Mapping of lead, magnesium and copper accumulation in plant tissues by laser-induced breakdown spectroscopy and laser-ablation inductively coupled plasma mass spectrometry ☆

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ABSTRACT

Laser-Induced Breakdown Spectroscopy (LIBS) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) were utilized for mapping the accumulation of Pb, Mg and Cu with a resolution up to 200 μm in a up to cm×cm area of sunflower (*Helianthus annuus* L.) leaves. The results obtained by LIBS and LA-ICP-MS are compared with the outcomes from Atomic Absorption Spectrometry (AAS) and Thin-Layer Chromatography (TLC). It is shown that laser-ablation based analytical methods can substitute or supplement these techniques mainly in the cases when a fast multi-elemental mapping of a large sample area is needed.

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1. Introduction

Diagnostic techniques, which enable at the same time both the monitoring of elemental contents and their spatial distribution within the examined material, offer at appropriate spatial resolution a powerful tool in various research areas, where chemical imaging of surfaces [1] or depth profile analysis [2] are required. Significant contribution of these techniques is appreciated e.g. in forensic analysis [3] and bio-medical applications [4].

More specifically, in plant sciences nowadays the microscopic inspection of biological objects by means of X-ray imaging techniques receive considerably interest [5]. X-ray microscopy and micro-radiography investigations usually make use of soft X-rays generated by plasma laser sources [6]. Synchrotron radiation micro-radiography uses harder radiations (1–100 keV), which is not limited to the so-called water window. In particular, the use of a monochromatic synchrotron radiation beam allows to select a very narrow spectral range and, by a specific optical set-up, to localize the absorption of a given chemical element in the observed sample [7].

Recently, laser-ablation based methods of analysis (LIBS, LA-ICP-MS) have been successfully utilized in spatially-resolved spectrochemical analysis of biological samples [8–10]. The relative simplicity of the experimental system, the possibility of single-shot multi-element analysis and the ability of excite multiple sample types with no or little sample preparation [11] represent the major advantages of these techniques and make them competitive with X-ray imaging techniques [12]. The possibility to detect heavy-metals in different parts of plant samples in their natural form, without difficult sample preparation [12–14] is important e.g. for phytoremediation [15]. Phytoremediation techniques represent an alternative and sustainable way to lower the contamination of environment by toxic metals, by utilization plant species. Plants are used to contain or drain toxic metals including radioactive isotopes. Selected plant species are prone to absorb and/or degrade organic compounds of various classes [16,17]. Several reasons exist for justification of the research and development in phytoremediation technology. Most of all, the costs can be much lowered because phytoremediation assumes to utilize well known agrotechnical approaches for the plant growing. The other

advantage of phytoremediation consists in the fact that it is the least harmful method, which uses naturally occurring organisms and preserves the natural state of the environment [18–20].

The results of LIBS and LA-ICP-MS measurements on plant samples have been linked to measurements with complementary techniques for validation, including X-ray techniques, Relaxation Weighted Magnetic Resonance Imaging, AAS and Atomic Emission Spectrometry (AES) [12–14]. It was shown that both, fs-LIBS [13] and ns-LIBS [14] techniques could potentially be used for identification of ions of the elements of interest within different plant compartments e.g. leaf vein and parts of leaf positioned between the veins. Moreover, analysis of plants by means of LIBS or LA-ICP-MS enables topochemical information with high spatial resolution and sensitivity. Affected material is fully removed with no or minimal collateral damage to the surrounding cells. This may be useful not only to monitor the accumulation in specific areas of the sample, but also to trace the time course of uptake of the elements of interest.

This work assesses the feasibility of using LIBS and LA-ICP-MS analyses for monitoring the distribution of Pb, Mg and Cu in leaves of _Helianthus annuus_ L. samples. _Helianthus annuus_ L. is considered as plant specie suitable for phytoremediation [21–28]. However, using _Helianthus annuus_ L. for this technology is at the very beginning due to the not well known physiological processes to contain heavy metal ions. Furthermore, the transport of the metal ions within a plant is also not clear yet. The main obstacle to study these processes is the impossibility to detect heavy metal ions in the given place at a plant. Using the common analytical instrument such as AAS or differential pulse voltammetry, whole plant parts have to be sampled, which can bring contamination to the sample in preparation phase and mainly do not allow the exact localization of elements. To overcome these obstacles it is possible to employ ion selective electrodes placed inside a plant [29,30]. The main disadvantages of these electrodes are the very high detection limits and the possibility to detect only one specific heavy metal ion. Laser-ablation based analysis methods represent a new, unique tool to detect heavy metal ions in the various plant parts without any samples preparation, _in situ_ and with sufficient sensitivity.

The choice of investigated chemical elements is motivated by the importance related to biological and environmental exposure. Magnesium(II) ions belong to essential parts of assimilation pigment in plants. The binding of Mg(II) to proto-porphyrin is catalyzed by an enzyme called magnesium chelatase [31]. Changes in chlorophyll content at plants treated with lead(II) ions have been investigated in last century [32]. It was found that if plants were treated with cadmium(II) and lead(II) ions, considerable decrease in chlorophyll content was determined [33,34]. Moreover decreasing activity of photosynthetic apparatus was observed with increasing concentration of lead ions and time of a treatment [35,36].

The overall concentration of the investigated nutrition elements and the contaminant within the sample was determined by AAS, after the spatially-resolved LIBS and LA-ICP-MS measurements. The chlorophyll content in the samples containing different amounts of lead was measured by TLC.

2. Experimental methods

2.1. Plants cultivation and sample preparation

Plants of common sunflower (_Helianthus annuus_ L.) were used in our experiments. After ten day long germination, plant seedlings were placed into vessels containing tap water and cultivated in Versatile Environmental Test Chamber (MLR-350 H, Sanyo, Japan) for 8 days. Further Pb-ethylenediaminetetraacetic acid (Pb-EDTA) was added to the cultivation solution at final concentrations of 0 (Control sample), 100, 250 and 500 μM. The sunflower plants placed in the vessels that contained tap water with addition of lead were grown for 5 days. Seven plants from each experimental group were harvested at certain time intervals during the experiment, and their roots were rinsed three times in distilled water and 0.5 M EDTA. Prior to their analysis each harvested plant was divided into leaves, stem and root. Other experimental details on preparation of Pb-EDTA or cultivation of plants are published by Vacek et al. [37] or Krizkova et al. [38], respectively.

2.2. Extraction of chlorophylls and their analysis

Above ground parts of plants (leaves and stem, app. 0.15 g of fresh weight) were processed according to protocol described by Stejskal et al. [39]. A content of chlorophyll in processed tissues was determined by TLC. Aluminium Silica gel 60 (Merck, Germany) was used. Isolation of fractions of chlorophyll was carried out according to Stejskal et al. [39]. Briefly, absorbance of the samples (2 ml) was measured at two wavelengths 663 nm and 645 nm. Wavelength
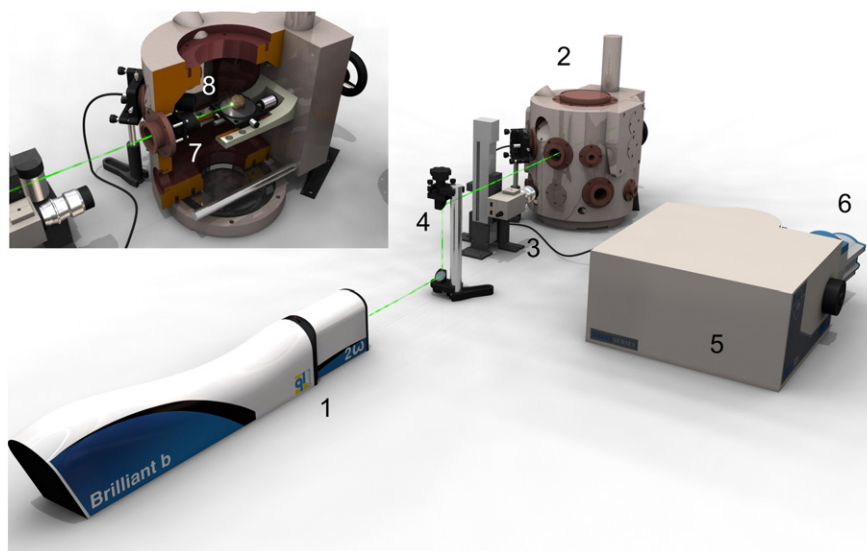

Fig. 1. The LIBS experimental setup 1 – Nd:YAG ablation laser with a module for second harmonic (532 nm) generation, 2 – interaction chamber, 3 – CCD camera for sample alignment, 4 – periscope, 5 – monochromator, 6 – ICCD camera. In the insertion, the inner view into the interaction chamber is shown with the micro-movement stage and 7 – focusing and 8 – collecting optics.
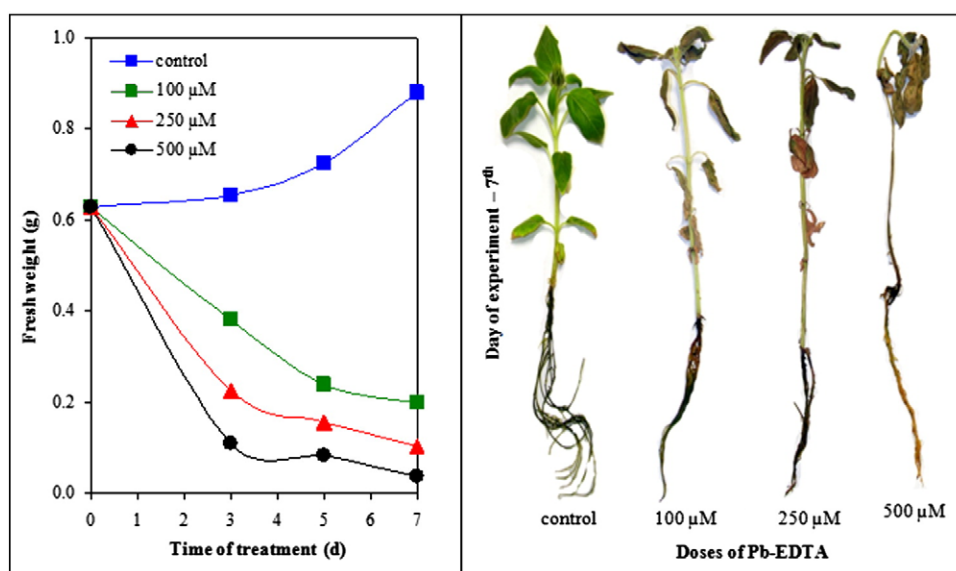
Fig. 2. Growth curves and pictures of the treated plants.

663 nm is used for quantification of *chlorophyll a* and 645 nm for *chlorophyll b*. Analyses were carried out on a spectrometer Hélios (Thermo Spectronic, Great Britain) in quartz cuvette against acetone.

2.3. Chemicals

All chemicals used were purchased from Sigma Aldrich (USA) in ACS purity unless noted otherwise. Acetone, benzene and isopropanol were obtained from Penta (Chrudim, Czech Republic). Silica plates were purchased from Merck (Germany).

2.4. LIBS experimental setup

The experimental arrangement of the utilized LIBS setup was detailed elsewhere [14]. Its 3D computer rendering is shown in Fig. 1. The LIBS micro-plasma was created using the second harmonic (532 nm) of a Nd:YAG laser system (Brilliant B, Quantel, France). The laser pulse width was ~5 ns and beam diameter 8 mm. The energy of the laser pulse was set by an energy meter (Field Master LM-P10, Coherent, USA) and its value was 10 mJ at the sample.

The sample was placed in the sample holder inside the ablation chamber (TESCAN, s.r.o. Czech Republic) to the stage with precision movements (2 μm in x, y and z direction). The LIBS analysis was performed in air at atmospheric pressure. The ablation spot was targeted and controlled for each shot by a CCD camera placed outside of the chamber.

The laser induced plasma was produced by focusing the laser beam with a 30 mm focal-length lens.

The LIBS plasma radiation was collected with quartz objectives and transported by a 3 m fiber optic system onto the entrance slit of the 0.32 m monochromator (TRIAX 320, Jobin Yvon, France). In this study, the grating 2400 g/mm of the monochromator and 50 μm entrance slit were used. As a detector an ICCD camera (Horiba, Jobin Yvon, France) was employed. The camera was triggered by the Q-switch signal of the laser.

2.5. LA-ICP-MS measurements

The LA-ICP-MS analysis was conducted by the UP-213 (New Wave Research, USA) laser ablation system coupled to the ICP-MS spectrometer (Agilent 7500, Agilent, USA). The ablation pattern was created by a 5th harmonic of the UP-213 Nd:YAG laser (213 nm). The energy of the laser pulse on the sample was ~0.3 mJ.

2.6. AAS measurements

AAS measurements were realized in order to determine overall amount of Mg and Pb in the samples. For solution analysis the leaf samples were milled and subsequently dissolved inside autoclave (ZA-1, JZD Pokrok) in the presence of nitric acid for 3 h at 140–150 °C.
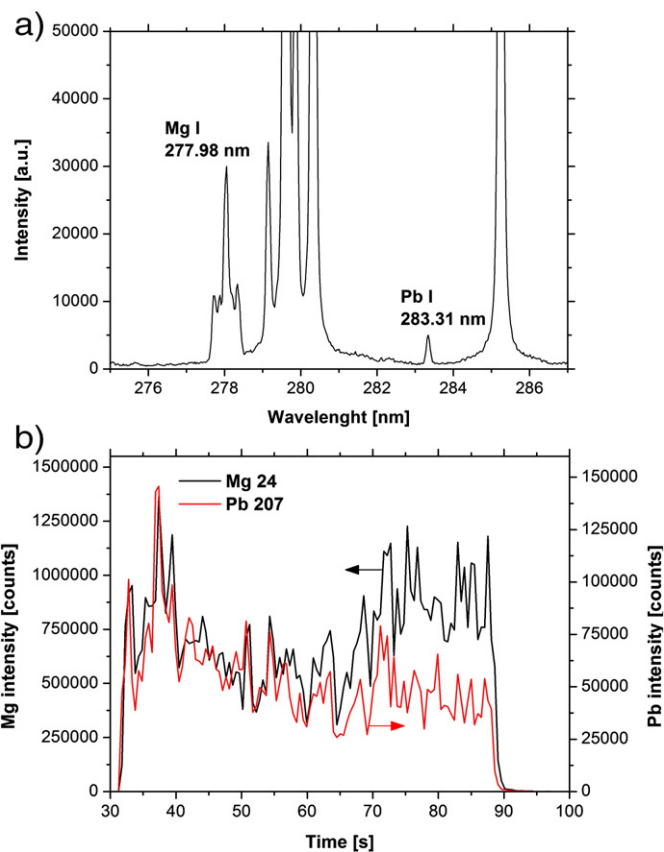

Fig. 3. a) The typical LIBS spectrum with the 277.98 nm Mg(I) and 283.31 nm Pb(I) lines used in the analysis and b) the section of LA-ICP-MS signal for Mg 24 and Pb 207 isotopes.

The calibration solutions for Pb were prepared in 5% HNO_3. During the measurements ammonium dihydrogen phosphate modifier was added to the 5 µl dose volume. The ET-AAS analysis was performed with Zeenit 650 (Analytik Jena) instrument.

The calibration solution for Mg was prepared in de-ionized water. The magnesium content was measured by flame AAS technique. In these measurements the NovAA 300 (Analytik Jena) was used.

3. Results and discussion

The growth curves of investigated *Helianthus annuus* L. plants treated with lead(II) ions are shown in Fig. 2a. The plants showed considerable growth depression depending on time of the treatment

and dose of lead(II) ions compared to control plants. Moreover at the end of the experiment (7th day) well noticeable necrotic changes were observed on plants treated with 500 µM of Pb-EDTA (Fig. 2b).

3.1. Mapping of the lead and magnesium content within the sample

The single-shot LIBS and the LA-ICP-MS analysis were performed on a 2×4.5 mm^2 leaf sections. The LIBS's ICCD detector was gated 1 µs after the Q-switch signal and the observation window was 10 µs. Typical LIBS spectrum and LA-ICP-MS signal are shown in Fig. 3. For Pb and Mg detection the 283.31 nm Pb(I) and 277.98 nm Mg(I) lines were used, respectively. For the mapping, the continuum background determined for each shot from five data points on both sides of the
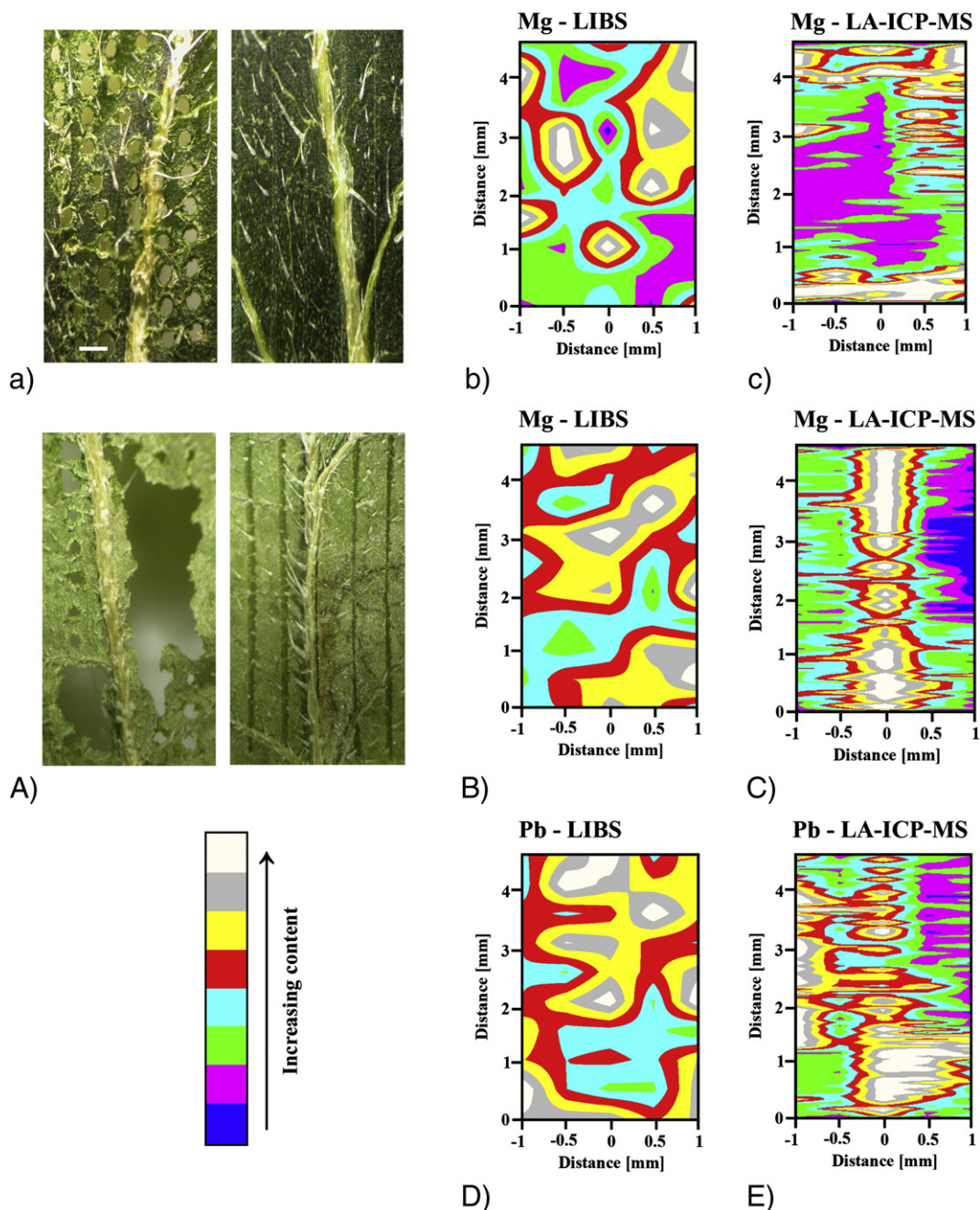

Fig. 4. Demonstration of the mapping capabilities of laser-ablation based methods. a) – the LIBS (left) and LA-ICP-MS (right) ablation pattern on the control sample, b) – the map of Mg obtained by LIBS and c) – by LA-ICP-MS analysis on this sample; A) – the LIBS (left) and LA-ICP-MS (right) ablation pattern on the 3-day 500 µM Pb-EDTA treated sample, B) – the map of Mg obtained by LIBS and C) – by LA-ICP-MS analysis on this sample, D) – the map of Pb from LIBS and E) – from LA-ICP-MS analysis. The length of the bar on the a) image is 500 µm.
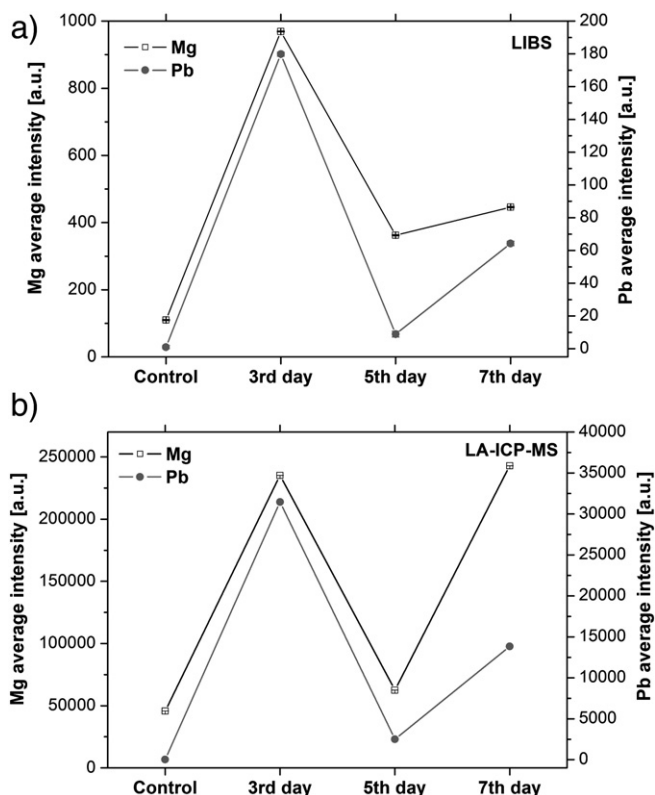
Fig. 5. Average intensities within the investigated leaf section derived from data obtained from a) LIBS and b) LA-ICP-MS analyses.

than the LA-ICP-MS crater, the possibility to create different ablation matrixes makes the LIBS analysis in our opinion more perspective for mapping. For more details please see also chapter 3.3.

3.2. Monitoring of the lead and magnesium accumulation

Fig. 5 demonstrates the capability of LIBS and LA-ICP-MS to signalize the trends in accumulation of different elements in selected structures of the sample. In order to trace the uptake of Mg and Pb in the whole investigated area of the leaf samples, a statistical analysis was carried out on the intensity data set from each sample. Because the measured intensity values within the whole investigated area of the leaves had a log-normal distribution [40,41] the average of the logarithm of intensity values was calculated. From these data (Fig. 5a) b)) we found that in the leaves of the plants the Mg content follows the changes in the lead content. This theory was proved also with AAS measurements (Fig. 6), where the Pb and Mg content was measured in the leaves of the given samples. Moreover, all of these three diagnostic techniques showed an increased amount of Pb and Mg in the leaves at the 3rd day of treatment.

The content of chlorophylls *a* and *b* in root and leaves of each investigated sample was determined by TLC (Fig. 7). Total content of chlorophyll (both chlorophylls *a* and *b*) enhanced markedly and reached the highest values at fifth day of the treatment at all plants then the total content of chlorophyll decreased at seventh day of the treatment compared to control (Fig. 7). In addition to chlorophyll measurement concentration of lead(II) ions in water, where the plants were treated, decreased, but concentration of magnesium(II) ions enhanced with the time of the treatment. The most considerable changes in concentration of these metals were from the fifth day of the treatment. This phenomenon probably relates with the affecting of magnesium chelatase activity by lead(II) ions. The chelatase probably incorporates lead(II) ions instead of magnesium(II) ions into chlorophyll structure.

If we compared the results obtained from LIBS, LA-ICP-MS and AAS analyses and TLC ones, we could conclude that the competition between Mg and Pb ions inhibits activity magnesium chelatase, which thus cannot incorporate Mg ions into newly synthesized chlorophyll molecules. More details will be published elsewhere.

3.3. LIBS for mapping of large area samples with high spatial resolution

The capability of LIBS for fast analysis of large area (cm×cm) samples with high spatial resolution is demonstrated in Fig. 8. The LIBS experimental system was improved in order to allow mapping square or rectangular areas within the sample. The measurements
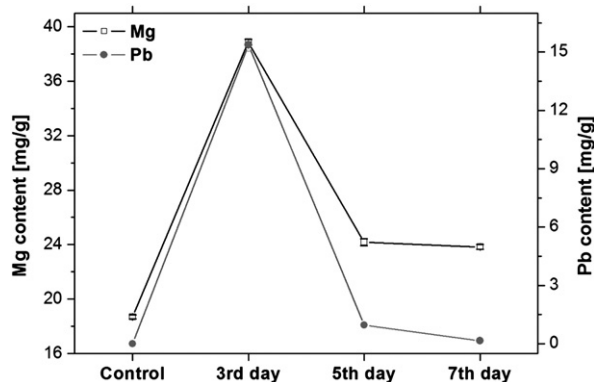
monitored spectral line by a linear background fit method was subtracted from the intensity value of every data point forming the spectral line. Subsequently the sum of intensities of the data points forming the selected peak (for appropriate chemical element) was calculated. In the LA-ICP-MS analysis the Pb 207 and Mg 24 isotopes were detected.

It should be noted that LIBS gives practically an instantaneous signal directly related to the location at which the single ablation event occurred. LA-ICP-MS involves sample transport and diffusion so that the signal produced in the mass spectrometer is not directly attributable to a specific location on the sample, without considerable care in the analysis.

The spatial distribution of lead and magnesium within the investigated areas is presented in Fig. 4. As an example the ablation patterns together with the maps of selected elements are shown for the Control and for 3 days 500 μM Pb-EDTA treated samples. The LIBS and LA-ICP-MS analysis was provided for appropriate treatment on the same leaf sample, in different but close positions around the central vein. For both methods it may be concluded that the diverse structures in the leaf –the central vein and the tissues around the vein structure with lower concentration of the investigated elements– can be distinguished. The (subjectively) more evident position of the central vein from LA-ICP-MS measurement that is observable mainly in Fig. 4C and E can be attributed to the different sampling mechanism. While in LIBS a regular matrix (Fig. 4a) and A) left images) is sampled, in case of LA-ICP-MS rows of ablation craters (Fig. 4a) and A) right images) are created and sampled. In case of sample shown in Fig. A, one of the LA-ICP-MS ablation rows is parallel with the central vein and is positioned very near/on it.

The spatial resolution of the LIBS is determined by the diameter of the ablation craters and by the distance between them. In the presented experiments the achievable resolution was limited to ~200 μm. However, here the LIBS ablation crater size was ~2x larger

Fig. 6. The overall concentration of Mg and Pb, derived from AAS measurements, in the leaves of *Helianthus annuus* L. samples in different days of treatment.
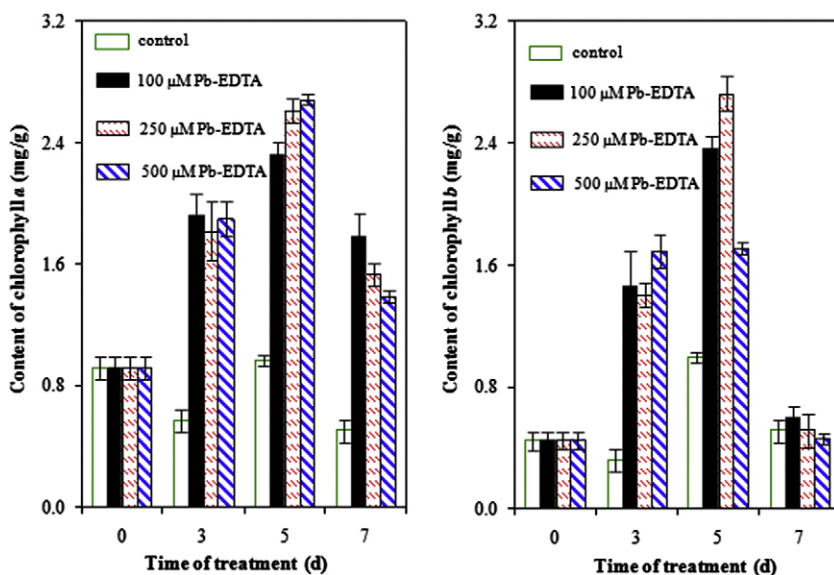
Fig. 7. Content of chlorophyll *a* and *b* in the leaves of the treated plants.

are fully automated, the parameters as number of ablation craters in *x* and *y* direction, distance between the centers of the craters and the number of shots to each position are set and controlled by a computer code. Moreover, the delay between the laser shots can be controlled, in order to take into consideration the readout time of the ICCD camera. Spectra can be recorded for each shot, and the possibility to apply several "cleaning" shot before recording the spectra is also incorporated into the program. a single-shot LIBS analysis of the natural Cu content in the 1×1 cm^2 leaf area of Control *Helianthus annuus* L. sample is shown in Fig. 8. For mapping of the Cu content the 324.75 nm Cu(I) line was used. The map was obtained from 20×20 single-shot spectra. The measurements took about 7 min.

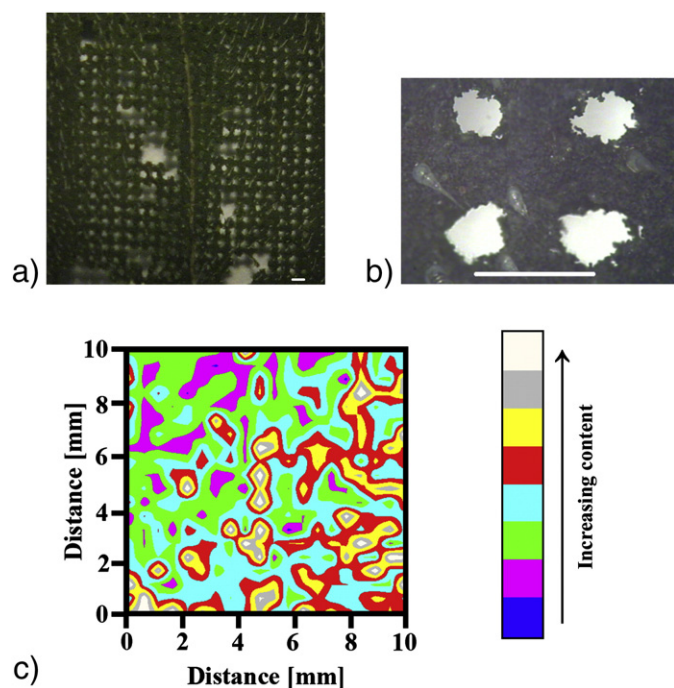**Fig. 8.** a) the photography of the 1×1 cm^2 ablation pattern b) the detail of four ablation holes and c) the map of Cu in the 1×1 cm^2 large sample analyzed by LIBS. The length of the bars on the a) and b) images is 500 µm.

Despite the number of advantages of laser-based techniques detailed above, the major problem that usually occurs during the analysis of plants –and more generally, for all biological samples [1]– i.e. the non-availability of reference standards should be stressed. For calcified specimen, e.g. bones and teeth standard matrices with quantifiable trace element concentration can be simulated [4], for soft tissue materials, including botanical specimens, this is normally not possible. The existing NIST powder leaf standards, e.g. strawberries, orchids and tea leaves do not give information about spatial distribution of elements. LIBS measurements presented in this paper gave the map of the intensity of a spectral line, which may be proportional to the local concentration but there was not attempt at calibration or a demonstration of linearity of response.

On the ongoing work we are targeting two tasks. First of them is to reduce the ablation crater size to the value under 100 µm by applying a special triplet (Sill Optics, Germany). The second, more complicated task is to incorporate our code with a module that allows automatic sample positioning to the focal distance of the focusing objective. The module was already tested on several samples with different reflection. Its inclusion to the existing code will allow monitoring wide variety of samples with complicated surface morphology with high-spatial resolution.

4. Conclusions

In summary, we have discussed the recent progress in the utilization of LIBS technique for mapping the accumulation of selected elements in leaves of sunflower (*Helianthus annuus L.*) samples. It was shown, that laser-ablation based analytical methods (LIBS, LA-ICP-MS) could be easily implemented in applications, in which the capability for spatially resolved analysis is required, e.g. to trace the possible migration of elements within plant samples. The results of LIBS and LA-ICP-MS analysis were compared with the outcomes from Atomic Absorption Spectrometry (AAS) and thin-layer chromatography (TLC). The capability of LIBS for fast analysis of large area (cm×cm) samples with high spatial resolution was also detailed.

References

[1] S. Pokrant, M. Cheynet, S. Jullian, R. Pantel, Chemical analysis of nanometric dielectric layers using spatially resolved VEELS, Ultramicroscopy 104 (2005) 233–243.

[2] K. Novotný, T. Vaculovič, M. Galiová, V. Otruba, V. Kanický, J. Kaiser, M. Liška, O. Samek, R. Malina, K. Páleníková, The use of zinc and iron emission lines in depth profile analysis in zinc-coated steel, Appl. Surf. Sci. 253 (2007) 3834–3842.

[3] K. Flynn, R. O'Leary, C. Roux, B.J. Reedy, Forensic analysis of biocomponent fibers using infrared chemical imaging, J. Forensic Sci. 51 (2006) 586–596.

[4] O. Samek, D.C.S. Beddows, H.H. Telle, J. Kaiser, M. Liška, J.O. Caceres, A.G. Urena, Quantitative laser-induced breakdown spectroscopy analysis of calcified tissue samples, Spectrochim. Acta Part B 56 (2001) 865–875.

[5] J.F. Adam, J.P. Moy, J. Susini, Table-top water window transmission X-ray microscopy: Review of the key issues, and conceptual design of an instruments for biology, Rev. Sci. Instrum. 76 (2005) 091301.

[6] L. Reale, A. Lai, I. Bellucci, A. Faenov, T. Pikuz, F. Flora, L. Spano, A. Poma, T. Limongi, L. Palladino, A. Ritucci, G. Tomassetti, G. Petrocelli, S. Martellucci, Microradiography as a tool to detect heavy metal uptake in plants for phytoremediation applications, Microsc. Res. Tech. 69 (2006) 666–674.

[7] J. Kaiser, L. Reale, A. Ritucci, G. Tomassetti, A. Poma, L. Spano, A. Tucci, F. Flora, A. Lai, A. Faenov, T. Pikuz, L. Mancini, G. Tromba, F. Zanini, Mapping of the metal intake in plants by large-field X-ray microradiography and preliminary feasibility studies in microtomography, Eur. Phys. J., D 32 (2005) 113–118.

[8] M.Z. Martin, S.D. Wullschleger, C.T. Garten, A.V. Palumbo, J.G. Smith, Elemental analysis of environmental and biological samples using laser-induced breakdown spectroscopy and pulsed Raman spectroscopy, J. Dispers. Sci. Technol. 25 (2004) 687–694.

[9] F.C. DeLucia, A.C. Samuels, R.S. Harmon, R.A. Walters, K.L. McNesby, A. LaPointe, R.J. Winkel, A.W. Miziolek, Laser-induced breakdown spectroscopy (LIBS): a promising versatile chemical sensor technology for hazardous material detection, IEEE Sens. J. 5 (2005) 681–689.

[10] J.S. Becker, J. Su, M.V. Zoriya, J. Dobrowolska, A. Matush, Imaging mass spectrometry in biological tissues by laser ablation coupled plasma mass spectrometry, Eur. J. Mass Spectrom. 13 (2007) 1–6.

[11] A.W. Miziolek, V. Palleschi, I. Schechter, Laser-Induced Breakdown Spectroscopy (LIBS) Fundamentals and Applications, Cambridge University Press, Cambridge, 2006.

[12] J. Kaiser, O. Samek, L. Reale, M. Liška, R. Malina, A. Ritucci, A. Poma, A. Tucci, F. Flora, A. Lai, L. Mancini, G. Tromba, F. Zanini, A. Faenov, T. Pikuz, G. Cinque, Monitoring of the heavy-metal hyperaccumulation in vegetal tissues by X-ray radiography and by femto-second laser induced breakdown spectroscopy, Microsc. Res. Tech. 70 (2007) 147–153.

[13] O. Samek, J. Lambert, R. Hergenröder, M. Liška, J. Kaiser, K. Novotný, S. Kukhlevsky, Femtosecond laser spectrochemical analysis of plant samples, Laser Phys. Lett. 3 (2006) 21–25.

[14] M. Galiová, J. Kaiser, K. Novotný, O. Samek, L. Reale, M. Malina, K. Páleníková, M. Liška, V. Čudek, V. Kanický, V. Otruba, A. Poma, A. Tucci, Utilization of laser induced breakdown spectroscopy for investigation of the metal accumulation in vegetal tissues, Spectrochim. Acta Part B 62 (2007) 1597–1605.

[15] U. Krämer, A.N. Chardonnes, The use of transgenic plants in bioremediation of soils contaminated with trace elements, Appl. Microbiol. Biotechnol. 55 (2001) 661–672.

[16] T. Macek, M. Mackova, D. Pavlikova, J. Szakova, M. Truksa, S. Cundy, P. Kotrba, N. Yancey, W.H. Scouten, Accumulation of cadmium by transgenic tobacco, Acta Biotechnol. 22 (2002) 101–106.

[17] T. Macek, M. Mackova, J. Kas, Exploitation of plants for the removal of organics in environmental remediation, Biotechnol. Adv. 18 (2000) 23–34.

[18] V. Supalkova, J. Petrek, J. Baloun, V. Adam, K. Bartusek, L. Trnkova, M. Beklova, V. Diopan, L. Havel, R. Kizek, Multi-instrumental investigation of affecting of early somatic embryos of spruce by cadmium(II) and lead(II) ions, Sensors 7 (2007) 743–759.

[19] P. Kotrba, T. Macek, T. Ruml, Heavy metal-binding peptides and proteins in plants. A review, Collect. Czechoslov. Chem. Commun. 64 (1999) 1057–1086.

[20] K. Francova, T. Macek, K. Demnerova, M. Mackova, Transgenic plants – a potential tool for decontamination of environmental pollutants, Chem. Lett. -Chem. Listy 95 (2001) 630–637.

[21] J.S. Garcia, P.L. Gratao, R.A. Azevedo, M.A.Z. Arruda, Metal contamination effects on sunflower (*Helianthus annuus* L.) growth and protein expression in leaves during development, J. Agric. Food Chem. 54 (2006) 8623–8630.

[22] M.S. Liphadzi, M.B. Kirkham, Availability and plant uptake of heavy metals in EDTA-assisted phytoremediation of soil and composted biosolids, S. Afr. J. Bot. 72 (2006) 391–397.

[23] L. Marchiol, G. Fellet, D. Perosa, G. Zerbi, Removal of trace metals by Sorghum bicolor and *Helianthus annuus* in a site polluted by industrial wastes: a field experience, Plant Physiol. Biochem. 45 (2007) 379–387.

[24] E. Nehnevajova, R. Herzig, G. Federer, K.H. Erismann, J.P. Schwitzguebel, Screening of sunflower cultivars for metal phytoextraction in a contaminated field prior to mutagenesis, Int. J. Phytoremediat. 7 (2005) 337–349.

[25] L. Szabo, L. Fodor, Uptake of microelements by crops grown on heavy metal-amended soil, Commun. Soil Sci. Plant Anal. 37 (2006) 2679–2689.

[26] S. Tandy, R. Schulin, B. Nowack, Uptake of metals during chelant-assisted phytoextraction with EDDS related to the solubilized metal concentration, Environ. Sci. Technol. 40 (2006) 2753–2758.

[27] C.H. Wu, T.K. Wood, A. Mulchandani, W. Chen, Engineering plant-microbe symbiosis for rhizoremediation of heavy metals, Appl. Environ. Microbiol. 72 (2006) 1129–1134.

[28] Y. Zhang, C. Banks, A comparison of the properties of polyurethane immobilised Sphagnum moss, seaweed, sunflower waste and maize for the biosorption of Cu, Pb, Zn and Ni in continuous flow packed columns, Water Res. 40 (2006) 788–798.

[29] H.H. Felle, P.K. Hepler, The cytosolic Ca2+ concentration gradient of Sinapis alba root hairs as revealed by Ca2+−selective microelectrode tests and fura-dextran ratio imaging, Plant Physiol. 114 (1997) 39–45.

[30] T. Sun, Y. Xu, P. Li, S.G. Yu, L.P. Yin, Non-invasive scanning ion-selective electrode technique and its applications to the research of higher plants, Prog. Nat. Sci. 17 (2007) 625–629.

[31] H.M. Rissler, E. Collakova, D. DellaPenna, J. Whelan, B.J. Pogson, Chlorophyll biosynthesis. Expression of a second Chl I gene of magnesium chelatase in *Arabidopsis* supports only limited chlorophyll synthesis, Plant Physiol. 128 (2002) 770–779.

[32] R. Hampp, K. Lendzian, Effect of lead ions on chlorophyll synthesis, Naturwissenschaften 61 (1974) 218–219.

[33] S. Phetsombat, M. Kruatrachue, P. Pokethitiyook, S. Upatham, Toxicity and bioaccumulation of cadmium and lead in Salvinia cucullata, J. Environ. Biol. 27 (2006) 645–652.

[34] S. Saygideger, M. Dogan, Influence of pH on lead uptake, chlorophyll and nitrogen content of Nasturtium officinale R. Br. and Mentha aquatica L, J. Environ. Biol. 26 (2005) 753–759.

[35] Y. Pinchasov, T. Berner, Z. Dubinsky, The effect of lead on photosynthesis, as determined by photoacoustics in Synechococcus leopoliensis (Cyanobacteria), Water Air Soil Pollut. 175 (2006) 117–125.

[36] M. Garnczarska, L. Ratajczak, Metabolic responses of Lemna minor to lead ions – I. Growth, chlorophyll level and activity of fermentative enzymes, Acta Physiol. Plant. 22 (2000) 423–427.

[37] J. Vacek, J. Petrek, R. Kizek, L. Havel, B. Klejdus, L. Trnkova, F. Jelen, Electrochemical determination of lead and glutathione in a plant cell culture, Bioelectrochemistry 63 (2004) 347–351.

[38] S. Krizkova, P. Ryant, O. Krystofova, V. Adam, M. Galiova, M. Beklova, P. Babula, J. Kaiser, K. Novotny, J. Novotny, J. Liska, R. Malina, J. Zehnalek, J. Hubalek, L. Havel, R. Kizek, Multi-instrumental analysis of tissues of sunflower plants treated with silver(I) ions – plants as bioindicators of environmental pollution, Sensors 8 (2008) 445–463.

[39] K. Stejskal, V. Diopan, V. Adam, J. Zehnalek, L. Trnkova, L. Havel, M. Galiova, R. Malina, K. Novotny, J. Kaiser, R. Kizek, Study of effects of lead ions on sugar beet, Lis. Cukrov. Repar. 124 (2008) 116–119.

[40] C. Reimann, P. Filzmoser, Normal and lognormal data distribution in geochemistry: death of a myth. Consequences for the statistical treatment of geochemical and environmental data, Environ. Geol. 39 (1999) 1001–1014.

[41] E. Limpert, W.A. Stahel, M. Abbt, Log-normal distributions across the sciences: keys and clues, BioScience 51 (2001) 341–352.