THE STUDY OF ZINC CONTENT IN SOME AERIAL PARTS OF AESCULUS HIPPOCASTANUM L. THROUGH AAS

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Abstract

Zinc is an oligoelement essential to life that can be found in every cell, it is a component of over 200 enzymes, it stimulates the activity of more than 100 enzymes, with an important role in DNA synthesis and in the growth and development of cells and tissues. Its implication in numerous systems has been highlighted by the study of its physiological changes in various pathological states: delay in growth and development, appetite decrease, delayed wound healing, alopecias, taste and smell disorders, male hypogonadism, difficulty in night vision, increased receptivity to infections, etc.

In the researches made on Aesculus hippocastanum L. (horse chestnut tree) in Bihor county, we had in view the determination of zinc content in leaves, flowers and fruis *according to the harvesting area, along three years of study, between 2005 – 2007. The samples were harvested from the same trees and in the same vegetation period in two different areas: Oradea (the city centre) and Adoni (an area with a low degree of pollution).*

Zinc can be found in horse chestnut tree in a quantity of approximately 13,68 – 110,28 µg/g, according to the results obtained through AAS. To assess the accuracy of these data, the standard deviation (S) and the confidence limits (of the safety level) have been calculated.

Key words: *Aesculus hippocastanum* L. (horse chestnut tree), zinc, atomic absorption spectrometry (AAS), standard deviation.

INTRODUCTION

The quantification of oligoelements in some plants represents a current subject for researchers on a worldwide level; they appeal to analytical methods of great performance, thus managing to overpass the difficulties connected to isolating the elements in the organis mould and protecting them by avoiding the losses through oxidation or the overdosing through pollution. The most important microelements are: zinc, copper, manganese, chromium, iron, boron, strontium, barium, lithium, molybdenum, arsenic, vanadium, bubidium, being presented in quantities between 0,00001 and 0,001% of the dry substance of the plant (Chappuis, 1991; http://www.zinc.org/info/zinc_crops; http://www.biocyclopedia.com).

The choice of analyses methods must take into account a series of parameters: the detection limit, sensitivity, exactity and the precision of the method; thus the absorption spectrometry correspond to a great extent, to the exigencies in the microanalyses area (Bojiță et al., 2003; Roman et al., 2007; http://www.sciencedirect.com).

The atomic absorption spectrometry is the method that gives excellent results in the establishment of microelements. The speed and sensitivity of this method has lead to its use in many laboratories, for a great number of tests.

The subject approached in this paper develops researches regarding the identification and quantification of the zinc, from the different aerial parts (leaves, flowers, fruits) of the native wild chestnut tree, experimental data related to the environment where it grows (Oradea and Adoni; Bihor county) (Horvath, 2009).

MATERIAL AND METHODS

During our study, we have collected different aerial parts of *Aesculus hippocastanum* L., from different areas (Oradea – the city center and Adoni-village in county Bihor), during the same periods of the year 2005-2007: leaves (50-100 pieces), flowers (20-50 pieces) of 1,5 – 2 kg fresh chestnuts fruits (Ciulei et al., 1995; Horvath, 2009). The vegetal organs were dried at room temperature, away from solar radiation and humidity, weighed and then, dried in the drying cabinet at 60 degrees Celsius, until they reached a constant mass. The studied vegetal material was collected during specific periods of vegetation, taking into consideration the fact that the humidity and type of soil, the period and age of plant, the morphological characteristics influence their content of zinc. The collection of samples has been made according to the technical conditions mentioned in specialized literature (Chappuis, 1991; Ciulei et al., 1995).

Table 1

Harvesting conditions of the vegetal material

Vegetal material	Harvesting place	Harvesting year	Harvesting date	Temperature °C
LEAVES	ORADEA,ADONI	2005	15.05 – 20.05	20 – 25°C
		2006	07.05 - 14.05	
		2007	25.04 - 30.04	
FLOWERS	ORADEA,ADONI	2005	26.05 – 31.05	20 – 25°C
		2006	07.05 – 14.05	
		2007	20.04 – 25.04	
FRUITS	ORADEA,ADONI	2005	16.10 – 23.10	16 – 21°C
		2006	07.10 – 14.10	
		2007	30.09 – 05.10	

We have used solvents and reactives of analytical purity: concentrated perchloric acid Merck, concentrated azotic acid Merck, perhydrol 30% Merck, stadard zinc solution 1000 mg/l Merck and double distilised water Merck.

0,200+/- 0,0001 g dried and shredded material is weighed. The sample is quantitatively transferred in a dry Erlenmayer glass flask capacity 100 ml.

Over the weighed material, we add 10 ml of concentrated perchloric acid. The balloon is covered with a watch window and is left at the room temperature until the next day. Afterwards, the sample balloons are put on an electric thermo-regulated electric range and heated at 150^{o}C. We continue the heating at this temperature until the removal of the concentrated azotic acid and until the bleaching of the solution. If the content of the balloon has not bleached, we add 1-2 ml perhydrol 30%. The filtered samples are brought to the line with double distilised water in recipients of 50 ml (Horvath et al., 2007; Seracu, 1986; Farmacopeea Română, Ed. X, 1993).

Wee used an atomic absorption spectrometer with a GBC Avanta flame and fix the aparata parameters typical for zinc: the wavelength λ = 213,86 nm, the intensity of lamp electric power: 10 mÅ, the width of the slit: 7mm, the mix for laminar and oxidative flame: acetylene/air (5 l/min / 0,8-1 l/min), the zinc lamp the apparatus command and the data processing through AVANTA software. The standardization of the apparatus is done with the help of a standard solution which contains 2 mg Zn/l.

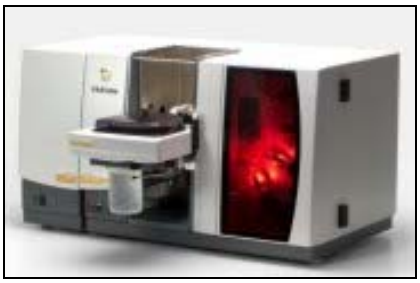

Fig. 1. Atomic Absorption Spectrophotometer GBC AVANTA

The sample prepared in the conditions described above is submitted to determination. *Witness sample* (M) – For every set of determinations a witness sample is done, which consists in bidistilled water, treated in the same conditions as the sample to be determined.

The stadard zinc solution – The zinc solution is prepared like this: 1g of metalic zinc is dissolved in 40 ml HCl concentrated and the volume is completed with bidistilled water to 1000 ml. From the standard solution left (1 g/l) a work solution is prepared through dilution (100 mg/l). From this solution the calibration standards are prepared and they have the following concentrations: 0,5mg/l; 1,0 mg/l; 3 mg/l; 5 mg/l; 10 mg/l. The device is calibrated and the final concentration of zinc, expressed in micrograms/g of analysed dried vegetal product.

The calculus of the concentrations

$$Zn\ \mu g/g = (A-M) \times V/m$$

where:

A and *M* – the values read on the apparatus screen for sample A and for the witness (M=0).

V – the volume of the graded balloon in which the exactly weighed sample was brought (50 ml).

m – the quantity of vegetal material powder weighed (0,2 g)

In order to compare the obtained values from the point of view of place, promotion period and the type of vegetal material, we considered the arithmetic mean of the values of the zinc concentrations determined for each studied aspect.

It is well known the fact that there is no laboratory where you can do a very high, number of analyses, since they are extremely expensive (consuming large quantities of reagents, different materials, energy, time, operators), but small series of determinations which furnish a number of analytical results equal with that of the measurements can be done. To evaluate the precision of these data, we can calculate the standard deviation (S) using the following relation (Bojiță et al., 2003; Ciulei et al., 1995; Farmacopeea Română, Ed. X, 1993; Pharmacopée Européune, Ed. 4, 1997; British Pharmacopeea, 2002; United States Pharmacopeea XXV, 2004; http://www.sciencedomain.org):

$$S = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \overline{x})^2}{n-1}}$$

x_i = the values of a result

$\overline{x}$ = the arithmetic mean of the analytical results

n = the number of results (in our case 3)

n-1 = the number of the freedom degrees

The arithmetic mean is calculated as follows:

$$\overline{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

In our case: $\overline{x} = \frac{x_1 + x_2 + x_3}{3}$

Therefore, considering for example the values of the zinc content in the leaves in Oradea, harvested in 2005, the value of the standard deviation will be:

$$S = \sqrt{\frac{(x_1 - \overline{x})^2 + (x_2 - \overline{x})^2 + (x_3 - \overline{x})^2}{2}}$$

$$S = \sqrt{\frac{(41{,}25-41{,}49)^2+(42{,}02-41{,}49)^2+(41{,}20-41{,}49)^2}{2}} = \sqrt{\frac{0{,}0576+0{,}2809+0{,}0841}{2}}$$

$$S = 0{,}460$$

The other values listed in the tables have been calculated identically.

It is important to underline the fact that to estimate the certainty of a result (represented through a number) to fit in the trust or confidence interval, we can calculate the trust limits of the mentioned interval. In the case of a limited number of analytical determinations, usually small (n = 3 – 5) for the characterization of the trust interval (of the safety level), we use the t distribution criterion and the relation (Bojiță et al., 2003; Roman et al., 2007):

$$x = \bar{x} \pm \frac{t \cdot S}{\sqrt{n}}$$

$\bar{x}$ = the arithmetic mean of the analytical n results
S = standard deviation
t = student test, which is a statistic factor depending on the number of freedom degrees (K = n-1) and the desired level of trust.

RESULTS AND DISCUSSION

The specialized literature mentions very few data referring to *Aesculus hippocastanum L.* tree and almost nothing referring to the one that grows up in this area of the country – and the evaluation of the chemical composition by the atomic absorption spectroscopic method is not cited. The pollution grade of the region in which the plant is growing up has got a great impact upon the metal loading of the aerial parts of *Aesculus hippocastanum L.* tree.

The obtained results are calculated for each element and the average of individual determinations (n=3) are specified in tables 2-4.

The zinc concentration increases in the leaves with approximately 140-170% both at the samples from Oradea and those from Adoni; important zinc concentrations for the samples harvested in 2007 (58,30 µg/g and 46,78 µg/g).

In the flowers from Oradea, on the contrary, there is a decrease of zinc concentration with approximately 9% and an increase with approximately 160% for the Adoni samples, whereas the values of zinc concentration in flowers varies between 110,28 µg/g and 46,10 µg/g.

Table 2

The variation of the Zn concentration in leaves of *Aesculus Hippocastanum L.* (µg/g) determined through AAS according to the area and the prelevation year

Vegetal material	Region / Year	Zn (µg/g plant) P1	Zn (µg/g plant) P2	Zn (µg/g plant) P3	Arithmetic mean (µg/g plant)*	Standard deviation S	Trust interval
LEAVES	Oradea / 2005	41.25	42.02	41.20	41.49	0.460	1.142
	Oradea / 2006	49.33	49.27	49.15	49.25	0.092	0.228
	Oradea / 2007	58.30	58.13	58.47	58.30	0.170	0.422
	Adoni / 2005	27.58	27.70	27.64	27.64	0.060	0.149
	Adoni / 2006	37.16	37.02	37.12	37.10	0.072	0.179
	Adoni / 2007	46.82	46.66	46.86	46.78	0.106	0.263

Table 3

The variation of the Zn concentration in flowers of *Aesculus Hippocastanum L.* (µg/g) determined through AAS according to the area and the prelevation year

Vegetal material	Region / Year	Zn (µg/g plant) P1	Zn (µg/g plant) P2	Zn (µg/g plant) P3	Arithmetic mean (µg/g plant)*	Standard deviation S	Trust interval
FLO-WERS	Oradea / 2005	110.16	110.36	110.32	110.28	0.106	0.263
	Oradea / 2006	100.06	100.14	100.10	100.10	0.040	0.099
	Oradea / 2007	94.65	94.43	94.12	94.40	0.266	0.661
	Adoni / 2005	46.11	46.03	46.16	46.10	0.066	0.163
	Adoni / 2006	59.26	59.18	59.16	59.20	0.053	0.131
	Adoni / 2007	76.02	75.72	75.12	75.62	0.458	1.138

Table 4

The variation of the Zn concentration in fruits of *Aesculus Hippocastanum L.* (µg/g) determined through AAS according to the area and the prelevation year

Vegetal material	Region / Year	Zn (µg/g plant) P1	Zn (µg/g plant) P2	Zn (µg/g plant) P3	Arithmetic mean (µg/g plant)*	Standard deviation S	Trust interval
FRUITS	Oradea / 2005	22.03	21.67	21.49	21.73	0.275	0.683
	Oradea / 2006	25.36	25.17	25.46	25.33	0.147	0.366
	Oradea / 2007	28.09	28.05	27.95	28.03	0.072	0.179
	Adoni / 2005	53.99	53.81	53.90	53.90	0.090	0.224
	Adoni / 2006	37.83	37.53	37.95	37.77	0.216	0.537
	Adoni / 2007	13.92	13.54	13.58	13.68	0.209	0.519

In the case of fruits, we obtained an increase of the zinc concentration with approximately 135% in the samples from Oradea (the maximum zinc concentration: 28,03 μg/g) and in the Adoni samples a decrease with approximately 26% (the minimum concentration 13,68 μg/g).

Generally, the registered results are variable and in literature, they can be found within valuable limits in what concerns the green plants (0,00001 and 0,001%).

The results of the calculus, that is the very low values of the standard deviation and the trust interval, also present in tables 2-4, demonstrate the exactity of determinations.

CONCLUSIONS

A high number of bibliographical data offer the necessary arguments and also explain the opportunity of approaching this study on *Aesculus hippocastanum L.,* present in two areas of Bihor county (Oradea and Adoni), which has not been studied from the point of view of it zinc content.

The identification and dosing of the zinc from the aerial parts (leaves, flowers, fruits, pollen) of *Aesculus hippocastanum L.*, from Bihor county, has been accomplished through a highly- performance method: the atomic absorption spectrometry.

As results from tables 2-4, the zinc concentration for the vegetal samples harvested in the two areas of Bihor County between 2005-2007 vary between 13,68 μg/g (present in fruits in Adoni, 2007) and 110,28 μg/g (present in flowers in Oradea, 2005).

In the analysed period the zinc concentration increases in 2005 < 2007 in leaves (Oradea), flowers (Adoni) and fruits (Oradea) – the average concentrations in 2005 – 2007 in leaves, flowers and fruits are higher for the trees in Oradea in comparison to those in Adoni. The average values for 2007 are situated between 60,24 μg/g (Oradea) and 45,36 μg/g (Adoni).

With the help of this modern method - the atomic absorption spectrometry – AAS – we quantified these oligoelements in some aerial parts of *Aesculus hippocastanum L.* of Bihor county – Transilvania, contributing to the completion of some important data on its chemical composition and to the signalling of some levels of pollution of the air, water and soil in this part of the country.

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