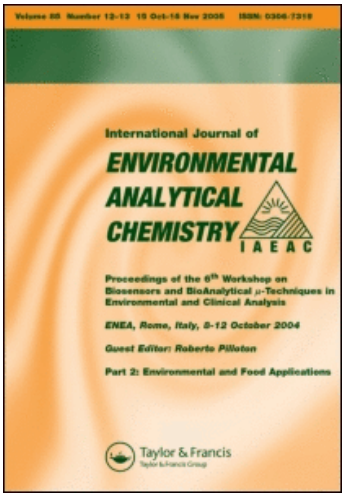

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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Anetta Hanć[a]; Danuta Barałkiewicz[a]; Aneta Piechalak[b]; Barbara Tomaszewska[b]; Barbara Wagner[c]; Ewa Bulska[c]
[a] Department of Trace Element Analysis by Spectroscopy Method, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland [b] Department of Biochemistry, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland [c] Laboratory of Basic Aspects of Analytical Chemistry, Faculty of Chemistry, Warsaw University, Warsaw, Poland

Online publication date: 22 September 2010

To cite this Article Hanć, Anetta , Barałkiewicz, Danuta , Piechalak, Aneta , Tomaszewska, Barbara , Wagner, Barbara and Bulska, Ewa(2009) 'An analysis of long-distance root to leaf transport of lead in *Pisum sativum* plants by laser ablation-ICP-MS', International Journal of Environmental Analytical Chemistry, 89: 8, 651 — 659
To link to this Article: DOI: 10.1080/03067310802559366
URL: http://dx.doi.org/10.1080/03067310802559366

Intern. J. Environ. Anal. Chem.
Vol. 89, Nos. 8–12, 15 July–15 October 2009, 651–659

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An analysis of long-distance root to leaf transport of lead in *Pisum sativum* plants by laser ablation–ICP–MS

Anetta Hanć[a], Danuta Barałkiewicz[a]*, Aneta Piechalak[b], Barbara Tomaszewska[b], Barbara Wagner[c] and Ewa Bulska[c]

[a]*Department of Trace Element Analysis by Spectroscopy Method, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland;* [b]*Department of Biochemistry, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland;* [c]*Laboratory of Basic Aspects of Analytical Chemistry, Faculty of Chemistry, Warsaw University, Warsaw, Poland*

(*Received 16 July 2008; final version received 8 October 2008*)

Laser ablation (LA) inductively coupled plasma mass spectrometry (LA–ICP–MS) has been applied to determine the nature of lead (Pb) distribution in plant tissues. Plants were cultivated hydroponically in Hoagland medium supplemented with 0.5 mM $Pb(NO_3)_2$. After 96 h, parts of root were cut off from *Pisum sativum* and analysed. The difference in the amount of Pb transported through the vascular tissues was expressed as the level of signal intensity. Mapping *in vivo* tissues reveals the metal pathway in the plant which may be particularly helpful in understanding of the mechanisms of heavy metal accumulation and transport in the plant.

Keywords: laser ablation; ICP–MS; lead; plant tissue; elemental distribution

1. Introduction

Heavy metals toxicity has become a major environmental and health problem. Metal concentration in soil ranges from <1 to even 100,000 mg kg^{-1}. This is mostly a result of anthropogenic activity, e.g. metallurgical industry, mining, agriculture and waste utilisation. Irrespectively of the source of heavy metals, enhanced level of many metals causes soil quality degradation and poor quality of agricultural products. Increasing content of heavy metals in the soil and in the surface and ground water also threatens normal functions of animals, plants and people. Lead (Pb) is one of the heavy metals occurring in soil that do not degrade but keep accumulating. The usual range of Pb concentration in soil is up to 200 mg kg^{-1}, but in some places it exceeds 700 mg kg^{-1} [1]. A tissue Pb concentration over 30 mg kg^{-1} is toxic to most of the plant species [2]. Dependent on soil pH, mobile Pb forms can make up from 9 to 12% of total Pb content, while Cd even up to 30%. Research on the ecosystem has indicated a strong link between plant tissue chemistry and soil chemistry. The concentration of elements in plants may be applied to monitor changes in environmental quality. Plants can take up and accumulate significant amounts of Pb without any visible changes in their habit or yield. Due to their ability to accumulate Pb in tissues, plants can be used in the process of soil remediation.

*Corresponding author. Email: danutaba@amu.edu.pl

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310802559366
http://www.informaworld.com

The most widely used variety of instrumental techniques for the determination of Pb in plant material are: atomic absorption spectrometry with flame (FAAS), inductively coupled plasma with optical emission spectrometry (ICP–OES) or inductively coupled plasma mass spectrometry (ICP–MS). In most cases the samples are dried and dissolved. An alternative to these methods is laser ablation (LA) linked with ICP–OES or ICP–MS. The major application of LA–ICP–MS is as a tool for the determination of element distribution in solid samples such as ancient manuscripts [3], geological materials [4] or ice cores [5]. However, there have been some studies on biological samples among which, most relevant to our studies are: those conducted on tree rings [6,7], tree barks [8], leaves [9] or root cap cells [10]. This technique is a useful tool for obtaining the information stored in the element concentration of plant material. The large surface area of barks from many tree species enables the effective accumulation of pollutants; hence the analysis of bark can provide useful information about the degree of pollution of a certain region. Narewski and co-authors [8] identified Al, Ca, Cd, Ce, Cr, Cu, Fe, Mn, P, Pb, S, Ti and Zn in barks using the LA–ICP–MS. This method can enhance the possibility of improving our knowledge about the elemental distribution in tissues on a micrometer scale [11]. It is a rather sensitive tool, with detection limits in the upper 1–100 $\mu g\,g^{-1}$ range. However, since the ionisation of elements is strongly dependent on the matrix and therefore rather difficult to calibrate, this technique can be considered only as a qualitative method [12].

The application of many spectroscopic methods can indicate the total concentration of elements in tissue, although it is difficult to localise trace elements precisely. Therefore, LA–ICP–MS technique was adopted to explore the possibility of determining elemental distribution in the tissues of roots of pea plants and to monitoring the level of elements such as S, Ca or P, which significantly influenced the heavy metal uptake and accumulation by plants.

The objective of the present work was to study the distribution of Pb in tissues of pea plant directly using the LA–ICP–MS methods. The influences of instrument operating conditions and preparation of each particular part of plant on the ion signals were investigated. This type of research could be used in speciation analysis applying the pinpointing of the metal pathway in a plant tissue, by the LA method, through the metal extraction from tissues, where it has been determined earlier.

2. Experimental

2.1 *Samples*

The plant material consisted of *Pisum sativum* from which morphologically selected seeds were first sterilised in sodium hypochlorite for 5 min, washed in distilled water, then sterilised in 75% ethanol for 5 min and again washed in distilled water. The seeds were soaked in bi-distilled water for 4 h. They were then germinated in the dark at 22°C for 4 days. Seedlings, from 4 to 5 cm long, were placed in a germination bed containing 1700 mL of 10× diluted Hoagland medium. The seedlings were cultivated for the next 96 h under controlled conditions. After 4 days the medium was replaced by a 100× dilution and 0.5 mM $Pb(NO_3)_2$ was added. Simultaneously, the control raising was conducted without a stress factor. After 96 h incubation the plants were rinsed twice for 5 min in 10 mM $CaCl_2$ and bi-distilled water and then 3 mm thick segments were cut from the roots, stems and leaves of the treated and control pea plants.

Table 1. Experimental parameters of LA–ICP–MS.

ICP–MS parameters	
Forward power	1050 W
Outer gas flow rate	15 L min^{-1}
Carrier gas flow rate	1.0 L min^{-1}
Auxiliary gas flow rate	0.9 L min^{-1}
Number of sweeps	3
Number of readings	1
Number of replicates	100
Measured mass	^{13}C, ^{208}Pb
Internal standard	^{13}C
LA parameters	
LA system	CETAC LSX-200
Wavelength	266 nm
Scan method	Single line scan
Focus	Sample surface
Laser energy	5.4 mJ
Repetition rate	5 Hz
Scan rate	25 μm s^{-1}
Spot size	50 μm

2.2 *Apparatus and conditions*

The experiments were carried out using the 266 nm Nd: YAG Laser Ablation system, CETAC LSX-200 (Cetac Technologies, USA) connected to ICP–MS (Model Elan 9000, Perkin–Elmer Sciex, Canada). The working parameters for the LA and ICP–MS are summarised in Table 1. The disadvantage of the LA–ICP–MS method is the semi-quantitive character of analysis due to the lack of standards with an identical composition to those of the measured samples. A standard reference material (NIST SRM 114 – a quartz plate) with a known Pb concentration was used.

3. Results and discussion

3.1 *Selection of LA conditions*

The results of the laser sampling depend on certain factors such as scan method, scan rate, laser energy level, repetition rate and spot diameter [13]. Those factors were important for the LA–ICP–MS analysis of plant samples. Effects of several parameters of the LA on the ion signals were investigated in the following experiments. In the preliminary experiments, various scan modes provided by LSX-200 were tested for the best signals of studied elements.

After evaluation, single line scan method was used. Additionally, effect of the scan rate on the ion signals was also studied. However, as the sample size was limited, a scan rate of 25 μm s^{-1} was selected in this study. Moreover, the effect of the laser energy on the ion signals was also studied. Results are shown in Figure 1. As presented, the signals increased with the increase of the laser energy level, but the best precision was obtained for an energy level of 5.4 mJ, which was selected in this study. Figure 2 shows the effect of the repetition rate on the ion signals. The repetition frequency ranged from 1 to 20 Hz. The level (5.4 mJ), the spot diameter (50 μm) and the scan rate (25 μm s^{-1}) were the constants. The measurement results were the most precise when the repetition rate was 5 Hz.

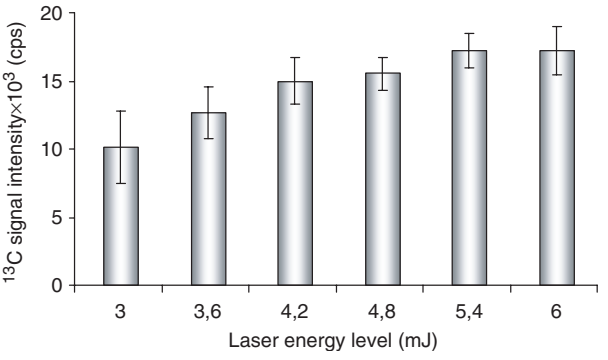

Figure 1. Effect of the laser energy level (instrument setting) on the intensity of the internal standard. Spot size was set to 50 μm. Values are means of three measurements ± SD.

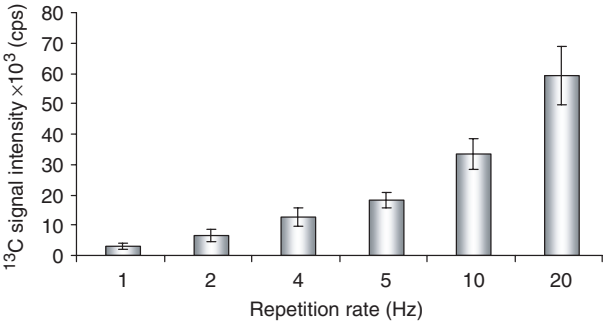

Figure 2. Effect of the repetition rate on the intensity of the internal standard. Spot diameter was set to 50 μm. Values are means of three measurements ± SD.

The measurement precision depended on applied repetition ranged from 12 to 30%. The plant material is very gentle, therefore a number of experiences were executed for various spot diameter. The application of spot diameter larger than 100 μm has caused the destruction of plant samples. The most precise and accurate results were obtained for the spot diameter equal 50 μm, and therefore this kind of spot diameter was applied in further research. If the studied material is gentle and thin, it is necessary to pay extra attention to laser energy and spot diameter, which is confirmed in Sekaran's investigations [12], who analysed the soft animal tissues. To compensate for signal fluctuation caused by the variation of the ablated sample, mass ^{13}C was used as a natural internal standard. Carbon is the most abundant element in pea samples and may be the most appropriate internal standard. Many workers have utilised ^{13}C in the analysis of biomaterials such as tree rings, rice, livers and kidneys [14–16]. Operation conditions are given in Table 1.

3.2 *Determination of Pb in pea (***P. sativum***) by LA–ICP–MS*

It is known that metal ions penetrate plants mainly through roots. It has been found that metal uptake is at first stopped on the root surface, then a large portion of metal penetrates the root and is bounded in the cell wall forming insoluble deposits, some of which are stored in the cell walls and other in intercellular spaces [17–19]. It has been

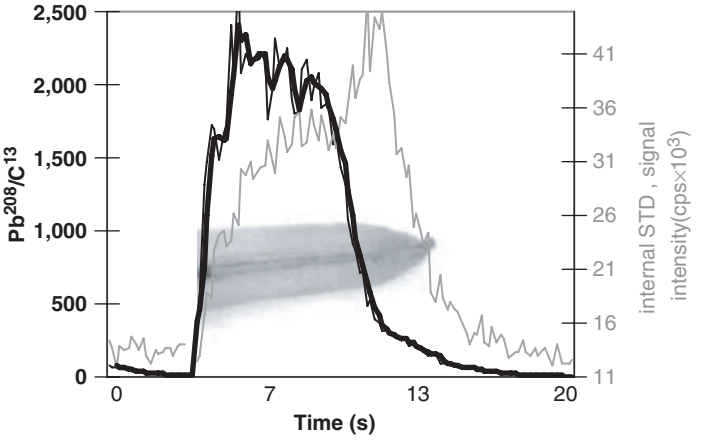

Figure 3. The Pb profile along the root tip of pea tissue. An arrow shows the root cap cell protection area, which blocks Pb penetration into conducting tissue, as a background a root apex has been shown.

revealed that in pea roots, 95% of the total amount of metal added is accumulated, irrespective of used $Pb(NO_3)_2$ concentration [20]. This is accomplished by restricting metal transport across the root endodermis (stele), and removing any mobile ions in the xylem by means of storage in cell walls and vacuoles, or binding by metal-binding proteins such as metallothioneins or phytochelatins [21].

The applied LA enabled an analysis of the migration and accumulation of absorbed metal in particular root tissue and also allowed the level of Pb transported to the aboveground parts of plant to be designated. In order to see how the different Pb is distributed in the tissues, it is necessary to scan a line on the surface of root cross-section. The laser ablated a line across a selected fragment of the samples, and the concentration of the chosen element in the vapour was measured. The difference in the amount of Pb in particular parts of plants was expressed as the level of signal intensity.

The investigation of Pb concentration was conducted in root apex and root hair zone. The root apex is composed of meristematic tissue, quiescent centre and root cap. Root cap cells, which cover the root apex protect meristematic tissue and enable toxic factor prevention. The analysis conducted by our research group reveals that root cap cells effectively protect root apex meristematic tissue. Figure 3 presents the intensity signal for Pb and internal standard (carbon ^{13}C), additionally the area of root apex meristematic tissue protecting cells has been marked. In most of the existing research, the Pb concentration in tissue has been estimated by using histochemical techniques. Seregin *et al.* [22] signified Pb presence in maize (*Zea mays*) root apex cells by using a Pb complex with dithisone after 24 h of plant raising with a 10^{-3} and 10^{-2} mol L^{-1} addition of $Pb(NO_3)_2$ concentration.

The signal intensity changes due to the presence of Pb at the cross-section through the root (Figure 4). The peak is located at the root surface and endodermis. It was found that the metal is bound in the cell wall forming insoluble deposits, while part of it is stored in intercellular spaces [23–25]. It was also determined that celluloses and lignin bound substantial amounts of Pb [26]. The presented research reveals a partial accumulation of absorbed Pb in the outer root tissue: rhizodermis and outer cortex [27]. The absorbed metal has been transported by the apoplast. An increase of Pb concentration in the

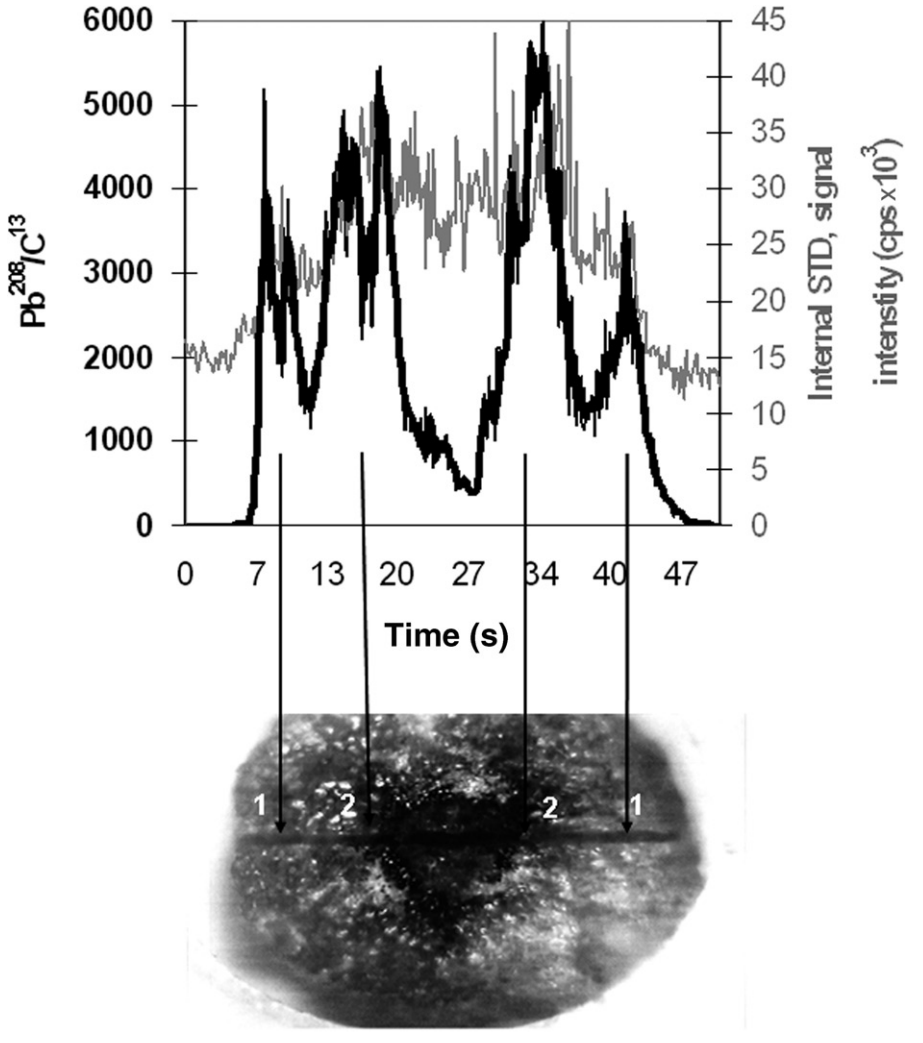

Figure 4. Laser ablation–ICP–MS spectra showing intensity of Pb and C signal due to spatial localisation of Pb at the cross-section through the root of *P. sativum* plant cultivated hydroponically for 96 h in 0.5 mM $Pb(NO_3)_2$. Root cross-section: rhizodermis and outer cortex (1) and xylem (2).

endodermis and only insignificant amounts of Pb in stele was observed. This accords with similar results which have been presented by several authors applying different methods [28,29]. The majority of plants have an endoderm barrier, protecting them from Pb penetration into conducting tissue and consequently preventing metal transport to the overground part of the plant. This has been confirmed by the results of the presented analysis of stem and leaves. The analysis of stem as well as root tissues was made in cross-section (Figure 5) and enabled the observation of potential Pb and other mineral compound transport from roots to stems. The signal increase indicating Pb presence was observed only on the stem surface. The lack of a signal increase for Pb in vascular tissue

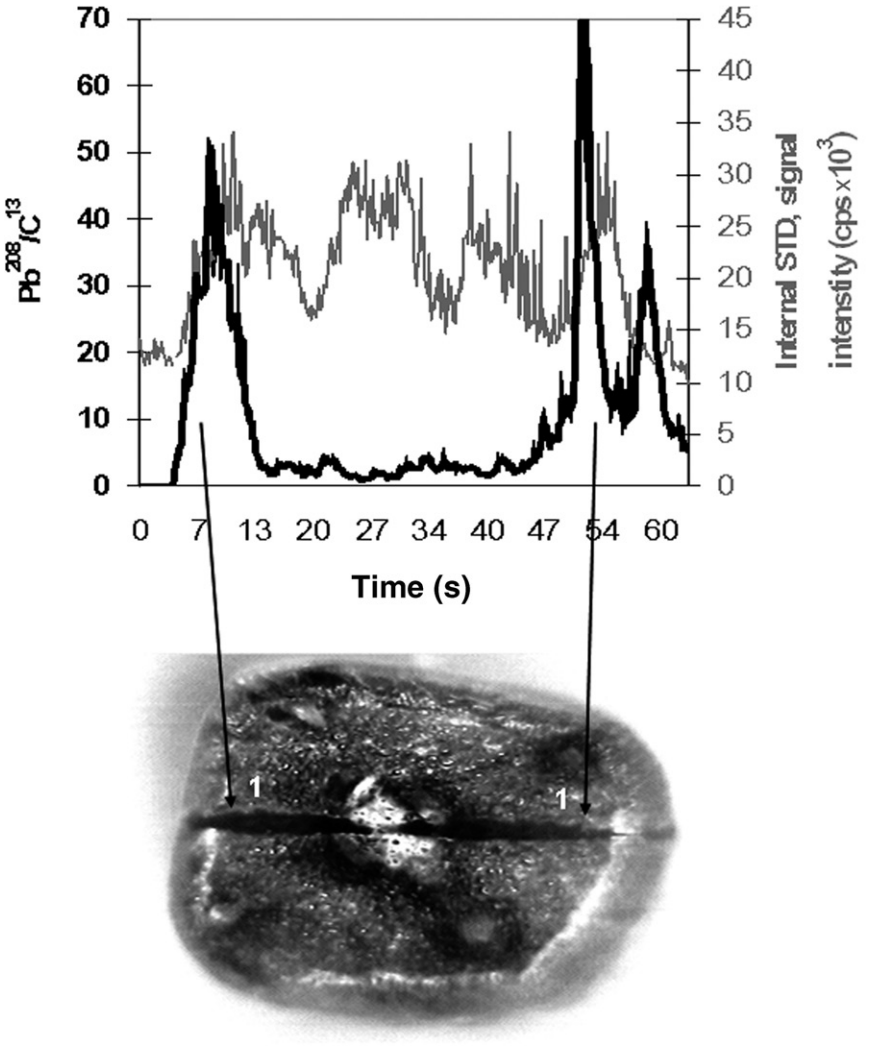

Figure 5. Laser ablation–ICP–MS spectra showing the Pb localisation along the cross-section through the stem of pea cultivated hydroponically for 96 h in 0.5 mM $Pb(NO_3)_2$. The photography shows the epidermis being marked (1).

area clearly indicates very limited transport to the above-ground parts of pea plants. There was no trace of Pb in leaf tissue, as shown in Figure 6. A recent study has reported that there are, however, few unique plants called hyperaccumulators that accumulate a high amount of metals in their stems. Such process is classified as natural phytoextraction. This group of plants can accumulate 1000 times more heavy metal than normal plants, and they are able to transport it to the above ground parts.

Spatial patterns in the chemical content of plants can be used to monitor changes in atmospheric conditions, soil chemistry and pollution history. LA–ICP–MS is an excellent approach for determining the chemical content in plant samples because of the high spatial

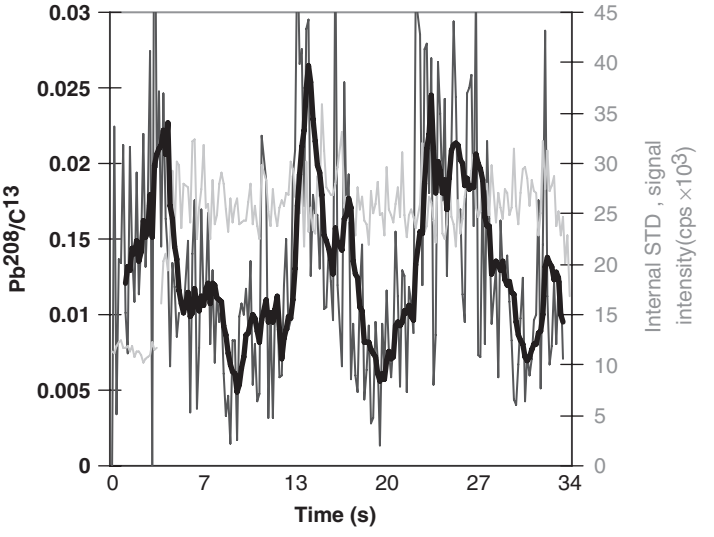

Figure 6. Laser ablation–ICP–MS spectra showing the Pb localisation in the leaf base of the pea plants cultivated hydroponically for 96 h in 0.5 mM $Pb(NO_3)_2$.

resolution provided by a focused laser beam. The excellent sensitivity of LA–ICP–MS allows measurements of many elements at very low detection levels. Mapping *in vivo* tissues allows an understanding of the metal pathway in the plant which would be of some value in the phytoremediation process.

4. Conclusion

The results presented in the current study as well as in previous findings revealed [20], that the highest quantities of Pb are accumulated in pea roots rather than in stem or leaf tissue. LA coupled to ICP–MS is the ideal technique for mapping trace elements in soft tissues which allows the analysis of Pb migration and accumulation in particular parts of the pea plant. The resolution of the method is effective enough to determine element variations in separate parts of vascular tissues. LA is a rapid, direct and comfortable method for measuring the changes in the level of uptake, accumulation and transportation of metals. This technique does not require drying or sample mineralisation, what considerably shortens time necessary for analyses. Moreover LA allows a simultaneous observation of changes in the level of significant elements such as S, Ca and P, which participate in heavy metal uptake and accumulation by plants. Aforementioned advantages of the LA–ICP–MS, no sample-size requirements and no sample preparation procedures, give this technique a great potential in environmental research. LA–ICP–MS is probably the best suited analytical method for such studies.

Acknowledgement

This work was financially supported by the grant KBN 1 T09D 057 30.

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