Metabolomic approach to the study of nanoparticles impact to barley plants

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Foreword

Welcome to CECE 2014. With this 11th CECE in a row we are entering the second decade of the conference. As in the previous year we start with lectures by young scientists (CECE Junior), followed by two days of invited lectures and poster sessions. This book of proceedings includes the program of all three days. This year the meeting is free of charge for all perticipants thanks to the financial support by the European Social Fund and the state budget of the Czech Republic (CZ.1.07/2.3.00/20.0182). Of course our original goal of "bringing together scientists who may not meet at specialized meetings, promote informal communication of researchers from different disciplines and map the current status of the fields shaping the bioanalytical science" remains intact. The organizers want to thank the invited speakers and all the participants and hope that you will enjoy the scientific presentations as well as personal contacts and informal discussions.

Brno, October 18, 2014

electrolyte and applied voltage on the separation of oligosaccharide derivatives were studied.

The best separation of ANDS derivatives was achieved in phosphate buffer in non-coated capillary. The ANDS derivatives were suitable for fast separation of selected oligosaccharides. The fastest separation in less than 1 minute was achieved in phosphate buffer (pH 2.5).

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P106 METABOLOMIC APPROACH TO THE STUDY OF NANOPARTICLES IMPACT TO BARLEY PLANTS

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Summary
Barley plants in the pots after formation of second leaves were exposed in the special chamber to elevated concentration of CdO nanoparticles. After three weeks exposure the content of primary metabolites was measured using metabolomic approach and compared with the control.

1 Introduction

Nanoparticles (NPs) are characterized by their small size (<100 nm) and large surface area, which confer specific physicochemical properties to them. NPs can be derived from natural or anthropogenic sources, such as engineered or unwanted/incidental NPs. The increasing applications and use of NPs are directly related to their release into all compartments of the environment. The toxicity and degradation of these compounds in the environment cannot be accurately assessed yet because it depends on the NPs type, their physicochemical properties, and also on the environmental media in which they partition and the respective conditions.

The effects of NPs have been described in a wide variety of organisms [1-3] however, interactions of NPs with plants have been poorly studied and so the general consequences of NPs exposure for plants remaining unclear. Very few NPs and plant species have been studied and most of the studies reported the effect of nanomaterials on the very early growth stages of the plants, especially on seed germination [4], root elongation and biomass [5]. Majority of papers have focused on phytotoxicity of metal based engineered nanomaterials, but surprisingly negligible interest is given to nanoparticles emitted from technological processes outside to the environment, inclusive of Cd and its oxides, which are abundantly present in ambient particles [6].

Transport of NPs through environment partition is the most critical parameter to evaluate NPs impact and it is expected that NPs which are released into the environment have a high mobility. As compared to algae or fungi, plants might also be exposed to NPs in atmospheric environment but the majority of studies conducted on plants thus far employed an aqueous solution [7, 8], agar [9, 10] or soil [11] rather than air media. Therefore the main goal of our study was to estimate the impact of CdO nanoparticles entering the young barley plants from the air, water and soil. To the best of our knowledge this is the first report on the effect of CdO nanoparticles entering plants from the atmosphere.

2 Experimental

2.1 Preparation of CdO nanoparticles

CdO nanoparticles (CdONPs) were generated continuously in-situ in a hot wall tube flow reactor, using an evaporation–oxidation–condensation technique in which a ceramic crucible containing a small amount of bulk cadmium was placed inside the ceramic work tube of a vertically orientated furnace (Carbolite TZF 15/50/610). The formed cadmium oxide nanoparticles were diluted with a stream of air (20 L/min) and used for whole experiment in dose-concentration chambers. The concentration of CdONPs used in experiment was stable at the concentration level $2.03 \pm 0.45 \times 10^5$ particles/cm^3. A particle size was in the range of 7-60 nm.

2.2 Experimental design of exposure to CdONPs

Barley seeds were germinated, planted into pots and after formation of second leaves the pots were transferred into experimental chamber. In each pot were three barley plants and for each variant there were 10 pots with barley. 30 plants were placed in control cage with no CdONPs and 90 plants were exposed to the same concentration

of CdONPs with following changes: in the first treatment (I_1) CdONPs affected only surface of the plants, in the second treatment (I_2) nanoparticles affected surface and the substrate of the plants and in the third treatment the last 30 plants (I_3) were completely exposed to the effect of CdONPs including plants surface, surface of the substrate and surface of the water into which the pots were immersed. Barley plants were exposed to CdONPs for three weeks, during this time irradiation was 150 μmol m^2 s^{-1} and illumination followed periods 12/12 hours.

After the experiment, leaves and roots were separated and part of the samples was frozen in liquid nitrogen for further metabolomic analysis. Another part of samples was dried and used for determination of Cd content and for electron microscopy study.

2.3 Analysis by liquid chromatography- mass spectrometry

Samples were analysed twice on HPLC-HRMS with positive and negative polarity of MS-Orbitrap. For the high performance liquid chromatography part, a Dionex Ultimate 3000 was used. The column used was a Hypersil Gold column 150mm x 2.1mm, 3μ. MS and MS^n experiments were performed using a LTQ Orbitrap XL-high resolution mass spectrometer equipped with a HESI II (Heated electrospray ionization) source.

3 Results and Discussion

Our established method for the metabolomic studies allows simultaneous analysis of 16 amino acids, 9 polyphenols, 7 acids of Krebs cycle, pentoses, hexoses, disaccharides and 2-deoxy-D-ribose from the group of saccharides, all mentioned compounds except polyphenols are plants primary metabolites.

Most of the results regarding content and composition of primary metabolites revealed large differences on both leaves and roots between control and CdONPs treatment. As an example it can be seen from the Fig. 1 large difference in amino acids composition in the leaves (control vs leaves exposed to different treatments). Two most abundant amino acids are tryptophan and phenylalanine most probably due to their role as precursors for biosynthesis of secondary metabolites. Similar picture was obtained also for the roots of affected plants. In contrary to our working hypothesis the main uptake of CdONPs was in the case of their complete exposure. Detailed study containing all data, including fatty acids profile, Cd content in the leaves and roots and materials from electron microscopy will be published elsewhere.

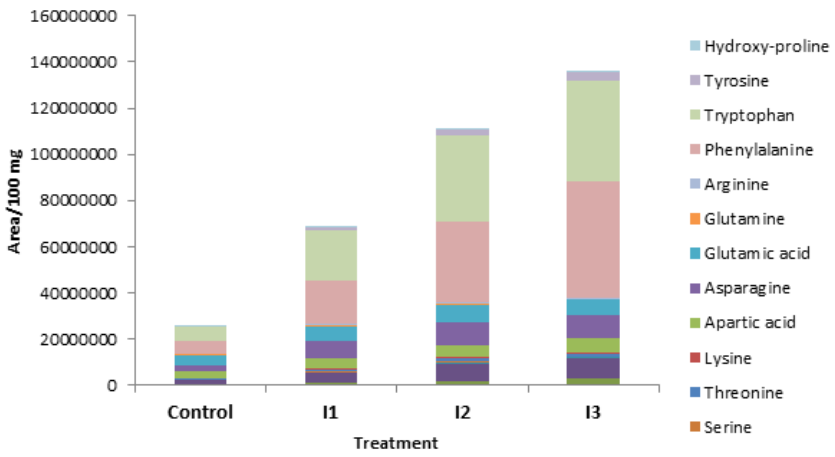

Fig. 1. Amino acids content and composition in the leaves exposed to CdONPs during different treatments (see experimental part). Values are means of 10 replicates.

4 Conclusions

The original hypothesis was that the nanoparticles enter the leaf through the stomata. This is not yet confirmed and it seems that the main uptake of CdONPs was through the roots from the soil and water. The results show differences on both leaves and roots between control and CdONPs treatment. The greatest effect was in the treatment I_3, in which plants were completely exposed to the effect of CdONPs including plants surface, surface of the substrate and surface of the water into which the pots were immersed.

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P107 IDENTIFICATION AND QUANTIFICATION OF AROMA COMPOUNDS OF SEA BUCKTHORN BERRIES

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Summary
Thirteen cultivars of sea buckthorn berries were tested for content of volatile aroma compounds using SPME-GC-FID method during two consequent years (2011-2012). In total 76 volatile compounds were identified: 26 alcohols, 13 aldehydes, 14 ketones, 9 acids and 14 esters. Alcohols, ketones and acids were quantitatively the most predominant group of compounds. Significant differences ($p < 0.05$) were found among varieties in both years, Krasavica cultivar was found as having the highest content of aroma compounds, stable during the monitored period.

1 Introduction

Fruits of sea buckthorn (*Hippophae rhamnoides* L.) belong to the most nutritious berry fruits, which seem to have preventive effects against many diseases, e.g. cardiovascular, mucosa and/or skin problems [1]. They are considered to be a good source of large number of bioactive substances like vitamins, carotenoids, phytosterols, organic acids, polyunsaturated fatty acids and some essential amino acids [2]. Besides the nutritional value, the sensory quality of fruits (especially flavour) is very important for consumers. Tang et al. [3] found astringency, sourness and bitterness as the main flavour attributes of sea buckthorn; its aroma was described variously, e.g. as strawberry-, peach-like [3], exotic fruit, pineapple and/or citrus fruit-like [1]. Despite highly acidic and exotic flavour, sea buckthorn berries have good potential for industrial production of various products like juice, tea, syrup, jam, jelly and many other nutritious products [2]. For this reason the pilot growing was started in Czech Republic several years ago.