15 sample and 6.56±1.36 units in the case of the Lps6 sample. Based on the chlorophyll and carotenoid content values, the Chl/Car and Car/Chl ratios were calculated, with the series of values presented in table 1. The ANOVA test confirmed the reliability of the data and the presence of variance in the data set, table 2.

**TABLE 1. The values of the content of photosynthetic pigments and the ratios between them, linden leaves**

| Trial | Lps | Chl | Car | Chl/Car | Car/Chl |
|---|---|---|---|---|---|
| Lps1 | 1 | 34.32 | 4.84 | 7.09 | 0.14 |
| Lps2 | 2 | 41.48 | 6.22 | 6.67 | 0.15 |
| Lps3 | 3 | 42.56 | 6.45 | 6.60 | 0.15 |
| Lps4 | 4 | 43.29 | 6.50 | 6.66 | 0.15 |
| Lps5 | 5 | 46.13 | 6.54 | 7.05 | 0.14 |
| Lps6 | 6 | 45.08 | 6.56 | 6.87 | 0.15 |
| Lps7 | 7 | 43.97 | 6.53 | 6.73 | 0.15 |
| Lps8 | 8 | 39.84 | 5.91 | 6.74 | 0.15 |
| Lps9 | 9 | 38.78 | 5.62 | 6.90 | 0.14 |
| Lps10 | 10 | 36.31 | 5.39 | 6.74 | 0.15 |
| Lps11 | 11 | 31.09 | 4.41 | 7.05 | 0.14 |
| Lps12 | 12 | 25.82 | 3.84 | 6.72 | 0.15 |
| Lps13 | 13 | 20.87 | 3.49 | 5.98 | 0.17 |
| Lps14 | 14 | 15.69 | 3.19 | 4.92 | 0.20 |
| Lps15 | 15 | 12.87 | 2.72 | 4.73 | 0.21 |
| SE | | ±10.92 | ±1.36 | ±0.72 | ±0.021 |

**TABLE 1. ANOVA single factor test for the analyzed data**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 10859.51 | 3 | 3619.837 | 118.8472 | 2.93E-24 | 6.229585 |
| Within Groups | 1705.642 | 56 | 30.45789 | | | |
| Total | 12565.15 | 59 | | | | |

Alpha=0.001

The variation in the content of photosynthetic pigments in relation to the position of the leaves on the shoot (Lps) was analyzed through regression analysis. Thus, the variation of Chl content was described by equation (1) under statistical safety conditions, according to $R^2$=0.991, p<0.001, F=426.3. The graphic distribution of Chl values in relation to Lps, and the graphic expression of equation (1) are shown in figure 2.

$$\text{Chl} = 0.02037x^3 - 0.854x^2 + 7.104x + 28.75 \quad (1)$$

where: x - Lps

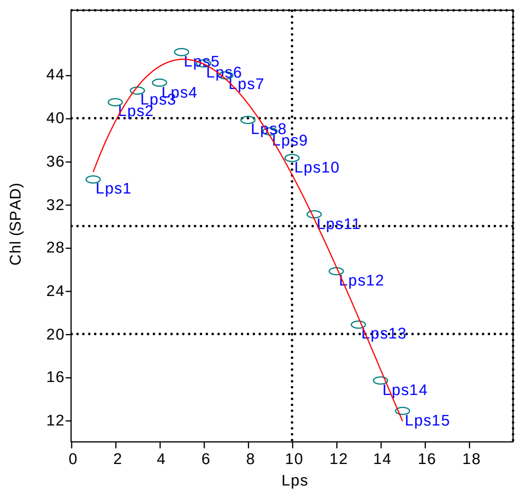



**FIGURE 2. The variation of Chl in relation to Lps, linden leaves**

The variation of carotenoid content (Car) in relation to Lps was described by equation (2) under statistical safety conditions, according to $R^2$=0.982, p<0.001, F=207.26. The graphic distribution of Car values in relation to Lps, and the graphic expression of equation (2), are shown in figure 3.

$$\text{Car} = 0.00497x^3 - 0.162x^2 + 1.237x + 3.983 \quad (2)$$

where: *x* - Car

The variation of the carotenoid content (Car) in relation to the chlorophyll content (Chl) was described by equation (3) under statistical safety conditions, according to $R^2$=0.998, p<0.001, F=1230.5. The graphic distribution of Car values in relation to Chl, and the graphic expression of equation (3) are shown in figure 4.

$$\text{Car} = -1.925E - 05x^4 + 0.00224x^3 - 0.091x^2 + 1.611x - 7.192 \quad (3)$$

where: *x* – Chl content

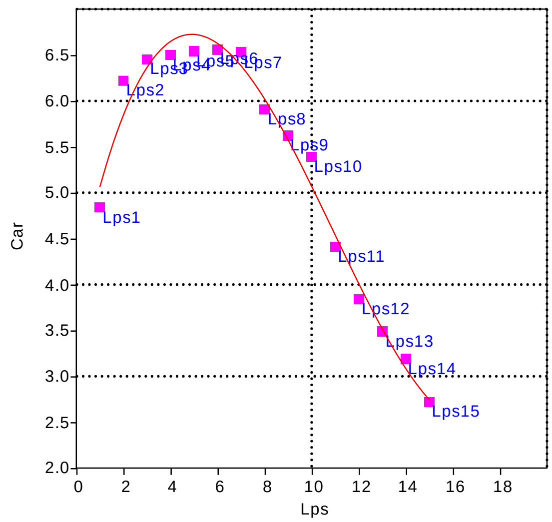



**FIGURE 3. The Car variation in relation to Lps, linden leaves**

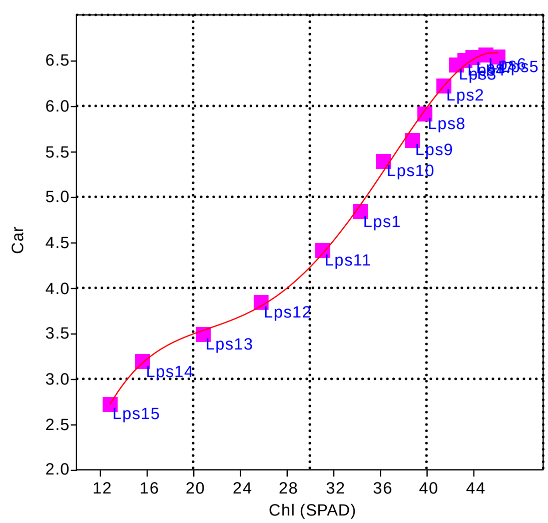



**FIGURE 4. Graphical distribution of Car values in relation to Chl, linden leaves**

The level of correlation between photosynthetic pigments (Chl, Car) and other parameters at the level of linden shoots was analyzed, some of which values were communicated in some previous studies (Rosu *et al*., 2022a,b).

The chlorophyll content (Chl) showed very strong, positive correlations with the carotenoid content (Car), r=0.987 (p<0.001), and strong, negative correlations with the position of the leaves on the shoot (Lps), r=-0.805 (p<0.001). The content of carotenoids (Car) showed very strong correlations with chlorophyll (Chl) and moderate, negative correlations with the position of the leaves on the shoot (Lps), r=-0.796 (p<0.001). Between the content of photosynthetic pigments (Chl, Car) and other parameters and indices determined in linden leaves and shoots, communicated in previous articles (Rosu *et al*., 2022a,b), different positive or negative correlations, were recorded, presented in the figure 5.

The variation of Chl in relation to Lps and Car, as a direct and interaction effect, was described by equation (4) under conditions of $R^2$=0.999, p<0.001. The graphic distribution of Chl in relation to Lps and Car is shown in figure 6, a and b.

$$\mathrm{Chl} = ax^2 + by^2 + cx + dy + exy + f \quad (4)$$

where: Chl – chlorophyll content;
*x* – Lps – leaf position on the shoot;
*y* – Car – carotenoid content;
a, b, c, d, e, f – coefficients of the equation (4);
a= -0.18116269;
b= -0.32336568;
c= 2.37814698;
d= 8.19244945;
e= -0.07732920;
f= 0

Based on PCA, the distribution diagram of Lps was obtained in relation to association with Chl, Car, Chl/Car, Car/Chl as biplot, figure 7. PC1 explained 87.905% of variance, and PC2 explained 11.927% of variance. The independent positioning in relaiton to parameters as biplot, was observed for the variants on the terminal positions of the shoot (Lps 12 to Lps 15).

Based on the Cluster analysis, the dendrogram in figure 8 was obtained, which shows the association of Lps based on similarity, in relation to the content of the photosynthetic pigments determined in the leaves (Chl and Car), under statistical safety conditions (Coph.corr.=0.857). Two clusters (C1 and C2) with several sub-clusters each resulted. Cluster C1 includes the leaves with a terminal position on the shoot (Lps12, Lps13, Lps14 and Lps15) where the lowest values of the content of photosynthetic pigments were recorded. Cluster C2 includes two sub-clusters (C2-a and C2-b) each with several sub-clusters. Sub-cluster C2-a includes variants Lps1, Lps8, Lps9, Lps10 and Lps11, which have intermediate values of photosynthetic pigments, and sub-cluster C2-b includes variants Lps2 to Lps7, with high values of Chl and Car pigments content. From the analysis of the Lps distribution in the obtained dendrogram, as well as based on the calculated SDI values, a high level of similarity was found between Lps4 and Lps7 (SDI=0.6806), followed by the Lsp3 and Lsp4 (SDI=0.7317), respectively by the Lsp5 and Lsp 6 (SDI =1.0502).

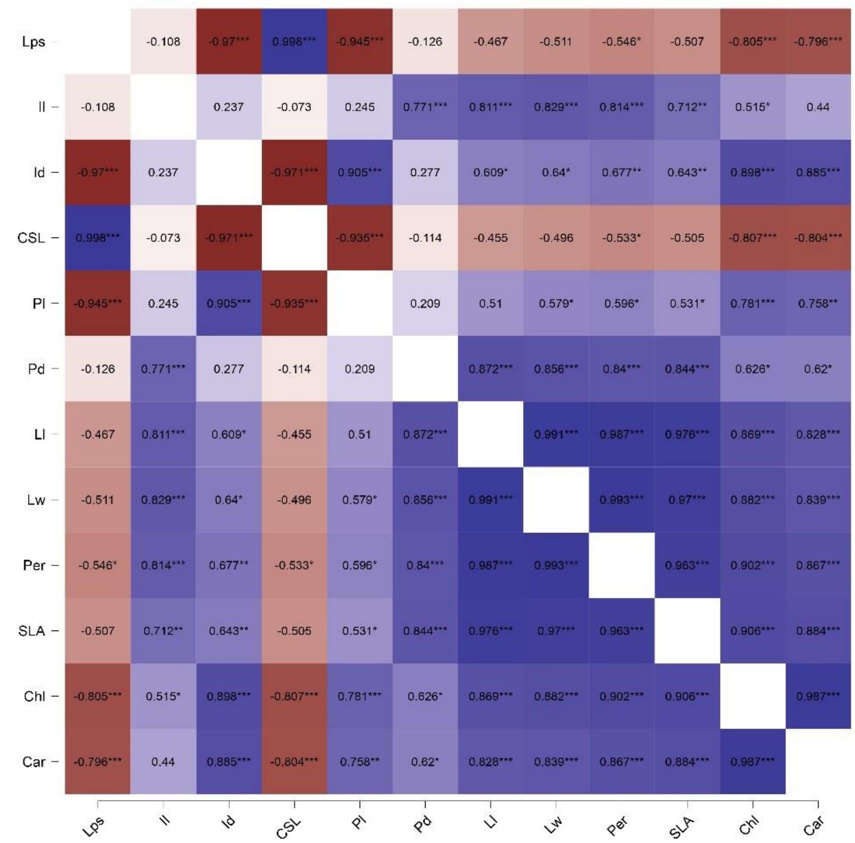
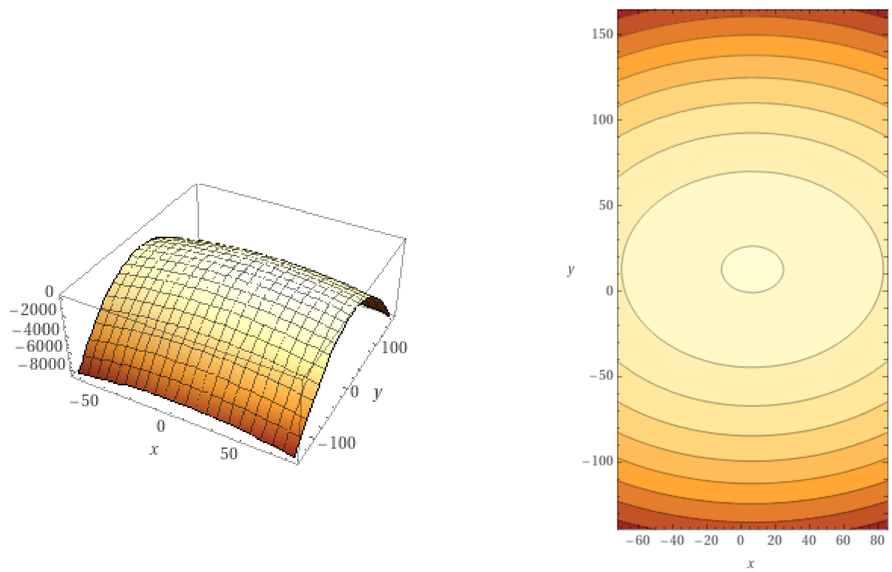



**FIGURE 5. Correlations between the chlorophyll pigments (Chl, Car) and other parameters at the level of linden shoots (Lps – leaf position on the shoot; Il – internode length; Id – internode diameter; CSL – cumulative shoot length; Pl – petiole length; Pd – petiole diameter; Ll – leaf length; Lw – leaf width; Per – perimeter; SLA – scanned leaf area, Rosu *et al*, 2022a,b); Chl – chlorophyll; Car – carotenoids**




a b

**FIGURE 6. The graphic distribution of Chl values in relation to Lps (x – axis) and Car (y – axis); a –3D model; b – model in the form of isoquants**

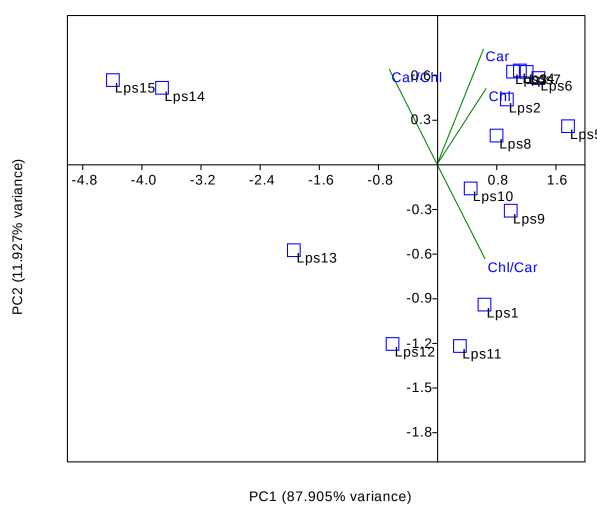



**FIGURE 7. PCA diagram, correlation, regarding the distribution of Lps in relation to considered parameters, as biplot (Chl, Car, Chl/Car, Car/Chl)**

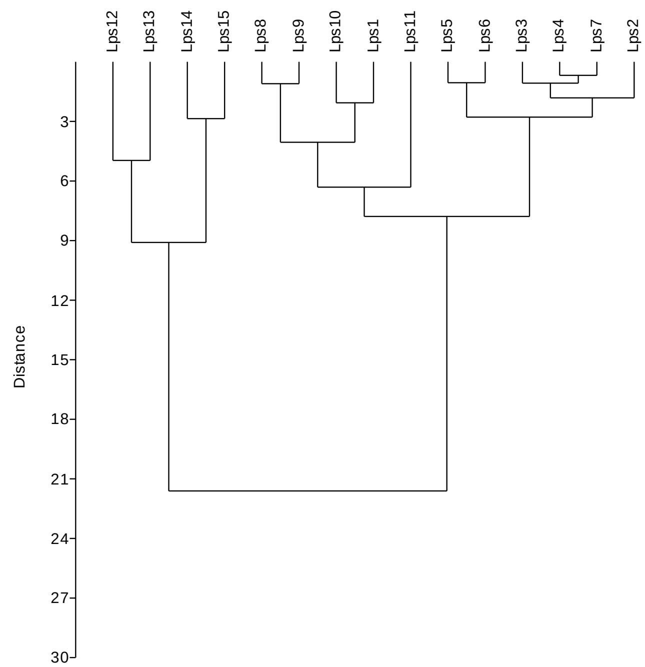



**FIGURE 8. Dendrogram of grouping of leaf samples (Lsp) based on Euclidean distances, in relation to the content of photosynthetic pigments (Chl and Car) in linden leaves, *Tilia tomentosa* Moench.**

The growth and development of the leaves in the successive order of appearance on the shoot, is a dynamic process, which requires time, and is influenced by genetic and environmental factors (Kwiatkowska and Dumais, 2003; Kalve *et al.*, 2014; Gleason *et al.*, 2018).

The accumulation, content and distribution of photosynthetic pigments in leaves along the length of the shoot, is differentiated in relation to the plant species, with the position of the leaves and in relation to the growth conditions, the provision of water and nutrients and has been studied from different perspectives (Borsuk and Brodersen, 2019).

The content of photosynthetic pigments in the leaves varies in relation to the spatial distribution of the leaves described by different models (Borsuk and Brodersen, 2019), and depending on the state of vegetation and nutrients of the plants, of photosynthetic processes, the level of distribution of mineral elements also varies in shoots, eg in vine (Sala and Blidariu, 2012).

Borsuk and Brodersen (2019) reported a high level of statistical confidence ($R^2$=0.94) for models that described the spatial variation of chlorophyll content in the different species studied.

Other models have been communicated, under statistical safety conditions, used to describe the variation of chlorophyll in relation to different plant species and study conditions (Hamblin *et al*., 2014; Lin *et al*., 2015; Li *et al*., 2019).

Contreras *et al*. (2013) reported a positive relationship between the variation in the content of chlorophyll (p=0.0051), carotenoids (p=0.0266) and the ratio of total chlorophyll/carotenoids (p=0.0188) with the green color of the leaves in different samples of Japanese cedar in relative to the season, but they did not record a variation in the ratio between chlorophyll a and b on the green level. The relationship between total chlorophyll and carotenoids was described by a linear relationship, regardless of the season, under statistical safety conditions ($R^2$=0.940 in the summer season, and $R^2$=0.880 in the winter season). Strong and moderate correlations were communicated between photosynthetic pigments and nutrients (NPK) in some plant species, and the regression analysis led to models that described with statistical certainty ($p<0.001$) the variation of Chl, Car and the ratio Chl/Car in relation to the nutrients (Sala, 2021).

The distribution of the content of chlorophyll and carotenoids in lime leaves, described in this study, agrees with other results from different studies in the field, which were the basis of the documentation of this article, and the statistical safety parameters used give the data and results statistical safety.

## CONCLUSIONS

The physiological indices considered in the present study, represented by the photosynthetic pigments (Chl, Car) and the calculated ratios (Chl/Car, Car/Chl), characterized with high fidelity the linden leaves in relation to their position on the annual growth shoots under the conditions of study.

Models of the type of polynomial equations of the 3rd degree were obtained through regression analysis and described with statistical certainty the variation of Chl and Car in relation to the position of the leaves on the shoot. The variation of Car depending on Chl, in relation to the position of the leaves on the shoot, was described by a polynomial equation of the 4th degree, also under statistical safety conditions.

The association of the leaf samples (Lps1 to Lps15) with the studied indices (Chl, Car) and the calculated ratios (Chl/Car, Car/Chl) was obtained by PCA, and the association of Lps based on affinity in relation to the values recorded for Chl and Car was obtained by Cluster analysis.

The obtained models can be extended in studies to other plant species, in order to evaluate the growth dynamics, as well as to species of higher economic interest, such as fruit trees.

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