Possibility of Surveying the Physiological Condition of Trees Growing in Urban Areas by Means of Total Phenol Content and Antioxidant Capacity Determined from the Leaves

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Abstract – The goal of the present research was to investigate how some specific chemical parameters of the leaves could reflect the physiological and health state of individuals of a given tree species (Norway maple, *Acer platanoides* L.) growing in an urban area. Two trees were considered for research, from which leves were collected and analysed. The trees originated not only from different parts of Sopron, Hungary but they could also be characterized with different living environments in terms of environmental load. Leaves were collected during the growth season between May and September at altogether nine occasions. The total phenol content and antioxidant capacity (DPPH assay) was determined from the leaves and the correlations of the two parameters were studied. Comparing the two parameters and investigating the environmental-load-sensitivity of the correlations new possibilites could open up for the characterization of the physiological state of the plant as whole and for the characterization and quantitation of the differences between control and sample individuals.

Keywords: correlation analysis / *Acer platanoides* / total phenols / DPPH assay / urban environment

1. INTRODUCTION

1.1. Outline and aims

The goal of the present research was to investigate how some specific chemical parameters of the leaves could reflect the physiological and health state of individuals of a given tree species (Norway maple, *Acer platanoides* L.) growing in an urban area. Two trees were considered for research, from which leaves were collected and analysed. The trees originated not only from different parts of Sopron, Hungary but they could also be characterized with different living environments in terms of environmental load. The control tree is located in the botanical garden of the University of West Hungary while the sample tree can be found at Csengery Street. The latter location can be characterized with heavy traffic and associated environmental load.

The total phenol content and the antioxidant capacity was determined from the leaves. As both of these parameters are stress sensitive, correlations between them can be suggested (TADHANI et al. 2007, TURKMEN et al. 2007). The principal idea was that different types of environmental impacts (i.e. different environmental conditions) could trigger biochemical processes of various type and extent which will result in the change of the level of the

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measured parameters as well as the parameters of the correlations between them. Comparing the two parameters and investigating the environmental-load-sensitivity of the correlations new possibilites could open up for the characterization of the physiological state of the plant as whole and for the characterization and quantitation of the differences between control and sample individuals (NÉMETH et al. 2009).

1.2. Materials

Two trees were considered for research, from which leaves were collected and analysed. The trees originated not only from different parts of Sopron, Hungary but they can also be characterized with different living environments in terms of environmental load. The control tree is located in the botanical garden of the University of West Hungary while the sample tree can be found at Csengery Street. The latter location can be characterized with heavy traffic and associated environmental load. Leaves were collected during the growth season between May and September at altogether nine occasions. At one sampling occasion seven leaves were collected from one tree from different parts of the foliage. Leaves were taken to the laboratory immediately after collection and processed further.

1.3. Methods

Leaves were extracted as follows: 0.5 g leaf was crushed and homogenized with 0.5 quartz sand in a mortar. 0.2 g of this mixture was extracted with 0.75 ml 80% aqueous methanol solution for 30 minutes in an ultrasonic bath. This was followed by centrifugation.

The determination of the total phenol content was carried out by spectrophotometry, using the method of Folin and Ciocalteu (SINGELTON – ROSSI 1965), applying quercetin as standard. Total phenol content was given in mmol/100g dry wood units.

Antioxidant capacity was measured using the DPPH-assay desribed in SHARMA – BHAT (2009). The value of the capacity was given as the IC_{50} value (concentration of the extract corresponding to the 50% inhibition level, ie. 50% of the DPPH oxidizes in the assay solution) in mg dry wood/ml extract units.

The dry mass content of the leaves was determined by drying 0.5 g of the sample leaves to constant weight at 105 ^{o}C in an oven.

2. RESULTS AND DISCUSSION

2.1. Total phenols content and antioxidant capacity

The mean values and standard deviations for the seven leaves at the sampling occasions are listed in *Table 1* (antioxidant capacity) and in *Table 2* (total phenol content). According to *Table 1* the leaves of the sample tree can be characterized with lower IC_{50} mean values (higher antioxidant capacity) than the leaves from the control tree. Standard deviation is usually high which means that there are large differences between the leaves at a given sampling time. The values of the IC_{50} show a clear increase in time in the case of both of the trees. This corresponds to decreasing antioxidant capacity of the tissues during the investigated vegetation period.

Regarding the total phenol contents, the leaves of the sample tree have higher levels of phenolic compounds than the leaves of the control tree. Standard deviation is significantly higher in the case of the sample tree. The mean total phenol contents decrease with time. The amount of this decrease is in the same order of magnitude for both of the trees.

The results highlight a possible connection between the two measured chemical parameters. The correlation between antioxidant value and total phenol content has already been discussed in the literature (TADHANI et al. 2007, TURKMEN et al. 2007).

Table 1. The mean values of the IC_{50} and the standard deviations for the seven leaf samples for each sampling occasion

	sample		**control**	
	IC_{50}	sd	IC_{50}	sd
June 2	0.0071	0.0038	0.0158	0.0046
July 13	0.0264	0.0234	0.0296	0.0138
July 27	0.0159	0.0131	0.0273	0.0113
August 9	0.0113	0.0067	0.0379	0.0216
August 24	0.0348	0.0204	0.0259	0.0060
September 14	0.0527	0.0341	0.0676	0.0241
	↓	↓	↓	↓
Group mean	0.0247	0.0169	0.0340	0.0136

Table 2. The mean values of the total phenol values and the standard deviations for the seven leaf samples for each sampling occasion

	sample		**control**	
	IC_{50}	sd	IC_{50}	sd
June 2	28.873	10.022	16.209	3.862
July 13	19.384	14.056	11.899	3.044
July 27	28.959	20.736	13.123	4.063
August 9	27.300	9.641	10.846	3.633
August 24	9.874	5.379	10.972	2.045
September 14	11.777	8.299	7.962	4.051
	↓	↓	↓	↓
Group mean	21.027	11.355	11.835	3.449

2.2. Correlation analysis

For analysing the possible relations between the two measured chemical features, linear correlations have been established between total phenol content and $1/IC_{50}$ value at each of the sampling occasions. To give an example for the analysis *Figure 1* depicts a typical linear correlation for the leaves (control and sample respectively) collected at June 2. The parameters of the equation (a: slope, b: intersection, R^2: coefficient of determination) have been calculated and evaluated.

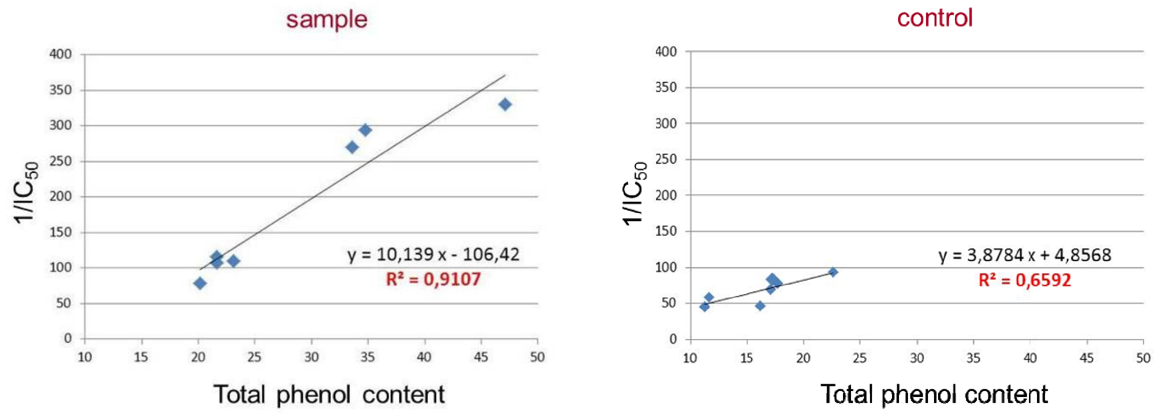

Figure 1. Linear correlation between total phenols and 1/IC_{50} values for the sampling occasion of June 2 (example)

Figure 1 shows clearly that there are pregnant differences between the respective parameters of the correlation equations for sample and control. The analysis has been carried out for each of the sampling occasions. Data is summed up in *Table 3*.

Table 3. The parameters of the linear correlations between total phenol content and 1/IC_{50} values for each sampling occasion. a: slope, b: intersection, R^2: coefficient of determination

		sample			**control**	
	a	b	R^2	a	b	R^2
June 2	10.139	-106.42	0.9107	3.878	4.858	0.6592
July 13	6.924	-41.16	0.8606	4.488	-13.833	0.7708
July 27	9.711	-101.89	0.8711	3.660	-6.038	0.9040
August 9	7.407	-78.44	0.9439	5.647	-23.163	0.5796
August 24	4.203	-1.93	0.8573	3.184	5.283	0.6154
September 14	2.046	3.31	0.8937	1.198	7.089	0.6017
	↓	↓	↓	↓	↓	↓
Group mean	6.738	-54.424	0.8896	3.676	-4.300	0.6885

It can be established that there are large differences between control and sample in terms of the *a*, *b* and R^2 values. For the sample, the values of *a* are higher, whereas *b* is generally lower as with the control. R^2 is higher in the case of the sample. According to these findings it can be stated that the two individuals can be distinguished according to the differences between the parameters of their correlation equations and their change in time.

Different types of environmental impacts could trigger biochemical processes of various type and extent which will result in the change of the level of the measured parameters as well as the parameters of the correlations between them. Comparing the two parameters and investigating the environmental-load-sensitivity of the correlations new possibilites could open up for the characterization of the physiological state of the plant as whole and for the characterization and quantitation of the differences between control and sample individuals.

By the further involvement of environmental parameters (e.g. sunshine duration, temperature, humidity, etc.) into the evaluation of the measured data the relations between the biochemical variables (state dependent linear regressions) and model establishment can further be refined, resulting in possible indicators for characterizing the relation between plant and its environment (SÁRDI ET AL. 1999, NÉMETH 2009, BOO ET AL. 2011).

3. CONCLUSION

Based on the measured data and the linear correlations between them, as well as on the temporal change of theses parameters the two individuals of the same tree species could be distinguished chemically. The cause for this could be tracked back to the differing environmental and physiological conditions under which these trees live. Besides of that the diverging genetic potentialities of the trees could not be excluded as a possible cause too, however. Further evaluations are required to find direct relations between the measured data and climatic conditions as well as air-pollution parameters.

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