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RGB COLOR PARAMETERS IN THE CHARACTERIZATION OF CHLOROPHYLL DEFICIENCY IN LEAVES. CASE STUDY: BIRCH

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ABSTRACT
This study aimed to test some spectral and physiological indices on the birch leaves (Betula pendula Roth.). The leaves for this study were harvested from the city of Timisoara, being grouped into 7 categories, depending on the chlorophyll content. Fresh and dry mass, but also leaf thickness were analyzed. RGB spectral indices were obtained after the probes were scanned. Statistical processing was represented by ANOVA single factor, regression, but also correlation analysis, PCA and cluster analysis. ANOVA confirmed the variance between data sets. Different strong positive or negative correlation coefficients values were obtained between the analyzed indices. After the completion of r correlation values, a very high positive correlation between dry and fresh masses (r=0.968) was observed. For chlorophyll estimation in relation with RGB color parameters second- and third-degree polynomial equations were utilized. In all the cases, third degree models assured a better estimation of chlorophyll content (Chl) as against second degree models, for the same color parameter. Very high negative correlation was obtained between chlorophyll index and R color parameter and between chlorophyll and G color parameter. Very high positive correlations were obtained also between R and G and between R and B values. PCA analysis conducted to two groups: PC1 explained 96.928% of variance, and PC2 explained 2.9718% of variance, with statistical safety being assured.
KEY WORDS: *Betula pendula, physiological indices, RGB color parameters, urban behavior*

INTRODUCTION

Plants grow in very variate environments and respond specifically to growth and development conditions (Yokoya *et al*., 1999; Tardieu, 2013; Kaiser *et al*., 2015; Lind *et al*., 2016).

Plant nutrition is a complex physiological process through which the plants take water and nutritive elements from the nutrition medium, transport and utilize them in metabolic processes for organic compounds synthesis (Marschner, 1995;

Mengel & Kirkby, 2001; Barker & Pilbeam, 2007).

The urban habitat is a hybrid ecosystem, strongly anthropic, but with a specific structure and composition of vegetation (individual plants or vegetal associations) (Manning, 2008; Lehmann *et al*., 2014; Threlfall *et al*., 2016; Drillet *et al*., 2020).

Specific conditions which characterize the urban environment are given by construction elements, the architecture, industrial areas, road network, parking lots, green zones, parks, manmade water bodies etc., with a specific manifestation of the associated indices NDVI, LST, UHI, UCI, SUHI etc. (Zhou *et al*., 2018; Aram *et al*., 2019; Orusa & Mondino, 2019; Herbei & Sala, 2020).

Plants were studied in relation with the urban ecosystem from different perspectives (Manning, 2008; Datcu *et al*., 2017; Solomou *et al*., 2019; Fineschi & Loreto, 2020).

Different plant species are present in the urban habitat, some of them natively and other introduced for decorative purposes (Gaertner *et al*., 2017; Kowarik & von der Lippe, 2018; Narango *et al*., 2018).

Soil and environment conditions of plants in urban areas were studied in relation with different substrate types, quality indices, garden management etc. (Lin *et al*., 2018; Tresch *et al*., 2018).

The plants exhibit different indices in relation with the growth medium and specific conditions of urban zones. Plant leaves show different phenomena in relation with nutrition and stress factors (Datcu *et al*., 2019; 2020).

Different analysis and study methods of leaves were promoted in relation with the nutrient support (Jivan & Sala, 2014), leaf area and pathogens attack (Drienovsky *et al*., 2017a,b; Candea-Crăciun *et al*., 2018),

The present study utilized imagistic analysis for the evaluation of chlorophyll deficit in birch leaves (*Betula pendula* Roth.) in urban environment conditions.

MATERIAL AND METHODS

This paper had the purpose to evaluate chlorophyll deficit in leaves through imagistic analysis by using the on RGB color parameters.

The biological material was represented by from *Betula pendula* Roth leaves. Normal and chlorophyll deficient leaves were randomly harvested figure 1.

The leaves were harvested from ornamental trees from the city of Timisoara. The analyzed leaves were grouped in 7 categories in relation with the chlorophyll content.

The chlorophyll content was determined through a nondestructive method, using a chlorophyll meter Konica Minolta SPAD-502Plus.

Color parameters were determined through imagistic analysis, based on the scanned images of leaves (Rasband, 1997). Spectral information was obtained for RGB color system.

Physiological indices of leaves - fresh mass (Fm), dry mass (Dm) and leaf thickness (Lt) were determined. Leaf weights were determined with an AXIS thermal balance (ATS 60, Poland), with a precision of determination of ±0.001g.

Experimental data was analyzed with ANOVA test, correlation analysis, regression analysis, PCA and Cluster analysis (Hammer *et al.*, 2001). Statistical safety parameters R^2, r, p and Coph. Corr were taken into consideration for the results evaluation.

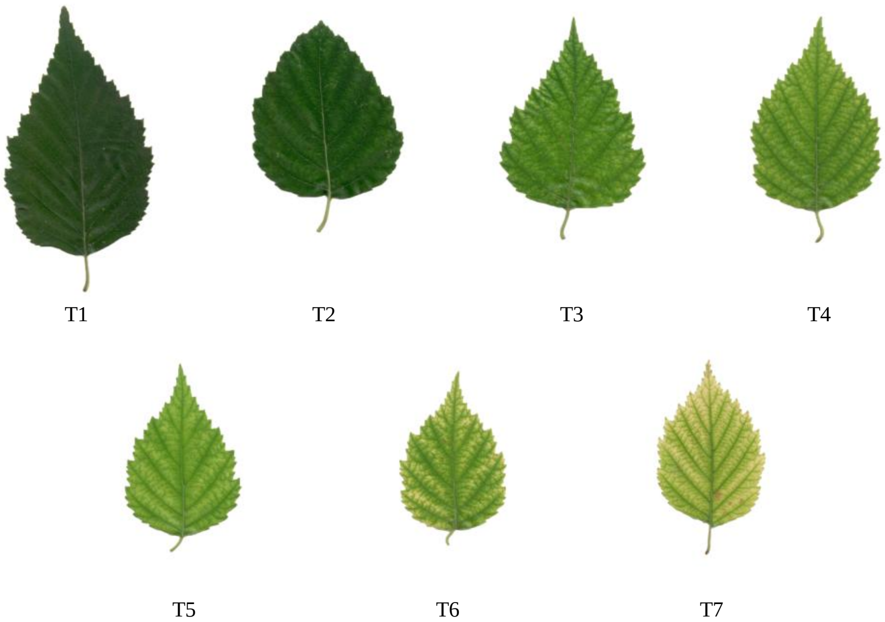

FIGURE 1. Birch leaves grouped by chlorophyll deficit

RESULTS AND DISCUSSIONS

After the analysis of birch leaves, physiological indices values for fresh mass (Fm), dry mass (Dm), leaf thickness (Lt), chlorophyll content (Chl) and color parameters RGB were obtained (Table 1). Fm index had values between 0.660 – 1.199±0.047 g and Dm index had values between 0.226 – 0.634±0.029 g.

Regarding leaf thickness, values between 0.183-0.314±0.008 mm were obtained. Chlorophyll content (Chl) varied between 4.83 – 51.45±2.95 SPAD units. Associated with chlorophyll content, the RGB color parameters presented specific values between 33.41 – 154.70±7.37 for R, 71.35 – 166.96±6.62 for G and 20.98 – 48.83±2.69 for B.

Single factor ANOVA test confirmed the presence of variation in the experimental data set and the results safety, in p<0.001, F>Fcrit, for Alpha=0.001 conditions, table 2.

TABLE 1. Values of physiological indices and RGB color parameters for birch leaves

Trial	Fm	Dm	Lt	Chl	Color parameter		
	(g)		(mm)	SPAD units	R	G	B
T1	1.199	0.634	0.314	51.45	46.65	71.35	35.94
T2	0.791	0.333	0.281	47.61	33.41	74.85	20.98
T3	0.712	0.262	0.272	26.86	80.26	125.04	33.81
T4	0.708	0.258	0.267	21.27	100.38	138.38	41.81
T5	0.372	0.148	0.228	20.06	105.76	148.15	43.66
T6	0.452	0.171	0.205	19.26	113.72	145.92	49.09
T7	0.660	0.226	0.183	4.83	154.7	166.96	69.81
SE	±0.047	±0.029	±0.008	±2.95	±7.37	±6.63	±2.69

TABLE 2. ANOVA single factor results for the analyzed data

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	106961.3	15	7130.754	18.48039	5.53E-22	2.854574
Within Groups	37042.1	96	385.8552			
Total	144003.4	111				

Alpha = 0.001

Correlation analysis conducted to r coefficient values presented in table 3. After the analysis of r correlation values, a very high positive correlation between Dm and Fm (r=0.968) was observed. Very high negative correlation were obtained between Chl index and R color parameter (r=-0.973), between Chl and G color parameter (r=-0.991). Very high positive correlations were also obtained between R and G (r=0.961) and R and B (r=0.938).

TABLE 3. Correlation table between physiological and color indices

	Fm	Dm	Lt	Chl	R	G	B
Fm		0.00035	0.04810	0.06910	0.14600	0.04452	0.44338
Dm	0.968		0.04260	0.03600	0.11010	0.02778	0.40267
Lt	0.758	0.771		0.00762	0.00598	0.01008	0.02377
Chl	0.718	0.786	0.888		0.00022	0.00001	0.01855
R	-0.610	-0.655	-0.898	-0.973		0.00055	0.00177
G	-0.766	-0.808	-0.874	-0.991	0.961		0.02753
B	-0.349	-0.378	-0.820	-0.838	0.938	0.809	

Comparative analysis of Chl variation depending on RBG color parameters by using different types of equations (second- and third-degree polynomial) conducted to the data from the Table 4.

After the analysis of the safety assurance parameters of equations (1) up to (6), table 4, it can be appreciated that the safest estimation of Chl was obtained based on G color parameter with a third-degree polynomial equation, graphical distribution can be seen in figure 2.

TABLE 4. Equations and safety assurance parameters for Chl variation depending on RBG color parameters

Estimated Indice	Color parameter	Equations	Eq No.	R^2	p	F test	RMSEP
Chl	R	$Chl = -0.0009772x^2 - 0.5681x + 69.39$	(1)	0.957	0.0018	44.421	3.19084
		$Chl = 2.064E-05x^3 - 0.004986x^2 - 0.05916x + 57.38$	(2)	0.959	0.0137	23.569	3.10137
	G	$Chl = -0.001375x^2 - 0.1281x + 66.16$	(3)	0.987	<0.001	158.62	1.71545
		$Chl = 0.0001184x^3 + 0.04259x^2 - 5.321x + 257.1$	(4)	0.997	<0.001	359.45	0.81214
	B	$Chl = 0.006096x^2 - 1.484x + 77.86$	(5)	0.712	0.0829	4.9458	8.24902
		$Chl = 0.0009671x^3 - 0.1234x^2 + 3.775x + 13.67$	(6)	0.732	0.2151	2.7371	7.95226

x – R values for Eq. (1), (2); *x* – G values for Eq. (3), (4); *x* – B values for Eq. (5), (6)

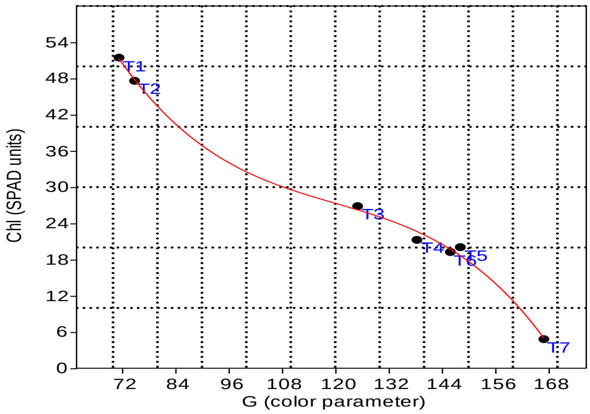

FIGURE 2. Graphical distribution of Chl in relation to color parameter G

Also, for Chl estimation in relation with RGB color parameters second- and third-degree polynomial equations were utilized. In all the cases, third degree models assured a better estimation of chlorophyll content (Chl) as against second degree models, for the same color parameter.

Fitting analysis of Chl measured values and the estimated one based on equation (4) conducted to linear equation (7) which described the estimated Chl valued in relation with the values of measured Chl values, with safety assurance conditions (R^2=0.997, p<0.001) and graphical distribution plotted in figure 3.

$$Chl_{Predicted} = 0.9986x - 0.0208 \quad (7)$$

where: x – $Chl_{measured}$

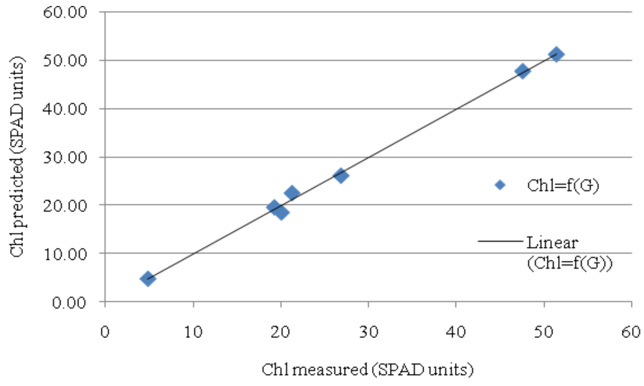

FIGURE 3. Fitting line between Chl predicted and Chl measured

PCA conducted to the distribution of the studied cases (T1 – T7) as in figure 4. PC1 explained 96.928% of variance, and PC2 explained 2.9718% of variance. After the analysis of the distribution of variance with by plot elements, the association of T1 variant with Chl, of T7 variant with B and R, of T4, T5 and T6 variants with Fm, Dm indices and G color parameter, and, on an independent position, T2 and T3 variants were placed.

Cluster analysis conducted to two different clusters, with statistical safety being assured, Coph. Corr=0.867. One cluster C1, which contains T1 and T2 variants, presented high chlorophyll content and high values of physiological indices values, for the studied indices. C2 has T3-T7 variants were grouped by affinity, being observed between T5 and T6 variants, SDI=9.923, table 5.

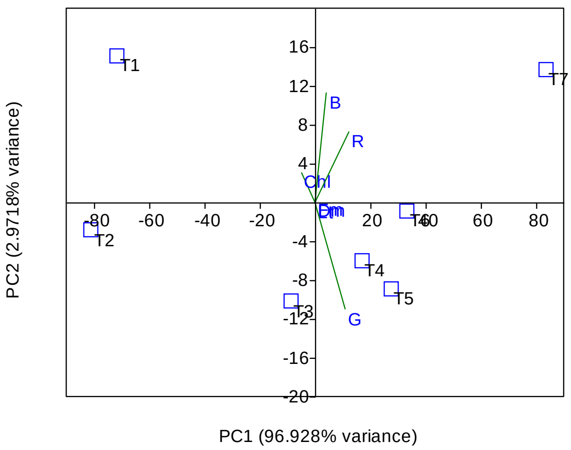

FIGURE 4. PCA diagram for variants distribution

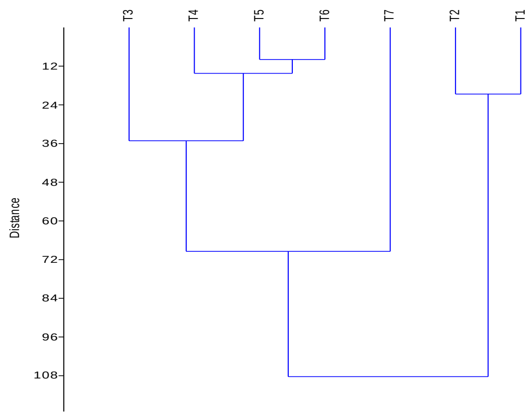

FIGURE 5. Dendrogram between the analyzed variants

TABLE 5. Similarity values and distance indices

	T1	T2	T3	T4	T5	T6	T7
T1		20.648	67.984	91.245	102.17	106.16	155.36
T2	20.648		72.864	98.228	109	114.43	165.56
T3	67.984	72.864		26.039	36.438	42.975	95.289
T4	91.245	98.228	26.039		11.376	17.086	69.439
T5	102.170	109.000	36.438	11.376		9.923	60.538
T6	106.160	114.430	42.975	17.086	9.923		52.532
T7	155.360	165.560	95.289	69.439	60.538	52.532	

Some important processes for plants, as flowering, germination, photosynthesis and biomass accumulation vary depending on color indices (Yeh & Chung, 2009; Vänninen *et al*., 2010).

In this study, high and moderate negative correlations were obtained between Dm and G (r=-0.808), but also between Fm and G (r=-0.766) and low correlations, negative, were obtained between Fm and R (r=-0.610) and Dm with G (r=-0.655). The values of correlation coefficients between Fm and B (r=-0.349) and between Dm and B (r=-0.378) had nonsignificant values. After the analysis of correlation coefficients between physiological indices Fm and Dm and RGB analyzed indices, the big importance of R and G specters into the synthesis of fresh substance and dry substance accumulation, when compared with B parameter. Similar results regarding the importance of RGB in plant photosynthesis process, fresh mass synthesis and dry mass accumulation were described by other authors. Red light, for example, has a massive role in developing photosynthesis devices while controlling the changes in light of the phytochrome devices (Urbonavičiūtė *et al*., 2007). On the other hand, blue light in general, required to promote normal development varies among species, developmental parameters within a species potentially responding to either the absolute or the relative amount of blue light (Dougher & Bugbee, 2001; Cope & Bugbee, 2013). There are also some studies which used imagining system and RGB indices to estimate leaf water content (Neilson *et al*., 2015). Variations in the concentrations of N, P, K, but also Ca and Mg contents are associated with varying spectra, including red, green, blue, and far-red components (Amoozgar *et al*., 2017).

CONCLUSION

This study main scope was to realize imagistic analysis for the evaluation of chlorophyll deficit in birch leaves (*Betula pendula* Roth.) for trees from an urban environment. Seven data groups were used depending on leaf chlorophyll content. Fresh and dry weights of leaves were very strong correlated. In all the cases of chlorophyll estimation in relation to the RGB color parameters, third degree polynomial models assured a better estimation of chlorophyll content (Chl) as against second degree models, for the same color parameter. Very high negative correlations were obtained between chlorophyll index and red (R), but also between chlorophyll index and green (G). Moreover, very high positive correlations were noticed between R and G and R and B indices. PCA analysis conducted on the two groups explained 96.928% of variance (PC1) and 2.9718% of variance (PC2) with statistical safety being assured (Coph. Corr=0.867).

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