DETERMINATION BY ATOMIC ABSORPTION SPECTROSCOPY OF THE CONTENT MANGANASE IN THE AERIAL PARTS OF PIC CHESTNUT (*AESCULUS HIPPOCASTANUM* L.)

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Abstract

Manganese is a widespread metal on the earth's crust, playing an essential role in plants, stimulating the enzyme activity (arginase, phosphorylase, catalase, peroxydase), enzymes which, in turn, stimulate the uptake of nitrogen, plant respiration, photosynthesis. The manganese content of plants varies between 18 and 1480 ppm as for the dry matter.

In the researches made on Aesculus hippocastanum L. (horse chestnut tree) in Bihor county, Romania, the purpose was the determination of manganese content in leaves, flowers and fruits *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).*

Manganese can be found in horse chestnut tree in a quantity of approximately 6.26– 119.85 µg/g of analysed dried vegetal product, according to the results obtained through AAS. In order 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), manganese, atomic absorption spectrometry (AAS), standard deviation.

INTRODUCTION

In plants, manganese interferes with the photosynthetic elimination of the oxygen derived from the photosynthesis reaction of water (Epel, Neumann, 1998; Tiţă, 1996). In leaves, the lack of manganese reduces carbon dioxide assimilation, starch and sugar content causing nitrate accumulation and reduces the turgescence of cells (source: http://www.organicfacts.net). The experiments made in our country have shown that manganese is involved in plant respiration intensity, in chlorophyll content in leaves, in *catalase* and *peroxydase* activity, all these processes showing lower values with a nutrition having manganese deficiency (Underwood, 1999; Zidenberger-Cherr et al., 1985; http://en.wikipedia.org/wiki/Manganese; http://www.eplantscience.com; http://www.organicfacts.net).

In humans, manganese acts as a regulator of enzymatic processes both during fetal development and during adult development. Manganese plays an extremely important role in supporting the hepatic and renal functions, in accelerating the burnings, in improving calcium absorption, its metabolism

and also it enhances iron and B vitamins absorption (Chirilă et al., 1987; Horvath, 2009).

Manganese is an enzymatic activator, it stabilizes manganese-dependent enzymes and it also replaces magnesium in some enzymatic processes (Chirilă et al., 1987; Mărgineanu, Miu, 1984). In an intact cell, there are small quantities of adenosine triphosphate ATP and adenosine diphosphate ADP, as an anion-free form, these substances being present as equimolecular complexes: Mg-ATP and Mg-ADP. The phosphate transfer on ADP is a reaction catalyzed by *pyruvate kinase* or *ATP-pyruvate phosphotransferase*; Mn^{2+} or Mg^{2+} being indispensable for these activities. Ions interferes with the formation of an enzyme complex; calcium ions (Ca^{2+}) have a competitive action with Mn^{2+} or Mg^{2+} ions, forming an inactive complex (Mărgineanu, Miu, 1984; Yong-Keun et al., 1996).

Manganese beneficially interferes with the glucydic metabolism, this oligoelement also being the activator of two enzymes involved in fructose metabolism, galactosyltransferase and fructose-bisphosphatase (Bucureasa, 2001; Chappuis, 1991), as for the body immunity, it is necessary in the proper synthethis of antibodies (Chappuis, 1991). It deeply affects the central nervous system activity and nerve functions (Lerebours, Galmiche, 1997), influencing the cerebral amine metabolism and the neuromediator metabolism (Chappuis, 1991).

The subject approached in this paper develops researches regarding the identification and quantification of the manganese, 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, Romania).

MATERIAL AND METHODS

The preparation of vegetal samples: in order to determine the manganese concentration through the atomic absorption spectroscopy (AAS), the vegetal material was collected during specific periods of vegetation, dried in the conditions mentioned in the specialty literature (room temperature, away from solar radiation and humidity, weighed and then dried in the drying room at 60°Celsius, until they reached a constant mass) and submitted to mineralization (Chappuis, 1991; Horvath, 2009; Horvath et al., 2007; Roman et al., 2007).

For this study, different aerial parts of *Aesculus hippocastanum* L. from different areas (Oradea – the city center and Adoni village in Bihor county, Romania) were collected during the same periods of the years 2005-2007: leaves (50-100 pieces), flowers (20-50 pieces) and fresh chestnuts fruits (1.5 – 2 kg) (Chappuis, 1991; Horvath, 2009).

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, 10 ml of concentrated perchloric acid were added. The flask is covered with a watch glass and is left at the room temperature for 24 hour. Afterwards, the sample flasks are put on an thermo-regulated electric range and heated at 150^{o}C. The heating was continued at this temperature until the removal of the 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 complete soluble samples are brought to the mark in graded balloons of 50 ml (Horvath et al., 2005; Horvath et al., 2007; Seracu, 1986).

The solvents and reagents used for this study were of analytical purity: concentrated perchloric acid Merck, concentrated nitric acid Merck, perhydrol 30% Merck, standard manganese solution 1000 mg/l Merck and double distilised water Merck.

Establishing the specific parameters of the GBC AVANTA spectrometer, we fix the aparata parameters typical for manganese:

- the wavelength λ = 279,48 nm
- the intensity of lamp electric power: 12 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 mangan lamp
- the apparatus command and the data processing through AVANTA software

The preparation of the solutions to be analysed: 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 standard manganese solution is prepared like this: 1g of metalic manganese is dissolved in 50 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: 0.5 mg/l; 1.0 mg/l; 3 mg/l; 5 mg/l; 10 mg/l. The device is calibrated and the final concentration of manganese, expressed in µg/g or ppm is calculated.

The calculus of the concentrations

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

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).

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

The determined manganese concentrations are expressed in micrograms/g of analysed dried vegetal product.

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 manganese 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.

RESULTS AND DISCUSSION

Generally, the oligoelements are absorbed by the plants as ions and they migrate towards the aerial parts of plants. The quantitative proportion of the chemical elements in the plants' body varies and depends of their concentration in soil, the metal type, vegetal species and the plant organ (source: http://www.eplantscience.com).

It is also worth to take into consideration the facts that the humidity, type of soil, the period, age of plant and their morphological characteristics influence their content of manganese (sources: http://www.plantnutrifert. org;http://www.researchgate.net/publication/233267440).

The obtained results are calculated for each element and the average of individual determinations (n=3) are specified in tables 1-3.

Table 1

The results of the determination of the Mn content (μg/g plant) in leaves according to the prelevation area and period

Item No.	Area	Prelevation year	Mn concentration (μg/g plant)	Standard deviation S	Trust interval
1.	Oradea	2005	107.24	0.035	0.086
2.	Oradea	2006	76.10	0.066	0.163
3.	Oradea	2007	18.88	0.140	0.348
4.	Adoni	2005	46.26	0.053	0.131
5.	Adoni	2006	81.23	0.092	0.228
6.	Adoni	2007	119.70	0.132	0.329

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

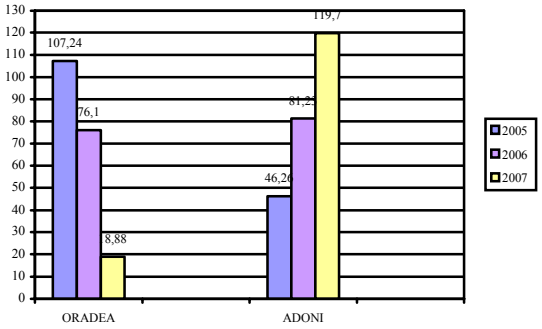

Fig. 1. The variation of the Mn concentration in leaves of *Aesculus hippocastanum* L. *(µg/g)* determined through AAS in 2005-2006-2007

The manganese content in leaves varies between 18.88-119.70 µg/g, the both minimal and maximal concentration being registered for 2007. The highest concentration is recorded for the samples from less polluted areas (Adoni), since for Oradea samples the concentration of manganese recorded a strong decrease.

Table 2

The results of the determination of the Mn content (µg/g plant) in flowers according to the prelevation area and period

Item No.	Area	Prelevation year	Mn concentration (µg/g plant)	Standard deviation S	Trust interval
1.	Oradea	2005	61.79	0.159	0.394
2.	Oradea	2006	56.08	0.066	0.163
3.	Oradea	2007	54.00	0.132	0.329
4.	Adoni	2005	40.43	0.040	0.099
5.	Adoni	2006	67.13	0.115	0.286
6.	Adoni	2007	86.65	0.229	0.569

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

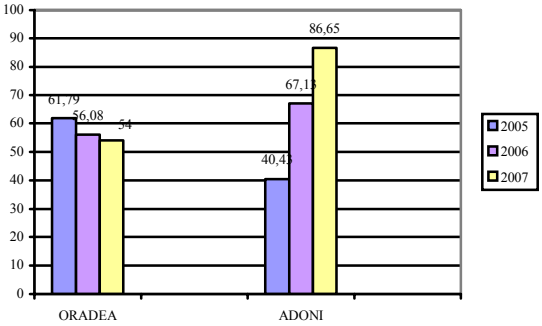

Fig. 2. The variation of the Mn concentration in flowers of *Aesculus hippocastanum* L. (µg/g) determined through AAS in 2005-2006-2007

The manganese content in flower samples from all areas varies between 40.43-86.75 µg/g. Since for Oradea samples the values are very near, an increase of about 50% was measured for Adoni samples.

Table 3

The results of the determination of the Mn content (µg/g plant) in fruits according to the prelevation area and period

Item No.	Area	Prelevation year	Mn concentration (µg/g plant)	Standard deviation S	Trust interval
1.	Oradea	2005	11.28	0.135	0.334
2.	Oradea	2006	12.01	0.050	0.124
3.	Oradea	2007	12.13	0.108	0.269
4.	Adoni	2005	6.32	0.053	0.131
5.	Adoni	2006	7.25	0.092	0.228
6.	Adoni	2007	8.32	0.201	0.499

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

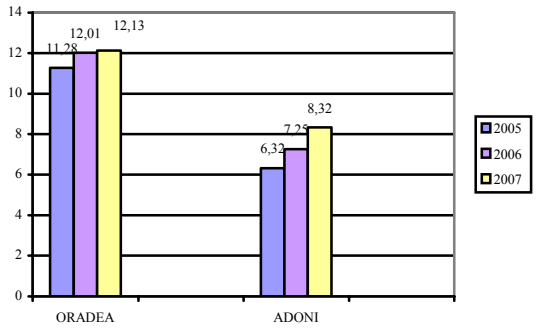

Fig. 3. The variation of the Mn concentration in fruits of *Aesculus hippocastanum* L. *(µg/g)* determined through AAS in 2005-2006-2007

Maximal concentrations of manganese (11.28-12.13 µg/g) were obtained from chestnut fruits from Oradea (2005-2007), since the Adoni samples were about 56% less concentrated in manganese.

CONCLUSIONS

The literature data 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 (Horvath, 2009; Horvath, Şerban, 2009; Horvath, Şerban, 2011; http://pubs.acs.org; http://www.scielo.cl).

The obtained results show a wide variation of manganese concentrations: horse chestnut leaves collected in Adoni, in 2007, contain 119.70 μg/g ant the ones collected in 2005, also in Adoni, contain 6.32 μg/g.

There is a decrease in manganese concentration in leaves and flowers harvested in Oradea with approximately 112%, and 90% respectively, while the manganese content in the samples of Adoni increases with approximately 270%, and 215% respectively. The maximum manganese concentration is found in the leaves from Adoni (119.70 μg/g), and the minimum manganese concentration is found in the sample from Oradea (18.88 μg/g), both samples being collected in 2007.

The flowers collected from Adoni in 2007 have the richest manganese concentration (86.65 μg/g), while the ones still coming from Adoni, but in 2005, have the poorest manganese concentration.

There is an increase of manganese concentration in horse chestnut fruit in all the samples with approximately 110% (Oradea) and with approximately 133% (Adoni). The manganese concentration limits are between 12.13 μg/g (Oradea, 2007) and 6.32 μg/g (Adoni, 2005).

The manganese concentrations (μg/g) decrease in 2005 – 2007 in leaves and flowers (Oradea) and increase in leaves, flowers and fruits (Adoni) and the average values in 2007 are: 28.34 μg/g (Oradea) and 71.56 μg/g (Adoni).

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