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Platinum uptake, distribution and toxicity in *Arabidopsis thaliana* L. plants

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ABSTRACT

Platinum (Pt) occurs at very low levels in parent rock and soils in unpolluted areas, however concentrations of this element in urban areas is steadily increasing. At the levels recorded in urban environments, Pt is not yet phytotoxic, but it already poses a threat to human health, particularly when present in airborne particulate matter. In this study an attempt was made to evaluate Pt(II) uptake, distribution and toxicity in *Arabidopsis thaliana* L. plants.

Arabidopsis thaliana plants were hydroponically grown with increasing Pt(II) concentrations in the range of 0.025–100 μM. Pt(II) was taken up by the roots and translocated to the rosette. At lower Pt(II) concentrations ($\leq$ 2.5 μM) hormesis was recorded, plant growth was stimulated, the efficiency of the photosynthetic apparatus improved and biomass accumulation increased. Higher Pt(II) concentrations were phytotoxic, causing growth inhibition, impairment of the photosynthetic apparatus, membrane injuries and a reduction in biomass accumulation. Exposure of *A. thaliana* to Pt(II) also resulted in an increased content of phytochelatins throughout the plant and glutathione in the rosette.

Uptake and translocation of Pt(II) to harvestable organs of *A. thaliana* suggests that species of higher biomass accumulation from the *Brassicaceae* family can probably be used for the phytoextraction of Pt-polluted sites.

1. Introduction

Platinum (Pt) is a rare noble element that occurs at concentrations of 0.14 μg kg^{-1} in unpolluted soils, 1.12 μg kg^{-1} in agricultural soils and 20.9 μg kg^{-1} in areas adjacent to roads (Zereini et al., 1997). Its chemical characteristics make it useful as a catalyst in a variety of chemical processes (Zereini et al., 2012), with the amount used in car catalysts, oncology, jewellery, the electrical and glass industries, polymer processing and pesticide production totalling around 200 t $year^{-1}$ (Sobrova et al., 2012; Zereini and Wiseman, 2010). Today, the main source of Pt emission is catalytic converters in vehicles (50.4% of total emissions in Europe) (Pawlak et al., 2014). Each one can emit 38–146 ng Pt km^{-1} due to rapid changes in oxidation reduction conditions, high temperatures and mechanical abrasion (Limbeck et al., 2007), resulting in this element's diffuse contamination in environmental concentrations up to hundreds of μg kg^{-1} (Reith et al., 2014; Sobrova et al., 2012). Elevated levels of Pt are recorded in soils, airborne particulate matter, roadside dust and vegetation, rivers, and coastal and oceanic environments (Ravindra et al., 2004; Wiseman et al., 2016 and references therein).

Environmental concentrations of Pt are already considered to pose a threat to human health (Ravindra et al., 2004). Occurring in the air and in street dust, Pt acts as a hypoallergenic compound and can be transported through the lungs and in the blood to other human organs (Zaray et al., 2004), while Pt from road dust can be soluble and consequently enters watercourses, sediments, soil and ultimately the food chain (Ravindra et al., 2004). Pt is principally emitted from catalysts in a metallic form or as oxides, however considerable quantities are converted in soil into bioavailable forms, mainly as chloro or organic complexes, and thus can be taken up by plants (Šebek et al., 2011; Pawlak et al., 2014). However, the solubility and mobility of Pt are dependent on the pH and the ability to form neutral or charged inorganic and organic complexes (Šebek et al., 2011). The presence of Pt has been reported in many cultivated and wild plant species, in concentrations ranging from a few μg kg^{-1} up to hundreds of μg kg^{-1}, depending on the plant species and their environments (Reith et al., 2014). Concentrations of Pt in plants correspond well with levels of the element in urban dust (Orecchio and Amorello, 2010) and soils (Hooda et al., 2007), however a substantial proportion of accumulated Pt, especially in leaves, may come from the air in dust deposition, and

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collection and adsorption on the external surface of plants (Hooda et al., 2007; Ravindra et al., 2004; Šebek et al., 2011). Plant species differ in the total amount of Pt taken up and in the proportion of Pt translocated to the aboveground parts, which is usually low (Kowalska et al., 2004; Mikulaskova et al., 2013; Nischkauer et al., 2013).

The natural properties of plants can be used to remove Pt from the environment *via* an environmental biotechnology called phytoextraction. However, before such phytotechnology can be applied in practice, knowledge is required about the biological basis of plant response to Pt at environmental concentrations. To the best of the authors' knowledge, complex data on the physiological basis of the plant response to Pt have not yet been presented in the literature. Therefore this study aimed to evaluate Pt(II) uptake, distribution and toxicity in model *Arabidopsis thaliana* L. plants.

2. Materials and methods

2.1. Plant material and growing conditions

Seeds of *Arabidopsis thaliana* L. Col 4, #N933 were purchased from the Nottingham Arabidopsis Stock Centre (NASC, UK). To ensure uniform germination, seeds were kept for three days in a refrigerator at 4 °C and sown onto multi-well plates (single well volume – 60 mL) filled with substrate (Universal Kronenerde) and mixed with sand in the proportion 2:1 v/v, with pH of 6.6. Plants were grown in a growth chamber (Simez Control s.r.o., Vsetin, Czech Republic) at a temperature of 20/18 °C (day/night) and relative humidity of 70%. The photosynthetic active radiation (PAR) at plant level was 200 $\mu E\ m^{-2}\ s^{-1}$ (MASTER SON-T PIA Green Power, Philips) with a 8/16 h day/night regime.

Five-week-old uniform plants at growth stage 1.12 (Boyes et al., 2001) were transferred to plastic containers. During transfer, the roots were gently rinsed with distilled water to remove substrate residues. Plants were then grown in hydroponic culture in 0.3 dm^3 of Hoagland's nutrient solution, modified according to Siedlecka and Krupa (2002). The solution was continuously aerated and replaced weekly. During the first week the nutrient solution was used at 0.5 strength, and thereafter the complete composition of macro- and microelements was applied. The pH of the growing medium was 6.2 (Multilevel 1 m, WTW, Germany). On day 14, when the nutrient solution was changed, Pt in oxidation state II was added in the form of $[Pt(NH_3)_4](NO_3)_2]$ at concentrations of 0.025, 0.25, 2.5, 5.0, 25, 50 and 100 μM. However, not all the concentrations were applied in each of the five experiments performed. The amount of N provided in Hoagland's solution was reduced by the amount of N added with Pt(II) salt. Control plants were grown in nutrient solution that did not contain Pt(II). During the experimental period the pH level was monitored daily (Multilevel 1 m, WTW, Germany) and an increase recorded from the initial 6.2–7.6 after 24 h and to around 8 at the time the nutrient solution was changed.

2.2. Pt(II) uptake from the growing medium and its concentration in the roots and rosette

To determine the uptake and accumulation of Pt(II) by *A. thaliana* plants, two experiments were performed with five biological replications each. Since the results obtained in both showed similar trends, the data presented here are from the experiment with a wider range of Pt (II) concentrations. Plants were exposed to Pt(II) at concentrations of 0, 2.5, 5.0 and 25 μM and grown in the conditions described in Section 2.1. Two weeks after the treatment, three uniform plants (biological replications) from each Pt(II) concentration and the control were harvested separately and divided into the rosette and roots. The roots were gently rinsed twice with distilled water, followed by rinsing with redistilled water to remove Pt(II). The biological material was immediately frozen in liquid nitrogen, freeze dried, ground in a mortar and stored at −80 °C until analysis.

2.2.1. Sample preparation

Three samples of about 0.2 g dry matter representative for each harvested plant were mineralised in a Speedwave MWS-3+ microwave digestion system (Berghof) with 3 mL ultrapure HCl (37%) and 1 mL concentrated HNO_3 (~65%, T) for 30 min at ~180 °C. After digestion, samples were cooled to ambient temperature and vented carefully. The solutions were evaporated in quartz crucibles until they were almost completely dry, and diluted to a volume of 10 mL with 0.5 M HCl. Samples were then subjected to sequential extraction using the procedure established and described by Połeć-Pawlak et al. (2005). The solutions obtained with different extracting solvents (A: 10 mM Tris-HCl, pH 7.4; B: 2% Driselase in solution A; C: 1% SDS in solution A; D: 10 mM ammonium acetate, pH 4.6) were centrifuged for 20 min at 10,000 rpm (600*g*) at 0 °C, and the supernatants filtered through 0.45 μm filters. The solutions obtained were diluted to a volume of 10 mL with 0.5 M HCl.

2.2.2. Quantification conditions

The total amount of Pt(II) was determined by inductively coupled plasma mass spectrometry (ICP-MS) (Model 7500a, Agilent Technologies, Tokyo, Japan) using 10 ng mL^{-1} indium as an internal standard, according to the modified procedure of Cyprien et al. (2008). Briefly, the calibration graphs to determine Pt(II) by ICP-MS were prepared in the range of 0.001–40 μg L^{-1}. All standard solutions were prepared in 0.3 mol L^{-1} HCl. The detection limit (DL) was calculated for two Pt(II) isotopes (194 and 195) from the standard deviation of ten measurements of blanks and was found to be 0.001 μg L^{-1}. ICP-MS measurement conditions (nebuliser gas flow 1.13 L min^{-1}, RF power 1200 W and lenses voltage 40 – 120 V) were optimised daily using a standard built-in procedure. The limit of detection was established for samples of plants not exposed to Pt(II) (control group) and was found to be 0.008 and 0.006 μg L^{-1} for roots and leaves respectively. The recovery of Pt(II) from plant tissue was established for 0.2 g samples of plants from the control group and exposed to 25 μM Pt(II) in growth medium. Samples were spiked with 4 and 40 ng of Pt(II) in the form of $[Pt(NH_3)_4](NO_3)_2]$ complex and the obtained recoveries were 90–92 and 102–105% respectively for the control group and 97–100 and 103–110% respectively for plants exposed to 25 μM Pt(II). The relative amount (%) of Pt(II) in each extract was established against the total amount of Pt(II) in the mineralised sample and post-extraction residue, in accordance with previous studies (Połeć-Pawlak et al., 2005). All the measurements with ICP MS were performed with three biological replicates derived from individual plants and each biological replication was the mean of five technical replications (n = 3).

2.3. Effect of Pt(II) on selected morphological, physiological and biochemical processes

In these experiments *A. thaliana* plants were treated with Pt(II) at concentrations of 0.025, 0.25, 2.5, 5, 25, 50 and 100 μM, and grown in the presence of Pt(II) in the conditions described in Section 2.1. Measurements were performed *in vivo* two weeks after application of Pt(II) or during harvest, which took place one week later. In all, three experiments were performed with five biological replications each. Since the results obtained in all showed similar trends, the data presented here are from the experiments with a wider range of Pt(II) concentrations and more parameters measured.

2.3.1. Parameters/processes measured during plant growth

During plant growth the efficiency of the photosynthetic apparatus and transpiration rate were measured. The efficiency of the photosynthetic apparatus was assessed on fully expanded, undamaged leaves from the middle part of the rosette. Plant gas exchange was measured using the LI-6200 Photosynthesis System (LI-COR, Inc., USA). Measurements of net photosynthesis and stomatal resistance were taken under ambient temperature (20 °C), humidity (70%) and irradiance