

## RESEARCH ARTICLE

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## Horse chestnut (*Aesculus hippocastanum* L.) as a biomonitor of air pollution in the town of 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 phytomonitoring with herbaceous species, trees, mosses and lichens for assessment of the anthropogenic factor in urban conditions. *Aesculus hippocastanum* L. was studied as a possible biomonitor of air pollution with heavy metals and toxic elements in the town of Plovdiv (Bulgaria). Concentrations of Cd, Cr, Cu, Pb, V and U in leaf samples from urban areas with different anthropogenic impact were compared. Motor transport was found to be the major source of contaminants. It was found the significant contribution of some factors as urban gradient, canyon-street effect and wind rose in forming the urban air quality.

**Key words:** *Aesculus hippocastanum*, biomonitoring, air pollution

## Introduction

The concentrations of many chemical elements in the atmosphere have been changed due to the anthropogenic activities. Most of trace metals in terrestrial ecosystems originate from atmospheric wet and dry deposition. Studies of their transport and mobilization have attracted attention from many years, and have shown that trace metals are persistent, widely dispersed in the environment, and interacting with different natural components, cause threat to human health and environment (Alcamo, 1992; Pacyna et al., 2007; Takuchev, 2011a,b).

Studies on atmospheric contamination have frequently been limited by high cost of instrumental monitoring methods, and difficulties in carrying out extensive sampling in time and space. Also, the instrumental monitoring technics lack information on impact of atmospheric pollutants on the living systems. Hence, there has been an increasing interest in using indirect monitoring methods based on a response of organisms that may act as bioaccumulators. The use of plants as passive biomonitors to complete the information on trace elements deposition from automatic gauges, obtain increasing

attention (Steinnes et al., 1994; Aničić et al., 2009a,b; Thöni et al., 2011). Relatively easy sampling, no need of complicated and expensive equipment, and integrated and accumulative behavior of the biomonitors in the time, contribute to the development of the biomonitoring of air pollution in the foreseeable future. An advantage of plants as biomonitors is that they provide information not only on quality/quantity of air pollutant concentrations, but also about air pollution effects on a living system. Providing a high density of sampling points, biomonitors are very effective for tracing maps of airborne metal contamination in the urban environments (Tomašević et al., 2001; Piczak et al., 2003; Tomašević et al., 2005; Culicov & Yurukova, 2006; Aničić et al., 2011).

Trees are very efficient in trapping atmospheric particles, mostly on their foliage (Nowak et al., 2006) and they have a special role in reducing the level of fine, "high risk" respirable particulates with the potential to have adverse effects on the environment and human health (Beckett et al., 2000; WHO, 2003). Leaves of various deciduous tree species have been studied for this purpose in urban areas with various successes (Tomašević et al., 2001; Piczak et al., 2003;

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Tomašević et al., 2005; Aničić et al., 2011). *Aesculus hippocastanum* L. is one of the most selected plants because of its large and palmately compound leaves, easy recognition and sampling, and also because it has been widely cultivated in streets and parks as an ornamental tree.

The specific location and topography of the town of Plovdiv, on the one hand, and the continuing high level of air pollution with heavy metals and toxic elements on the other, are a prerequisite for the application of the methods of biomonitoring. However, until now, in Plovdiv have been conducted single phytomonitoring studies and they were concerning only individual point sources (Dushkova & Ninova, 1977, 1981, 1982; Dimitrova, 2000; Dimitrova & Yurukova, 2005; Hristeva et al., 2011).

The aim of this study was to: (1) evaluate the reliability of *Aesculus hippocastanum* as a possible biomonitor by quantifying some heavy metals and toxic elements like Cd, Cr, Cu, Pb, V and U in leaf samples from urban areas with different anthropogenic impact; (2) assess the contribution of some factors as urban gradient, canyon-street effect and wind rose for the forming of the urban air quality.

## Materials and Methods

### Study area and sampling sites

Plovdiv (42°9'N, 24°45'E) is situated in south-central part of Bulgaria, in the southern part of the Plain of Plovdiv, on the two banks of the Maritsa River. It is the second-largest city after the capital Sofia with a population of over 338 000 inhabitants (as of February, 2011). Inside the city proper are six syenite hills, 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.

Plovdiv has a humid continental climate and a huge temperature range between summer and winter. Average annual temperature is 12.3°C with maximum in July (32.3°C) and minimum in January (6.5°C). Average relative humidity is 73%, highest in December (86%) and lowest in August (62%). Gentle winds (0 to 5 m s<sup>-1</sup>) are predominant in the city, winds with speed of up to 1 m s<sup>-1</sup> represent 95% of all winds during the year. The prevailing wind direction is from west, rarely from east.

According to the urban gradient theory, six areas with different level of anthropogenic pressure were selected (GPS Garmin eTrex Vista HCx): 1 – Nature monument “Bunardzhik”; 2 – Park “Lauta”; 3 – Neighborhood “Hristo Smirnenski”; 4 – Vegetable Crops Research Institute “Maritsa”; 5 – Railway station “Trakiya”; 6 – Park “Otdih i kultura” (Figure 1).



Figure 1. The town of Plovdiv and location of the sampling areas

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**Sampling and sample preparation**

At each sampling site were chosen at least two chestnut trees (diameter 45-55 cm), growing at similar light conditions and mineral nutrition, from 5 to 10 m away from intense traffic. 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 20-30 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. At laboratory conditions plant material for chemical analyses was air dried for two weeks, ground to a powder and homogenized.

**Chemical analysis**

About 1 g ground plant material was treated with 5 ml 65% nitric acid (Merck) for 24 h at room temperature. Samples were treated for 5 min at 600 W (Microwave Digestion System CEM MDS 81D) 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. For full digestion of the organic matter, samples were treated for 10 min again at 600 W. The filtrate was diluted with double distilled water up to 50 ml.

The content of Cd, Cr, Cu, Pb, V and U was 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 Rh5 ppb final concentration in the solutions. Calibration standards Multy VI (MERCK) were freshly prepared from 1 to 1000 ppb in 0.05% HNO<sub>3</sub> (p.a.).

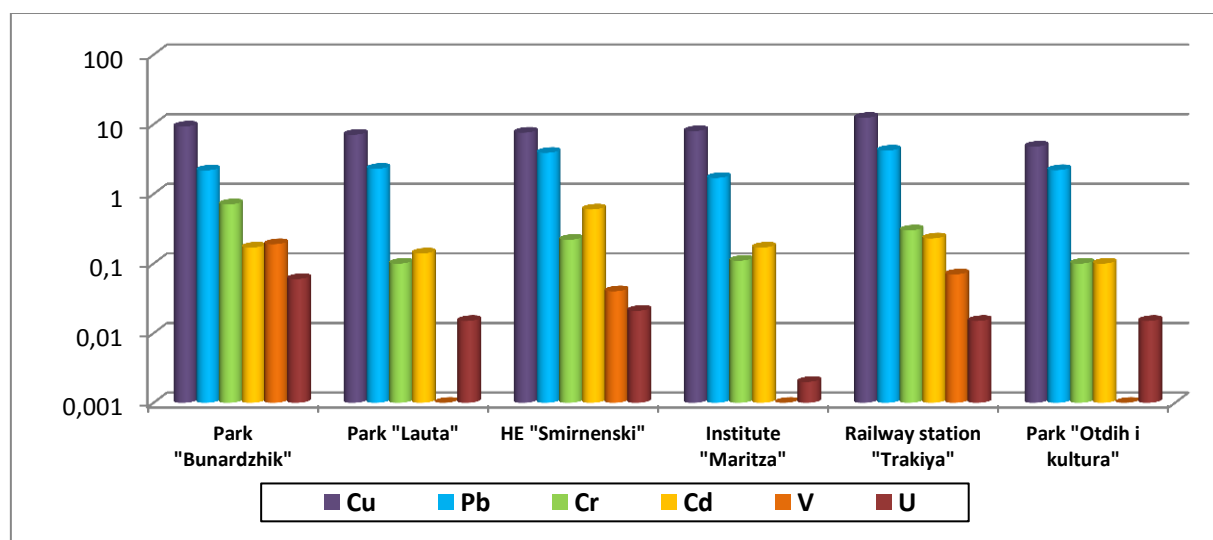
**Statistical analysis**

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**

The results from the chemical analyses of collected leaved samples are presented in Figure 2. Average concentration of the studied elements in the *Aesculus hippocastanum* leaves, collected from the town of Plovdiv were in the descending order as follows: Cu ( $8.2 \pm 0.04 \text{ mg.kg}^{-1}$ ) > Pb ( $2.75 \pm 0.03 \text{ mg.kg}^{-1}$ ) > Cr ( $0.25 \pm 0.01 \text{ mg.kg}^{-1}$ ) > Cd ( $0.24 \pm 0.05 \text{ mg.kg}^{-1}$ ) > V ( $0.05 \pm 0.01 \text{ mg.kg}^{-1}$ ) > U ( $0.012 \pm 0.001 \text{ mg.kg}^{-1}$ ).

Aničić et al. (2011) have given the concentration of Cr ( $0.53 \mu\text{g.g}^{-1}$ ), Cu ( $12.3 \mu\text{g.g}^{-1}$ ) and Pb ( $1.5 \mu\text{g.g}^{-1}$ ) in chestnut leaves, sampled in three parks from the urban area of Belgrade in May, 2006.



**Figure 2.** Concentrations of analyzed elements ( $\text{Log}_{10}$  values)

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Yilmaz et al. (2006) have reported the concentrations of Pb ( $0.11 \mu\text{g.g}^{-1}$ ), Cd ( $0.07 \mu\text{g.g}^{-1}$ ) and Cu ( $0.48 \mu\text{g.g}^{-1}$ ) for leaves of same plant species, collected from the region of Trace, Turkey (Edirne, Luleburgaz, Chorlu and Tekirdagi). Excluding the copper and chromium concentrations in Belgrade samples, all these data showed from 3 to 25 times lower values in comparison with our results. So, it can be concluded that the air quality in Plovdiv is rather worsening.

**Copper**

Although copper is an essential enzymatic element for normal plant growth and development, it can be toxic at concentrations above  $25 \text{ mg.kg}^{-1}$  (Allen, 1989). Kabata-Pendias & Pendias (2001) also showed normal Cu content in plant tissues ranging from 5 to  $20 \text{ mg.kg}^{-1}$ , and values below  $2\text{-}5 \text{ mg.kg}^{-1}$  – as a deficit of this element.

In our study, minimal content of this element we found in leaf samples from Site 7 ( $4.8 \pm 0.3 \text{ mg.kg}^{-1}$ ). Maximal Cu concentration was measured in chestnut leaves from Site 3 – Railway Station “Trakiya” ( $12.4 \pm 0.4 \text{ mg.kg}^{-1}$ ), followed by Site 5 – Nature monument “Bunardzhik”, with  $9.3 \pm 0.4 \text{ mg.kg}^{-1}$ . These values were 10% higher in comparison with copper concentration in *Quercus robur* leaves ( $11.3 \text{ mg.kg}^{-1}$ ), collected near two busy crossroads in Sofia, the biggest city in our country (Doncheva-Boneva, 2000).

**Lead**

Lead is regarded as very hazardous for the biota. Normal concentrations in plants given by Kabata-Pendias & Pendias (1992), are  $0.1\text{-}10 \text{ mg.kg}^{-1}$ . Izraely (1989) showed that the amount of lead in leaves of deciduous and coniferous vegetation in Europe varies between  $1.5$  and  $2.1 \text{ mg.kg}^{-1}$ . According this criterion, it could be concluded that the air in Plovdiv, assessed by foliar uptake in chestnut leaves, is polluted with Pb, as the highest value obtained was  $4.2 \pm 0.1 \text{ mg.kg}^{-1}$  (Railway Station “Trakiya”), i.e. 2.5 fold higher than the average value for Europe and 3 fold lower in comparison with lead concentrations in oak leaf samples from Sofia given by Doncheva-Boneva (2000).

Previous data for lead atmospheric concentrations in Plovdiv (Tarnovska et al., 2003) were quite higher than those, obtained in our study. This might be a consequence of diminished usage of leaded gasoline in favor of the unleaded kind, similarly of the concentration trends, reported for other European cities: Belgrade (Tomašević & Aničić, 2010), Warsaw (Dmuchowski & Bytnerowicz, 2009), Rome (Gratani et al., 2008).

**Chromium**

Chromium is also well known as a very toxic element. Kabata-Pendias & Pendias (2001) reported that its normal values are between  $0.1$  and  $0.5 \text{ mg.kg}^{-1}$ , and concentrations  $> 5 \text{ mg.kg}^{-1}$  are very phytotoxic.

Our study revealed that chromium concentration in leaf samples from the central part (Natural monument “Bunardzhik”) exceeds more than 2.5 fold the concentrations in the other sampling sites. Average chromium content in Plovdiv leaf samples ( $0.25 \pm 0.01 \text{ mg.kg}^{-1}$ ) is 2 fold lower in comparison with data from Belgrade (Tomašević & Aničić, 2010).

**Cadmium**

Cadmium is a very toxic element to all living organisms. It is taken up by plants primarily from the soil through the roots and from the air through the leaves. Generally, it is accepted that the normal Cd concentrations in plants are  $0.2\text{-}0.8 \text{ mg.kg}^{-1}$  and toxic levels are defined as  $5\text{-}30 \text{ mg.kg}^{-1}$  (Kabata-Pendias & Pendias, 1992). According these criterions, the area of Plovdiv is not highly polluted by Cd since its content in all samples did not exceed the upper limit:  $0.6 \pm 0.03 \text{ mg.kg}^{-1}$  in Site 6,  $0.23 \pm 0.01 \text{ mg.kg}^{-1}$  in Site 3 and below  $0.2 \text{ mg.kg}^{-1}$  in the other studied locations.

**Vanadium**

Some authors have reported that V act as a specific catalyzer of nitrogen fixation and can partially replace molybdenum in this function. Welch & Cary (1975) have found an average content in the plant tissues below  $0.002 \text{ mg.kg}^{-1}$ .

In three of selected sites – Site 1, Site 2 and Site 7 – the concentration of vanadium in the collected leaf samples were below the given in the literature limit. Highest value we found in samples from Nature monument “Bunardzhik” ( $0.19 \pm 0.01 \text{ mg.kg}^{-1}$ ), followed by Railway Station “Trakiya” ( $0.07 \pm 0.01 \text{ mg.kg}^{-1}$ ). Our data were higher in comparison with those from Welch & Cary (1975), but two times lower of vanadium level in chestnut leaves from Belgrade (Tomašević & Aničić, 2010).

**Uranium**

Like the other radionuclides, in elevated amount uranium can be toxic for all living organisms. It persists as a dust in the air and through wet and dry atmospheric deposition reaches the earth. It was found that plants absorb it primarily through the roots and keep it there.

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Maximal uranium concentration ( $0.06 \pm 0.006 \text{ mg.kg}^{-1}$ ) we found in chestnut leaves from Site 5 (Nature monument “Bunardzhik”), which belongs to the real city center and borders with one of the major traffic arteries in Plovdiv. This concentration was 3 to 4 times greater when compared to the rest of sampling sites.

Statistical analysis revealed only one strong positive correlation between chromium and vanadium. These two chemical elements are considered indicative of vehicle emissions (Garty et al., 1985) and partly associated with tire and brake abrasion, so this correlation is consistent with the fact that in Plovdiv urban traffic is the main anthropogenic source of airborne trace metals (Atanassov et al., 2006). Cluster analysis also pointed Cr-V association, and the similarity between Cu-U, and Pb-Cd (Figure 3).

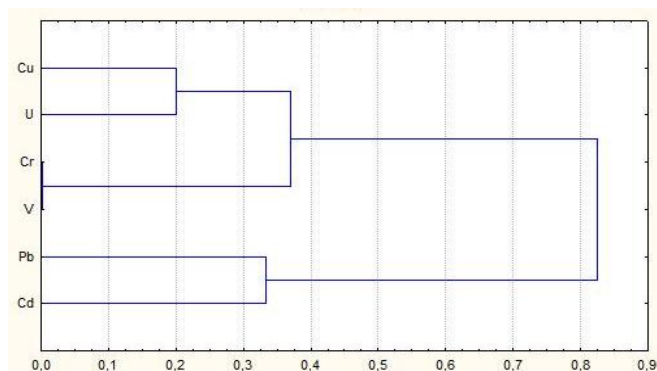


Figure 3. Cluster analysis by elements

Although no definite trend for all elements examined, the area of the Railway Station “Trakiya” (Site 3) has emerged as the most heavily loaded with Cu, Pb and U, followed by Nature monument “Bunardzhik”. Probably, these results are due to location of Site 3 (east of downtown) and, according to the rose of the winds of Plovdiv, most of them blow from west to east, that they play an important role in the transport of air pollutants in that direction. It should not be neglected and the emissions of non-ferrous plant, situated nearby.

According to the urban gradient theory, the pollution level must decrease from the center to the periphery of the town (Alberti et al., 2001). Our results confirm this to some extent (Figure 4). Most likely this is due to the specific characteristic sand topography of Plovdiv. The presence of large lawns and abundant planting trees on main roads, tall buildings and other obstacles (like hills) prevent dispersion of emitted pollutants in this area. They contribute to the retention of pollutants in the surface air layer, recirculation

and deposition. Concentrations in this situation will also depend on the speed and direction of wind turbulence characteristics of the road network (number of streets, intersections, traffic lights), traffic load, etc.

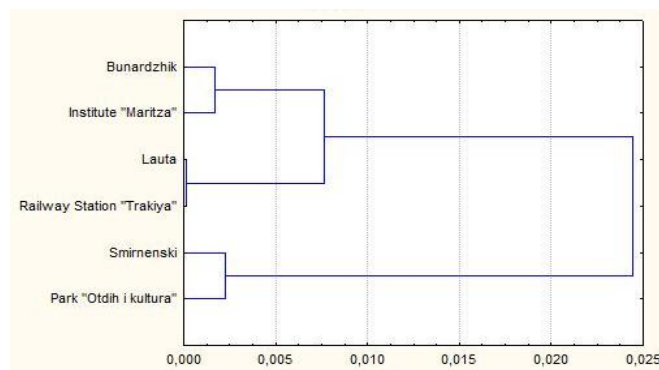


Figure 4. Cluster analysis by sampling sites

Our results confirm the statements given by Atanassov et al. (2006), that only about 5% of the air pollution found in Plovdiv is from industrial origin, while the remaining 95% are emitted from a variety of sources and mainly by the transport.

## Conclusions

Air quality biomonitoring was used to determine the air pollution levels in urban environments and concentration measurements can be compared with the national and international guideline values. This study presents an attempt to assess air quality in Plovdiv urban area and identify the pollution sources. *Aesculus hippocastanum* was confirmed as an effective and reliable plant species for this purpose.

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