Utilization of the Laser Induced Plasma Spectroscopy for monitoring of the metal accumulation in plant tissues with high spatial resolution

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We report on the utilization of Laser Induced Plasma Spectroscopy for monitoring of the lead and magnesium accumulation in lettuce (*Lactuca sativa* L. var. *capitata*) leaf samples. The capability of this analytical method is compared with the results obtained by laser-ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS). The total amount of magnesium as essential element and lead as pollutant is determined by atomic absorption spectrometry (AAS) in the target samples.

Keywords spectroscopy methods, plants, phytoremediation, heavy metal

1. Introduction

1.1 Interactions between plants and element ions

Plants grow and develop in the environment containing all elements from the periodic table [1]. Further, the plants live on a place for tens of years without any chance to escape from polluting environment [2,3]. Such selective strain of the environment had to result in developing of various protective mechanisms. Both organic and inorganic (e.g. heavy metals) compounds enter to a plant mainly via two "gates": a) leaves and b) roots.

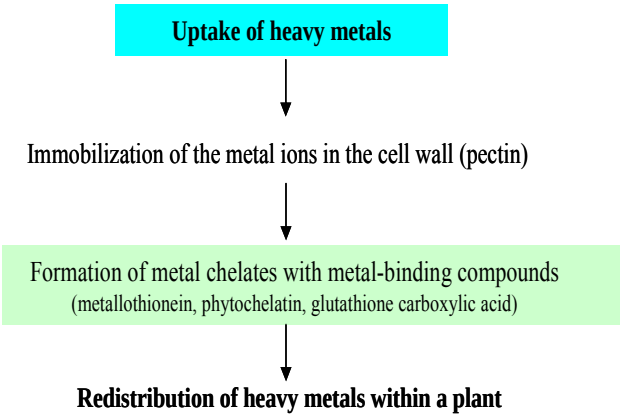

Fig. 1 General figure of elements ions distribution within a plant.

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It is known that different plant species have developed various strategies for uptake, distribution or redistribution of the heavy metals (Fig. 1). These strategies enable plants to maintain vitally important homeostasis of these elements. Moreover, there have been observed plant species, which were able to spread in the new niche thanks to these strategies [4-6].

A cell wall is the first selective barrier for entering of heavy metals into a plant. To our knowledge heavy metals can be bounded into the cell wall by whole battery of chemical compounds, most of all, by polysaccharides as pectin. The binding of heavy metals to the cell wall is not well regulated. If the heavy metals break through the cell wall, their interactions with other, groups of more specific compounds can be observed. Both low and high molecular compounds rich in –SH moieties regulate levels of heavy metals inside the cell [7,8]. Investigating of these compounds is mostly aimed on glutathione and phytochelatins. Nevertheless, monitoring of distributing of heavy metals and these heavy-metal binding thiols is almost impossible using conventional analytical instruments [9,10]. Spectroscopic methods can help in the solving of this task [11].

1.2 Spectroscopic analysis

The analysis of biological micro-particles, like aerosols and cells are receiving increased interest in the recent years [12]. Beyond the capability of identification of these particles, additional information about interactions at atomic/molecular level is also often highly needed. For example, information on where specific ions are stored within plants is of extreme interest in plant science itself, and in the context of a biological secondary environmental monitor. Thus, a technique enabling high spatial and lateral distribution of elements within individual plant cells/different cell layers would give plant scientist a very powerful tool to possibly answer many of the open questions of plant metabolism. Advanced laser-based techniques for spectrochemical analysis allows fast and accurate study of different materials both for analysis in the laboratory and industrial environments [13]. The laser-generated patterns consisting of precisely ablated microcraters can be utilized among others also for mapping the distribution of different elements in plant tissues.

1.3 Laser-induced plasma spectroscopy

The system for laser-induced plasma spectroscopy (LIPS) (also called laser-induced breakdown spectroscopy – LIBS) consists of three main parts – an ablation laser, detector and focusing and collection optics. This technique utilizes the high power densities obtained by focusing the radiation from a pulsed, fixed frequency laser (usually Nd:YAG) to generate a luminous micro-plasma in the focal region of an analyte. The energy density in the focal region can reach values up to GW/cm^2. Part of the laser pulse energy is used to ablate the sample of interest; subsequently the material in the plasma core is vaporized and atomized. The plasma temperature can reach several electron volts and the plasma is typically highly ionized. In a good approximation, the plasma composition is representative to the analyte's elemental composition [13].

The micro-plasma emission is analyzed by spectrometer. In the LIBS analysis the emission from singly-ionized and neutral atomic lines are used. These lines are observable as the plasma cools - for the typical LIBS plasmas 0.5 – 1 μs after the laser pulse reaches the target. Before this time a continuum emission (due the Bremsstrahlung) prevails in the spectrum. The detection gate pulse width is usually several μs. As the plasma further cools molecular recombination occurs [13]. We should note that these "optimal" values depend on the temporal evolution of the LIBS plasma that is determined mainly by the laser pulse energy, pulse length and by the analyte´s material.

Laser spectroscopic methods allows to reach high spatial (this is normally limited by the size of the laser beam diameter) and depth resolution (in the range of several tens of nanometers). Detection limits can be estimated usually in the range of ppm.

2. Materials and methods

2.1 Plants cultivation and sample preparation

Leaves of lettuce variety Deon (*Lactuca sativa* L. var. *capitata*) were used in our experiments. Lettuce seeds were germinated on wet filter paper in special vessels at 25 ± 2 °C in dark. After 20 days, letttuce 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 $\mu E.m^{-2}s^{-1}$) at a temperature 22 °C and humidity 65 %. After that, Pb-EDTA was added to the cultivation solution at final concentrations of 0.5 mM and 1 mM. The lettuce plants placed in the vessels that contained tap water with addition of Pb-EDTA were grown for five days. Four plants from each experimental variant were harvested at certain time intervals during the experiment, and their roots were rinsed three times in distilled water and 0.5 M EDTA. In addition, each harvested plant was divided into leaves and root.

2.2 Experimental methods

The schematic of the table-top LIBS setup utilized for experiments is shown in Fig. 2.

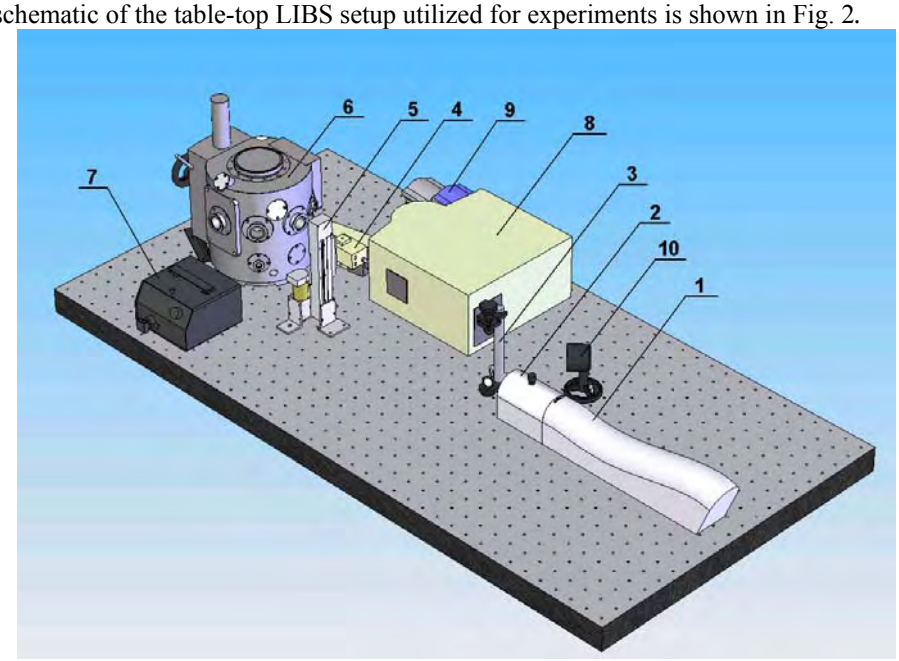

Fig. 2 Schematic of the LIBS experimental setup. 1 – Nd:YAG laser, 2 – module for the second harmonic generation, 3 – periscope, 4 – CCD camera for sample positioning, 5 – linear movement, 6 – interaction chamber, 7 – halogen lamp, 8 – spectrograph, 9 – ICCD camera, 10 – energy detector head.

The sample was placed to the sample holder inside the ablation chamber (Tescan) to the stage with precision movements (2 μm 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 LIBS micro-plasma was created using the second harmonic (532 nm) of a Nd:YAG laser system (Quantel, Brilliant B). The laser pulse width was ~5 ns and beam diameter 8 mm. The energy of the laser pulse (10 mJ at the sample) was set by an energy meter (Coherent Field Master, LM-P10). The laser

induced plasma was produced by focusing the laser beam with a 16 mm focal-length glass doublet (Sill Optics). Typical LIBS ablation patterns are shown in Fig. 3.

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

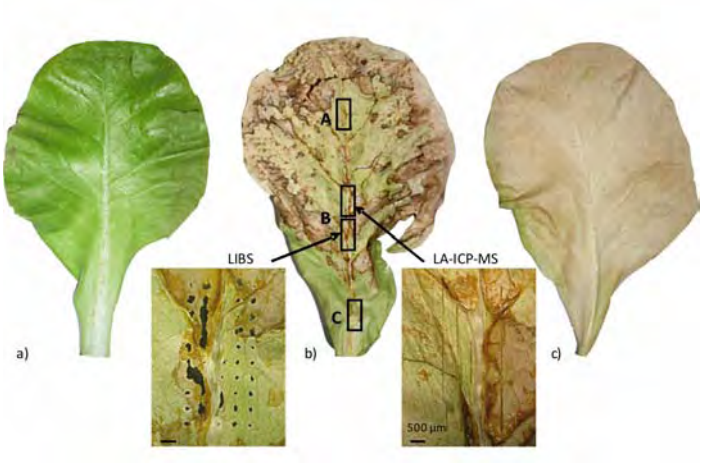

Fig. 3 Photography of the analyzed a) 0 (Control), b) 0.5 mM and c) 1 mM Pb-EDTA treated lettuce (*Lactuca sativa* L. var. *capitata*) leaf samples. The LIBS and LA-ICP-MS examination was performed in the areas indicated by rectangles A, B and C in all three samples. In the insertion the typical LIBS and LA-ICP-MS ablation patterns are shown.

For the LA-ICP-MS analysis the New Wave Research laser ablation system (UP-213) coupled to the ICP-MS spectrometer (Agilent 7500) was utilized. In this case the ablation pattern was created by a 5th harmonic of the UP-213 Nd:YAG laser (213 nm). The LA ablation pattern is shown in Fig. 3.

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 hours at 140 – 150 °C.

The calibration solutions for Pb were prepared in 5% HNO_3. During the measurements ammonium dihydrogen phosphate modifier was added to the 20 µl dose volume. The AAS analysis was preformed 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

Distribution and motion of elements ions within plants are still not clear yet [14]. Further different plant species differ in metabolism of ions and in tolerance to the higher doses of toxic elements and

Fig. 4 Photography of lettuce plants after 96 hours expositing to 0 and 1 mM Pb-EDTA. The control plants does not show any hallmarks of abiotic stress. The plant treated with the heavy metals lost marked amounts of chlorophyll and turgor in the leaves.

compounds [15-18]. We aimed on investigation of influence of Pb-EDTA complex on lettuce plants. It was published that chemicals chelating heavy metals enable them easily passing through cells structures [14]. Here, we found out that the control plants did not show any growth abnormalities during the experiments as it is shown in Fig. 4. Marked changes in the habit of the plants exposed to Pb-EDTA were observed already after 24 hours of the exposition. The leaves loose tonus and were lazy after 48 hours of the exposition. Moreover, chlorosis and decreasing in content of chlorophyll occurred. Between 96 and 120 hours of the exposition the plants had not any chlorophyll and had colourfully changed leaves (Fig. 3 and Fig. 4). Roots of the plants treated with the heavy metal were also damaged, whereas in the case of 1 mM Pb-EDTA treated sample only main root part remained without any other smaller side root

systems. Considerable decrease in weight (for more than 50 %) of plants treated with Pb-EDTA was determined. The decrease can be associated with reducing of water content in the plant tissues and with other metabolic changes [8,19].

3.1 LIBS and LA-ICP-MS measurements

The capability of laser-assisted diagnostic methods for monitoring of Mg and Pb in the investigated lettuce samples is demonstrated in Fig. 5. The single-shot LIBS and the LA-ICP-MS analysis were performed on a 4.5 × 2 mm leaf sections. The LIBS's ICCD detector was gated 1 μs after the Q-switch

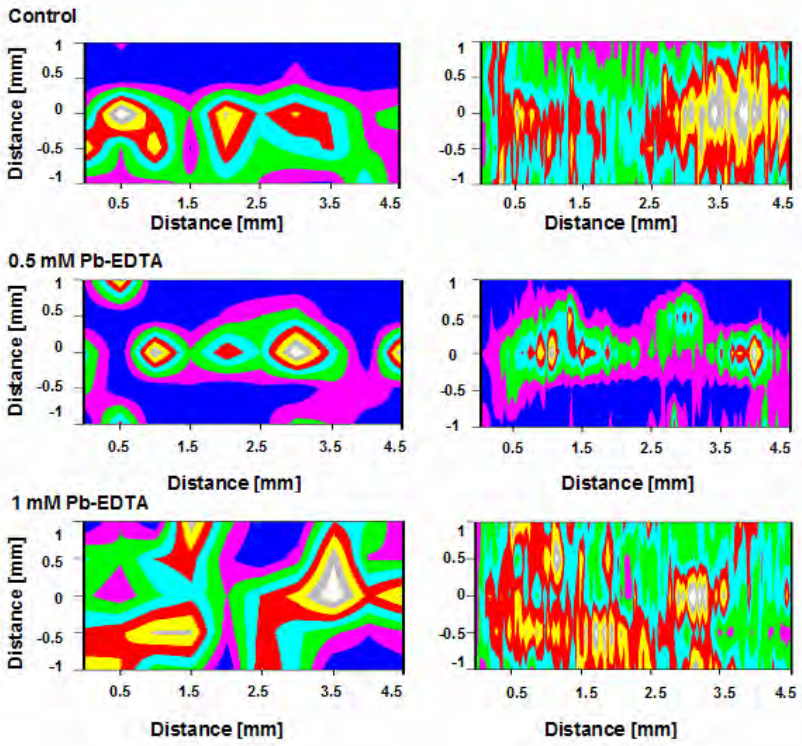

Fig. 5 The map of the Mg distribution in the Control and the Pb distribution in the treated leaves sections obtained by LIBS (left column) and LA-ICP-MS analysis (right column).

signal and the observation window was 10 μs. For Pb detection the 283.31 nm Pb(I) line was used. In the case of Control and 0.5 mM Pb-EDTA treated samples the Mg content was monitored using the 285.22 nm Mg(I) line. This line had a very week intensity in the case of 1 mM Pb-EDTA treated sample, thus the 278.04 nm Mg(I) line was used.

For each of the analyzed spectra from different ablation crater on the sample the background was subtracted and the peak area of emission lines for Pb and Mg calculated.

To ensure the analysis of the appropriate area on the sample, the LA-ICP-MS ablation pattern was positioned nearby the LIBS ablation pattern (see Fig. 3). As the example the maps obtained at the central area (B in Fig. 3) are shown in Fig. 5.

We observed higher content of Mg in the vein structures of Control and higher Pb content in the same structure of tissues from sample treated with 0.5 mM Pb-EDTA. The central vein is clearly observable in all investigated areas within the leaves (Fig. 3). In the case of 1 mM treated sample the lead is spread more homogenously within the sample, and there is only a slight increase of the lead signal on the central vein for both LIBS and ICP-MS (see Fig. 5).

However LIBS and LA-ICP-MS are not absolute methods [13], the higher Pb amount with increased treatment was evident on the higher intensity of the monitored spectral lines using LIBS and in higher counts using LA-ICP-MS. Within a leaf the higher concentration of the pollutant was detected on the part near to the petiole (part C in Fig. 3).

Concerning the spatial distribution of the Mg and Pb investigated in leaves from the plants treated with 0.5 mM and 1 mM Pb-EDTA, the parts with increased Pb and Mg content corresponded. However, we should note that the overall Mg content decreased with higher Pb dose (see also Chapter 3.2).

3.2 AAS measurements

The results of the AAS measurements are summarized in Fig. 6. The total amount of Mg is decreasing considerably for tissues from the plants treated with 1 mM Pb-EDTA. On the other hand content of Pb increased with increasing time of exposition and dose of the toxic element. At the highest dose of Pb the

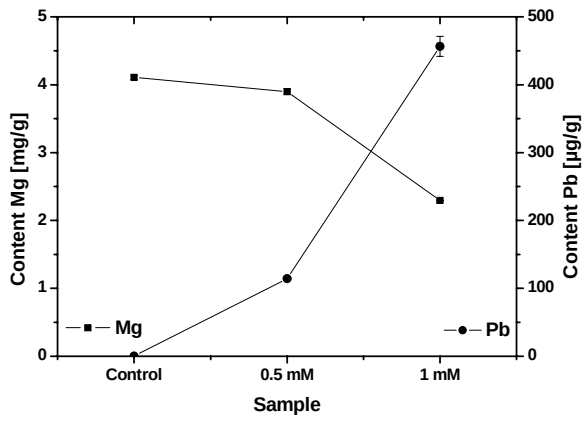

Fig. 6 Magnesium and lead content in the investigated 0 (Control), 0.5 mM and 1 mM Pb-EDTA treated lettuce (*Lactuca sativa* L. var. *capitata*) leaf samples.

content of this element was about 500 µg per g of fresh weight. The gradual decrease in the content of Mg in the target samples was interesting for us. This phenomenon is probably associated with competition between lead and magnesium ions and can result in chlorosis of the leaves relating to decay of chlorophyll structure (Fig. 7).

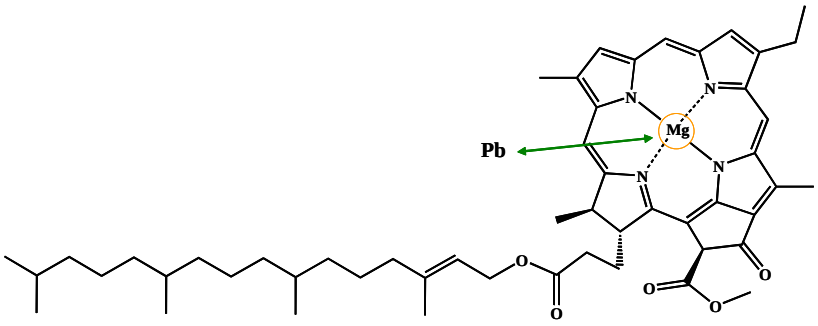

Fig. 7 Structure of chlorophyll with central atom of magnesium, which can be replaced by lead.

4. Conclusions

We reported on the possible utilization of laser ablation based diagnostic methods for monitoring of the lead and magnesium accumulation in lettuce (*Lactuca sativa* L. var. *capitata*) leaf samples. The capability of LIBS was compared to the results obtained by LA-ICP-MS. The total amount of magnesium and lead was determined by atomic absorption spectrometry (AAS) in the target samples.

The measurements showed that both of investigated laser ablation based techniques can be utilized for the analysis of the plant tissues. Using LIBS analysis the simplicity of the method foresees different application "*on-site*" that can be important for example for environmental monitoring and subsequent environmental remediation. LA-ICP-MS can be utilized mainly "*in-situ*" for monitoring of elements with lower detection limits in samples of interest.

The possibilities connected to obtain standard materials for laser-ablation based techniques (e.g. LIBS or LA-ICP-MS) are presently under investigation in our laboratories. The potential to utilize of methods based on X-ray radiation – X-ray microradiography and X-ray microtomography for the standard data for elemental distribution in plant samples is also studied [20].

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