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SEM AND EDAX MORPHOLOGICAL OBSERVATION OF THREE ORNAMENTAL SPECIES OF THE GENUS MAGNOLIA

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ABSTRACT. The purpose of this study is to reveal certain preliminary aspects related to the morphology and ultrastructure of vegetative and reproductive organs in three species of Magnolia. The study will be extended by quantifying the particles of dust or other impurities adhering to the leaves, so as to show the amount of pollution characteristic of the areas from which the plant materials were sampled and are to be investigated. For that purpose, we will proceed by identifying the presence of certain reference metals by means of EDAX analyses. The detailed description of both structure and ultrastructure in Magnolia species is intended as relevant so as to enable a comparison to previous researches regarding the decorative trees/shrubs in question. Its purpose is to facilitate communication among researchers and practitioners, in the event that they wish to undertake studies at tissue, cell or even molecular level. For the purposes of generating a detailed description, several studies were carried out via optical microscopy and scanning electron microscopy. Scanning electron microscopy (SEM) will allow for in-depth study, resulting in an ample examination of the structure of vegetative and reproductive organs of trees yielding special ornamental value. This preliminary study is to be completed by pursuing the analysis of the other vegetative organs. The *Magnolia kobus* flower has much more particularities than that of the hybrids: a greater number of stamens, carpels, ovary chambers, pollen grains; this species also presents with poorly pigmented reproductive organs. Following the micro-elemental EDAX analysis of organic matter represented by leaves collected from different species of the genus Magnolia - *Magnolia kobus, Magnolia x soulangeana, Magnolia liliiflora* -, we were able to determine the presence or absence of those chemical elements which present a potential polluting effect. The chemical elements of significance in urban pollution which were determined by EDAX analysis were Al and Cl.

Keywords: Magnolia kobus, *Magnolia x soulangeana*, *Magnolia x "Susan"*, *Magnolia liliiflora*, scanning electron microscopy (SEM), EDAX

INTRODUCTION

The Magnoliaceae include a large variety of trees and shrubs of great interest, such as ornamental tree species (Zanoschi et al., 1996) planted and acclimatized in our country with great success, which add true landscape value to the embellished areas (Coandă & Radu, 2006). So far, in Romania, 13 species are grown as ornamental plants, one of which is a hybrid (Zanoschi et al., 1996). The richest collection of magnolias in our country can be found at Simeria Arboretum, which includes 10 species belonging to the aforementioned genus (Coandă & Radu, 2006). Among the members of the Magnolia family, we proposed to study three species, namely *Magnolia kobus*, as well as two hybrids: *Magnolia x soulangeana* "Soulange-Bodin" and *Magnolia x "Susan"*.

The selected species have the following origins: *Magnolia kobus* is native to Japan (Gilman et al., 1994; Leins el al., 2007; Groza, 2010), while the other two hybrids are the result of crossbreeding: *Magnolia x "Susan"* (*Magnolia stellata 'Rosea' x Magnolia liliiflora 'Nigra'*) and *Magnolia x soulangeana* "Soulange-Bodin" (*Magnolia denudata x Magnolia liliiflora*) (Stănescu et al., 1997; Dirr, 1998; Mohan & Ardelean, 2010).

The Magnolia are known as species of high ornamental value (Dumitru-Tătăreanu et al., 1960; Ardelean and Mohan, 2006; Mohan and Ardelean, 2010), the flowers of which display special decorative characteristics (Paşcovschi et al, 1954). The leaves of certain Magnolia species add an exotic touch to the landscape with the help of their quite unusual coloration and size (Dirr, 1998), while their seeds would attract anyone's attention, as they resemble earrings suspended from the intensely coloured cones - a true curiosity of the plant world (Mohan & Ardelean, 2010; Stănescu et al., 1997).

As entomophilous plants, primarily pollinated by insects, Magnolias are safe to grow in urban areas, as their pollen does not act as an allergen (Ianovici, 2007, 2008).

By using scanning electron microscopy, we can study these previously introduced and acclimatized plants from the perspective of specific characteristics of the tissues, reproductive and vegetative organs. However, by using the special EDAX detector, we can identify, based on the radiation X (specific to each chemical species) emitted by the sample, the chemical composition of the sample studied, both qualitative and quantitative. We can even render distribution maps for

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every chemical element in the study area. We analyse the adaxial and abaxial surface of the leaf slides, determining the size, granulometric distribution, morphology and chemical composition of individual particles (Tomasević et al., 2005). We identify the manganese (Fernando et al., 2006) and sulphur and its speciation forms (Zeng et al., 2013; Yarmolinsky et al., 2013) hyper-accumulations on the leaf, as well as the distribution of calcium ions (Kerton et al., 2009). We can confirm the capability to biosynthesise gold (Badri Narayanan & Saktivel, 2008) or silver (Arunachalam and Annamalai, 2013) nanoparticles by using leaf extracts. This type of analysis was used to identify the biological production of silver nanoparticles using Magnolia leaf extracts (Song et al., 2009).

MATERIALS AND METHODS

The plant material for the samples was collected from downtown Arad, the Micalaca area and the Simeria Arboretum- Dendrological Park.

Three samples of *Magnolia x soulangeana, Magnolia kobus, Magnolia x "Susan"* were examined (figures 3, 4, 5). A one year-old stem was analyzed. The obtained samples were excised and placed in Eppendorf tubes. The samples were then examined using scanning electron microscopy (SEM) for the purposes of studying anatomical and ultrastructural particularities.

SEM

An image is obtained via scanning electron microscopy (SEM) by detecting and measuring the electronic flows dispersed or issued (secondary electrons) from the surface of the sample preparation under investigation, organ fragments or even the specimen being studied (Hug et al., 2003).

Samples of *Magnolia x soulangeana, Magnolia kobus* and *Magnolia x "Susan"* were excised and placed in Eppendorf tubes in 2.7% Glutaraldehyde for 6 hours at 4° C (Crăciun et al., 1996). They were sputter-coated with gold, three times (4 nm thickness/deposition) (Stokes, 2008). The anatomical studies were made using Fei Quanta 250 SEM, while macroscopic images of their natural habitat were taken with a Nikon Coolpix L22.

EDAX

The organic matter used in micro-elemental analysis is represented by leaves collected from different species belonging to the genus Magnolia: *Magnolia kobus, Magnolia x soulangeana, Magnolia liliiflora*. The organic matter harvesting points were the Arad city centre and the central area of the city (two harvesting points), respectively the central area of Timisoara (figures 1, 2).

During the EDAX analysis, all samples were examined without metallization, being fitted on conductive aluminum brackets with double sided adhesive carbon discs. The EDAX analysis parameters were the following: HV examination mode, 20 kV acceleration voltage, EDAX detector, spot 5, 10 mm WD. Each assessment involved 6600 reiterations/sample.

Fig.1. Sample harvesting point for the species *Magnolia x soulangeana* and *Magnolia kobus*, Timisoara, Pestalozzi St.

Fig.2. Sample harvesting point for the species *Magolia x soulangeana* and *Magnolia kobus, Magnolia liliiflora*, Arad: 1. Revolutiei Blvd, 2. Tudor Vladimirescu St., 3. Calimanesti St.

RESULTS AND DICUSSIONS

The result of the SEM analyses was the ultrastructural description of different plant segments: gynaecium, ovary chambers, stamen (anther, filament) (Figures 3, 4, 5, 6 and 7).

As it we also notice in figures 3, 4 and 5, such shrubs feature a special ornamental value, mainly due to their specific floral coloration, which both delights visually and provides anchorage into a space of mental peace; that is to say, it transports the viewer to a small "Garden of Eden". The tepals of these species are: white (Ciocârlan, 2000, Azuma et al., 2001, Mohan & Ardelean, 2010, Groza, 2010), many of them hanging in groups at the top of branches in *Magnolia kobus* (figure 3B); pink-purple, sometimes white at the inside and with reddish median nerves at their base (Mohan and Ardelean, 2010), as it is the case with *Magnolia x soulangeana "Soulange-Bodin"* (figure 3A); pink-purple-reddish on the outside and lighter one the inside (Groza, 2010) in the case of the large-sized flowers of *Magnolia x "Susan"* (figure 3C). According to the location of floral elements, magnolias are spirocyclic flowers (Xu et al., 2006), with free floral elements (Ianovici et al., 2011). The first three tepals are relatively smaller (Ciocârlan, 2000, Mohan & Ardelean, 2010); numerous short, wide filaments and long, twisted anthers; polymeric androecium (Erbar et al., 2007); polycarpellary apocarpous gynaecium (Ianovici, 2009).

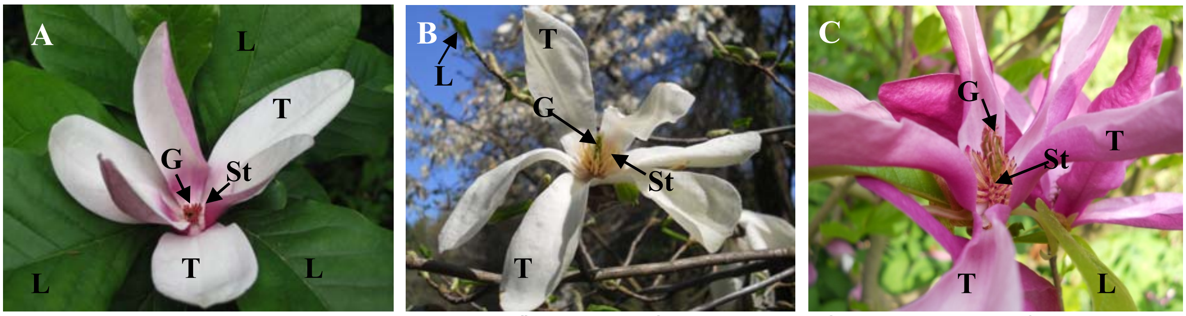

Fig.3. Flower morphology (12MP) ”: T – tepal, G – gynaecium, St – stamen, L- leaf
A - Magnolia x soulangeana “Soulange-Bodin”, B - Magnolia kobus, C - Magnolia x “Susan”

Magnolias have several carpels, each forming a separate pistil. The central portion of a Magnolia flower showing the central cluster of pistils surrounded by stamens is presented in figures 4-A, B, C. *Magnolia kobus* reveals numerous stamens which become wider and thicker; the widening is evident for *Magnolia kobus* in figure 5B, more so for *Magnolia x "Susan"* (figures 6C, 7C) and even more apparent for *Magnolia x soulangeana "Soul.-Bod."* (Figures 4A and 5A).

The *Magnolia k*obus gynaecium reveals large egg cells. The ovary chambers of *Magnolia x "Susan"* more frequently reveal two egg cells (figure 6C). *Magnolia kobus* presents a great number of carpels (figure 7B). Placentation is parietal (figures 6-A, B, C).

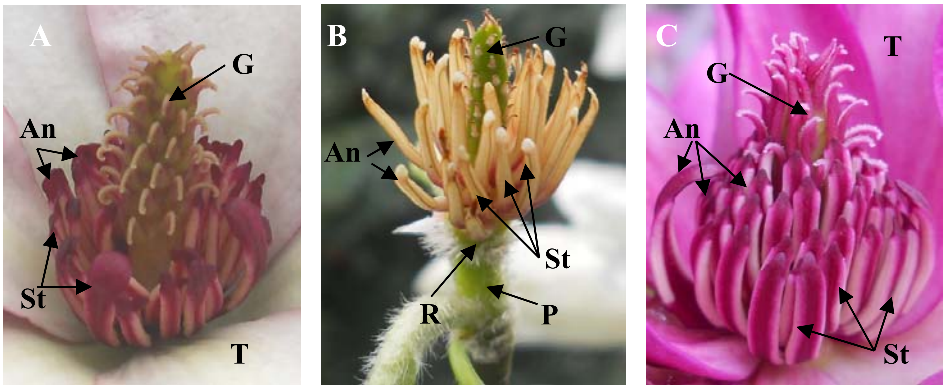

Fig. 4. Reproductive organs of the flower (12MP zoom 5,3x): T – tepal, G – gynaecium, An – anther, St – stamen, R – receptacle, P- peduncle
A- *Magnolia x soulangeana "Soul.-Bod.",* B - *Magnolia kobus, C - Magnolia x "Susan"*
B-

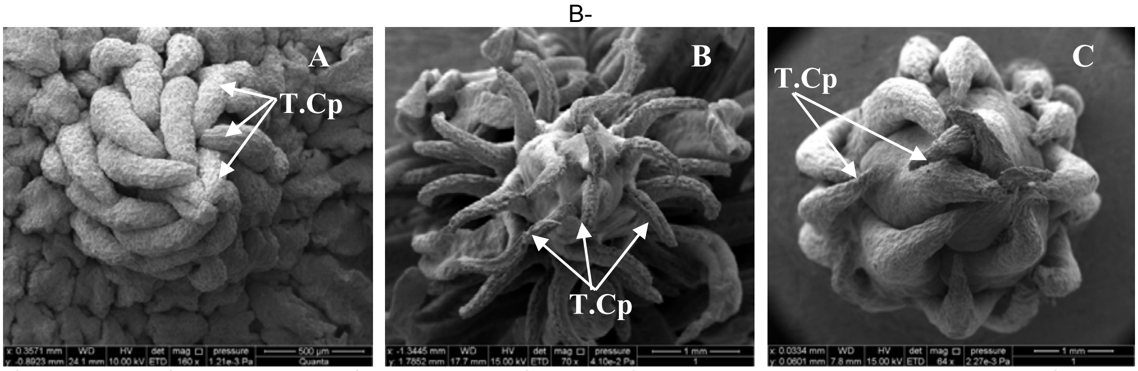

Fig.5. SEM images of the gynaecium: Cp – carpels, T.Cp – top of carpel. (A – mag. 160x, B - mag. 70x, C – mag. 64x)
A-Magnolia x soulangeana “Soul.-Bod.”, B-Magnolia kobus, C-Magnolia x “Susan”

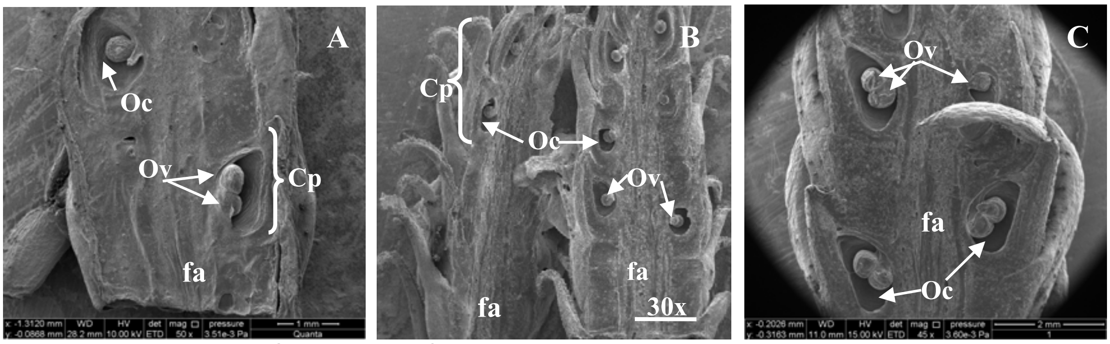

Fig. 6. SEM images of longitudinal sections through the gynaecium:
Ov – ovule, O – ovary, Cp – carpels, Oc – ovary chambers, St – stamens, fa –floral axis
A - *Magnolia x soulangeana "Soul.-Bod."*, B - *Magnolia kobus*, C - *Magnolia x "Susan"*

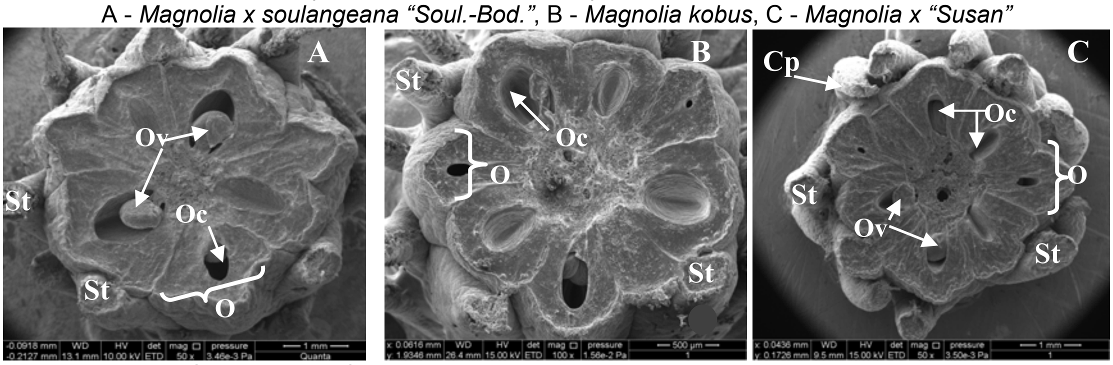

Fig. 7. SEM images of transverse sections through the gynaecium/ovary chambers:
Ov – ovule, O – ovary, Cp – carpels, Oc – ovary chambers, St - stamens
A-*Magnolia x soulangeana*, B-*Magnolia kobus*, C-*Magnolia x "Susan"*

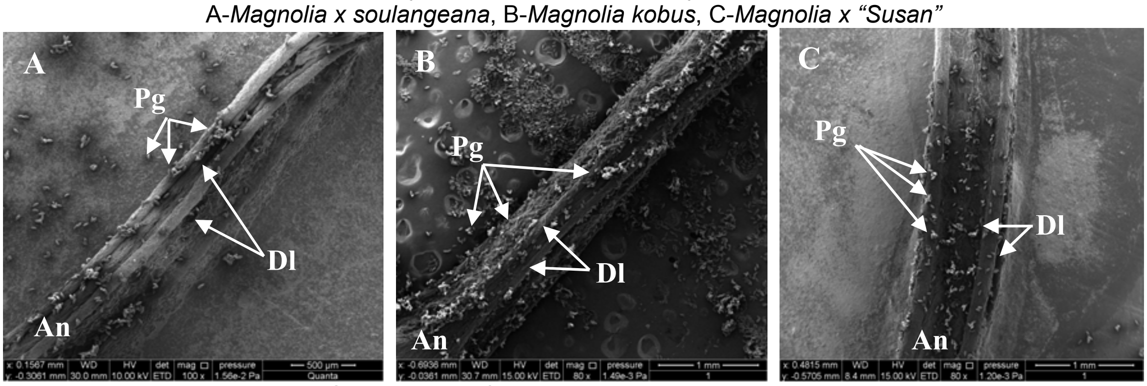

Fig. 8. SEM images of the middle anthers with pollen grains:
An – anther, Dl – dehiscence lines, Gr.p - pollen grains. (A – mag.100x, B,C – mag.80x)
A-*Magnolia x soulangeana "Soul.-Bod."*, B-*Magnolia kobus*, C-*Magnolia x "Susan"*

In all three cases, there are multiple dehiscence lines on the anther. *Magnolia x soulangeana* (figure 8A) reveals less pollen grains and deeper hollows. The anther has a slightly irregular surface. In *Magnolia kobus*, the anther has a harsh surface (figure 8B). *Magnolia x "Susan"* presents a strongly folded anther surface and its fold is highly pleated (figure 8C).

The terminal buds are large, revealing 1-2 scales (Zanoschi et al., 1996). The bud surface is entirely different in *Magnolia kobus* in comparison to the other two species – in size, as well as presenting a higher density of cataphyll hairs (figures 9, 10). The observed hairs spread uniformly on the surface of the protective sheath. The orientation of the hairs, both on the cataphyll of terminal buds and lateral buds, is oblique, as they extend from the base of the bud towards the tip. The color of the hairs is silver-white (figure 9-A, B).

Fig. 9. Stems with buds: A - Magnolia x soulangeana "Soul.-Bod.", B - Magnolia kobus, C - Magnolia x "Susan" (12MP)

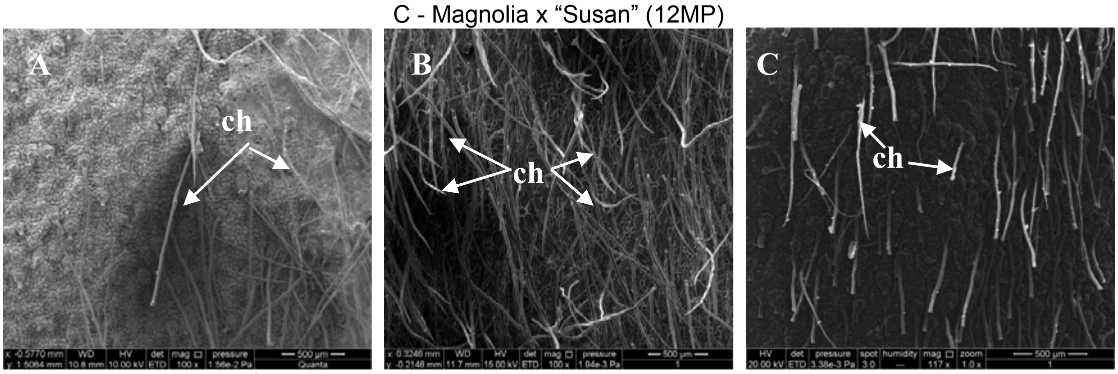

Fig. 10. SEM images of the surface of the buds (100x): ch - cataphyll hairs.
(A,B- mag.100x, C – mag.117x)
A-Magnolia x soulangeana "Soul.-Bod.", B-Magnolia kobus, C-Magnolia x "Susan"

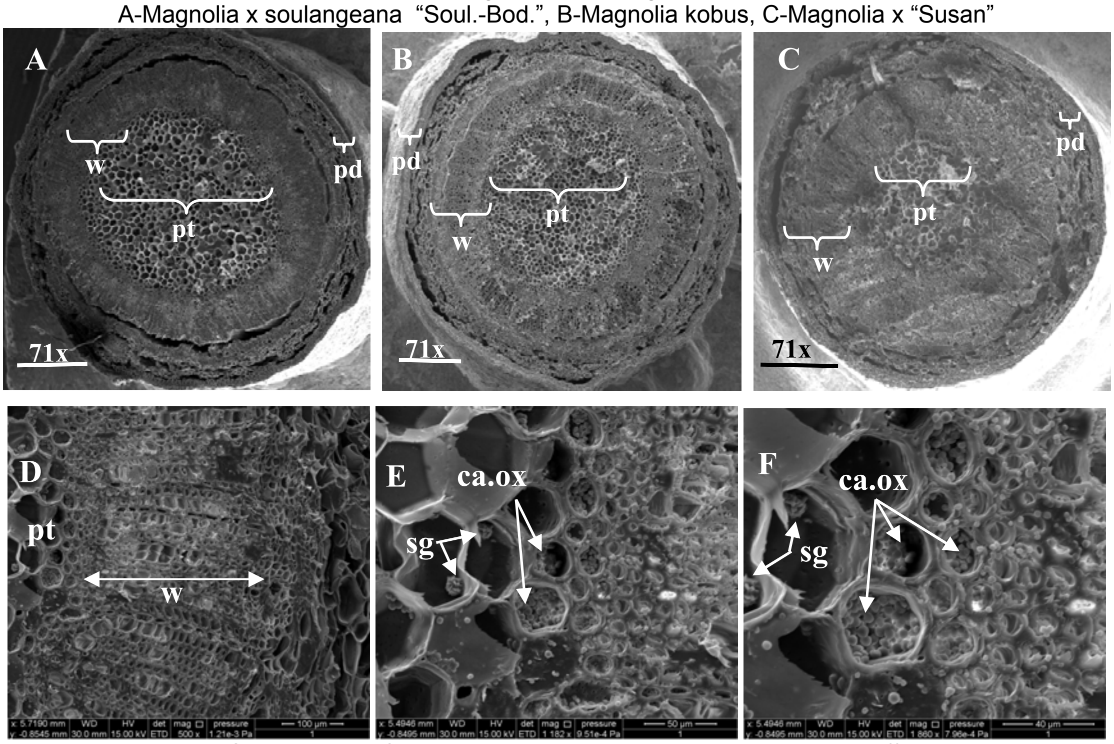

Fig. 11. SEM images of the transverse section through *Magnolia sp. offshoots*:
w – wood, lm.s – secondary wood, pt – pith, pd – periderm, ca.ox - calcium oxalate crystals, sg - starch grains
A, D, E, F - *Magnolia x soulangeana "Soul.-Bod."*, B - *Magnolia kobus*, C - *Magnolia x "Susan*

Studying the images of transverse sections featured in figure 11, we notice the following: *Magnolia x "Susan"* reveals a weak pith, but a prominent wood profile (figure 11C). *Magnolia kobus* presents a thicker periderm (figure 11B). In *Magnolia x soulangeana "Soul.-Bod."* (Figure 11A), we identify large quantities of starch grains (Figure 11 E, F) and calcium oxalate crystals (figure 11E) forming at the border between primary wood and pith (figure 9D).

As a result of the EDAX analysis on the adaxial surface level of the foliar limb in *M. kobus, M. x soulangeana, M. liliiflora*, we were able to determine the presence of the following chemical elements: C, O, Mg, P, S, K, Ca, Cl, Al (figures 12, 13, 14, 15, 16, 17).

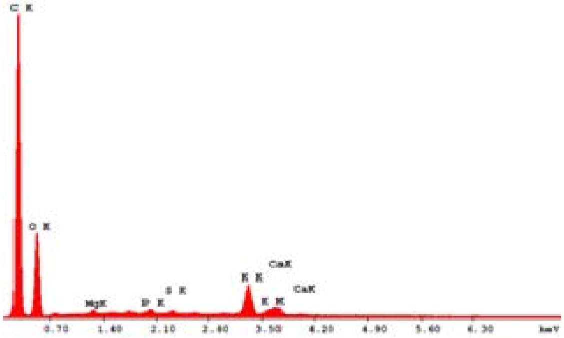

Fig. 12. Measurements spectrum resulting from the EDAX analysis and the chemical elements identified at the adaxial surface level of the foliar limb in *M. kobus* (harvesting point - Arad, Micalaca neighborhood, Calimanesti St.).

Weight % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	75.89	22.08	0.17	0.37	0.14	0.15	0.95	0.25

Atomic % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	81.44	17.79	0.09	0.18	0.06	0.05	0.31	0.08

Table 1. The semi-quantitative expression of the chemical elements identified in the fig. 12 spectrum was achieved by calculating Weight % by Element and Atomic % by Element.

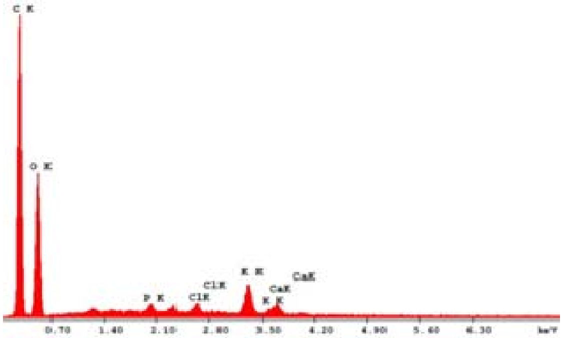

Fig. 13. Measurements spectrum resulting from the EDAX analysis and the chemical elements identified at the adaxial surface level of the foliar limb in *M. kobus* (harvesting point – Timisoara, Pestalozzi St.).

Weight % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	75.89	22.08	0.17	0.37	0.14	0.15	0.95	0.25

Atomic % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	81.44	17.79	0.09	0.18	0.06	0.05	0.31	0.08

Table 2. The semi-quantitative expression of the chemical elements identified in the fig. 13 spectrum was achieved by calculating Weight % by Element and Atomic % by Element.

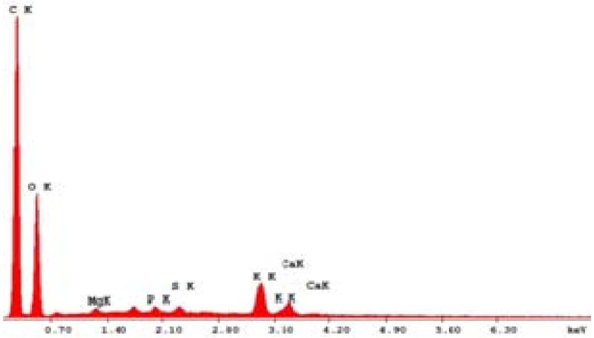

Fig. 14. Measurements spectrum resulting from the EDAX analysis and the chemical elements identified at the adaxial surface level of the foliar limb in *M. x soulangeana* (harvesting point - Arad, Tudor Vladimirescu St.).

Weight % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	75.89	22.08	0.17	0.37	0.14	0.15	0.95	0.25

Atomic % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	81.44	17.79	0.09	0.18	0.06	0.05	0.31	0.08

Table 3. The semi-quantitative expression of the chemical elements identified in the fig. 14 spectrum was achieved by calculating Weight % by Element and Atomic % by Element.

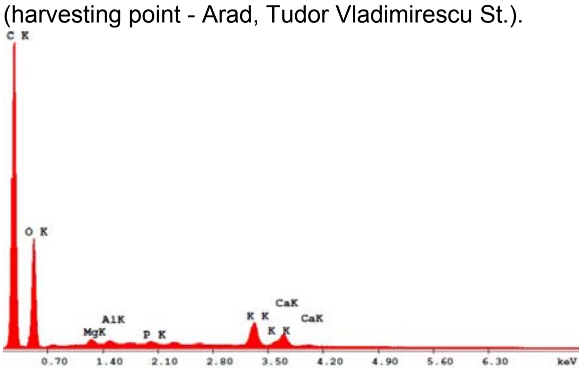

Fig. 15. Measurements spectrum resulting from the EDAX analysis and the chemical elements identified at the adaxial surface level of the foliar limb in *M. x soulangeana* (harvesting point - Arad, Micalaca n., Calimanesti St.).

Weight % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	75.89	22.08	0.17	0.37	0.14	0.15	0.95	0.25

Atomic % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	81.44	17.79	0.09	0.18	0.06	0.05	0.31	0.08

Table 4. The semi-quantitative expression of the chemical elements identified in the fig. 15 spectrum was achieved by calculating Weight % by Element and Atomic % by Element.

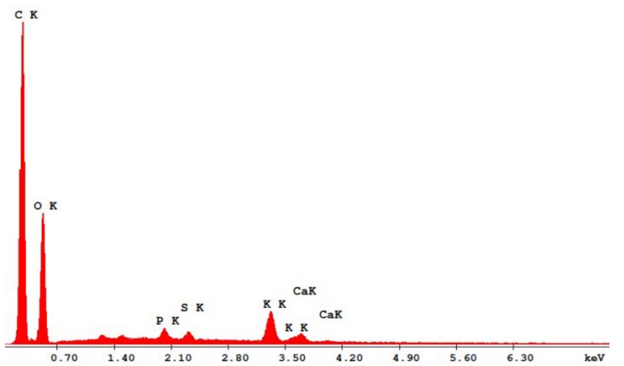

Fig. 17. Measurements spectrum resulting from the EDAX analysis and the chemical elements identified at the adaxial surface level of the foliar limb in *M. liliiflora* (harvesting point - Arad, Revolutiei Blvd).

Weight % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	75.89	22.08	0.17	0.37	0.14	0.15	0.95	0.25

Atomic % by Element

Filename	C K	O K	MgK	AlK	P K	ClK	K K	CaK
genspc.spc	81.44	17.79	0.09	0.18	0.06	0.05	0.31	0.08

Table 6. The semi-quantitative expression of the chemical elements identified in the fig. 17 spectrum was achieved by calculating Weight % by Element and Atomic % by Element.

The distribution of the chemical elements identified at species level was the following: *Magnolia kobus*: C, O, Mg, P, S, K, Ca, Cl; *Magnolia x soulangeana*: C, O, Mg, P, S, K, Ca, Al; *Magnolia liliiflora*: C, O, Mg, Al, P, Cl, K, Ca. The qualitative differences in determining the chemical elements are represented by the presence of traces of Cl in M. kobus and *M. liliiflora* and the absence thereof in *M. x soulangeana*, respectively the presence of Al in *M. x soulangeana* and *M. liliiflora*.

The presence of polluting chemical elements, correlated to the harvesting point, presents as follows: we identified S, P, Al, Cl in the Arad city centre, as well as the central area of the city, where Cl is, however, absent. The presence of polluting chemical elements, correlated to the harvesting point, when comparing the two cities reveals that Al is present in Arad, yet absent in Timisoara.

The semi-quantitative expression of the chemical elements revealed elevated values of C, O (expressed in percentages), ranging between 75.89-81.44% C, while the 17.79-22.08% difference expressed in percentages represents trace quantities of chemical elements Al, S, P, Cl, Mg, Ca, K.

A different research path would involve focusing the investigation on the transmission electron microscopy (TEM). By means of transmission electron microscopy (TEM), an image is formed from the interaction of the electrons transmitted through the specimen. In the case of this type of microscopy, the main steps (the accuracy of which highly impacts the compilation of certain data, as closely as possible to the real condition of living cells), are as follows: sampling, stabilization, dehydration, infiltration and inoculation, capsulation, shaping, cutting, contrasting, examination and digital recording of images, followed by further computer processing.

CONCLUSIONS

This preliminary study must be completed by performing certain analyses of the other vegetative organs.

The *Magnolia kobus* flower reveals far more particularities than those of the hybrids: a greater number of stamens, carpels, ovary chambers, pollen grains, poorly pigmented reproductive organs. The anthers have a threadlike appearance, with a discreet recurved tip, in all three species.

Between the two hybrids, *Magnolia x soulangeana "Soul.-Bod."* presents the lesser amount of hairs. Also, Magnolia kobus shoots have a thicker periderm, as compared to the other studied hybrids.

As a result of the imaging analysis via electronic microscopy techniques (SEM and ESEM), as well as the micro-elemental analysis (EDAX) of the organic matter represented by leaves collected from different species of the genus Magnolia – *M. kobus, M. x soulangeana, M. liliiflora*, we were able to determine the presence or absence of chemical elements which present a potential polluting effect.

The chemical elements of significance in urban pollution, as determined via EDAX analysis, were Al, P, S, Cl.

Using the EDAX type of analysis and detection, we can easily determine the chemical elements responsible for pollution in ornamental species of decorative plants of economic significance and found in the dendroflora of urban agglomerations.

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