DETERMINATION OF IRON CONTENT IN SOME AERIAL PARTS OF AESCULUS HIPPOCASTANUM L. THROUGH AAS

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Abstract

Although required by plants in small amounts, iron is involved in many important compounds and physiological processes in plants. Thus, iron influences plant photosynthesis, nitrogen metabolism and oxidative phosphorylation, and catalyzes the chlorophyll and carotenoid pigments biosynthesis. Iron is absorbed in the form of ions by the root system of terrestrial plants and by the whole body of aquatic submerged plants. According to the literature, the iron content of plants varies between 20 and 300 µg/g of dry vegetal material.

This paper presents the research we have done on Aesculus hippocastanum L. (horse chestnut tree) samples harvested in Bihor County, Romania. Our purpose was the determination of iron content in leaves, flowers and fruits during three years of study (2005–2007) according to the harvesting area. The samples were harvested from the same trees and in the same vegetation period in two different areas: Oradea (downtown) and Adoni (an area with a low degree of pollution).

According to the results obtained through AAS, the horse chestnut content of iron varies between 71.385– 144.59 µg/g. The standard deviation (S) and the confidence limits (safety levels) have been calculated in order to assess the accuracy of these data.

Key words: *Aesculus hippocastanum* L. (horse chestnut tree), iron, Atomic Absorption Spectrometry AAS, standard deviation

INTRODUCTION

The role of iron in plants is as basic as it can get: in the absence of iron the plants can not produce chlorophyll, can not get oxygen and will not be green. iron for plants can come from a number of sources. one of them is ferric oxide, a soil chemical that gives to dirt a distinctive red color. the living plants are able to absorb iron from this chemical (http://www.ncbi.nlm.nih.gov, http://www.drt.com.tr/doctoferro/iron.aspx, http://www.nutrition-and-you.com/chestnuts.html).

Iron is involved in the manufacturing process of chlorophyll which gives the necessary oxygen to plants as well as the healthy green color. this is why the plants with an iron deficiency, or chlorosis, show a sickly yellow color to their leaves. iron is also required for certain enzyme functions in many plants (http://www.gardeningknowhow.com, https://en.wikipedia.org/wiki/iron_deficiency, http://www.plantnutrifert.org, http://edis.ifas.ufl.edu/).

Iron is essential to the metabolism of the mitochondria and chloroplast, and recent discoveries showed the importance of iron for the transport and homeostasis at the intracellular level (http://www.ncbi.nlm.

nih.gov, http://www.organicfacts.net, http://www.pthorticulture.com, http://www.spectrumanalytic.com).

MATERIAL AND METHOD

The preparation of vegetal samples

The samples were harvested from the same trees and in the same vegetation period (2005-2007) in two different areas: Oradea (the city centre) and Adoni (an area with a low degree of pollution). In order to determine the iron concentration through the atomic absorption spectroscopy AAS, the vegetal mass after drying in the standard conditions mentioned in the literature and bringing to the adequate shredding degree was submitted to mineralization (Table 1) (Horvath, 2009).

Table 1

Harvesting conditions of the vegetal material (Horvath, 2009)

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	

0.200 ± 0.0001 g dried and shredded material was weighed and quantitatively transferred into a 100 mL dry Erlenmayer glass flask. Over the weighed material, 10 mL of concentrated nitric acid and 2 ml concentrated perchloric acid was added. The flask was covered and left at room temperature overnight. After that, the sample was heated at 150ºC until the removal of the nitric acid and the bleaching of the solution. When the sample has not bleached, 1-2 mL of 30% hydrogen peroxide were added. The complete soluble samples were brought to the mark in a graded flask of 50 ml (Seracu, 1986; Horvath et al., 2007; Horvath et al., 2011; FR X-Farmacopeea Română, Ediţia a X-a, 1993; British Pharmacopeea, 2002).

Establishing the specific parameters of the GBC AVANTA spectrometer. The typical parameters for iron have been set:

- the wavelength λ = 248.30 nm
- the intensity of electric power of lamp: 10 mÅ
- the width of the slit: 7 mm

- the mix for laminar and oxidative flame: acetylene/air (5 L/min/ 0,8-1 L/min)

- the iron lamp

- the apparatus command and the data processing through AVANTA software.

The standardization of the apparatus was done with the help of a standard solution which contains 2 mg Fe/L.

The preparation of the solutions for analysis

The sample prepared in the conditions described above was submitted to determination.

Witness sample (M) – For every set of determinations a witness sample consisting in bidistilled water treated in the same conditions as the sample for analysis was done.

The standard iron solution – 1g of iron was dissolved in 40 mL of concentrated hydrochloric acid and the solution was filled to a volume of 1000 mL using bidistilled water. From the standard solution (1000 mg iron/L), a work solution was prepared through dilution (100 mg/L). From this solution the calibration standards of the following concentrations: 0.5mg/L; 1.0 mg/L; 3 mg/L; 5 mg/L; 10 mg/L were prepared. The device was calibrated and the final concentration of iron, expressed in µg/g or ppm was calculated.

The calculus of the concentrations

$$\textbf{Fe } \mu g/g = (A-M) \times V/m$$

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 flask in which the exactly weighed sample was brought (50);

m – the quantity of vegetal material powder (0.2 g).

The determined iron concentrations are expressed in micrograms/g of analysed dried vegetal product (Seracu, 1986; Horvath et al., 2007; Horvath et al., 2011; Pharmacopée Européeune, Ed.4, 1997; United States Pharmacopeea XXV, 2004).

In order to compare the obtained values concerning the place, prelevation period and the type of vegetal material, we considered the arithmetic mean of the values of the iron concentrations.

RESULTS AND DISCUSSION

It is well known that it is difficult to make a very high number of analyses, since they are extremely expensive (large quantities of reagents, 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, the standard deviation (S) can be calculated as follows:

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

x_i = the value of a result

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

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

n-1 = the number of the freedom degrees (Bojiţă et al., 2003; Roman et al., 2007).

The arithmetic mean is calculated as follows:

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

It is important to underline the fact that to estimate the certainty of a result (represented by a number) to fit in the trust or confidence interval, the trust limits of the mentioned interval can be calculated. 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 (Bojiţă et al., 2003):

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

$\bar{x}$ = 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.

Table 2

Determination of the iron content (µg/g plant) in leaves

Area	Prelevation year	Fe concentration (µg/g plant)*	Standard deviation S	Trust interval
ORADEA	2005	101.24	0.460	1.142
ORADEA	2006	105.76	0.092	0.228
ORADEA	2007	121.43	0.170	0.422
ADONI	2005	97.36	0.060	0.149
ADONI	2006	99.12	0.072	0.179
ADONI	2007	107.39	0.106	0.263

* the arithmetic mean of a number of 3 determinations (n=3).

The results of the determination of the iron content in the leaves of *Aesculus hippocastanum* L. according to the prelevation area and the prelevation period are presented in table 2 and figure 1.

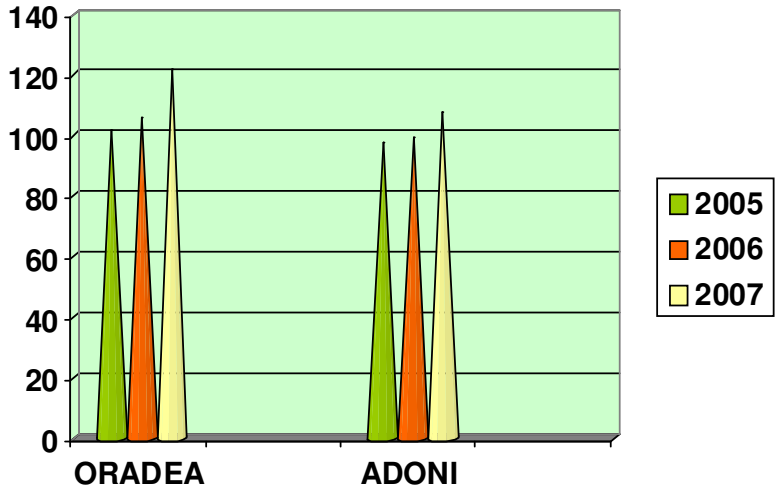

Fig. 1. The variation of the iron content in leaves of *Aesculus hippocastanum* L.

The results of the determination of the iron content in the flowers of *Aesculus hippocastanum* L. according to the prelevation area and the prelevation period are presented in table 3 and figure 2.

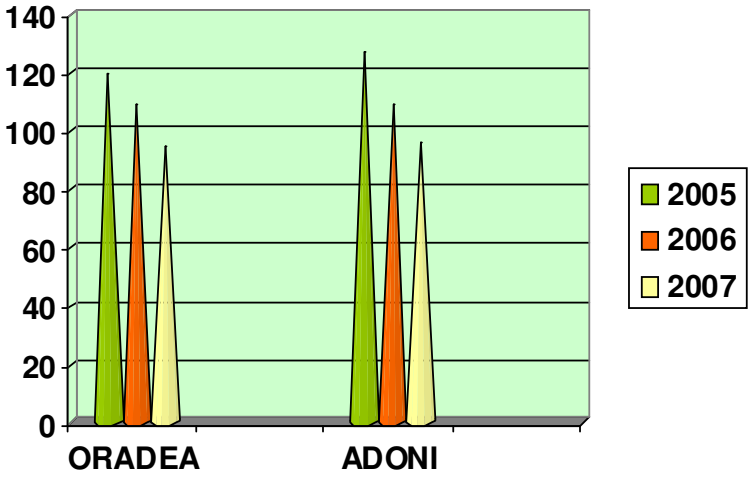

Fig. 2. The variation of the iron content in flowers of *Aesculus hippocastanum* L.

Table 3

Determination of the iron content (µg/g plant) in flowers

Area	Prelevation year	Fe concentration (µg/g plant)*	Standard deviation S	Trust interval
ORADEA	2005	119.55	0.106	0.263
ORADEA	2006	109.10	0.040	0.099
ORADEA	2007	94.63	0.266	0.661
ADONI	2005	126.90	0.066	0.163
ADONI	2006	109.21	0.053	0.131
ADONI	2007	95.62	0.458	1.138

* the arithmetic mean of a number of 3 determinations (n=3).

The results of the determination of the iron content in the fruits of *Aesculus hippocastanum* L. according to the prelevation area and the prelevation period are presented in table 4 and figure 3.

Table 4

Determination of the iron content (µg/g plant) in fruits

Area	Prelevation year	Fe concentration (µg/g plant)*	Standard deviation S	Trust interval
ORADEA	2005	71.38	0.275	0.683
ORADEA	2006	87.74	0.147	0.366
ORADEA	2007	123.81	0.072	0.179
ADONI	2005	71.81	0.090	0.224
ADONI	2006	95.98	0.216	0.537
ADONI	2007	144.59	0.209	0.519

* the arithmetic mean of a number of 3 determinations (n=3).

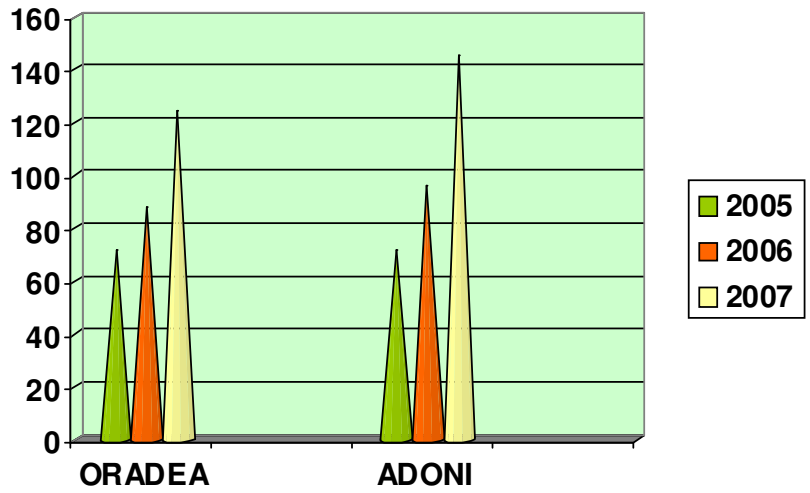

Fig. 3. The variation of the iron content in fruits of *Aesculus hippocastanum* L.

CONCLUSIONS

As can be seen in the tables 2-4, the iron concentration of the vegetal samples harvested between 2005-2007 in two areas of Bihor County vary between 71.38 µg/g (Oradea, fruits, 2005) and 144.59 µg/g (Adoni, fruits, 2007) and is in the range of iron standard for dried vegetable products.

The iron concentration of fruits increases with approximately 140-185% both for the samples from Adoni and those from Oradea; important iron concentrations for the samples harvested in 2007 in the fruits are 123.81 µg/g for Oradea and 144.59 µg/g for Adoni.

The results of the calculation which show very low values of the standard deviation and the trust interval, also presented in tables 2-4, demonstrate the exactity of determinations.

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