Qualitative Detection of Mg Content in a Leaf of *Hedera helix* by Using X-ray Radiation From a Laser Plasma Source

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KEY WORDS X-ray contact microradiography; plant leaves; magnesium target; leaf details; phytoremediation

ABSTRACT In this article, a method to reveal the presence of Mg content inside the different parts of leaves of *Hedera helix* is presented. In fact a sample of a *Hedera helix*'s leaf, commonly characterized by a green and a white side, is analyzed under X-ray radiation. The presence of two zones with different colors in the *Hedera helix*'s leaf has not been explained. In this connection, there are presently three hypotheses to explain the characteristic double-color appearance of the leaf. The first hypothesis suggests a different cytoplasmic inheritance of chloroplasts at the cell division, the second a different allelic composition, homozygote and heterozygote, between the two zones, and finally the third the action of a virus which changes the color properties in the *Hedera*'s leaves. The resulting effect is a different content of "something" between the green and the white side. We utilized X-ray radiation, obtained from a plasma source with a Mg target, to image *Hedera helix* leaves and we found that the green side of the leaf is highlighted. We may suppose that the reason why the X-rays from a Mg plasma source, allow us to pick up the green side is probably due to the greater presence of the amount of Mg (from chlorophyll or other complexes and/or salts) in the two sides, green and white, of the leaf. *Microsc. Res. Tech. 71:459–468, 2008.*

INTRODUCTION

The most common techniques of imaging samples are mainly concerned with the morphological analysis of the cellular structure and ultrastructure at different resolution.

As is well known, that standard optical microscopy can produce images of the sample with a resolution of about 0.2 μm, whereas by the transmission electron microscopy (TEM) and scanning electron microscopy (SEM) it is possible to obtain a resolution of about 0.2 nm and 10 nm, respectively. However, it is worth noting that both the TEM and SEM requires a significant modification of the sample through different treatments; specifically dehydration and reaction with electron dense salts (i.e. osmium tetraoxide) for the TEM and special treatments of the sample's surface for the SEM (Everhart and Hayes, 1972).

Within the last few years a new method of sample analysis, based on the direct observation of the sample under X-ray radiation is now being applied, which enables the observation of the samples in a condition close to their physiological state, thus preventing unwanted artifacts. (Albertano et al., 1997a,b; Bollanti et al., 1998; Cotton et al., 1992, 1995; Fletcher et al., 1992; Panessa-Warren et al., 1989, 1991).

It should be noted that X-ray radiation is mainly obtainable through synchrotrons and laser generated plasmas.

The importance of the X-ray based method basically resides in the possibility of selectively imaging the presence of different chemical elements.

In fact, it is well known, the presence of a specific chemical element in the sample can be revealed by exposing the sample to X-ray radiation with a wavelength range including the absorption edge of that element.

Using a laser generated plasma source, it is possible to use the chemical element to be detected as the target for the laser beam thus producing X-ray radiation with a wavelength spectral range, which usually includes the L-edge or K-edge wavelength from the target ele-

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ment. (Albertano et al., 1997a,b; Bollanti et al., 1998; Cotton et al.,1992, 1995; Fletcher et al., 1992). Hence, using different chemical elements as the target, makes it possible to observe in the X-ray detector where these metals have accumulated within the sample (Kaiser et al., 2005, 2007; Reale et al., 2004, 2006, in press).

We note that the detector is usually a CCD camera or an X-ray sensitive photo resist like polymethyl-methacrylate (PMMA). Also a photographic film selectively sensitive to X-rays (RAR 2492 film) is widely used.

Moreover, the procedure is further enhanced by using monochromatic X-ray synchrotron radiation (Howells et al., 1991), which can be tuned to the desired X-ray wavelength (Howells et al., 1991; Kaiser et al., 2004).

In this way it is possible to select the wavelength corresponding exactly to the L-edge or K-edge of the chemical element under inspection. Then, in the X-ray image of the sample it is possible to localize the biological sites where this chemical element absorbs X-rays within the sample (Howells et al., 1991).

As a consequence, interest in the use of, the X-ray radiation based technique for the imaging of biological samples has increased rapidly. (Albertano et al., 1997a,b; Bollanti et al., 1998; Cotton et al., 1992, 1995; Fletcher et al., 1992.). A variety of methods have been developed: contact X-ray microscopy, X-ray contact microradiography, projection microscopy with zone plate lenses, and diffraction microscopy.

Contact X-ray microscopy is used to observe a sample within the water window wavelength range of 2.4–4.3 nm. Through this spectral range, carbon is not transparent to the X-ray radiation while oxygen in water is, and hence a natural contrast appears in the X-ray image on the X-ray photo sensitive substrate.

In the X-ray contact microradiography, the final image is obtained using different spectral ranges corresponding to the spectral range relative to the L-edge or K-edge of a specific chemical element, which is frequently outside of the water-window (Kaiser et al., 2005, 2007; Reale et al., 2004, 2006, in press).

In this article, a method of imaging samples of *Hedera helix* leaf by contact microradiography on the photographic film RAR 2492 is presented.

The *Hedera helix* leaf is characterized by having different colors on its two sides, one being white and the other green. Such a characteristic coloration is due to the different content of "something" which gives the green color.

Some theories explain such a difference by means of a different chlorophyll content, hypothesizing a cytoplasm inheritance of chloroplasts during the cell division, or a different allelic composition in the two areas: homozygote and heterozygote, respectively, corresponding to the green and white side (Gorsic, 2000), or also the presence of a virus that changes the color properties of the chlorophyll in the leaf white side. But there could be other contributory reasons for this difference in color.

In this article we report on the observation on the *Hedera helix* leaf by X-ray radiation and describe the preliminary investigations carried out in order to establish whether the aforementioned difference, between the two leaf sides might be due to a different content of magnesium (Mg). To this end, by using the X-ray radiation produced by a XeCL laser plasma source on a Mg target we verified the possibility of reproducing the typical two colors (green and white) of the *Hedera* leaf in the resulting X-ray radiographic image, thus demonstrating that the different absorption of the X-ray radiation in the two areas (and so the two colorations) originate from a different concentration of Mg possible present either in chlorophyll or in other molecules containing Mg or Mg salts.

MATERIALS AND METHODS

Characteristics of the Plasma X-ray Source

The experimental apparatus for the X-ray microradiography, shown in Figure 1, is rather simple: the leaf is placed inside a dark box (the holder) protected by an aluminum filter and is exposed to the X-ray radiation from a laser plasma source; a RAR X-ray film is placed just 1 mm behind the leaf.

The laser plasma source used for the leaf imaging is located at the ENEA research Center of Frascati, Italy. It is driven by a high-power Excimer laser emitting in the UV spectral range, which has a pulse energy of about 1.3 J and a pulse duration of 9 ns, thus allowing us to obtain an X-ray source suitable for Soft-X-Ray Contact Microradiography of plant leaves (Kaiser et al., 2005, 2007; Reale et al., 2004, 2006, in press). The laser radiation was focused by a lens on a Magnesium (Mg) tape target placed in the middle of a vacuum chamber (Bollanti et al., 1995, 1996, 1998). The corresponding X-ray radiation has a wavelength in the range of 0.7–1.0 nm including the K-edge of the Mg target itself at 0.951 nm, with an X-ray fluence of about 0.07 mJ/cm^2/shot.

Furthermore, the X-ray source diameter is $\sim$30 μm due to the rather high divergence of the laser and to the low numerical aperture (NA $\sim$ 0.16) of the focusing lens. The distance from the plasma source and the holder is d = 34 cm.

For the exposure of the samples 1,000 shots of the laser system have been applied to obtain the microradiographies.

As is well-known, the energy of soft X-ray radiation is higher than the energy of any chemical bound, and hence the microradiographies are in principle able to yield the map of the single chemical elements within the sample independently of their chemical bonds.

In fact, the inner electrons in the L-edge of K-edge of each atom absorb the X-ray radiation. Since the absorption involves the atoms individually and independently of their chemical bonds, this technique may in principle provide information about the number of atoms/cm^2 that are present inside the sample.

Such an argument is also reinforced by the fact that using a laser plasma source of X-ray radiation with a target of an element, present in the sample, it could, in principle, be possible to detect the presence of that element in the sample. In fact the spectral range of the X-ray radiation produced in the plasma mainly lies to the left of the L-or K-edge of the target element, where the absorption of that element is particularly high (Kaiser et al., 2005, 2007; Reale et al., 2004, 2006, in press).

As an example, Figure 2 shows the emission and absorption spectra of Mg targets. Evidently, the wave-

length emission range is 0.7–1.0 nm while the wavelength absorption range is 0.1–1.0 nm. Both of them include the K-edge value for Mg, while the emission spectra lay to the left side of the absorption spectra wavelengths, where the absorption of Mg is highest.

Then, one can say that in this region of the spectrum the absorption of the Mg present in the leaf should be high and therefore it should be possible to localize this element within the leaf.

The Soft-X-ray Beamline

The X-ray absorption spectroscopy was performed on the leaf samples at the Mg K-edge (1,310 eV). The measurements were carried out at the DAΦNE soft X-ray beam-line (1–3 keV), located at the INFN National Laboratory of Frascati. The DAΦNE double storage ring collider for electrons and positrons was operated with an electron beam energy of 0.51 GeV and a mean electron current $I > 1$ A. The X-ray monochromator, equipped with a pair of KTP ($KTiOPO_4$, $2d = 10.95$ Å) crystals, in the "boomerang" geometry was used to ensure a fixed exit mode while scanning the whole energy range. The energy resolution is of about 1.0 eV at 1.3 keV. The Mg K-edge X-ray absorption spectrum of an MgO sample was measured for energy calibration in transmission mode. Also, spectra from all the samples were taken in transmission mode. Nitrogen-filled ionization chambers were used to monitor the incident (I_0) and the transmitted intensity (I_t) of the beam.

The samples were fixed on a translation manipulator and introduced into the evacuated sample chamber at about 10^{-4} mbar. The spectra were collected at room temperature in the energy range (1.300–1.325) eV with 0.4 eV step width and 1 s acquisition time per step.

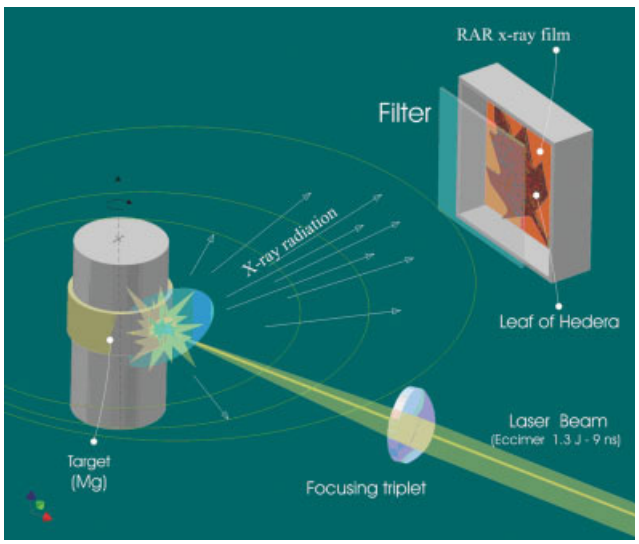

Fig. 1. Experimental system for X-ray contact microradiography using a laser generated plasma to excite Mg X-rays. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Sample Preparation, Analyses, and Microradiographies

Young leaves of *Hedera helix* were exposed to X-ray radiation.

The leaves have not been treated at all. They have only been dehydrated by alcohol gradient (50, 70, 80, 95%, and absolute) in order to avoid any absorption of X-rays by the natural water content.

The leaves have been divided in four groups according to the leaf's size in order to analyze leaves of roughly the same age. For each group the dry weight, thickness, and surface density average values of the leaves have been evaluated for both the green and white sides. The surface density X (g/cm^2) is the mass of contaminant material per unit of leave surface. It can be calculated as:

$$X = (1/\mu)\ln(1/T) \qquad (1)$$

where T is the ratio of the transmission of the treated sample with respect to the control sample and μ is the mass absorption coefficient (in units of cm^2/g) of the contaminant material at the central energy value of

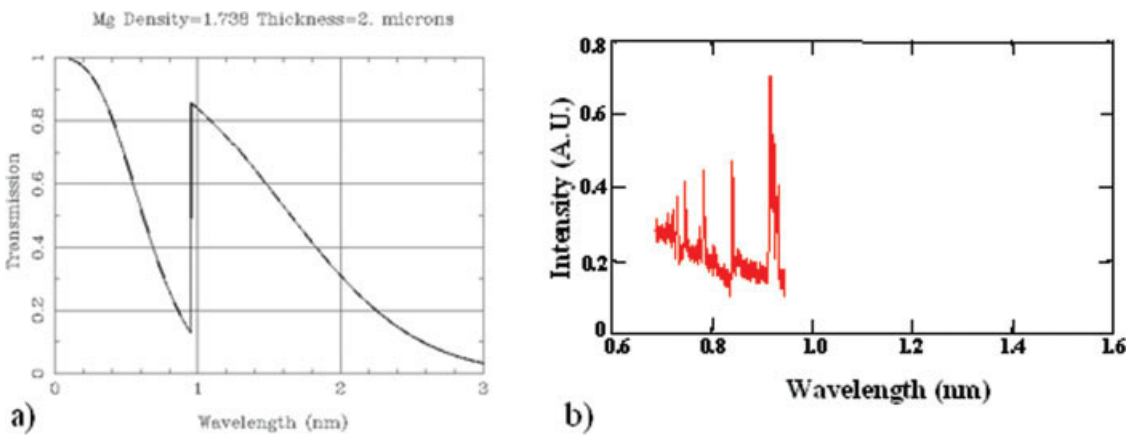

Fig. 2. Spectral range of transmission (**a**) and of emission (**b**) of X-ray radiation produced by a plasma sources using a Mg foil target. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

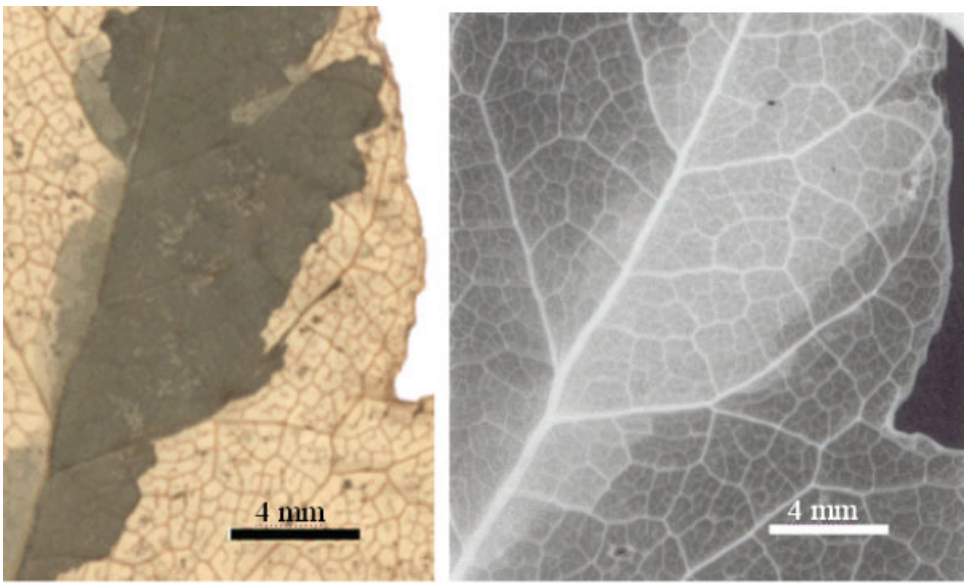

Fig. 3. Leaf of *Hedera helix*, (**a**) photographic image of a sample dehydrate with alcohol, (**b**) same sample imaged with X-ray radiation.

the spectrum (Kaiser et al., 2007). Several sections of a fixed area (1.9 cm^2) were cut according to the size (and then the age) of each leaf from either the green or the white side. The weight, the thickness, and the surface density of each of the 1.9 cm^2 sections cut in each leaf for the green or the white side have been evaluated in both dry and wet conditions, and then averaged according to the number of sections for each leaf, thus obtaining the average weight, thickness, and surface density of the leaves in each of the four different groups for both dry and wet leaves.

These means, we cut a number of small round samples of 1.9 cm^2 from the green part and an equal number from the white part of the leaves. The green and white areas occupy leaf domains extended through the thickness of the leaf (Fig. 3). We measured the parameters (dry weight, thickness, and surface density) averaging among the number of the round sample sections from green and white part, respectively. We do this for each leaf of the four different groups according which the leaves are separated for their surface area and age (in this sense each of the four groups consist of leaves grouped together because of their surface follows in one of four different range of area).

The collected data of weight, thickness, and surface density were compared with an ANOVA analyses (6.0 version of SPSS) to establish any statistically significant difference between the green and the white side areas (see later).

These measurements have been done to support the microradiographic results.

In fact, some of the leaves were exposed to 1,000 shots of the X-ray laser plasma radiation source. The laser beam was focused on a magnesium target and the X-ray sensitive detector was the RAR Film 2492 located behind the leaf sample.

The dehydrated leaves and the radiographic film (a RAR Film 2492) were held in the leaf and film holder. The distance between them was dictated by the thickness of the film loading support in the holder of 1 mm.

The X-ray microradiographies have been obtained according to the previously described experimental scheme with a target of Magnesium with 1,000 shots at the excimer laser Hercules of ENEA with pulse duration of 9 ns. The energy of the laser was 1.3 J while the X-ray fluence was about 0.07 mJ/cm^2/shot.

After exposure to X-rays, the films were developed in Ormano Bromor ST-50 1:4 diluted and fixed in Ormano Superfix F205 1:4 diluted 5 min each.

Eventually, the dehydrated leaf samples were observed at visible wavelengths by a scanner of 600 dpi resolution (EPSON), while the microradiographs of the leaves on exposed RAR film 2492 were imaged by a scanner with 2,000 dpi resolution for radiographic films (CanonScan FS4000US) (Fig. 3).

Moreover, the RAR films were also observed under an optical microscope with the objective lens 40× and 100× to obtain different magnification of either the leaf's white or green side X-ray image.

Atomic Absorption Spectrometry Measurements

For solution analysis the white part and green part of the leaf samples were dissolved inside autoclave (ZA-1, JZD Pokrok) in 5 mL of nitric acid for 2 h at the temperature 140°C. The calibration solutions for Mg were prepared in 5% HNO_3.

The Mg content was measured by flame atomic absorption spectrometry (AAS) technique using the NovAA 300 (Analytik Jena) instrument by taking separate samples from the green part of the leaves and the white part of the leaves.

TABLE 1. ANOVA (F values) for dry weight, surface density, and thickness of the green and white side of four different groups of Hedera helix leaves divided according with size (and than age)

	Weight (mg)		Surface density (mg/cm^2)		Thickness (cm)	
Leaf size	Green side	White side	Green side	White side	Green side	White side
1	22.67	10.17	10.05	4.59	0.02	0.01
2	25.55	12.18	9.65	4.64	0.02	0.01
3	25.25	11.00	9.82	4.21	0.02	0.01
4	26.00	11.33	10.17	4.44	0.02	0.01
F (color)	46.099[a]		204.04[a]		163.94[a]	
F (leaf size)	0.345 n.s.		0.115 n.s.		0.64 n.s.	
F (interaction)	0.044 n.s.		0.200 n.s.		0.002 n.s.	

The values listed for the leaf size are the means inside each different group.
n.s., non-statistically significative.
[a]Statistically significative.

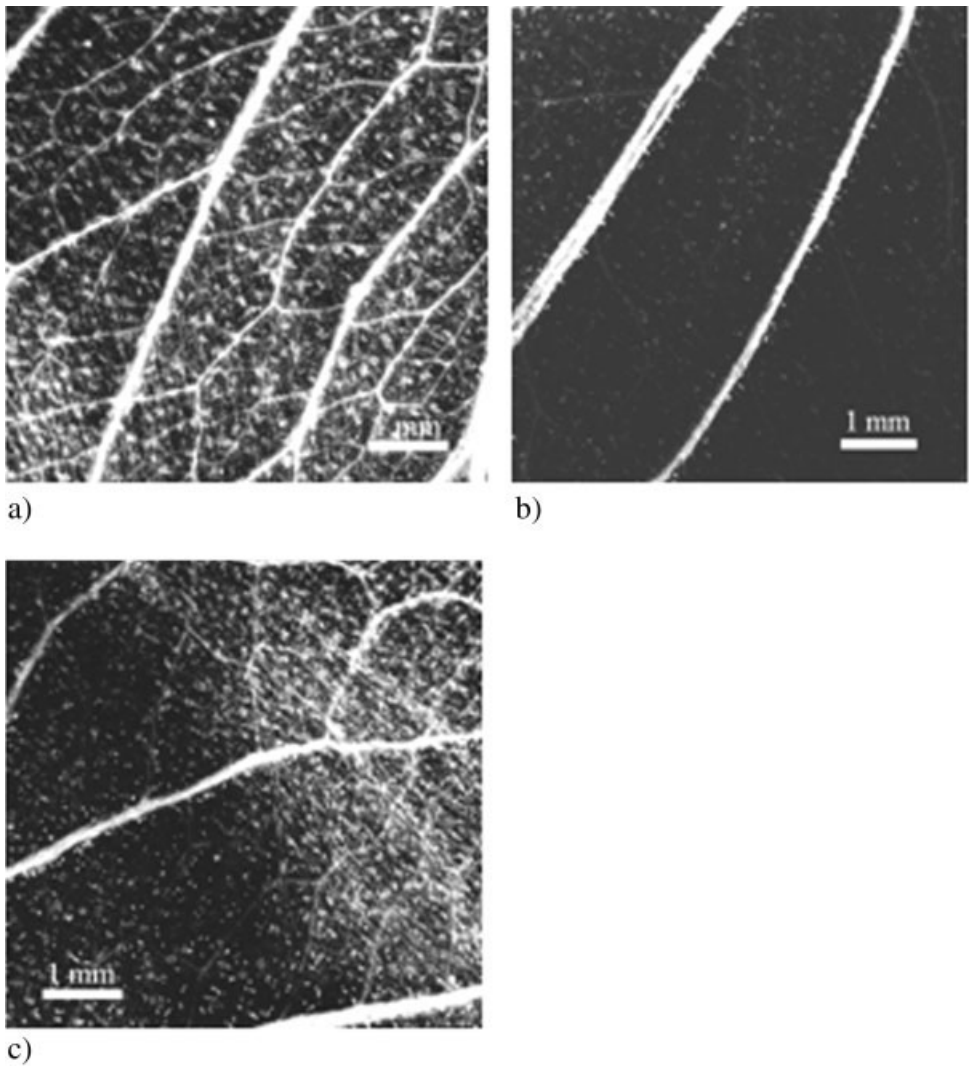

Fig. 4. Microradiograph negatives of leaves of *viola tricolor*: (**a**) control; (**b**) treated with acetone; (**c**) control and treated areas together in the same leaf: the treated area is the half surface on the bottom left of the figure.

RESULTS AND DISCUSSION

As remarked in the Introduction, the main aim of this article is to confirm the suspected difference in the natural Mg content in the green and white side of a *Hedera helix* leaf by observing the half leaf samples with the soft X-ray radiation emitted by a laser plasma source. Indeed, microradiographies of leaf samples have been

produced through the experimental arrangement described in Materials and Methods (see also Fig. 1).

The image of a *Hedera helix*'s leaf at the visible spectrum wavelength and the corresponding microradiography on a RAR film 2492 are shown in Figure 3. This is the whole leaf where the green and white parts that go through the whole leaf thickness can be clearly distinguished (Fig. 3a).

In the X-ray image with a resolution of 2,000 dpi it becomes evident where the X-ray absorption in the sample has been higher (white region of the microradiography), the relevant area observed in the visible range being green, and where the absorption has been lower (dark regions of the microradiography) corresponding to the white region in the leaf. In this case the white and the green areas go through of the whole leaf thickness. In the transmission images we can detect different absorption of the radiation created on the magnesium target by white and green areas.

The different tones of the X-ray image could mainly be due to two reasons. They could arise, for instance, from a different thickness of the white and green sides of the leaf (hypothesis 1). Alternatively, they could originate from a different content of Mg present in chlorophyll, Mg salts, or Mg associated macromolecules, in the two sides of the leaf. In other words, the green side could be have a higher Mg concentration than the white side, and the green side would display a higher absorbance of X-radiation, resulting into a lower density region in the negative's image (hypothesis 2).

However, it should be also pointed out that if the difference in the two micrograph densities, darker and whiter, were due only to a difference in the side thickness, there should be an area of intermediate thickness between the two sides and so a smooth passage between the dark and white regions in the image. Actually, as we see, the separation line between the two regions is steep (Fig. 3b).

As mentioned earlier, for the statistical analysis the leaves have been divided in four groups according to the their size in order to analyze leaves of approximately the same age.

Here, we have been enclosed the ANOVA analyses only for the dry leaf data since water in the hydrated samples does not permit an exact comparison of the various parameters. In fact the weight and the thickness of the hydrated leaves changes significantly during the experiment compared to the dehydrated leaves.

The ANOVA test results conducted with the four groups of leaves are shown in Table 1. The results are statistically significant for the leaf weight, surface density, and thickness when considering only the color effect, while when considering only the leaf size (related to leaf age) the difference is not significant whereas if color and leaf size are considered together then the difference is significant.

This analysis also reveals that the values of the weight, the surface density, and the thickness of the green areas are on average about twice the corresponding values for the white areas.

These results appear to confirm the hypothesis of a difference in the thickness between the two leaf sides, as previously suggested in hypothesis 1. On the other hand, this observation does not disprove hypothesis 2

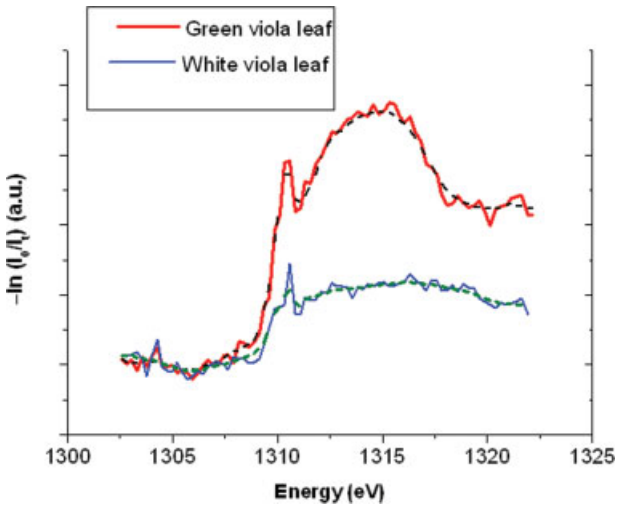

Fig. 5. Absorption spectra (equivalent to the optical density in the X-rays) of the tricolor green leaf (upper curve) and of the tricolor decolored leaf (bottom curve). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

concerned with a difference in Mg concentration between the green and white side, since the difference in microradiograph negative density does not correspond to the leaf thickness. Therefore, both hypotheses about the different densities in the microradiograph negative are potentially acceptable. Moreover, from the biological point of view, it is not clear why the green side should be thicker than the white side.

To understand if hypothesis 2 might significantly contribute to the change of X-ray absorbance between the green and white side, we performed a completely different experiment, in which the complications due to a possible variation of thickness were eliminated.

For this new experiment, some leaves of a plant of *Viola tricolor* have been collected and treated with acetone, which removes the chlorophyll and its associated Mg. The Mg present in chlorophyll is only a small part of the Mg present in the leaf. Nonetheless, this slight change in Mg concentration in the green side of the leaf should reduce the corresponding X-ray absorption in the microradiograph.

The microradiographs of three different *Viola* samples, obtained, as before, using X-ray radiation from a plasma source with a Mg target, are shown in Figure 4. Figure 4a shows the detail of a microradiography of an intact, i.e., green, leaf that was only air dried. We can see that there are many spots in the leaf, which have absorbed the X-rays. The second, Figure 4b, shows the detail of a leaf treated with acetone to remove chlorophyll. This treated leaf appeared completely white in the visible light range while the corresponding X-ray image, in Figure 4b, clearly shows reduced X-ray absorption by the leaf. It is interesting to note the absence of white spots indicating X-ray absorbance; also the dark background seems uniform but still contains some fine white spots.

The third leaf sample in Figure 4c has been treated with acetone only on the left half of the leaf. In the microradiography, shown in Figure 4c, two distinct

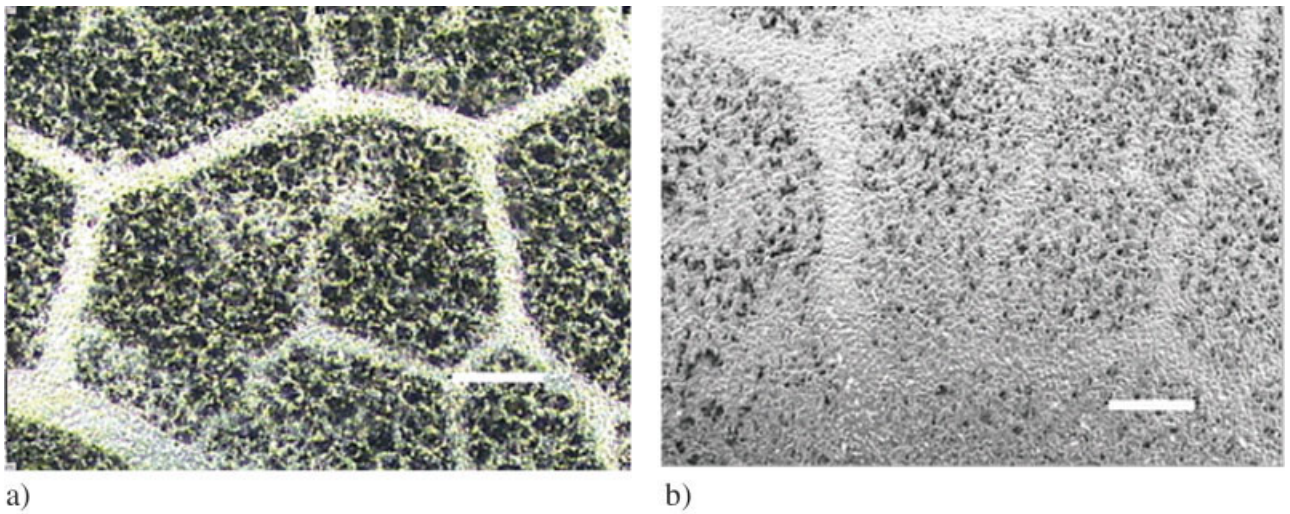

Fig. 6. Leaf of *Hedera helix*, negative image on a RAR film observed at an optical microscope, with a 40× objective, (**a**) detail of the white side, (**b**) detail of the green side (bar = 40 μm).

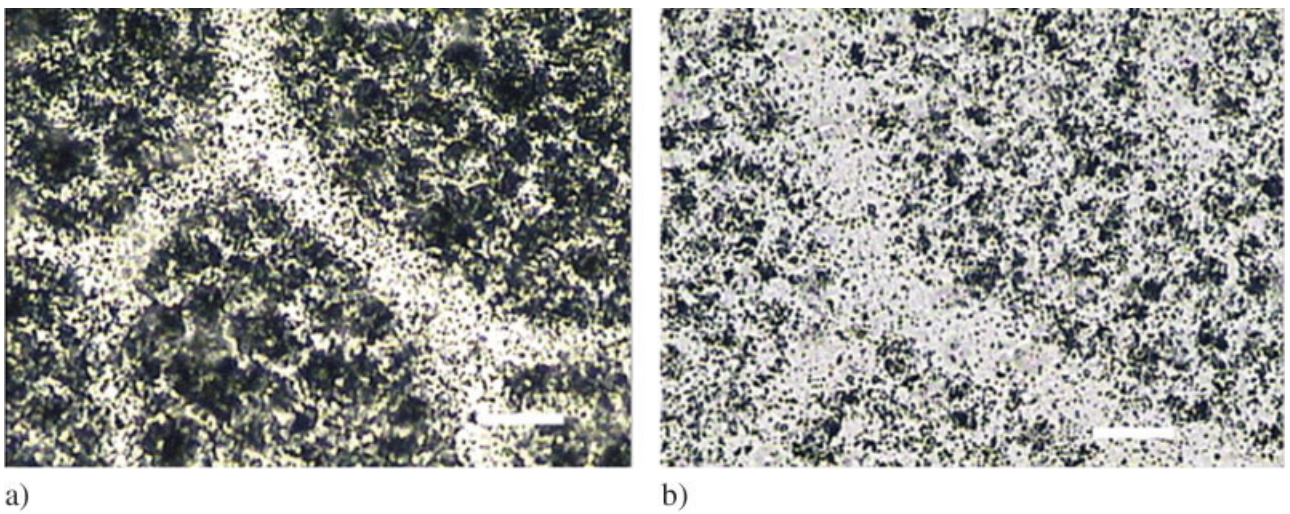

Fig. 7. Leaf of *Hedera*, negative image on a RAR film observed at an optical microscope, observed with a ×100 objective, (**a**) detail of the white side, (**b**) detail of the green side; (bar = 8 μm).

regions are clearly visible. This means that removing chlorophyll and its associated Mg, and perhaps other Mg associated organics can strongly influence the resulting image in the X-ray microradiograph. Also, this image clearly demonstrates that there is no thickness effect responsible for the effects in Figure 3.

It is worth noting that it was not possible to treat with acetone directly the *Hedera* leaves because of the presence on their surface of a thin layer of wax which does not allow the acetone to easily penetrate the leaf surface.

To be more confident with the results illustrated earlier, the X-rays transmission spectra at the Mg K-edge of the two *Viola tricolor* leaves have been measured at the synchrotron DaΦne of Frascati by using monochromatic radiation as shown in Figure 5.

In this figure the absorption spectra (ln (I_0/I_t)) are reported as a function of energy for the green leaf and for that extracted with acetone: a lower absorption of the extracted leaf than the unextracted green one can be observed. For comparison purposes, the XANES spectra of both samples were subtracted by the pre-edge absorption background using a linear function. The bad signal-to-noise ratio of the data depends on the high thickness of the leaf itself and on the low statistics used for the measurements.

Turning back to the experiment with *Hedera helix*, Figure 6 shows the details of the microradiographies corresponding to the white (a) and green (b) sides of this leaf; the two images are obtained by observing the microradiographies by an optical microscope with 40×

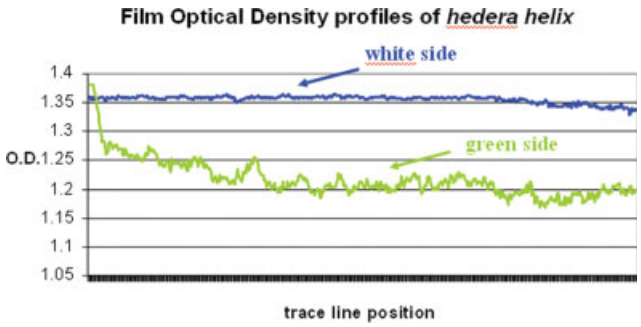

Fig. 8. Optical density profiles in the microradiograph negatives of the two sides of a leaf of *Hedera helix*. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

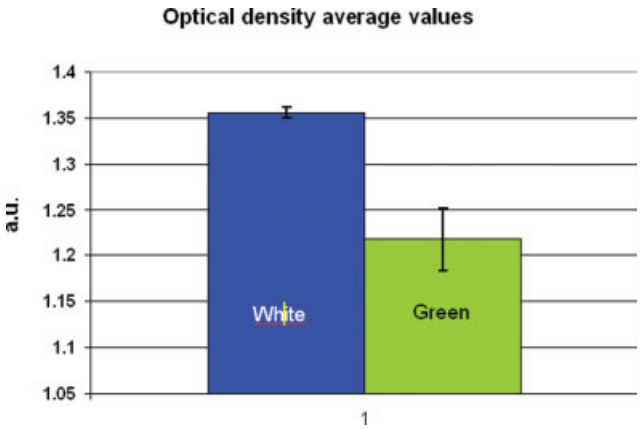

Fig. 9. Optical density average values in the microradiograph negatives of the two sides of a leaf of *Hedera helix*. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

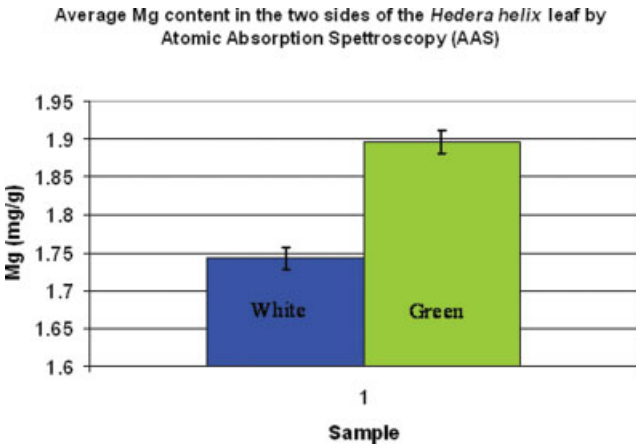

Fig. 10. AAS measurements showing the average Mg content in the green and white sides of the *Hedera helix* leaves. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

magnification objective lens. It is interesting to note how the absorption of the leaf increases in the detail corresponding to the green side (b). The gray-scale level of the microradiography of the leaf tissue is almost undistinguishable from that in the vein areas, and the space among veins is completely covered by white spots almost undistinguishable from the background. On the contrary for the white area (a) it seems that there are among the veins some X-ray dense lines, which can be understood as microveins. As we see, among them there are black spots quite similar to granules of poorly absorbing material. In the green side of the leaves (b) among these microveins the granules show a different content of some Mg containing substance (and so X-ray dense). Also they are not uniform in size, but completely cover the space producing a uniform background. Since this microradiography is obtained with X-ray radiation from a Mg target the X-ray dense granules presumably include structures with a higher Mg content than the poorly absorbing ones.

Observing the details of the leaf of *Hedera helix* by an optical microscope with a 100× objective, Figure 7, the same situation can more sharply be observed due to the higher magnification. In fact, it is possible to identify the same white X-ray dense areas very close to each other in the green side of the leaf giving a uniform background as it is clearly visible in the detail (b) of Figure 7 and a rather less diffused, dark and poorly absorbing zones in the white side as it is visible in the detail (a) of the same Figure 7. Unfortunately smaller details cannot be observed, since the resolution is limited by the emulsion of the photographic film.

Figures 8 and 9 show the optical density values of the microradiography negative for the green and white areas of the leaf determined from the gray-scale levels in the microradiograph negative digitized by a scanner. The grayscale levels are converted into O.D. values through the scanner calibration curve obtained by scanning a test Kodak film with known optical density values.

This optical density profiles (of the white and green side), obtained on the microradiographies by averaging the OD (x,y) along the vertical coordinate (y), are shown in Figure 8; while their average values (that is the O.D. value averaged on the whole area) are shown in Figure 9.

From the average values shown in Figure 9, it is evident that for the white side of the leaf the absorption of the sample is low and the photographic film becomes darker in the microradiograph, while for the green side of the leaf, the absorption of the sample is higher and the corresponding zone in the microradiograph has a lower density.

This quantitative measurement of the film darkness is not simply understandable in terms of the aforementioned difference of thickness between the green and white regions of the leaf.

Finally, we performed measurement on the same leaves observed at X-rays with the AAS technique. The results are obtained by averaging among various samples and are shown in Figure 10. It seems that the amount in Mg (mg/g) is higher in the green side of the leaf. It is note worthy that these results show the opposite behavior in comparison with the optical density measurements of the microradiographs which also confirm that the green side shows a higher presence of Mg than the white side. In fact the whiter region microradiograph is darker and the less dark negative, corresponds to the higher Mg content in the green side.

These last results again demonstrate that the difference of the absorption at X-rays in the two sides, white and green, is also due to a different chemical content of "something" which shows a higher content in the green side and which is probably the whole amount of Mg in molecules, in chlorophyll, and salts.

CONCLUSION

We have applied a technique to obtain microradiographs of biological samples with X-ray radiation with the intent of detecting a sample difference in chemical composition.

We have exposed leaves of *Hedera helix* to X-ray radiation obtained from a plasma source by using a Mg target in order to directly detect the different Mg content of the well-known double color sides, green and white, in the leaves of this plant.

Measurements were made to check if the different absorbing areas in the microradiography of the *Hedera helix*'s leaf are due to a different chemical concentration of magnesium.

First, four different groups of leaves have been chosen according to their size (greater age greater size and thickness), and leaf dry thickness, weight, and surface density (g/cm^2) have been compared.

Second, images of the microradiographs have been observed by an optical microscope at different magnifications to show the different shapes and structures visible in the two green and white leaf areas.

Third, the O.D. values of the microradiograph negatives for the green and white side of the leaves have been measured.

Fourth, a green leaf of *Viola tricolor* has been extracted with acetone and exposed, along with the still green leaf, to the X-rays from the plasma source (with a target of Mg with emission including the K-edge of Mg), and to the monochromatic radiation of the edge of Mg (around 1310 eV) from the synchrotron radiation source of LNF.

Finally, measurements by AAS have been performed on various leaves of *Hedera helix* for the exact determination of the whole amount of Mg in the green and white leaf sides, by averaging among the different *Hedera* leaves utilized for the exposure to X-rays.

The obtained results seem to prove that the different chemical composition causes a difference in the X-ray absorption in the two regions, green and white, of the leaf of *Hedera helix*. This confirms the X-ray based technique for the element (metals) analysis of biological samples. In fact, in principle it is possible to pin point any element in a sample on the basis of its selective absorption of X-ray radiation.

Previous results showed that we can detect the intake of contaminants with plant leaves and proved the possibility to apply this technique for phytoremediation studies and to detect the natural heavy metal (lead) content in any plant (Kaiser et al., 2005, 2007; Reale et al., 2004, 2006, in press).

Much more selective absorption has been actually obtained by using the synchrotron radiation which allows one to select the desired wavelength; in fact, the X-ray synchrotron radiation is emitted in a very narrow spectral range, and so it is monochromatic; in this way, one could selectively choose specific wavelength ranges, in particular those relevant to the K- or L- edge of absorption of a given chemical element. Further investigations will be devoted to the use of monochromatic X-rays from synchrotron radiation to image biological samples and eventually to perform further chemical analyses as well.

In fact, it is possible to select a wavelength value very close to or exactly the same as that of the K-edge or L-edge of any element of interest and, by the dual energy analysis, either to localize the element or to quantitatively measure it. A dual energy analysis is the subtraction between two images of the same sample obtained at two slightly different wavelengths opposite to each other with respect to the absorption edge of a specific chemical element. This technique will allow not only for the chemical mapping of the sample, but also to increase the sensitivity to the chemical abundance by an order of magnitude or more, so that the amount of such chemicals can be estimated in treated and nontreated leaves.

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