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STUDY OF VARIATIONS IN ELEMENT CONCENTRATIONS IN HORSE CHESTNUT LEAVES

Some trace and major elements have been determined in leaves of horse chestnut (*Aesculus hippocastanum*) tree by inductively coupled plasma atomic emission spectrometry. The samples were collected from places with various levels of environmental pollution during a period of few months. Variations of some element concentrations in the chestnut leaves over the period when trees are in leaf with time and location have been observed. The results obtained have been discussed taking into account the application of chestnut tree leaves as a bioindicator of environmental pollution.

1. INTRODUCTION

In the last years, more and more attention has been paid to environmental analysis. The reasons of this state of affairs can be itemized as follows: clear understanding of relation between human health and life conditions on one hand and degree of environmental contamination on another one, common knowledge about continuous rise of environmental pollution and development of highly advanced and reliable analytical techniques. In addition to that, effective methods to prevent and eliminate danger of pollution have been developed and tested. A strong interest is focused on transport of pollutants in the environment. Bioconcentration, degradation, accumulation, deposition in sediments and uptake of both organic compounds and metal ions by organisms are recently investigated by a great number of scientists busy in different fields. A wide variety of environmental samples and materials has been analysed (see, e.g. [1]–[3]). The concentration of trace elements, especially toxic metals, have been measured quite often in various plants. Recently, studies of environmental pollution

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based on the analysis of some metals in tree leaves have been reported [4]–[8]. KIM et al. [6] analysed the concentrations of Cd, Cu, Pb and Zn in chestnut leaves.

Method of inductively coupled plasma atomic emission spectrometry (ICP-AES) introduced more than twenty years ago has become an advanced and very popular technique. The ICP-AES method has strongly attracted the attention and interest of specialists from various areas as an excellent tool of quick and multielement analysis. It can be used in a very wide range of element concentration from ppb (or below) to percents. Advantages of the argon ICP-AES method have well been documented in many papers and monographs (see, e.g. [9]–[11]).

In the present study, results of determination of some trace and major elements in leaves of horse chestnut tree by the argon inductively coupled plasma atomic emission spectrometry are reported. The horse chestnut tree was selected taking into account that this tree is very common and one can easily find it in many various parts of Europe. The study has been undertaken to investigate variations of element concentrations in the chestnut leaves and to evaluate applicability of this biological material for biomonitoring of natural environment.

2. EXPERIMENTAL

2.1. SAMPLE COLLECTION

Foliage was collected in the city of Wrocław, which can be treated as an example of a large urban area. The horse chestnut leaves were taken from three different sites of the city: high traffic area (HTA), medium traffic area (MTA) and park area (PA). One chestnut tree was chosen in each location. Every sample was composed of five to six leaves growing on various branches about 2 meters above the ground level. Samples were collected four times: on May 1st, June 1st, September 16th and October 9th, 1994.

2.2. SAMPLE PREPARATION FOR MEASUREMENTS

Leaves were dried at temperature of 80 °C during 24 hours. About 3 g of dry matter was obtained from 10 g of the leaves collected. The dry sample was digested to prepare solutions for measurements of element concentrations. A successful digestion of biological material leading to homogeneous solution is a critical point in many spectrochemical elemental analyses for trace element determination [12]–[14] by inductively coupled plasma and atomic absorption spectrometry. In the present study two digestion methods have been used: a classical wet digestion hot plate technique and microwave heating under high pressure in closed vessels.

Conventional wet (hot-plate) procedure. A sample of 1 g was weighted in a 50 cm^3 Pyrex glass beaker and digested with HNO_3 with addition of hydrogen per-

oxide until the end of dissolution process. The $HNO_3:H_2O_2$ ratio was 6:1. Another acid mixture used for digestion was HNO_3 and H_2SO_4 (2:1). After digestion the solution was transferred into calibrated flask and diluted to 50 cm^3 with deionized water.

Microwave dissolution. A high-pressure apparatus (MDS-1S Plazmatronika) was applied here for digestion of powdered leaves. A sample of 0.2 g was weighted and placed into a Teflon vessel, then 3 cm^3 of HNO_3 were added and the vessel was closed. The sample was heated for 20 min. After cooling (25 min) the solution was transferred into a 25 cm^3 calibrated flask and diluted to volume with deionized water.

2.3. CHEMICALS

High purity reagents (ARISTAR and SpectrosoL) and deionized water (of the resistivity equal to 18.3 $\Omega \cdot cm^{-1}$ resistivity, Barnstead EASY pure RF) were used for sample solution preparation. Multielement standard solutions were prepared from 1000 $\mu g/cm^3$ Merck standards for AAS and 100 $\mu g/cm^3$ SPEX standards for ICP.

2.4. METHOD OF MEASUREMENTS

Measurements of element concentrations in digested leaves were performed by means of the inductively coupled plasma atomic emission spectrometry. A sequential ICP-AES spectrometer (Jobin Yvon JY38S) was used for the measurements. The instrumental parameters and experimental conditions are given in table 1.

Table 1

Instrumental and operating conditions – ICP-AES sequential system JY 38S

Plasma	
Generator frequency	40 MHz
R.F. power	1.0 kW
Nebulizer	pneumatic, Meinhard C-type
Spray chamber	Scott type
Argon flows	outer gas, 12.0 dm^3/min
	intermediate gas, 0.3 dm^3/min
	sheath gas, 0.2 dm^3/min (0.8 for alkaline)
Sample uptake	1.0 cm^3/min
Monochromator	
Focal length	1 m
Grating	double, 4320/2400 groves/mm
Software	JY 4.02 version

3. RESULTS AND DISCUSSION

In the first step of study, effectiveness of the digestion methods used here was investigated and compared. As it was reported earlier (see, e.g. [12]–[14]), we observed that high pressure microwave procedure is very efficient, generally more effective than the classical wet digestion method. The advantage of the microwave digestion in closed vessels lies in a lower risk of contamination and of volatilisation losses. Considerably shorter time of sample dissolution is an additional profit of this procedure. Therefore, the microwave procedure was applied in final measurements.

Considering possible spectral line interferences and sensitivity of lines, two spectral lines of each element were chosen for first measurements. Usually, agreement between concentrations measured from two lines was quite satisfactory. Final results from one line (the 'better' line) have been presented here. The selection of lines was based on analysis of spectral characteristics of the analysed sample. The elements determined here and their lines used for final analyses are presented in table 2.

T a b l e 2

Elements and their lines
used for final measurements

Element	λ [nm]
Cr	205.55
P	213.62
Zn	213.86
Cd	214.44
Pb	220.35
Fe	238.20
Mn	259.37
Mg	279.55
Cu	324.75
Ca	393.37
Al	396.15
Na	588.99
K	766.49

In the first step, concentrations of all elements were determined on the basis of calibration curves obtained using SPEX multielement standard solutions. We found that major elements in the analysed samples of horse chestnut leaves are as follows: K, Ca, Mg, P, Na (which are typical matrix elements in biological samples) and Fe and Al. In final measurements of major elements, highly diluted solutions of the digested samples were used to eliminate possible matrix effects. Concentrations of the

major elements in the horse chestnut leaves related to sampling time and locations have been presented in tables 3 and 4.

Table 3

Concentrations (dry mass percentage) of major elements
determined in chestnut leaves
(collected from various locations) versus time

Element	May 1st		June 1st		September 16th		October 9th	
	min	max	min	max	min	max	min	max
Ca	0.86	1.3	0.52	1.82	2.5	3.1	2.2	3.3
Mg	0.31	0.42	0.26	0.55	0.46	0.60	0.48	0.70
P	0.42	0.59	0.21	0.29	0.14	0.19	0.12	0.20
Na	0.025	0.043	0.032	0.050	0.039	0.058	0.039	0.052
K	1.4	2.4	0.96	1.5	0.86	1.9	1.1	2.2
Al	0.010	0.026	0.017	0.028	0.032	0.091	0.050	0.076
Fe	0.030	0.054	0.037	0.073	0.064	0.145	0.090	0.219

Table 4

Concentrations (dry mass percentage) of major elements determined in
chestnut leaves (collected in various time) depending on tree locations

Element	Heavy traffic area		Medium traffic area		Park area	
	min	max	min	max	min	max
Ca	0.86	2.80	0.51	3.2	1.3	3.4
Mg	0.25	0.49	0.36	0.58	0.42	0.70
P	0.12	0.55	0.14	0.62	0.16	0.44
Na	0.032	0.058	0.025	0.045	0.039	0.050
K	1.20	2.4	0.86	2.2	1.0	1.5
Al	0.026	0.063	0.010	0.076	0.011	0.091
Fe	0.054	0.219	0.037	0.161	0.030	0.119

Major elements, especially easily ionised elements (EIE) such as Ca, Na, K or Mg may cause considerable changes in analytical signals of traces. In the ICP-AES method, interelement effects are usually poor. Nevertheless, at high EIE concentrations the interelement effects can be significant (see, e.g. [15]–[17]). In this study, in order to reduce possible interelement effects and to ensure a good accuracy and reliability of measurements, standard solutions used for trace element determination have

Table 5

Comparison of the concentrations of some elements measured based on calibration curve and by standard addition methods (µg/g of dry mass)

Element	Calibration curve method	Standard addition method
Cr	1.82	1.88
Pb	11.2	11.4
Mn	27.1	29.2

been prepared in a special way. Such elements as Ca, K, Mg and Na were added to every standard solution so that the EIE concentrations in standards and samples analysed were the same or similar. The standard solutions prepared in this way allowed us to prepare calibration curves for trace analysis. The results derived from the calibration curves have been compared with those obtained from the standard addition method. A quite good agreement, as can be seen for Cr, Pb and Mn in table 5, indicates discrimination of matrix effects and correctness of measurements based on calibration curves. Values of the trace element concentrations for samples of leaves collected in various time and from various locations of trees are given in tables 6 and 7.

Table 6

Concentrations of trace elements (µg/g of dry mass) in chestnut leaves (collected from various locations) versus time

Element	May 1st		June 1st		September 16th		October 9th	
	min	max	min	max	min	max	min	max
Zn	45.6	51.2	22.3	48.3	31.9	72.8	48.3	91.9
Cr	0.99	1.82	1.14	4.42	1.87	4.61	1.79	7.38
Cd	0.28	0.96	0.2	0.87	0.19	0.99	0.50	0.89
Pb	5.0	11.3	6.0	25.4	9.8	27.0	12.7	43.4
Cu	29.3	42.5	20.3	34.6	23.4	85.7	30.5	159
Mn	23.5	181	16.2	230	13.3	325	25.0	333

A wide variation in element concentrations depending on leaf age (sampling time) and location of examined tree has been observed. This variation occurs both in the concentrations of trace and major elements. Seasonal changes in the concentrations of some elements in chestnut leaves are shown in figures 1–6. Some elements, i.e. Ca, Mg, K, P, Fe, Al and Pb, have shown large concentration variations (increase or decrease) depending on sampling time and location. Concentrations of some elements as, for example, Na seemed to be independent of such variables as time and location, but scattering of results was considerable. Figure 6 proves that Cd concentration has not changed in straight-forward way. The highest concentrations of Pb and Fe were observed in highly polluted area, which is characteristic of lead.

Table 7

Concentrations of trace elements (µg/g of dry mass) in chestnut leaves
(collected in various time) in various tree locations

Element	Heavy traffic area		Medium traffic area		Park area	
	min	max	min	max	min	max
Zn	36.4	89.5	22.3	74.5	34.6	67.3
Cr	1.82	7.38	0.99	1.87	1.06	2.58
Cd	0.19	0.87	0.10	0.89	0.35	0.99
Pb	11.3	43.4	5.00	30.8	6.53	12.7
Cu	31.5	72.1	20.3	159	30.5	85.7
Mn	27.2	37.7	12.7	23.8	181	333

The results obtained by us have been compared with those obtained by KIM et al. [6] (table 8). With the exception of cadmium, concentrations of other elements in chestnut leaves analysed were considerably lower than those reported by KIM [6]. The increase in lead accumulation with time was observed both in the present study and in paper [6]. In our investigation, we were not able to observe a relationship between the decrease in the Zn and Cu concentrations and sampling time as it was reported by KIM et al. [6]. More complex and advanced studies are necessary to state whether the uptake of Zn and Cu from soil can be considered as the main source of these elements in chestnut leaves as it was suggested previously [6].

Table 8

Comparison of mean concentrations
(µg/g of dry mass) in chestnut leaves

Element	This study	KIM et al. [6]
Cd	0.55	0.197
Cu	53	129
Pb	17	294
Zn	54	299

4. CONCLUSIONS

The inductively coupled plasma atomic emission spectrometry has been found to be both applicable and very suitable method for quick determination of major and trace elements in such a biological material as tree leaves. The sensitivity of the ICP-AES measurements has been sufficient to investigate variations of element concentrations in horse chestnut leaves.

A crucial point of environmental analysis may be sample preparation, and the microwave high pressure and high temperature digestion procedures appear to be a very good choice.
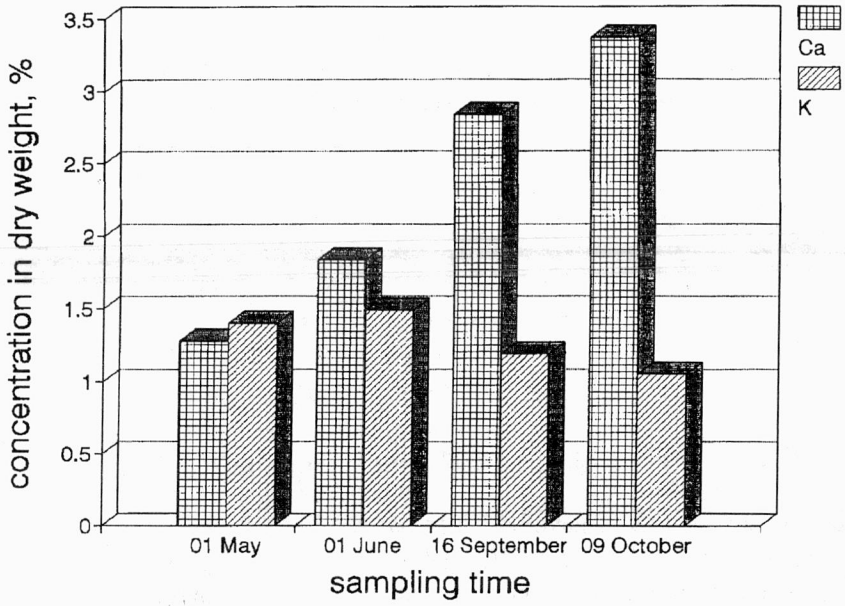
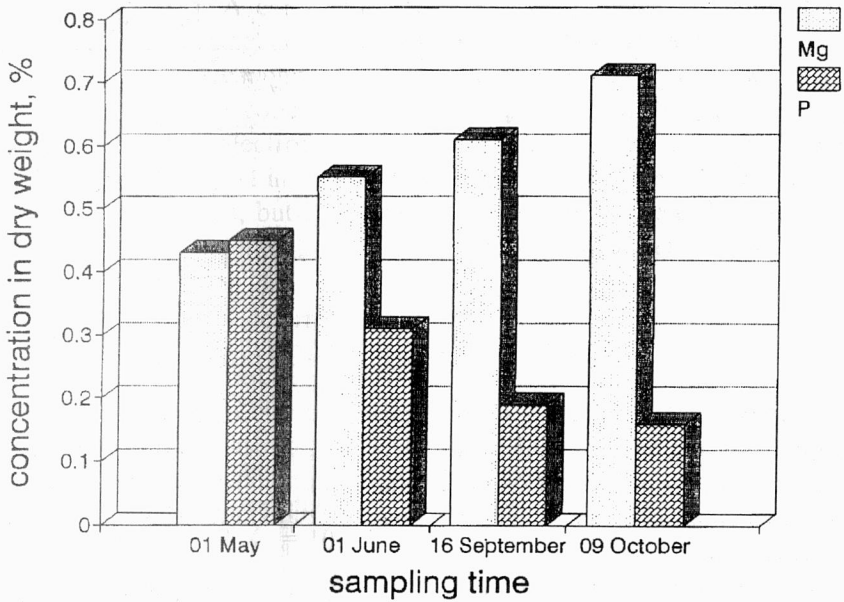Fig. 1. Ca and K concentrations in horse chestnut leaves in park area

Fig. 2. Mg and P concentrations in horse chestnut leaves in park area
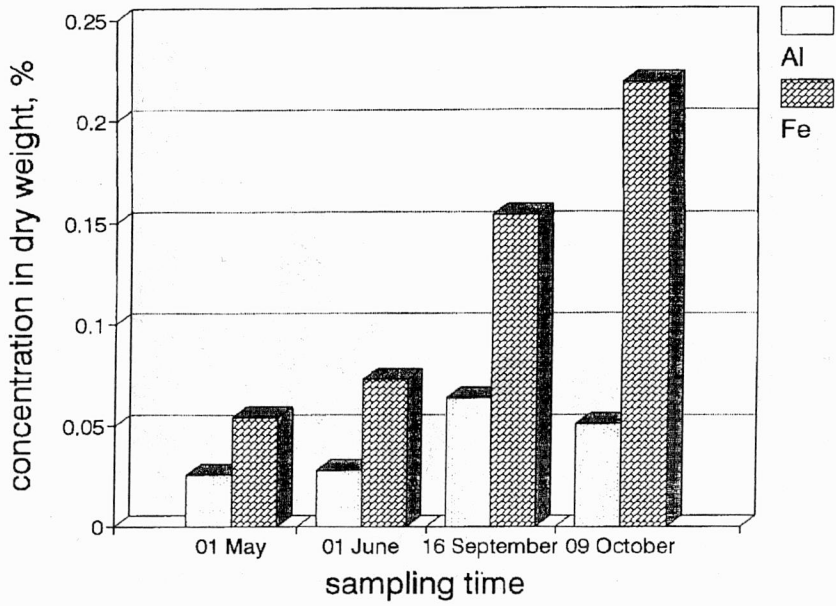
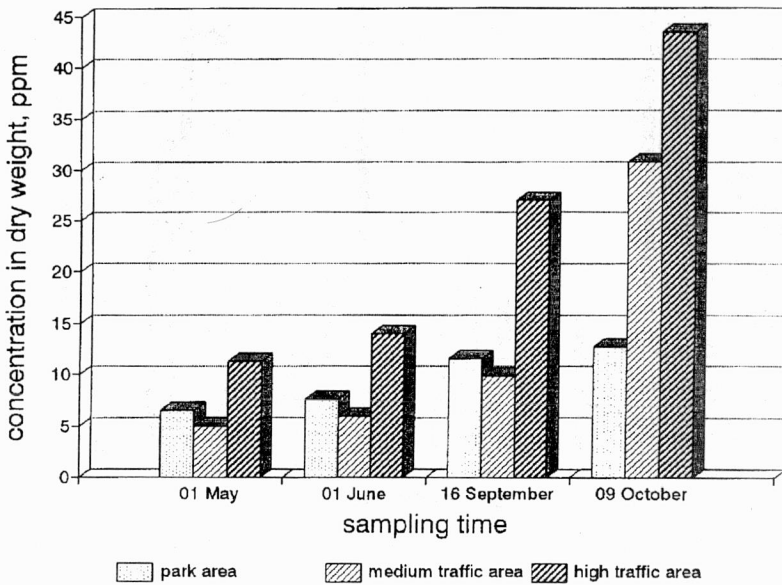Fig. 3. Al and Fe concentrations in horse chestnut leaves in park area

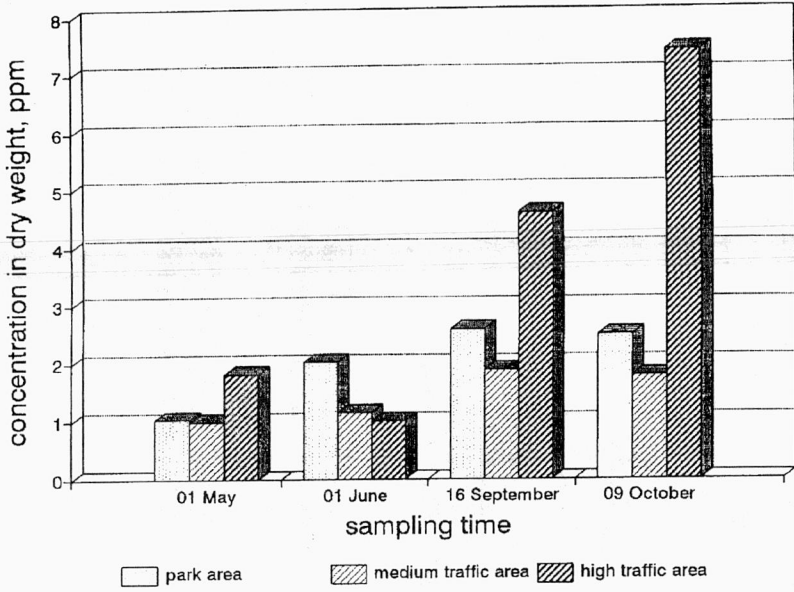Fig. 4. Pb concentration in horse chestnut leaves versus sampling time and location

Fig. 5. Cr concentration in horse chestnut leaves versus sampling time and location
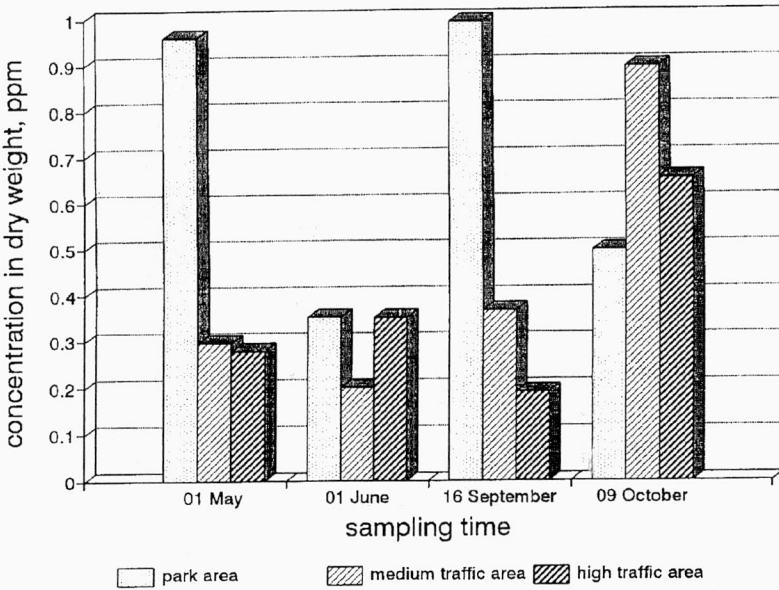

Fig. 6. Cd concentration in horse chestnut leaves versus sampling time and location

It seems that the horse chestnut tree leaves are sensitive to environmental changes. Concentrations of some elements in the leaves have shown considerable seasonal variations which indicates that some precautions have to be taken in comparing environmental pollution in quite different places. The horse chestnut tree leaves may be a bioindicator allowing monitoring of environmental pollution caused by some elements, e.g. lead, but in the case of other elements, e.g. cadmium, such conclusions are not justified so far.

More advanced and extended studies of element concentration variations in selected plants depending on environmental conditions have been undertaken.

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BADANIA ZMIAN STĘŻENIA PIERWIASTKÓW W LIŚCIACH KASZTANOWCA

Wybrane pierwiastki śladowe i główne oznaczono w liściach kasztanowca metodą emisyjnej spektrometrii atomowej z użyciem indukcyjnie sprzężonej plazmy (ICP-AES). Próbki pobierano z miejsc o różnym stopniu skażenia środowiska w okresie kilku miesięcy. Zmierzono zmiany stężenia pierwiastków w zależności od czasu i lokalizacji rosnących drzew kasztanowca. Omówiono otrzymane wyniki z punktu widzenia zastosowania liści tego drzewa jako bioindykatora skażenia środowiska.