SD 665

Plant Biology ISSN 1435-8603

SHORT RESEARCH PAPER

Palladium exposure of barley: uptake and effects

F. Battke[1], K. Leopold[2], M. Maier[2], U. Schmidhalter[1] & M. Schuster[2]

1 Department für Pflanzenwissenschaften, Technische Universität München, Wissenschaftszentrum Weihenstephan, Lehrstuhl für Pflanzenernährung, Freising, Germany

2 Department Chemie, Technische Universität München, Lehrbereich Anorganische Chemie, AK Analytische Chemie, Garching, Germany

Keywords
Abiotic stress; barley; exhaust gas catalytic converter; graphite furnace atomic asbsorption spectrometry; nano particles; palladium.

Correspondence
F. Battke, Department für Pflanzenwissenschaften, Technische Universität München, Wissenschaftszentrum Weihenstephan, Lehrstuhl für Pflanzenernährung, Am Hochanger 2, 85350 Freising, Germany.
E-mail: florian.battke@t-online.de

Editor
M. Hawkesford

Received: 21 March 2007; Accepted: 28 May 2007

doi:10.1111/j.1438-8677.2007.00017.x

ABSTRACT

Motor vehicles are now equipped with exhaust gas catalytic converters containing rare metals, such as palladium (Pd), platinum and rhodium, as catalytic active materials, leading to significantly increased emission of these metals. Compared with platinum and rhodium, low concentrations of Pd have been shown to have more serious effects on cells and organisms. In the present study, uptake of Pd by barley and behaviour of Pd nanoparticles in nutrient solutions used to grow plants were observed in order to develop a model of Pd exposure of plant systems. Pd determination was performed using a selective separation and pre-concentration procedure, which was further developed for this study, and coupled to graphite furnace atomic absorption spectrometry. The results show that uptake of Pd depends on Pd particle diameter. Compared to other toxic metals, like mercury, Pd causes stress effects in leaves at lower concentrations in nutrient solutions. Furthermore, Pd particles are dissolved at different rates, depending on size, in the nutrient solution during plant growth.

INTRODUCTION

Palladium (Pd) is only present at very low concentrations in undisturbed environments. Since the geogenic background concentration of Pd is significantly lower than the concentration of other non-essential toxic elements like mercury, lead or cadmium, Pd may not yet have affected biological systems. Motor vehicles are now equipped with exhaust gas catalytic converters that contain rare metals like Pd, platinum and rhodium as the catalytic active material, and emission of these metals has recently increased significantly. Therefore, on the verges of frequently-used roads, environmental concentrations of these metals now exceed geogenic background concentrations by factors of 10^5 to 10^6 and they are continuing to accumulate (Hoppstock & Sures 2004). In 1997, 60% of petrol-driven motor vehicles sold in Europe had exhaust gas catalytic converters containing Pd. As Pd emissions have increased rapidly, it is therefore of interest to determine the effects of Pd on biological systems. Initial studies showed that Pd is more mobile in soil than platinum and uptake rates are significantly higher (Zimmermann & Sures 2004), *e.g.* in grass samples from roadsides, the Pd content can reach 1.31 $\mu g \cdot g^{-1}$ (Schuster *et al.* 2000). Compared with platinum and rhodium, a lower concentration of Pd causes more serious effects to cells and organisms (Sures *et al.* 2006).

Platinum and rhodium accumulation and effects on biological systems have been studied previously; however, similar analyses of Pd have not been performed, even though platinum and rhodium in catalytic converters are now being substituted by Pd. This is probably because of serious difficulties in ultra-trace analysis of Pd in complex matrices (Sutherland 2007). Sensitive routine methods, *e.g.* inductively-coupled mass spectrometry, that are suitable for platinum determination, suffer from strong

interference of matrix constituents in Pd determination. Other analytical techniques, *e.g.* graphite furnace atomic absorption spectrometry (GFAAS), are not sufficiently sensitive for Pd determination in biological samples.

Since Pd (II) is only soluble in aqueous solutions at low pH (<3) with an excess of sulphate or nitrate and Pd emitted from exhaust gas catalytic converters consists of nanoparticles of different diameters supported on aluminium oxide, studies were carried out with nutrient solutions containing Pd nanoparticles in determined diameter ranges. Synthesis of these particle suspensions was performed as described by Mucalo *et al.* (1991). In the present study, uptake of Pd in barley and behaviour of Pd nanoparticles in nutrient solutions during growth of plants were observed as a model for Pd exposure of botanic systems. Pd determination was performed by a modified selective separation and pre-concentration procedure (Schuster & Schwarzer 1998), coupled to GFAAS.

MATERIAL AND METHODS

Preparation of Pd particle suspensions

The study was performed with two Pd particle suspensions differing in the diameter range of the particles: a series with colloidal Pd nanoparticles with a diameter range of 1–12 nm (series A), and a series with insoluble Pd particle agglomerates of about 1 μm diameter (series B). The general preparation procedure was similar to that described by Mucalo *et al.* (1991). Both series were prepared as described below (Table 1). An aliquot of a Pd standard solution [$Pd(NO_3)_2$ in 0.5 M HNO_3, 1000 mg Pd l^{-1}, Merck, Darmstadt, Germany] was diluted in 500 ml ultrapure water, obtained by de-ionization in a Milli-Q Gradient System (Millipore GmbH, Schwalbach, Germany). A defined stoichiometric excess of a freshly prepared 0.03 M sodium boron hydride (Merck) solution (0.022 g $NaBH_4$ in 20 ml ultrapure water) was then added to series A under vigorous stirring at a temperature of 0–5 °C. For series B, the procedure was done under gentle stirring at room temperature.

The addition rate of the reductant is decisive for the size of the resulting particles. For series A, an addition rate of 50 μl reductant s^{-1} is optimal, whereas for series B, a rate of 1 μl reductant s^{-1} is necessary. Black Pd particles were formed immediately after addition of reductant, but the suspensions were further stirred for about 1 h to confirm complete reduction of Pd and formation of elemental particles. Samples of about 2 ml of the Pd particle suspensions were then investigated by transmission electron microscopy (TEM) for size determination. A series of investigations (including high-resolution TEM and selective area diffraction) was further performed to characterize the particles in detail (Leopold *et al.* 2008) and to confirm that the surface of the nanoparticles consists of Pd^0.

Cultivation of barley plants

Barley plants were cultivated *in vitro* using a floating hydroponic system according to Battke *et al.* (2003). Seeds of barley (*Hordeum vulgare* L. cv. Barke) were placed on a floating layer formed from polypropylene beads in 2000-ml glass beakers containing 350 ml of nutrient solution. The nutrient solution was prepared by adding nutrient salts according to modified Hoagland E medium (Cowgill & Milazzo 1989) to the Pd suspensions described above. Nutrient salts were dissolved in aliquots of Pd suspensions to prevent changes in Pd concentration. The final concentration of nutrient salts in the nutrient solution was 5% of Hoagland E medium normal concentration, to minimize chemical interactions between nutrient salts and Pd. All chemicals were of highest commercially available purity. Beakers were sealed with Parafilm (American Can Company, Greenwich, USA) and placed in a climate-controlled chamber with 14 h light (+22 °C)/10 h dark (+18 °C). For each Pd concentration and control, two identical beakers were prepared.

After 1 week, barley plants were harvested by cutting off leaves approximately 5 mm above the floating layer. Leaves and aliquots of nutrient solution were immediately put in Falcon™ tubes (BD Biosciences, San Jose, CA, USA) and deep-frozen at −20 °C. For measuring leaf length (of primary leaves), some whole plants were excavated from the floating layer. Leaf length was determined as the length between the seed grain and the tip of the leaf.

Table 1. Preparation of Pd particle suspensions for both series A and B. The reagents are combined for preparation of Pd solutions of determined Pd concentration.

resulting Pd content in nutrient solution (exposure concentration) [μmol·l^{-1}]	volume of Pd standard solution (μl) diluted in 500 ml ultrapure water	added volume of 0.03 m $NaBH_4$ solution (μl)
50	2500	1250
40	2000	1000
30	1500	750
20	1000	500
10	500	250
8	400	200
6	300	150
4	200	100
2	100	50
1	50	25

Investigation of Pd content in nutrient solutions

Palladium concentration analysis in nutrient solutions of series B was performed with a 4100 ZL atomic absorption spectrometer equipped with a AS-71 auto-sampler (Perkin-Elmer, Wellesley, USA). Standard instrumentation parameters were applied according to the recommendations of Perkin-Elmer for Pd analysis.

Palladium standard solutions used for calibration of GFAAS were freshly prepared by dilution of a stock solution (1000 mg Pd l^{-1}) with 0.25 M nitric acid. The determination limit derived from the calibration function according to the method described by Funk *et al.* (1992) was 3.51 µg Pd l^{-1}. For sampling, an aliquot of about 2 ml of the Pd particle-containing nutrient solution of series B was filtered through a 0.2-µm filter. The filtered solution was then investigated by GFAAS for its 'dissolved' Pd content. The measured 'dissolved' Pd concentration is the sum of actual dissolved and/or colloidal Pd (nanoparticles <0.2 µm). The dissolved Pd content of the nutrient solution of series A was not investigated since the particles are too small for microfiltration.

Investigation of Pd content in barley leaves

Sampling and sample pre-treatment

Barley leaf material was dried for at least 24 h at 120 °C to constant dry weight, and milled for 50 min at 80 rpm in a S1000 ball mill (Retsch, Haan, Germany). Complete decomposition of the milled barley sample was performed by pressurized microwave-assisted digestion at 1000 W for 25 min in a mixture of concentrated nitric acid and hydrogen peroxide. The microwave sample preparation system (MULTIWAVE; Perkin-Elmer, Überlingen, Germany/Anton Paar, Graz, Austria) was equipped with up to six tetrafluorometoxil (TFM) vessels. The TFM liners of the MULTIWAVE system were charged with 100 mg of milled sample material, 5 ml HNO_3 (65%, Merck) and 2 ml H_2O_2 (VSLI, 31%, Merck). This reagent mixture has an extremely low Pd blank value since it evolves a GFAAS signal intensity of 0.0009 ± 0.0001 A-s which is more than five-times lower than the detection limit of the method. Solid particles attached to the wall of the TFM liner were rinsed off with the digestion reagents. After thorough mixing of solid sample material and liquid reagents, digestion (see Table 2) was performed in a microwave oven at a maximum temperature of 220 °C and a maximum pressure of 30 bar. After cooling, the digestion solutions were transferred into 25-ml glass flasks and filled with ultrapure water. For each plant sample two independent digestions were performed and each digest was analysed four times by the procedure described below.

Table 2. Microwave oven program for complete decomposition of the sample material.

step No.	energy [W]	ramp time/ hold time [min]	fan
1	500–1000	5	1
2	1000	25	1
3	0	15	3

Table 3. Instrumentation parameters for Pd GFAAS measurement.

Instrument	Perkin Elmer 4100 ZL
Lamp type, power	HCL, 30 mA
Wavelength	247.6 nm
Slit width	0.7 nm (low)
Signal measurement	Peak area
Integration time	5 s
Baseline offset correction (BOC) time	2 s
Read delay	0 s
Background correction	Zeeman

Pd pre-concentration and measurement procedure

Palladium analysis was performed with a SIMAA 6000 GFAAS equipped with an AS-71 auto-sampler (Perkin-Elmer) with the instrumentation parameters listed in Table 3. Due to the very low Pd concentration in barley samples (ng·g^{-1}), samples were pre-concentrated before GFAAS measurement. The Pd detection device was coupled to a fully automated selective pre-concentration system for Pd, as described by Schuster & Schwarzer (1998). This flow injection analysis system (using an FIAS-400 equipped with an AS-90 auto-sampler; Perkin-Elmer) was slightly modified for this study, but the basic principles remain the same: Pd is selectively complexed in a special micro-column for enrichment and separation from the sample matrix, providing undisturbed ultra-trace Pd analysis. This was confirmed by successful recovery from four unexposed barley samples spiked with 0–32.5 ng Pd g^{-1} prior to digestion. The resulting recovery rate was 100 ± 9%. Standard Pd solutions for calibration were freshly prepared by stepwise dilution of stock solutions (1000 mg Pd l^{-1}) with 1.4 M nitric acid. The detection limit derived from the calibration function according to Funk *et al.* (1992) for Pd in barley leaves was 3.275 ng Pd g^{-1}.

RESULTS

Barley plants grown on nutrient solutions with smaller (series A) and larger (series B) Pd particles were comparatively assessed. After 1 week, exposure of barley plants to Pd particles of small diameter (series A) causes significant effects on leaf length growth. With increasing concentration of Pd in the nutrient solution, leaf length decreased significantly and variability of leaf lengths increased (Fig. 1). This decrease in leaf length is significant, 105–115 mm after exposure to 50 µm Pd, compared to leaf lengths of unexposed plants of 120–125 mm (n = 10). Furthermore, with increasing Pd concentration (>10 µm) in the nutrient solution, leaves become rigid and slightly convoluted.

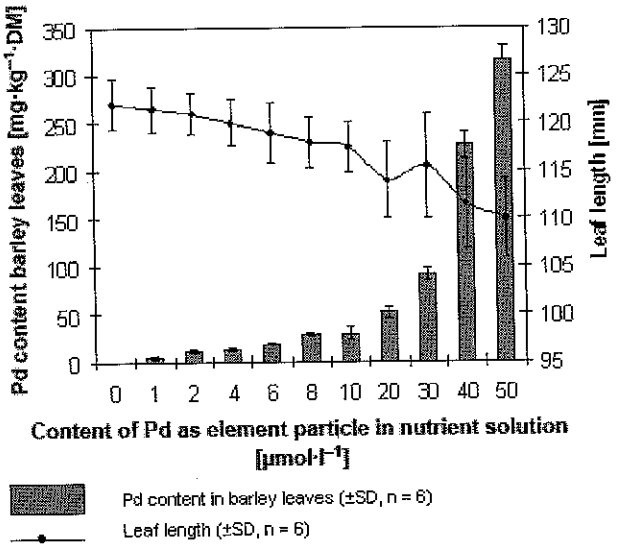

Fig. 1. Leaf lengths and Pd content in leaves 1 week after expsoure to Pd particles in series A (diameter 1–12 nm).

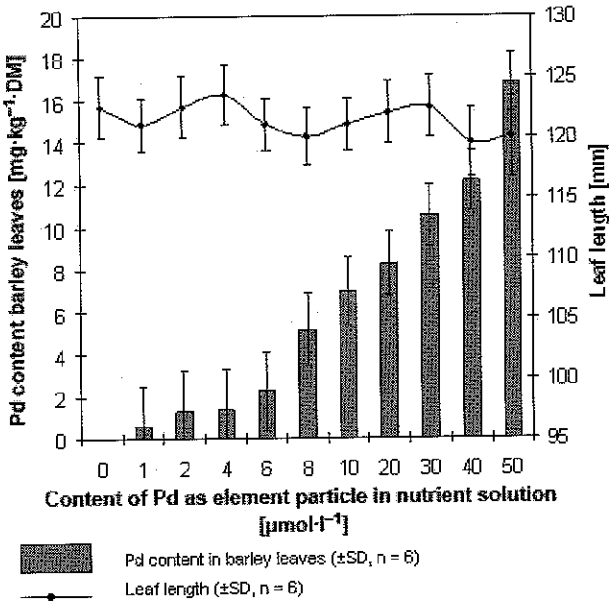

Fig. 2. Leaf lengths and Pd content in leaves 1 week after expsoure to Pd particles in series B (diameter 1 nm).

Exposure to Pd particles of larger diameter (series B) did not cause detrimental effects at low concentrations. Only exposure to high concentrations (≥40 µm) of Pd caused a slight decrease in leaf length and increased variability of leaf length when compared to controls (Fig. 2). No rigid or convoluted plants were observed in series B.

The Pd content of barley leaves in series A (small particles) was significantly higher than in series B (larger particles), resulting in a lower Pd concentration in barley leaves exposed to large Pd particles (Figs 1 and 2).

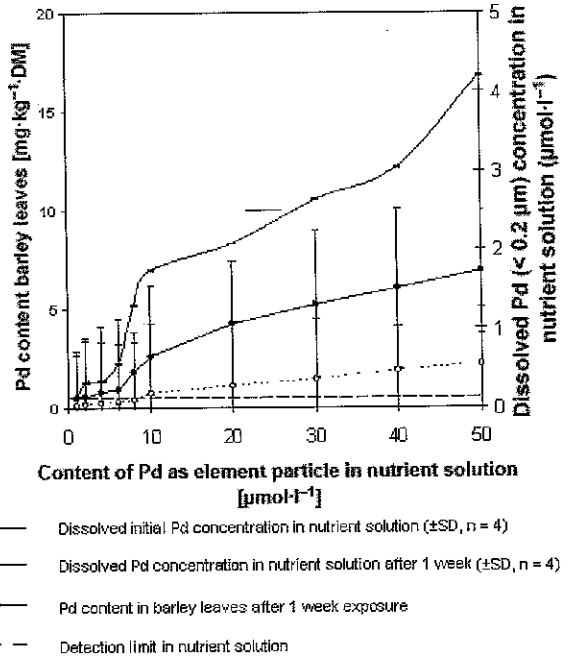

Fig. 3. Dissolved Pd (<0.2 µm) concentration in nutrient solution after 1 week and Pd content of barley.

The nutrient solutions for the series with the larger Pd particles (series B) were investigated for 'dissolved' Pd content, *i.e.* Pd in the solution after filtration through 0.2-µm filters. The 'dissolved' Pd content in the nutrient solution is the sum of actually dissolved and/or colloidal Pd smaller than 0.2 µm. The Pd content was measured before exposure and after 1-week growth of the plants. Figure 3 gives measured values for dissolved Pd in the nutrient solution of series B compared with Pd content in the leaves after a 1-week exposure. The amount of dissolved Pd increased in the 1-week experiment at all Pd concentrations. Furthermore, the amount of Pd taken up correlates well with 'dissolved' Pd, namely available Pd of the nutrient solution. Nutrient solution with 50 µm Pd particles in series B has a concentration of about 1.7 µm 'dissolved' Pd after 1 week, and barley leaves grown on this solution contain 17 mg Pd kg^{-1} dry matter (DM). A comparable Pd content in barley leaves grown on nutrient solution with Pd particles of series A was 6 µm. However, barley leaves grown on nutrient solution with a concentration of 2 µm Pd particles in series A only contained 12 mg Pd kg^{-1}DM.

DISCUSSION

The results clearly show that barley is able to take up Pd via the roots. During exposure to small particles with diameters of 1–12 nm, similar to those emitted from exhaust gas catalytic converters, Pd uptake is five- to 15-fold higher than in plants exposed to particles of about 1 µm (Figs 1 and 2). In principle, uptake of Pd can occur in two ways: (i) Pd nanoparticles might be taken up directly by plant roots and be transported to leaves in the

xylem, (ii) however, Pd is chemically relatively reactive, *i.e.* Pd in the nutrient solutions (*e.g.* EDTA, chloride content) is partially oxidized by dissolved oxygen (M. Maier, unpublished data) to soluble Pd (II). In addition, increased availability of Pd might be caused by exudates from barley root. Thus, Pd can also be taken up as dissolved Pd (II) via the plants roots. Control nutirent solution, without barley plants, had a comparable level of dissolved Pd particles after 1 week in the growth cabinet. The Pd dissolution process in suspensions with smaller particles (series A) should lead to more dissolved Pd in the nutrient solutions since the active specific Pd surface of smaller particles is higher. Thus, the different uptake rates of series A and B can be explained by both forms of Pd uptake.

Uptake of Pd in series B is only 17 $mg \cdot kg^{-1}$ DM in leaves at Pd concentration of 50 μm in the nutrient solution, which is comparable to exposure in series A at Pd concentration of 6 μm in the nutrient solution. Therefore, the small effects on leaf length growth and plant habit at 50 μm Pd in the nutrient solution in series B is reasonable, since at 6 μm Pd in the nutrient solution in series A, significant effects on leaf length growth and plant habit are also not observed. Hence, exposure to high ≥40 μm Pd particles in series B causes Pd content in leaves to correspond to exposure to relatively low 1–6 μm Pd particles of series A. It is likely that particles of series B are too big for direct uptake via roots and/or conduction in the xylem and that only a small amount of Pd is dissolved, giving a relatively small active specific Pd surface to these particles. For uptake of Pd in series B, the 1-μm diameter Pd particles and observed dissolved Pd content in nutrient solutions correlates well with the amount of Pd taken up by the leaves (Fig. 3). However, exposure to 50 μm Pd in series B gives a concentration of 1.7 μm 'dissolved' Pd in the nutrient solution after 1 week, and barley leaves grown on this solution contain about 17 mg Pd kg^{-1}. Compared to an exposure to 2 μm Pd in series A (leaf content after 1 week of only 12 mg Pd kg^{-1}), the content of Pd in leaves is higher. It seems that a concentration of 1.7 μm 'dissolved' Pd obtained from a 50 μm Pd particle suspension in series B after 1 week of exposure contains more available Pd than a 2 μm Pd particle suspension in series A. This might suggest that Pd is taken up preferentially in the ionic, 'dissolved' form.

Regarding the uptake of Pd at exposures to particles in series A, uptake increases disproportionately to increasing Pd concentration in the nutrient solution. This might be the effect of a plant protection system that is depleted at high Pd concentrations. Initially, significant effects are observed at a Pd concentration of 10 μm in the nutrient solution: leaf length growth decreases, becoming increasingly variable, and leaves are rigid and slightly convoluted. These are typical signs of plant abiotic stress. Strong effects of Pd exposure may correlate with high Pd transfer coefficients and availability, as described by Schäfer *et al.* (1998).

Compared to plant ionic mercury Hg (II) exposure, uptake of Pd particles is lower at equal concentrations in the nutrient solution, that were observed in similar conditions by Battke *et al.* (2003). However, Pd causes effects on plant development at even lower levels. The first significant effects appear at Pd concentration of 10 μm in the nutrient solution, giving a Pd content of about 30 $mg \cdot kg^{-1}$ DM in leaves. Mercury exposure causes the first visible effects at 30 μm Hg (II) in the nutrient solution, giving a Hg content of about 250 $mg \cdot kg^{-1}$ DM in leaves (Battke *et al.* 2003).

REFERENCES

Battke F., Schramel P., Ernst D. (2003) A novel method for in vitro culture of plants: cultivation of barley in a floating hydroponic system. *Plant Molecular Biology Reporter*, **21**, 405–409.

Cowgill U.M., Milazzo D.P. (1989) The culture and testing of two species of duckweed. In: Cowgill U.M., Williams L.R. (Eds), *Aquatic Toxicology and Hazard Assessment.* ASTM STP 1027, Philadelphia ASTM.

Funk W., Dammert V., Donnevert G. (1992) *Qualitätssicherung in der Analytischen Chemie.* VCH, Weinheim: p. 26.

Hoppstock K., Sures B. (2004) Platinum group metals. In: Merian E., Anke M., Ihnat M., Stoeppler M. (Eds), *Elements and Their Compounds in the Environment*, 2nd edition. Wiley-VCH, Weinheim: 1047–1068.

Leopold K., Maier M., Schuster M. (2008) Preparation and characterization of Pd/Al_2O_3 and Pd nanoparticles as model material for biological exposure studies. *Science of the Total Environment*, in press.

Mucalo M.R., Cooney R.P., Metson J.B. (1991) Platinum and palladium hydrosols: characterisation by X-ray photoelectron spectroscopy and transmission electron microscopy. *Colloids and Surface*, **60**, 175–197.

Schäfer J., Hannker D., Eckhardt J.-D., Stüben D. (1998) Uptake of traffic-related heavy metals and platinum group elements (PGE) by plants. *The Science of the Total Environment*, **215**, 59–67.

Schuster M., Schwarzer M. (1998) A new online column separation and preconcentration system for the selective determination of trace and ultratrace levels of palladium. *Atomic Spectroscopy*, **19**(4), 121.

Schuster M., Schwarzer M., Risse G. (2000) Determination of palladium in environmental samples. In: Zereini F., Alt F. (Eds), *Anthropogenic Platinum-Group Element Emissions.* Springer Verlag, Berlin, Heidelberg: 173–182.

Sures B., Singer C., Zimmermann S. (2006) Biological effects of palladium. In: Zereini F., Alt F. (Eds), *Palladium Emissions in the Environment: Analytical Methods, Environmental Assessment and Health Effects.* Springer, Heidelberg: 489–499.

Sutherland R.A. (2007) Platinum-group element concentrations in BCR-723: a quantitative review of published analyses. *Review, Analytica Chimica Acta*, **582**, 201–207.

Zimmermann S., Sures B. (2004) Significance of platinum group metals emitted from automobile exhaust gas converters for the biosphere. *Environmental Science and Pollution Research*, **11**, 194.