

Investigation of heavy-metal accumulation in selected plant samples using laser induced breakdown spectroscopy and laser ablation inductively coupled plasma mass spectrometry

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Abstract Single-pulse Laser-Induced Breakdown Spectroscopy (LIBS) and Laser-Ablation Inductively Coupled Plasma Mass-Spectrometry (LA-ICP-MS) were applied for mapping the silver and copper distribution in *Helianthus Annuus* L. samples treated with contaminant in controlled conditions. For Ag and Cu detection the 328.07 nm Ag(I) and 324.75 nm Cu(I) lines were used, respectively. The LIBS experimental conditions (mainly the laser energy and the observation window) were optimized in order to avoid self-absorption effect in the measured spectra. In the LA-ICP-MS analysis the Ag 107 and Cu 63 isotopes were detected. The capability of these two analytical techniques for high-resolution mapping of selected trace chemical elements was demonstrated.

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1 Introduction

Laser-ablation based analytical methods offer a simple and fast tool of elemental analysis. The LIBS technique allows

real-time multi-element analysis in-situ and even remotely [1]. The advantages of this method, i.e., usually none or very simple sample preparation consisting of several laser pulses in order to remove the oxidized layer from the sample surface [2], together with the capability of analysis with high spatial resolution [3] make it possible to utilize it in a wide-range of applications [1]. Basically, the achievable spatial resolution of LIBS is determined by the size of the laser craters produced within the material [3]. Among the LIBS applications the monitoring of accumulation of selected chemical elements in different structures of plant species [4] is important for both, to identify ion storage and trafficking within different plant compartments, which is still not fully explained [5], and for the environmental protection to find appropriate species for phytoremediation [6–8]. This method of environmental cleaning uses plants for containing, degrading or eliminating of toxic metals including radioactive isotopes and several groups of organic compounds [9, 10].

The advantages of LA-ICP-MS over conventional solution nebulization ICP-MS or other micro-sampling analytical techniques have been discussed by many authors as reported by Russo et al. [11]. Similarly to the LIBS technique, the analysis of solid samples with LA-ICP-MS is direct, requires little preparation (a flat surface may be required if the entire sample is to be probed) and both conducting and non-conducting solid material can be analyzed. Recent biological and environmental applications of this technique can be found in [12–17].

In this work, we assessed the feasibility of LIBS analyses for measuring the silver and copper distribution in leaves of *Helianthus Annuus* L. samples. The results of the LIBS analyses are compared with the outcomes of the LA-ICP-MS measurements. The choice of investigated chemical elements is motivated by the importance related to biological

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and environmental exposure. Silver ions are highly toxic for aquatic ecosystems, but their exact mechanism of action is still unclear. In this work, we investigated the effecting of embryonic spruce culture by Ag single ionized species [18]. Copper is a micronutrient that has roles in photosynthesis, respiration, antioxidant activity, cell wall metabolism, and hormone perception. Excess Cu is toxic and therefore its delivery has to be tightly regulated [19].

2 Experimental

2.1 Plants cultivation and sample preparation

Plants of a common sunflower (*Helianthus Annuus* L.) were used in our experiments. The sunflower seeds were germinated on wet filter paper in special vessels at $25 \pm 2^\circ\text{C}$ in the dark (box Chirana, Czech Republic). After 10 days, plant seedlings were placed into vessels containing tap water and cultivated in Versatile Environmental Test Chamber (MLR-350 H, Sanyo, Japan) for eight days with 14 hours long daylight per day (maximal light intensity was about $100 \mu\text{E m}^{-2} \text{s}^{-1}$) at a temperature 22°C and humidity 65%. Further, silver (AgNO_3) was added to the cultivation solution at final concentrations of 1 mmol l^{-1} . The sunflower plants placed in the vessels that contained tap water with addition of silver were grown for five 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 and root. The leaves and root section were frozen and the analysis was performed on the fresh (frozen) samples.

2.2 LIBS and LA-ICP-MS measurements

The schematic of the utilized LIBS setup is shown in Fig. 1. The LIBS micro-plasma was created using the second har-

monic (532 nm) of a Nd:YAG laser system (Brilliant B, Quantel, France). The laser pulse width was $\sim 5 \text{ ns}$ and beam diameter 8 mm. The energy of the laser pulse (10 mJ at the sample) was set and controlled by an energy meter (Field Master LM-P10, Coherent, USA).

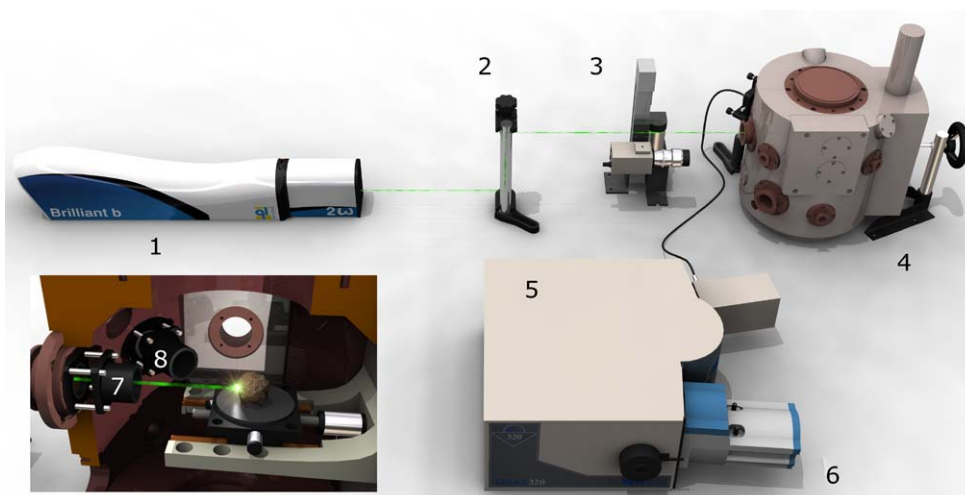
The sample was placed to the sample holder inside the ablation chamber (TESCAN s.r.o., Czech Republic) to the stage with precision movements ($2 \mu\text{m}$ in x , y , and z direction). The LIBS analysis was performed in air on atmospheric pressure. The ablation spot was targeted and controlled for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

The laser induced plasma was produced by focusing the laser beam with a 16 mm focal-length glass doublet (Sill Optics, Germany). 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^{-1} of the monochromator and $50 \mu\text{m}$ 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.

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). A laser fluence of 3 mJ cm^{-2} was used and helium carrier gas flow through the ablation cell of 0.6 l min^{-1} was applied. The volume of ablation cell was 20 cm^3 . The measurements were performed using ICP-MS instrument (Agilent 7500CE, Japan) with dynamic reaction cell, and the RF power was 1350 W. The optimization procedure of the LA-ICP-MS was performed using a standard reference material (NIST SRM 612).

Most LIBS applications are based on using Nd:YAG lasers operated at wavelengths 1064 nm and 532 nm that

Fig. 1 The LIBS experimental setup 1—Nd:YAG ablation laser, 2—periscope, 3—CCD camera for sample alignment, 4—interaction chamber, 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



correspond to the fundamental and the second harmonic frequencies, respectively. Radiation of this wavelength contributes to plasma heating when a nanosecond pulse is applied. The instrumentation in these experiments was optimized by preliminary measurements. The used 532 nm laser wavelength has an advantage for high-resolution mapping due to better focusing properties in comparison with 1064 nm. The fifth harmonics corresponding to 213 nm and the carrier gas (helium) were utilized for LA-ICP-MS system due to the lower fractionation effect of the small ablated particles and better crater formation at these experimental conditions.

3 Results and discussion

The single-shot LIBS and the LA-ICP-MS analysis were performed on a $3 \times 5 \text{ mm}^2$ or $3 \times 3.7 \text{ mm}^2$ 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. 2. For Ag and Cu detection the 328.07 nm Ag(I) and 324.75 nm Cu(I) lines were used, respectively. The selected Ag(I) line was reported by Fichet et al. [20] as an appropriate for detect trace Ag contained in uranium and plutonium dioxides. This spectral line was used also for measuring the concentration of Ag in aqueous solution as reported by Schmidt et al. [21]. The selected 324.75 nm Cu(I) line is used frequently in LIBS analysis. As it was shown by Freedman et al. [22] the Cu(I) transition at 324.75 nm can be utilized to detect Cu in a relatively low concentration. Because the content of copper ions in the investigated *Helianthus Annuus* L. sample is of natural origin only, their concentration is very low compared to content of silver ions (Ag was added to cultivation medium).

In order to properly relate the detected Ag(I) emission lines intensities with the species amount, the evaluation of the self-absorption was performed for all recorded single-shot LIBS spectra. The 328.07 nm and 338.29 lines of the Ag(I) atoms that are detectable in the selected spectral range have almost the same transition probability, but the degeneracy of the upper level of the 328.07 nm transition is 4 while that of the 338.29 nm line is 2. This means that the intensity ratio of these two lines (Fig. 2(a)) should be 2 if there were no re-absorption effect [23]. The LIBS experimental conditions (mainly the laser energy and the observation window) were optimized in order to avoid self-absorption effect in the measured spectra.

For the mapping the selected LIBS spectra were processed in the following way: background was subtracted (for each shot) and the area under the selected peak (for appropriate chemical element) was calculated. In the LA-ICP-MS analysis the Ag 107 and Cu 63 isotopes were detected.

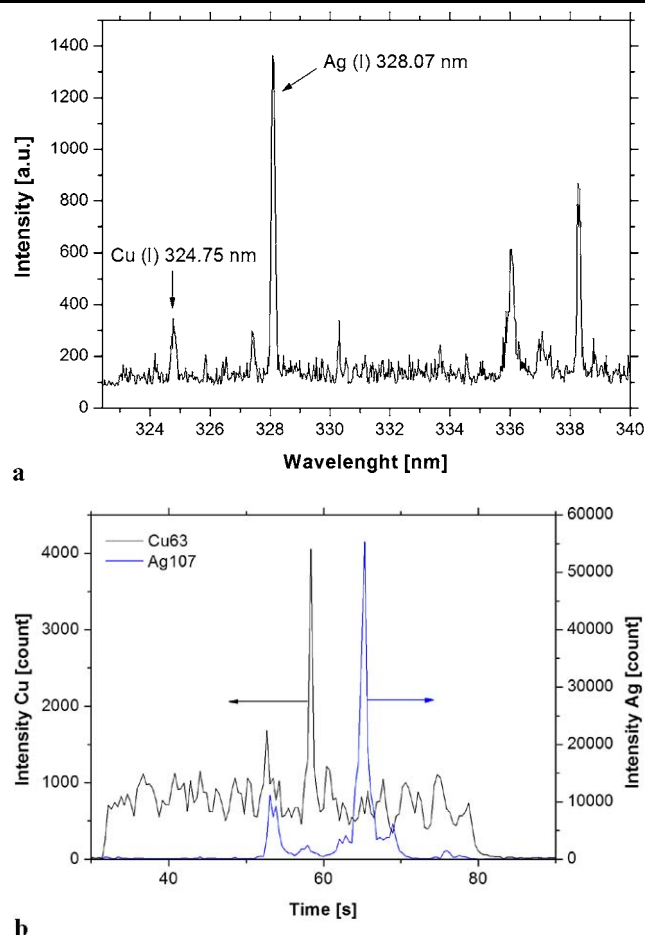
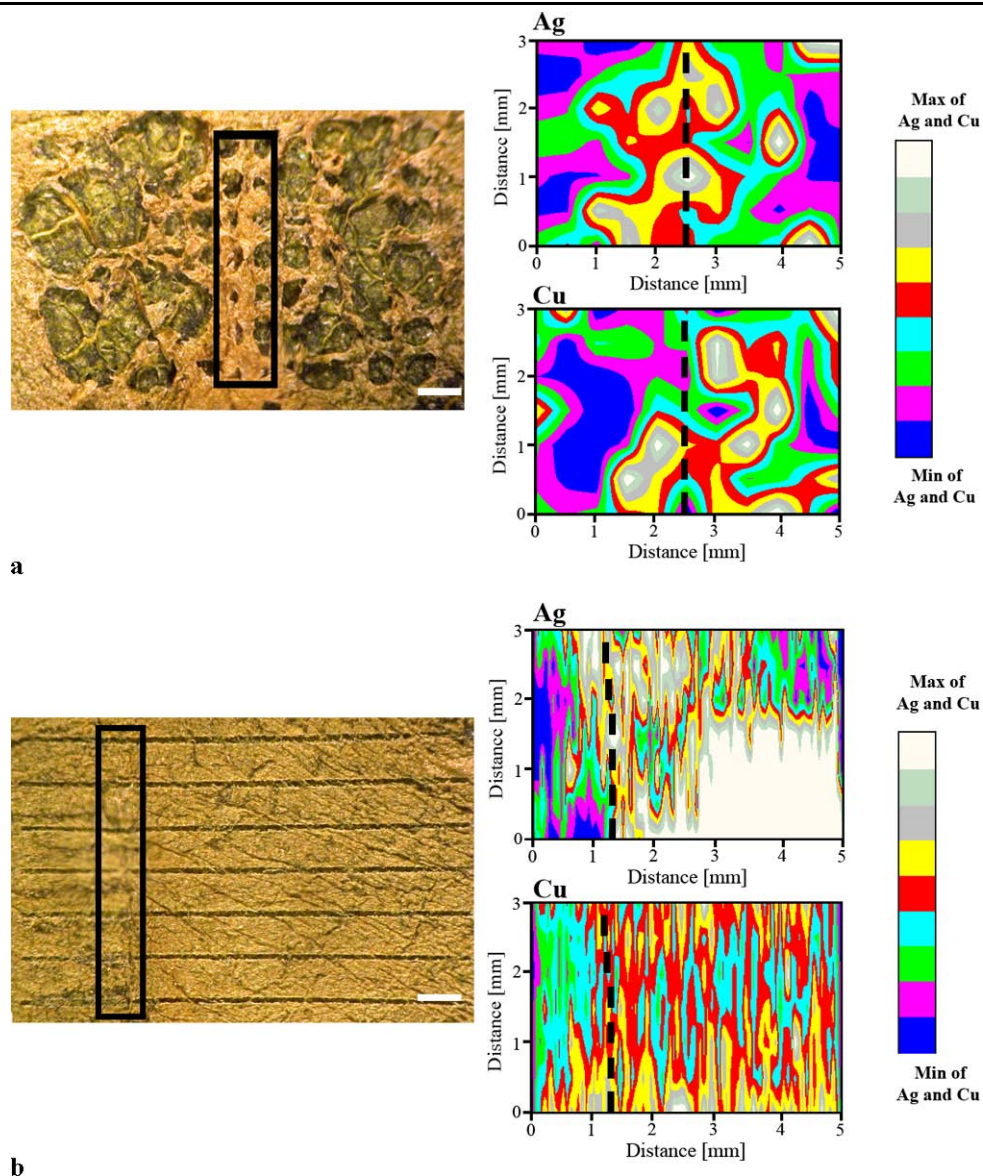


Fig. 2 (a) The typical LIBS spectrum with the 328.07 nm Ag(I) and 324.75 nm Cu(I) lines used in the analysis and (b) the section of LA-ICP-MS signal for Ag and Cu

Basically, the achievable spatial resolution of LIBS and LA-ICP-MS is determined by the size of the laser craters produced within the material. The typical LIBS craters created in these experiments have a diameter of $\sim 250 \mu\text{m}$. The LA-ICP-MS pattern consisted of ablation craters of $\sim 110 \mu\text{m}$ in diameter.

Higher content of Ag was observed in the vein structures of investigated samples utilizing both analytical methods. As an example, in Fig. 3(a) the LIBS ablation pattern of the 4 days 1 mmol l^{-1} AgNO_3 treated sample is shown together with the map of Ag and Cu accumulated in the sample. In Fig. 3(b) the LA-ICP-MS pattern is presented, together with the appropriate maps obtained by processing the LA-ICP-MS signal. The pattern was obtained from the same sample in different position. The lack of easily recognizable vein system in this part of the sample caused the rather homogenous LA-ICP-MS signal; however, the vein on the left part of the image is clearly visible. In Fig. 4 the results of the LIBS and LA-ICP-MS analysis of 5 days 1 mmol l^{-1} AgNO_3 treated sample is presented. The increase of the Ag

Fig. 3 (a) LIBS and (b) LA-ICP-MS ablation pattern created on a $3 \times 5 \text{ mm}^2$ leaf section of the 4 days 1 mmol l^{-1} AgNO_3 treated *Helianthus Annuus* L. sample together with the map of Ag and Cu accumulated in the sample. The position of the central vein of the investigated leaf is marked with a *box* and with a *dashed line* on the photograph and on the maps, respectively. The length of the bar is $500 \mu\text{m}$. The color scale of the signal for the appropriate element is linear



signal in the central vein area of the sample on the map of Ag is observable.

The increased Ag content in the central vein and generally in the vein system was clearly visible from the first day of treatment in all the investigated leaves. The Cu is spread more homogeneously (see also maps in Figs. 3 and 4) within the sample, and there is only a slight increase of the copper signal on the central vein for both LIBS and LA-ICP-MS.

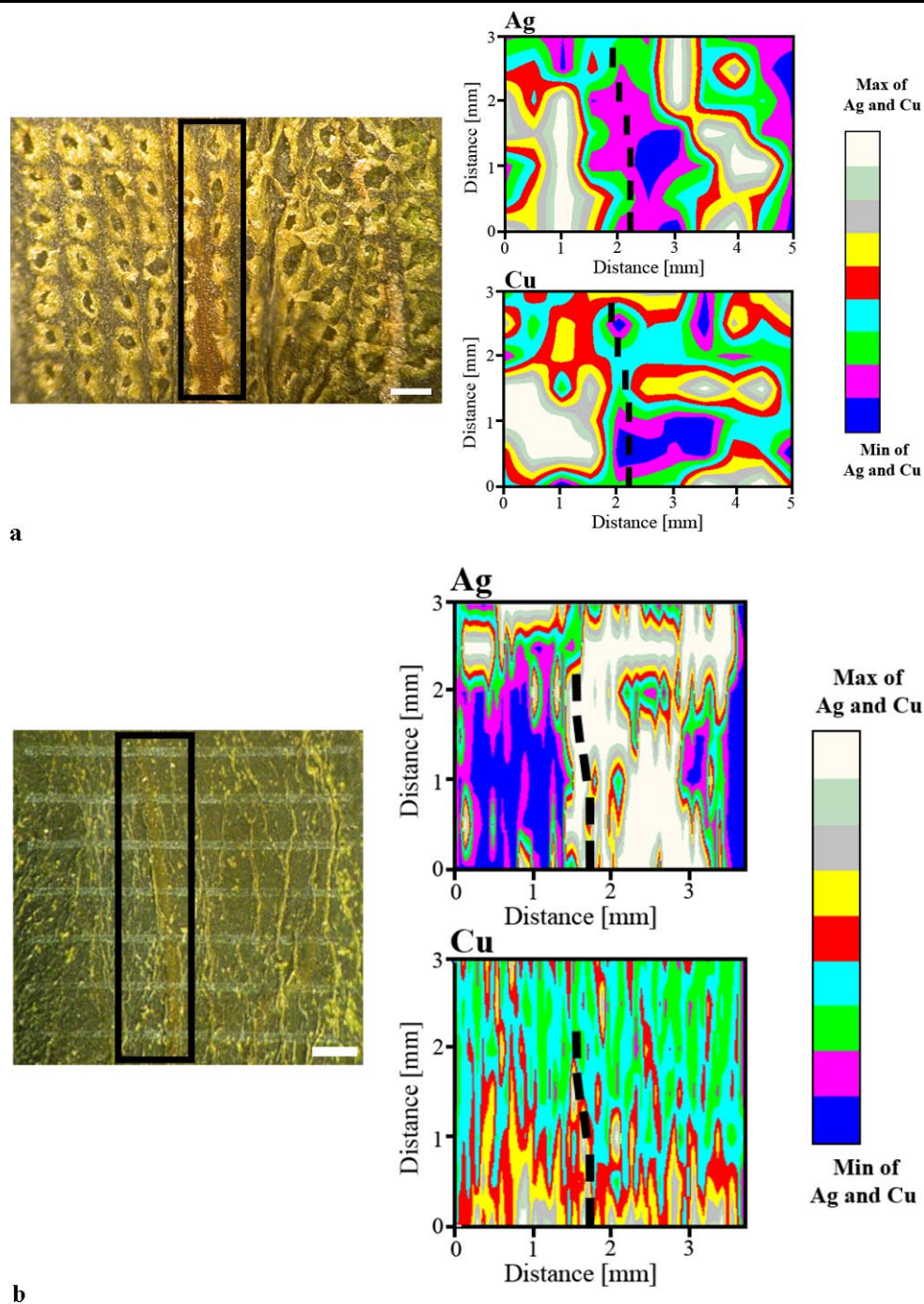
We should also note that it is next to impossible to prepare standard samples from leaves for calibration of LIBS of LA-ICP-MS for quantitative measurements. The existing NIST powder leaf standards, e.g., strawberries, orchids, and tea leaves, do not give information about spatial distribution of elements. Consequently, there is an urgent need for a standard technique which can offer spectro-chemical

analysis with a sufficient lateral and spatial resolution. As we have demonstrated earlier [8] with the aid of techniques based on soft X-ray laser plasma generation or using the synchrotron X-ray radiation it should be possible to develop standards. In our future work, we are planning to use the internal standardization method for LIBS so that qualitative analysis for monitoring heavy metals in plants will be feasible.

4 Conclusions

In summary, we have demonstrated the ability of two laser-ablation based analytical methods, LIBS and LA-ICP-MS to mapping the distribution of silver and copper in the leaves of *Helianthus Annuus* L. samples. It was shown that LIBS

Fig. 4 (a) LIBS and (b) LA-ICP-MS ablation pattern created on a $3 \times 3.7 \text{ mm}^2$ leaf section of the 5 days 1 mmol l^{-1} AgNO_3 treated *Helianthus Annuus* L. sample together with the map of Ag and Cu accumulated in the sample. The length of the bar is $500 \mu\text{m}$. The position of the central vein of the investigated leaf is marked with a *box* and with a *dashed line* on the photograph and on the maps, respectively. The color scale of the signal for the appropriate element is linear



and LA-ICP-MS analysis could be implemented and used in applications, in which the capability for spatially resolved analysis is required, e.g., to trace the possible migration of elements within botanical samples.

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