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Biomonitoring Study of Air Pollution with Betula pendula Roth., from Plovdiv, Bulgaria

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Abstract. The present study is a small part of a program for application the methods of passive and active biomonitoring with tree, herbaceous, moss and lichen species for assessment of the anthropogenic factor in urban conditions. All reported results here are preliminary. *Betula pendula* was studied as a possible biomonitor of air pollution in Plovdiv. Eight sampling sites in the urban roadside, city center and suburban areas were investigated. Chlorophyll content was determined as essential and sensitive physiological parameter. The concentrations of 26 micro- and macroelements were analyzed by FAAS and ICP-MS. Maximum for chlorophyll was found in the birch leaves from west part of the town, minimum – in these from north part. More significant variations were detected for Ni, Mn, B, Cr, Co, Fe, Bi, Cd, Al, Zn. Highest concentrations of 12 elements were found in the samples, collected from the central area of Plovdiv.

Key words: biomonitor, *Betula pendula,* chlorophyll *a* and *b*, macroelements, microelements.

Introduction

Urbanization is one of the most drastic changes that can be imposed on an environment (MOLLOV & VALKANOVA, 2009). Urban ecosystems are comprised of diverse land uses including commercial, industrial, residential, transport, recreational, agricultural and nature areas, resulting in different habitats for plants, animals and human within urban landscape. Urban habitat quality results the integration of different abiotic and biotic components, such as air, soil and water quality, microclimate and the presence of vegetation.

The use of plants as passive biomonitors to complete the information on trace elements deposition from fully or semi-automatic gauges, commonly used in current pollution monitoring programs, obtain increasing attention. This reliable, versatile and inexpensive method can assist us on the subject of health and environmental protection against potentially hazardous trace elements. Providing a high density of sampling points, the biomonitors are very effective for tracing maps of airborne metal contamination in the urban environments (KLUMPP *et al.*, 2009; BAYCU *et al.*, 2006).

An advantage of plants as biomonitors is that they are effective collectors which reflect the accumulated effect of environmental pollution and accumulation of toxicants from atomspheric pollution (deposition, binding and solubility of metals on the leaf surface) and soil pollution (concentration and bioavailability of elements in soil).

Different biomonitors have been used for evaluation of the distribution of heavy metal pollution: mosses and lichens (ANIČIĆ *et al.*,

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2009; CULICOV & YURUKOVA, 2006; GONZALEZ *et al.*, 1996; STEINNES, 1993), grasses (KLUMPP *et al.*, 2009), many trees as chestnut (YILMAZ *et al.*, 2006), maple, linden, willow, birch (PICZAK *et al.*, 2003), poplar (DJINGOVA *et al.*, 1995), oak (MONACI *et al.*, 2000).

Betula pendula (Betulaceae) was successfully used as a biomonitor in many studies for assessment of the pollution level in different countries like Russia (KOZLOV *et al.*, 1995), England (MAHER *et al.*, 2008; MATZKA & MAHER, 1999; REY & JARVIS, 1998), Germany (FRANZARING *et al.*, 2006), Finland (RIIKONEN *et al.*, 2005; JUUROLA, 2003; PÄÄKKÖNEN *et al.*, 1998). It had been widely planted all over the town of Plovdiv as a ruderal ornamental tree and fulfills all basic criteria about selection of a species as a biomonitor, given by WITTIG (1993).

The aim of this study was to evaluate the reliability of *Betula pendula* as biomonitor by quantifying inorganic leaf content (26 trace elements, toxic and heavy metals) and concentrations of chlorophylls *a* and *b* as essential and sensitive physiological parameters.

Material and methods

Study area and sampling sites

The town of Plovdiv (42° 9' N, 24° 45' E), one of the most populated city of Bulgaria (over 365 000 inhabitants on 102 km^2), was selected as a study area. It includes several industrial zones, densely populated central area, some moderately populated areas around it, wide network of busy streets and train tracks, big parks and other green yards.

The climate in Plovdiv is temperate with mild influence from the Mediterranean Sea and a huge temperature range between summers and winters. The average annual temperature is 12.3°C with maximum in July (32.3°C) and minimum in January (6.5°C). The average relative humidity is 73%. It is highest in December (86%) and lowest in August (62%). The total precipitation is 540 mm – the wettest months of the year are May and June with an average precipitation of 66.2 mm, while the driest is August with an average of 31 mm. Gentle winds (0 to 5 m s^{-1}) are predominant in the city, winds with speed of up to 1 m s^{-1} represent 95% of all winds during the year. The prevailing wind direction is from west, rarely from east.

The sampling sites was selected as follows: 1 – Vegetable Crops Research Institute "Maritsa", 2 – Park "Lauta", 3 – Railway station "Trakiya", 4 – Ruski Boulevard (sidewalk), 5 – Nature monument Bunardzhik, 6 – Housing estate "Smirnenski", 7 – Park "Otdih i kultura", 8 – near Foreign language school, at the NW end of the town (Fig. 1).

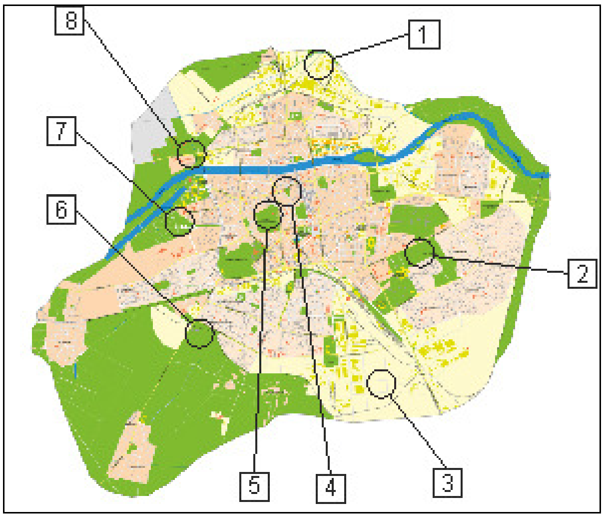

Fig. 1. Sampling sites in Plovdiv (Bulgaria)

Sampling and sampling preparation

At each sampling site were chosen at least two birch trees (diameter 20-25 cm), growing at similar light conditions and mineral nutrition. They must have been from 5 to 10 m away from intense traffic, except site 4 – Ruski Bul., where trees were on the sidewalk, up to 1 m from the roadway. Sampling period was 13-14 June 2010. Leaves were sampled from the lower part of the tree crown at the 2.5–3 m height in all directions. Usually 20-40 fully expanded leaves were collected and a composite sample was prepared for analyses. All the samples were stored in clean, labeled, polyethylene bags, closed tightly to avoid contamination during transportation. Determination of chlorophyll content at laboratory conditions was carried out immediately after sampling. Plant material for additional analyses was air dried for two weeks, ground to a powder and homogenized.

Chlorophyll analysis

Pigment analysis followed SHLYK (1965). Spectrophotometric reading of photosynthetic pigments was performed after extraction with 90% acetone (SPECOL 11 absorption spectrophotometer) at the Faculty of Biology, University of Plovdiv "Paisii Hilendarski". All the analyses were conducted in three replications.

Concentrations of chlorophyll (chl) *a* and *b* were calculated as follows:

$C_a = 9.784 \times E_{662} - 0.990 \times E_{644}$

$C_b = 21.426 \times E_{644} - 4.650 \times E_{662}$

$C_{a+b} = 5.134 \times E_{662} + 20.436 \times E_{644}$,

where C_a – concentration of chl *a* in mg l^{-1}, C_b – concentration of chl *b* in mg l^{-1}, C_{a+b} – total content of chlorophylls in mg l^{-1}.

The received results were recalculated in mg g^{-1} fresh weight:

$C_a' = (C_a \times V \times R) \times g^{-1}$

$C_b' = (C_b \times V \times R) \times g^{-1}$

$C_{a+b}' = (C_{a+b} \times V \times R) \times g^{-1}$,

where C_a – concentration of chl *a* in mg l^{-1}, C_b – concentration of chl *b* in mg l^{-1}, C_{a+b} – total content of chlorophylls in mg l^{-1}, V – volume of extract in l, R – dilution (if it was necessary), g – starting fresh weight of the sample.

Chemical analysis

About 1 g ground plant material was treated with 5 ml 65% nitric acid (Merck) for 24 h at room temperature. The wet-ashed procedure was assisted by a Microwave Digestion System CEM MDS 81D. Samples were treated for 5 min at maximum power (600 W) in closed vessels. After cooling for 1 h at room temperature, vessels were opened and 2 ml nitric acid and 3 ml 30% hydrogen peroxide were added and were left to react for another 1 h. Vessels were closed and treated by the Microwave Digestion System for 10 min again at 600 W for full digestion of the organic matter. The filtrate was diluted with double distilled water up to 50 ml.

The elements Zn, Fe, K, Mg, Mn, Na, Cu and Ca were determined by FAAS method using Atomic Absorption Spectrometer PERKIN-ELMER 4000 (flame air - acetylene). The sample solutions with dilution factors from 50 till 250 were additionally spiked with La as releasing agent - 0,05% La for Zn, Fe, K, Mg, Mn, Na, Cu, and 1% La for Ca. Calibration standards Multy VI (MERCK) from 0.5 up to 40 ppm were used for different elements.

The content of Be, B, Al, S, P, Cr, V, Co, Ni, As, Se, Sr, Mo, Cd, Hg, Pb, Bi and U were determined by inductively coupled plasma mass spectrometry (ICP-MS) using instrument Agilent 7700 ICP-MS (2009), DF 1000. All samples, blanks and standards were spiked with internal standards - Ge 50 ppb and Rh 5 ppb final concentration in the solutions. Calibration standards Multy VI (MERCK) were freshly prepared from 1 to 1000 ppb in 0.05 volume% HNO_3 (p.a.) Monostandard of Hg 100 ppt was also used in the calibration. Signals of suitable isotopes for the tested elements have been measured twice in both modes - without and with helium gas collision cell.

Statistical analysis

For evaluation of determined concentrations a descriptive statistical analysis was applied. For grouping the studied elements a cluster analysis was used (Unweighted pair-group average linking and Pearson's index distance measure) and the relationships between the contents of individual elements in collected leaf samples were tested using Spearman rank correlation coefficients. For all statistical analysis the STATISTICA 7.0 statistical package was used (STATSOFT INC., 2004).

Results and Discussion

Chlorophyll content

Chlorophyll *a* content varied between 1.32 and 1.89 mg g^{-1} within the study areas, whereas, chl *b* content varied from 0.54 to 1.52 mg g^{-1} (Fig.2). Maximums of chl *a*, chl *b* and total chlorophyll content have been observed in the sample from site 7. Chlorophyll *a/b* ratio varied from 1.41 to 2.44 (Table 1). It had highest value in leaves from site 1 as a result of minor anthropogenic impact and lowest in leaves from site 7, in negative correlation with chlorophyll content.

The lowest concentrations of chlorophyll have been observed in the sample from site 1, the sampling site situated in the NE end of Plovdiv and being most distant from the central part of the town. The higher concentrations in other seven sites are due to the intensive anthropogenic activity.

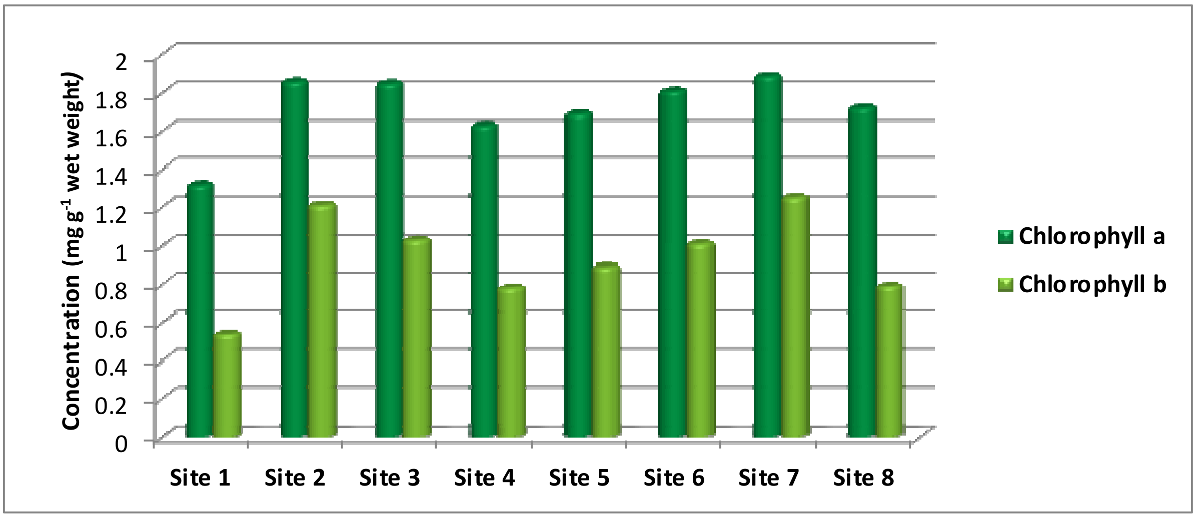

Fig.2. Comparison of chlorophyll concentrations in birch leaves from different sampling sites

Table 1. Levels of chlorophyll in birch leaves (mg g^{-1} wet weight**)**.

Sampling site	**Chl *a***	**SD**	**Chl *b***	**SD**	**Chl *a+b***	**SD**	**Ratio chl *a/b***	**SD**
1	1.32	0.22	0.54	0.14	1.86	0.13	2.44	0.19
2	1.86	0.22	1.21	0.35	3.07	0.13	1.53	0.36
3	1.85	0.05	1.03	0.12	2.88	0.03	1.79	0.15
4	1.63	0.60	0.78	0.31	2.42	0.04	2.09	0.05
5	1.69	0.10	0.89	0.10	2.58	0.06	1.90	0.10
6	1.81	0.04	1.01	0.07	2.83	0.02	1.79	0.09
7	1.89	0.09	1.25	0.21	3.14	0.06	1.51	0.21
8	1.72	0.12	0.79	0.14	2.51	0.07	2.18	0.21

The birch leaves from roadside trees (sample from site 4) showed quite low content of pigments, followed by the leaves from site 5. That can be explained with stress reaction of plant and the degradation of chlorophyll (ALI, 1991). The studied pigment had increased levels as a compensatory mechanism towards enhanced concentrations of air pollutants. It could be supposed that reaching of determined pollution level interrupted photosynthesis process, followed by chlorophyll degradation.

Similar results about pigment concentrations in birch leaves, sampled in June from 7 years old trees (in Sofia, Bulgaria), had been reported by IVANOVA & VELIKOVA (1990). In cited publication the average chlorophyll *a* and *b* concentrations were 1.65 mg g^{-1} wet weight and 0.92 mg g^{-1} wet weight, respectively. The average chl *a* content, found in this study, was 1.72 mg g^{-1} (4% higher) and the average chl *b* content was 0.94 mg g^{-1} (2% higher).

Inorganic content

The results from the chemical analyses of collected leaved samples are presented in Table 2. Under detection limits were some elements like Be, As, Se, Mo, Hg (0.1, 0.5, 0.6,

Table 2. Mean values and RSD of micro- and macroelements in birch leaves.

Sampling site	B mg kg^{-1}	RSD %	Al mg kg^{-1}	RSD %	V mg kg^{-1}	RSD %	Cr mg kg^{-1}	RSD %	Co mg kg^{-1}	RSD %
1	36	2.4	40	0.8	0.11	2.6	0.31	7.2	0.11	3.8
2	31	1.4	54	9.5	0.14	2.1	0.44	2.3	0.14	4.0
3	29	1.1	57	14.6	0.14	3.3	0.43	3.9	0.14	2.1
4	61	1.4	75	14.9	0.17	9.1	0.58	18.2	0.11	9.4
5	13	2.9	75	1.2	0.27	1.1	1.2	2.6	0.13	3.0
6	20	2.4	102	6.0	0.26	2.6	0.91	15.6	0.11	3.0
7	44	1.2	36	16.5	0.12	6.3	0.27	6.1	0.39	1.5
8	51	3.2	36	0.6	0.11	2.3	0.39	7.0	0.22	2.1

Table 2. Continued…

Sampling site	Ni mg kg^{-1}	RSD %	Sr mg kg^{-1}	RSD %	Cd mg kg^{-1}	RSD %	Pb mg kg^{-1}	RSD %	Bi mg kg^{-1}	RSD %
1	0.48	3.6	81	0.4	0.17	3.5	1.17	0.8	0.72	-
2	0.61	1.2	73	0.4	0.36	2.3	3.34	0.7	0.68	-
3	0.59	4.7	72	0.2	0.34	1.7	3.27	0.7	0.66	-
4	2.3	1.1	39	1.7	0.12	2.4	1.31	1.3	1.73	15.3
5	0.65	4.7	43	0.2	0.25	5.5	2.66	1.1	0.71	-
6	1.4	1.4	32	1.3	0.28	0.6	3.04	0.7	0.54	-
7	1.7	1.9	34	0.8	0.18	3.6	1.36	1.5	0.67	-
8	0.41	7.1	41	1.3	0.13	5.2	1.24	0.9	0.70	-

Table 2. Continued…

Sampling site	Zn mg kg^{-1}	RSD %	Cu mg kg^{-1}	RSD %	Na mg kg^{-1}	RSD %	Fe mg kg^{-1}	RSD %	Mn mg kg^{-1}	RSD %
1	104	0.9	4.5	4.6	30.2	1.0	89.3	1.8	85	1.0
2	102	1.0	5.1	4.1	29.8	0.8	97.9	2.0	44	0.7
3	142	0.8	4.9	3.9	27	2.7	103.4	1.8	44	0.6
4	85	1.5	3.8	4.6	26.6	0.7	115.3	1.2	56	0.9
5	140	0.9	5.3	8.4	47.3	1.4	248.8	2.5	187	1.6
6	122	0.9	4.7	8.3	25.9	1.0	177.4	3.3	34	1.1
7	122	0.9	3.4	11.8	31.7	1.5	77.7	1.9	44	0.8
8	237	0.7	6.1	9.7	27.7	1.0	97.9	2.6	57	1.1

Table 2. Continued…

Sampling site	P %	RSD %	S %	RSD %	Mg %	RSD %	K %	RSD %	Ca %	RSD %
1	0.21	1.0	0.29	5.2	0.31	0.5	1.62	0.5	1.5	0.8
2	0.19	0.8	0.29	5.6	0.26	1.6	1.25	1.2	1.7	1.6
3	0.23	0.4	0.30	4.0	0.45	1.9	1.00	1.2	1.3	0.9
4	0.15	1.6	0.27	4.9	0.23	1.1	1.31	3.2	1.1	0.8
5	0.25	0.8	0.28	2.6	0.26	1.6	1.10	0.6	1.2	0.7
6	0.16	0.4	0.25	3.6	0.29	1.2	1.01	1.4	1.1	1.5
7	0.25	0.7	0.28	2.9	0.37	0.7	1.10	1.6	1.2	0.7
8	0.28	0.9	0.27	4.5	0.32	1.1	1.55	1.5	1.1	0.9

0.4, 0.05 mg kg^{-1}, respectively) and they are not showed in this table. Exceptions were determined for As (0.5 mg kg^{-1}) in site 4, Hg (0.05 mg kg^{-1}) in sites 2 and 3, also for Mo (0.45 mg kg^{-1}) in site 7 and (0.86 mg kg^{-1}) in site 4. The concentrations of uranium were between 0.01 and 0.02 mg kg^{-1} in all cases, higher in sites 4, 5 and 6, and are not presented too.

The average concentration of macro- and microelements in the *Betula pendula* leaves, collected in the town of Plovdiv, was in the descending order as follows: Ca (1.3%)>K (1.24%)>Mg (0.31%)>S (0.28%)>P (0.22%)>Zn (132 mg kg^{-1})>Fe (126 mg kg^{-1})>Mn (69 mg kg^{-1})>Al (60 mg kg^{-1})>Sr (52 mg kg^{-1})>B (36 mg kg^{-1})>Na (30.8 mg kg^{-1})>Cu (4.7 mg kg^{-1})>Pb (2.18 mg kg^{-1})>Ni (1.01 mg kg^{-1})>Bi (0.80 mg kg^{-1})>Cr (0.56 mg kg^{-1})>Cd (0.23 mg kg^{-1})>V (0.16 mg kg^{-1})>Co (0.17 mg kg^{-1})>U (0.01 mg kg^{-1}).

The maximums of 6 elements were measured in birch leaves from the sidewalk of Ruski Boulevard (B, Ni, Bi, As, Mo and U) and from the Nature monument "Bunardzhik" (V, Cr, U, Na, Fe and Mn). These two sampling sites, situated along one of the major traffic arteries in Plovdiv, differed only by the greenbelt which separated "Bunardzhik" from the road.

The maximums of 4 elements (Cd, Hg, Pb and Ca) were detected in sample from site 2 (Park Lauta). This highest content of pointed elements could be due to a carting speedway (open about 3 years ago). Three highest concentrations were obtained in site 3 – near the Railway station Trakiya (S, Mg, Hg) and in site 8 (Zn, Cu, P).

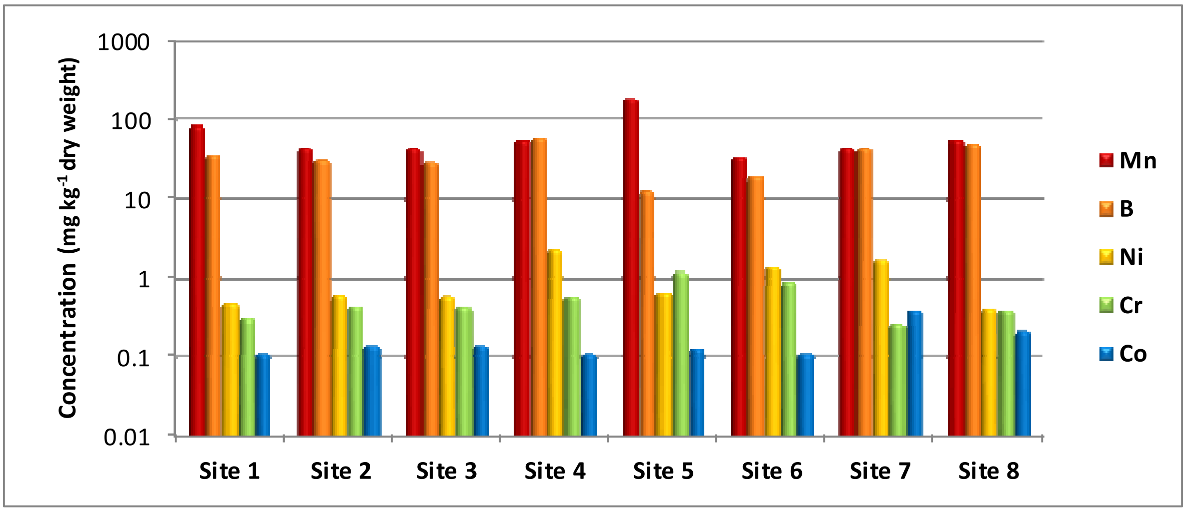

Fig. 3. Comparison of Ni, Mn, B, Cr and Co concentrations in the birch leaves from different sampling sites

Most significant variation (6-fold) were detected for nickel - from 0.41 mg kg^{-1} at site 8 to 2.3 mg kg^{-1} at site 4 and for manganese - from 34 mg kg^{-1} at site 6 to 187 mg kg^{-1} at site 5. Boron, chromium and cobalt varied 4-5 times: B - from 13 mg kg^{-1} at site 5 to 61 mg kg^{-1} at site 4, Cr – from 0.27 mg kg^{-1} at site 7 to 1.2 mg kg^{-1} at site 5, and Co - from 0.11 mg kg^{-1} at sites 1 and 6 to 0.39 mg kg^{-1} at site 7 (Fig. 3).

Sulphur was the biogenic element which varied insignificantly in the birch leaves from the selected sampling sites. PICZAK *et al.* (2003) studied element content, analyzed by ICP-AES method, in birch leaves, collected in June in Wrocław, Poland. The content of Cr (0.50 μg g^{-1}), Al (45.9 μg g^{-1}), Ca (12300 μg g^{-1}), Mg (2240 μg g^{-1}) are similar with the concentrations found by us. Lead found in our study is slightly higher while cadmium is 5 times lower in comparison with the birch Polish leaves.

Statistical evaluation

The cluster analysis divided the studied elements in two major groups (Fig. 4). The first group consisted of only three elements – B, Ni and Bi, which appeared to have similar deposition levels in the leaves. The second major group was further separated into two subgroups. The first one clustered Al, V, Cr, Fe, Na and Mg and the second group was segmented into two smaller clusters – (Co, Mg, Zn, P, Cu and K) and (Sr, Ca, S, Cd and Pb).

The elements Ca, Mg, K, P and S (together with N and Cl, the concentrations of which were not determined) represented the main inorganic components in plants. For birch leaves this fact was confirmed by the statistical analysis, which clustered them into one subgroup (according the uniformity and similarity of distributions of these elements). Zinc, being a key functional microelement in plant physiology, also had similar accumulation

The Spearman's rank correlation analysis revealed some significant correlations between the analysed chemical elements (Table 3). The content of Fe, which resulted primarily from the abrasion of vehicle brake linings, was closely correlated with Al, Cr and V concentrations. High correlation coefficient was found between Pb and Cd, which are released during the combustion of fuel. Therefore, motor vehicle traffic may represent the main emission source for these groups of pollutants.

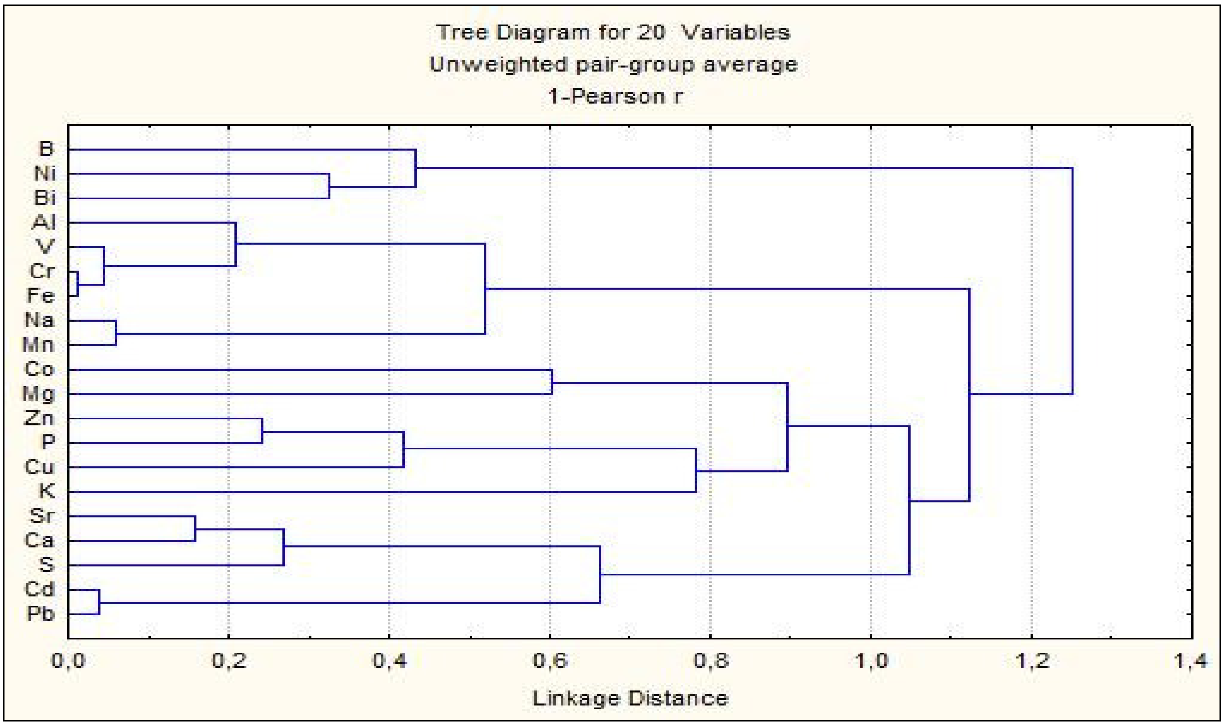

Fig. 4. Tree diagram for analyzed chemical elements
(Cluster analysis, Unweighted pair-group average, Pearson's index)

Based on this correlation analysis, it could be supposed the existence and the effect of different synergistic and antagonistic relationships between chemical elements. That was clearly observed for Cd and B, K and Pb, as well as Co and Al, where a negative correlations were strongly expressed (r>0.7) Close positive correlations (r>0.8) were observed with the plant macroelements Ca and S, Ca and Sr, as well as S and Sr, P and Zn, P and Co (in this case r>0.74).

Conclusions

Pigment response to urban air pollution was studied in birch leaves from 8 sampling sites in Plovdiv; highest chlorophyll values were observed at medium level of urbanization and road traffic pressure, probably as a result of enhanced protection against stress, which seemed to be not efficient in conditions of strong anthropogenic impact.

Inorganic characteristic of leaf samples tended to indicate high values in dense traffic and industrialized areas and low values in the less urbanized areas. Correlation coefficients were calculated for each element-element combination; many positive and three negative relationships were found.

Table 3. Spearman correlations between micro- and macroelements in birch leaves (positive significant correlations are marked in grey, negative – in pink), p<0,05.

Element	B	Al	V	Cr	Co	Sr	Cd	Pb	Bi	Zn	S
Al	-0.530										
V	-0.590	0.896									
Cr	-0.548	0.892	0.916								
Co	0.184	-0.721	-0.422	-0.528							
Sr	-0.119	-0.265	-0.349	-0.190	-0.061						
Cd	-0.738	0.241	0.313	0.238	0.160	0.238					
Pb	-0.643	0.446	0.530	0.405	0.037	0.095	0.929				
Bi	0.476	-0.096	-0.120	0.024	-0.344	0.357	-0.690	-0.595			
Zn	-0.287	-0.236	-0.145	-0.096	0.506	-0.084	0.096	-0.120	-0.407		
Fe	-0.539	0.891	0.885	0.958	-0.513	-0.252	0.144	0.299	-0.012	0.096	
Mn	0.098	-0.198	-0.136	0.024	-0.126	0.464	-0.512	-0.586	0.805	0.123	
P	-0.036	-0.642	-0.400	-0.395	0.741	0.084	-0.084	-0.323	-0.084	0.801	
S	-0.206	-0.307	-0.288	-0.352	0.231	0.812	0.473	0.376	-0.012	0.018	
K	0.635	-0.473	-0.582	-0.347	-0.148	0.371	-0.659	-0.731	0.778	-0.319	-0.085
Ca	-0.282	-0.273	-0.248	-0.282	0.127	0.822	0.577	0.454	-0.012	-0.216	0,900

The passive biomonitoring with *Betula pendula* proved to be a simple and reliable tool for assessing and monitoring the air pollution in Plovdiv. In this way, biomonitoring with tree leaves will contribute additional information to the routine monitoring program in urban areas. Data of this investigation represented the pollution situation in a specific growing season. It will be necessary to make repetitions for obtain information about temporal trends and to demonstrate the increasing or decreasing environmental relevance of air pollutants.

In conclusion, our study fully supports the view that *Betula pendula* can be a usefull biomonitor of air pollution as it is a commonly distributed species, the leaves are easy to sample and show clear response to differences in air quality. Therefore, future studies are needed to establish such correlations, and to find out if other tree species are more sensitive to air pollution, and thus are better suited for passive biomonitoring.

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