TOXICITY ASSESSMENT OF PHOTON-UPCONVERSION NANOPARTICLES AND THEIR BIOIMAGING BY USING LASER-INDUCED BREAKDOWN SPECTROSCOPY IN *BRASSICA OLERACEA* L. PLANT

Sára Střítežská[1], Pavlína Modlitbová[1]*, Pavel Pořízka[1,2], Jozef Kaiser[1,2]

[1]Central European Institute of Technology (CEITEC) Brno University of Technology, Purkyňova 123, 612 00 Brno, Czech Republic
[2]Faculty of Mechanical Engineering, Brno University of Technology, Technická 2896/2, 616 69, Brno, Czech Republic
**e-mail: pavlina.modlitbova@ceitec.vutbr.cz*

1. INTRODUCTION

The increasing use of many types of nanoparticles (NPs) in commercial products as well as in many research areas has been found to lead to the NP-accumulation in the environment and within the food chain [Rodrigues 2016]. It poses an additional potential toxic effect on various organism/tissues at the cellular level [Magnuson 2011]. The evaluation of NPs toxicity, bioaccumulation, and translocation in diverse organisms is an extremely challenging task [Modlitbová 2020]. The precise localization of NPs through the plant tissues is of a paramount importance to reveal the relationship between the exact location of NPs and its toxic effect.

In this study, the rare-earth elements-doped photon-upconversion nanoparticles (UCNPs) are studied. They are composed of $NaYF_4$ nanocrystal and doped with Yb^{3+} and Tm^{3+} ions; a carboxylated silica shell is added on the surface to increase the chemical stability and reduce the releasing of free ions [Modlitbová 2019].

Brassica oleracea plant was exposed to the selected contaminants, UCNPs suspension in two nominal concentrations 50 μg UCNPs/mL. After a 72-h exposure, we monitored several macroscopic end-points, and plants were dried, molded, and epoxide-glued onto the glass slides.

The LIBS method was used to determine the permeation of Y, Yb, or Tm through the whole plant. The photon-upconversion laser microscaning was used as a complementary technique confirming the presence of Y, Yb, and Tm in the plants in the form of UCNPs [Sedlmeier 2016].

2. EXPERIMENTAL

2.1. Plant exposure

Seeds of cabbage (*Brassica oleracea* L.) were germinated for 48 hours in Petri dishes with the bottom covered with filtration paper in Milli-Q water. Seedlings with similar

size (approximately 1 cm root length) were selected into the toxicity test. Plants were exposed for 72 hours under the lighting cycle of 15 h light/9 h darkness at room temperature (22 ± 1 °C). The experiment consisted of a control group (Milli-Q water) and UCNPs suspension in nominal concentration 50 μg UCNPs/mL (10 μg Y/mL + 4.4 μg Yb/mL). Each exposure group contained 12 replicates of test plants.

Plants after exposure were washed with milli-Q water, dried and molded at room temperature for seven days and then glued by epoxide onto the glass slide. First the plants were photographed using an optical microscope, then photon up-conversion microscans (only in UCNP-treated plants) and LIBS measurements were performed.

2.2. LIBS measurements

The LIBS Discovery system (CEITEC, Czech Republic) was used for all experiments. Briefly, this set-up consists of a nanosecond laser (CFR 400, Quantel, France; 20 Hz, 532 nm, 10 ns), UV grade collecting optic (F2, SOL instruments, Belarus), an optical fiber (core diameter 400 μm, Thorlabs, US), the Czerny-Turner spectrometer (Shamrock, Andor, UK), and a sCMOS camera (iStar, Andor, UK).

The settings used during the LIBS analysis were optimized in our previous work [Modlitbová 2019] and only slight modifications were made. 1 μs gate delay, 15 μs integration time, 20 mJ pulse energy and 20 Hz repetition rate were used. The lateral resolution was 100 μm based on the crater diameter.

The emission lines found most appropriate for monitoring were Y II 437.49 nm, Yb I 398.80 nm, and Tm I 409.42 nm. After a background subtraction using a moving minimum, the data were imaged in the form of 2D maps.

3. RESULTS AND DISCUSSION

LIBS maps were constructed for selected elements contained in UCNPs, Y (Y II 437.49 nm), Yb (Yb I 398.80 nm), and Tm (Tm I 409.42 nm). These maps were completed by photon-upconversion microscans to confirm the presence of selected elements in the NPs form. The distribution of elements in *B. oleracea* exposed to 50 μg UCNPs/mL is shown in **Figure 1**.

The UCNPs could transfer from the root via the stem into the leaves by vascular bundles [Modlitbová 2019]. However, the main bioaccumulation storage organ was root, as is easily visible on 2D LIBS maps. In the tested concentration of 50 μg UCNPs/mL, UCNPs the found behaviour in plant translocations was: root >> stem > leaves. The detected signal from stem and leaves was negligible.

As visible in **Figure 1.**, the 2D maps of spatial Y, Yb, and Tm distribution obtained by LIBS method showed very similar sensitivity in comparison to photon up-conversion microscans. Moreover, LIBS method could easily detect the NPs without any luminescence, or NPs whose luminescence quench. Also, LIBS offers the bioimaging of selected elements in their ionic form.

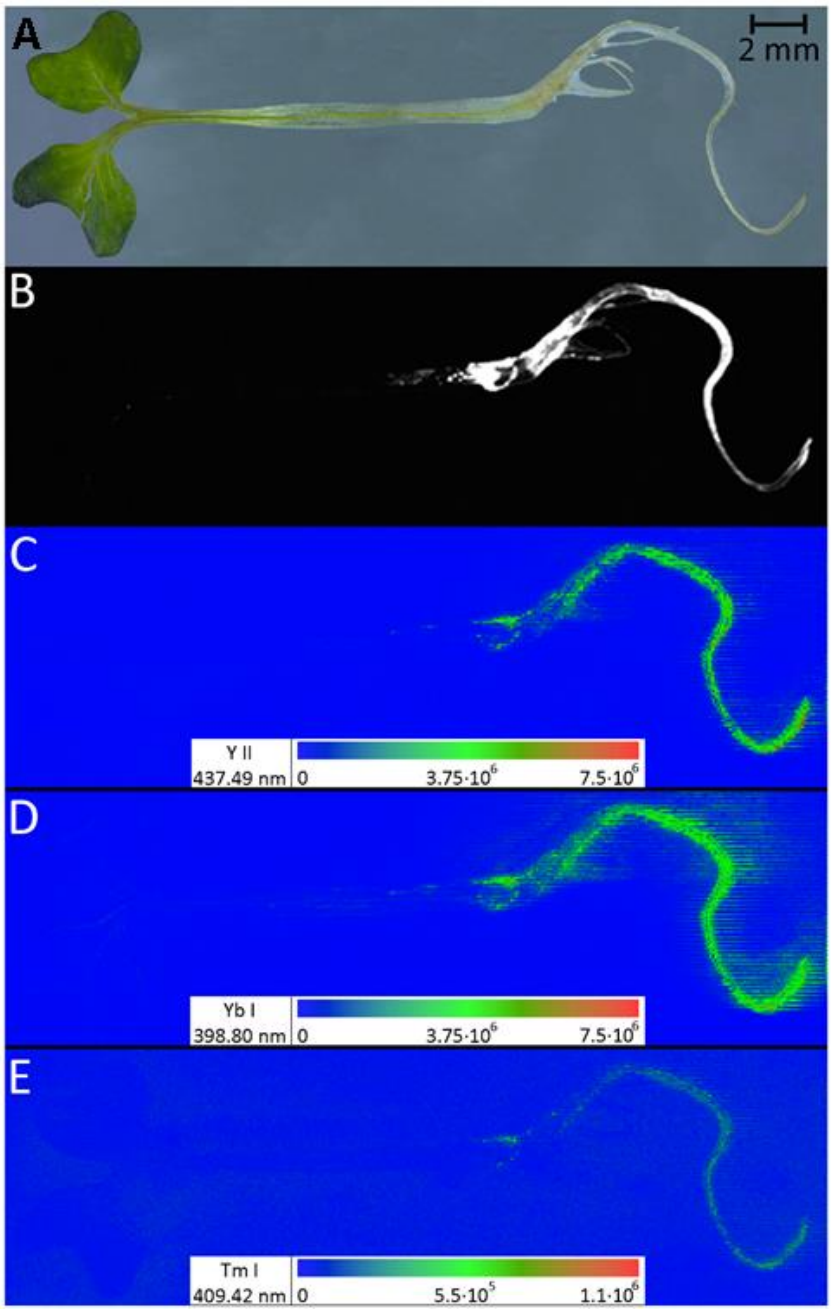

Figure 1. *Brassica oleracea* after 72-h exposure to 50 µg UCNPs/mL suspension, A: photography of plant, B: photon-upconversion microscan of plant, C: 2D LIBS map of spatial yttrium distribution (Y II 437.49 nm), D: 2D LIBS map of spatial ytterbium distribution (Yb I 398.80 nm), E: 2D LIBS map of spatial thulium distribution (Tm I 409.42 nm). The scales show the intensity of emission lines.

4. CONCLUSIONS

As a general conclusion, we can say that it is particularly beneficial to use LIBS for a fast screening in common phytotoxicity tests if information about the site of bioaccumulation, translocation, up-take routes as well as about the contaminants' trophic transfer is needed. All this information is valuable to phytotoxicity research, particularly because it is critically important to understand these processes to protect the quality of global ecosystems. LIBS can predominantly serve as a fast screening method for a large number of samples or as a complementary method providing other details to the complex analysis. Moreover, LIBS could be used in the field conditions to get a preliminary look before the laborious and not trivial sample selection, collection, storing, and transfer for a laboratory analysis.

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