Study of the influence of platinum, palladium and rhodium on duckweed (*Lemna minor*)

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Abstract

OBJECTIVES: Road traffic pollutants and the residues of cytostatics that are widely used in anti-cancer therapy are a significant sources of platinum group elements (PGE; Pt, Pd and Rh) in environment. These metals can migrate into sewage and thus pollute surface waters. The purpose of our study was to evaluate the effect of $PtCl_4$ on the antioxidant and enzymatic activity of duckweed (*Lemna minor*), a bioindicator of the aquatic environment.

METHODS: The study was performed using a 7-day conventional test based on the OECD 221 (CSN EN ISO 20079) – *Lemna* sp. Growth Inhibition Test. We also conducted a microbiotest to analyse the effects of $PtCl_4$, $PdCl_2$ and $RhCl_3$ on the morphology and vegetative growth of colonies of this plant and compared their inhibitory effects during the microbiotest.

RESULTS: We observed inhibition of colony growth and clear morphological changes. Antioxidant and enzymatic activities increased with platinum doses increased. The $168hEC_{50}$ of $PtCl_4$ was 12.16 μM (95% confidence interval = 9.88–14.44) and the $168hEC_{50}$ of $PdCl_2$ was 50.39 (95% confidence interval = 23.83–76.96). The greatest inhibition of growth by $RhCl_3$ was observed at 25 μM.

CONCLUSIONS: The obtained results suggest that L. minor phytotoxicity tests should be widely used in the biomonitoring.

Abbreviations:

PGE	- platinum group elements
OECD 221	- *Lemna* sp. growth inhibition test
CSN EN ISO 20079	- Water quality - Determination of the toxic effect of water constituents and waste water on duckweed (*Lemna minor*) - Duckweed growth inhibition test
FRAP	- Ferric Reducing Antioxidant Power
ABTS	- chromophore 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulphonic acid)
AST	- aspartate aminotransferase
ALT	- alanine aminotransferase
96hEC50	- 96 hours effective concentration, which caused 50% of growth inhibition, calculated with the assessment of average specific growth rate
168hEC50	- 168 hours effective concentration, which caused 50% of growth inhibition, calculated with the assessment of average specific growth rate

ORIGINAL ARTICLE

INTRODUCTION

Vehicular traffic in urban centres contributes large amounts of pollutants to the environment (Guney *et al.* 2010). The introduction of exhaust catalysts led to a significant decline in emissions (24–35%), and environmental concern now focuses on platinum group elements (PGE; Pt, Pd and Rh), which are used as active components in automobile catalysts (König *et al.* 1992). They are also used in dental alloys and anti-cancer drug therapy (Dubiella-Jackowska *et al.* 2009; Ravindra *et al.* 2004).

About 40–90% of the heavy metal burden in European freshwater systems comes from agriculture, erosion or urban runoff from non-point sources (Scherer *et al.* 2003). Due to their allergenic and cytotoxic potential, their content and behaviour in different samples should be monitored. Duckweed (*Lemna minor*), a bioindicator of ecotoxicological changes in the aquatic environment, is widely used for phytoremediation because of its ability to accumulate heavy metals (Radic *et al.* 2010a; Tel-Or & Forni 2011), and has been previously investigated in this context by authors Supalkova *et al.* (2008) and Stejskal *et al.* (2007) in a studies with cisplatin. The influence of $PtCl_4$ on *Lemna minor*, *Pseudokirchneriella subcapitata and Folsomia candida* was further studied in Bednarova *et al.* (2012) and Nemcova *et al.* (2012).

Metal phytotoxicity results in oxidative stress, in which the normal balance between reactive oxygen species (ROS) and antioxidants in all aerobic cells is disturbed (Razinger *et al.* 2007). ROS formation is enhanced and the capacity of the antioxidative system is often increased. However, ROS production could exceed scavenging capacity and lead to metabolic disruption, loss of function, and tissue destruction (Juknys *et al.* 2012; Schmid *et al.* 2007). The induction of antioxidant enzymes has been suggested to play an important metabolic role under conditions of metal stress (Radic *et al.* 2010a; Razinger *et al.* 2007).

The aim of this study was to expand our knowledge of the phytotoxicity of PGE. We focused on $PtCl_4$ and the antioxidant and enzymatic parameters of duckweed. Simultaneously we performed a microbiotest to compare the impact of $PtCl_4$, $PdCl_2$ and $RhCl_3$ on vegetative growth of *L. minor* using polystyrene macroplates.

MATERIAL & METHODS

Experimental organisms

Plant material was obtained from a culture collection held at the ecotoxicological laboratory of the University of Veterinary and Pharmaceutical Sciences Brno and was adapted for the test. The adaptation and the experiments were performed under the conditions specified in the OECD 221 (CSN EN ISO 20079) – *Lemna* sp. Growth Inhibition Test.

Lemna sp. growth inhibition test

To assess the enzymatic and antioxidant parameters of duckweed we conducted a standard growth experiment. Duckweed was cultivated in SIS (Swedish standard medium) for 7 days under continuous warm fluorescent lightning (6,500–10,000 lx) at 24±2 °C, using a static method (without renewal of the test solution). Each replicate initially comprised 100 ml SIS (150 ml beaker) and 10 fronds of duckweed. A frond is an individual leaf-like structure. Beakers containing the test plants were covered with food foil, following the concentration of 0, 1, 10 or 100 M of $PtCl_4$. Plant samples were taken on the 2nd, 4th and 6th day to determine the effects of the heavy metal treatments on antioxidant and enzymatic activity.

Lemna sp. – Growth Inhibition Test: microbiotest

The effect of heavy metal treatment on the vegetative growth of duckweed was assessed using a microbiotest. Polystyrene macrotitration plates comprising six wells with a flat bottom and a cover, and a maximum volume of 15 ml, were used as cultivation vessels. Each well was 4 cm in diameter. The design of the macrotitration plates means that the plants had free access to oxygen. The test sample volume was 10 ml and five fronds of duckweed were added at the beginning of the test. We used five replicates for each test concentration and the controls, and three solutions of PGE (Sigma-Aldrich, USA): $PtCl_4$, $PdCl_2$, $RhCl_3$ at concentrations 0, 1, 5, 10, 25, 50 and 100 μM. The objective of the test was to quantify substance-related effects on the vegetative growth of duckweed over a period of 7 days. Assessment was based on the average specific growth rate expressed by frond number. To quantify substance-related effects, growth in the test solutions was compared with that of the controls, and the concentration resulting in 50% growth inhibition was designated the $168hEC_{50}$.

Spectrophotometric measurement

Plant samples for spectrophotometric measurement were weighed (approximately 0.1 g of fresh weight) and transferred to test tubes (2 ml) (Eppendorf, Germany), to which liquid nitrogen was added. The samples were frozen to disrupt the cells. Each frozen sample was transferred to a mortar and ground for 1 min with a pestle. Then, 2 ml of 0.2 M phosphate buffer (pH 7.0) was added and the sample was homogenised also with a pestle for 5 min. The homogenate was transferred to a new test tube and further homogenised by shaking on a Vortex–2 Genie (Scientific Industries, USA) at 4 °C for 15 min. The homogenate was centrifuged (15 000 rpm for 15 min at 4 °C using a Universal 32 R centrifuge (Hettich-Zentrifugen GmbH, Germany). The supernatant was filtered through a membrane filter (0.45 μm Nylon filter disk, Millipore, USA) prior to analysis (Supalkova *et al.* 2007a; Supalkova *et al.* 2007b).

Spectrophotometric measurement was carried out using an automated chemical analyser BS-200 (Mindray,

China). Reagents and samples were placed in a cooled sample holder (4 °C) and automatically pipetted directly into plastic cuvettes. Incubation proceeded at 37 °C and the mixture was consequently stirred. Washing steps using distilled water (18 mΩ) were performed in the midst of the pipetting. Contamination was reduced using this rinsing system, which involved rinsing the dosing needle as well as the stirrer with MilliQ water.

Protein determination – Pyrogallol red reaction

Reagent R1 (100 mM succinic acid, 6.94 mM sodium benzoate, 0.12 mM sodium molybdate, 2.09 mM sodium oxalate) in a volume of 200 µl was pipetted into a plastic cuvette along with 20 µl of sample for protein determination. Pyrogallol red with sodium molybdate is bound in the complex with proteins in the succinic buffer at pH 2.5. This complex results in a shift in the absorption peak from 460 nm (agent) to 600 nm (complex). The absorbance at wavelength λ=605 nm was measured after 10 minutes of incubation at 37 °C. The absorbance values indicated the absorption by the reagent itself and absorbance after 10 minutes of incubation with the sample.

Determination of antioxidant activity using the FRAP method

The FRAP method (Ferric Reducing Antioxidant Power) is based on the reduction of complexes of 2,4,6-tripyridyl-*s*-triazine (TPTZ) with ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), which are almost colourless, and eventually slightly brown in colour. This chemical forms blue ferrous complexes after its reduction. The method has its limitations, especially in measurements conducted below non-physiological pH values (3.6). In addition, this method is not able to detect slowly reactive polyphenolic compounds and thiols (Jerkovic & Marijanovic 2010; Ou *et al.* 2002).

A 1:10 mM solution of TPTZ (m=0.07802 g/25 ml), in 40 mM of hydrochloric acid, a 2:20 mM solution of ferric chloride hexahydrate (m=0.13513 g/25 ml) in ACS water, and a 3:20 mM acetate buffer, pH 3.6 (weight of sodium acetate trihydrate is 0.27216 g in 100 ml ACS water, adjusted to the desired pH using HCl) were mixed in a 1:1:10 ratio. This reagent can be used for 7 days if stored at 4 °C in the dark. Two hundred and forty-five microlitres of reagent were pipetted into a plastic cuvette with the subsequent addition of 5 µl of sample (gallic acid, Trolox®). Absorbance was measured for 1,520 seconds at λ=578 nm (primary absorbance) and λ=630 nm (secondary absorbance). The differential absorbance (primary absorbance – secondary absorbance) is used to calculate the antioxidant activity (Sochor *et al.* 2010).

Determination of antioxidant activity using the ABTS test

The ABTS radical method is one of the most widely used assays for determining the concentration of free radicals. It is based on the neutralization of a radical-cation arising from the one-electron oxidation of the synthetic chromophore 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•): $ABTS^{\bullet} - e^{-} \; ABTS^{\bullet+}$. This reaction is monitored spectrophotometrically by the change in the absorption spectrum. Results obtained using this method were converted to Trolox® concentration and are described as "Trolox® Equivalent Antioxidant Capacity" (TEAC). For chemically pure compounds, TEAC is defined as the micromolar concentration of Trolox® equivalents demonstrating the same antioxidant activity as a tested compound (at 1 mM concentration) (Re *et al.* 1999).

Seven mM ABTS• (m=0.03841 g/10 ml) and 4.95 mM potassium peroxodisulphate (m=0.01338 g/10 ml) were mixed and dissolved in ACS water. The solution was then diluted with ACS water in a 1:9 v/v ratio (10 ml was quantitatively transferred into a 100 ml calibrated flask and diluted). The solution was incubated for 12 hours in the dark. A 245 µl volume of reagent was pipetted into a plastic cuvette with the subsequent addition of 5 µl of sample (gallic acid, Trolox®). Absorbance was measured at λ=670 nm after 1,520 seconds. The antioxidant activity was calculated from values measured before the decrease in the absorbance (224th second of measurement – A_{224}) and the last measurement (1,520th second of measurement – A_{1520}), according to the following formula: $A = A_{1520} - A_{224}$.

Determination of AST

Determination of aspartate aminotransferase (AST) involves a two-step reaction. In the first reaction catalysed by AST, aspartate is converted to oxalacetate. In the following reaction malate dehydrogenase (MDH) reduces oxalacetate to malate, accompanied by the oxidation of NADH to NAD^+. AST activity is determined kinetically and is based on the rate that the NADH concentration decreases during the reaction, as measured by the decrease in absorbance at 340 nm. The catalytic concentration of ASAT is proportional to the decrease in absorbance. One hundred and fifty microlitres of solution R1 (80 mM Tris buffer pH 7.8, 240 mM L-aspartate, 1200 U/l MDH) were pipetted into a plastic cuvette with the subsequent addition of a 15-µl sample. This solution was incubated for 270 seconds. Subsequently, 30 µl of solution R2 (15 mM 2-oxoglutarate, 0.18 mM NADH) was added and the solution was incubated for 90 seconds. Measurement of absorbance then took place, lasting for 180 seconds. The mean decrease in absorbance per minute was calculated.

Determination of ALT

Determination of alanine aminotransferase (ALT) involves a two-step reaction. In the first reaction catalysed by ALT, alanine is converted to pyruvate. In the following reaction lactate dehydrogenase (LDH) reduces pyruvate to lactate, accompanied by the oxidation of NADH to NAD^+. ALT activity is determined kinetically

and is based on the rate of decrease in NADH concentration during the reaction, as measured by the decrease in absorbance at 340 nm. The catalytic concentration of ALT is proportional to the decrease in absorbance.

One hundred and fifty microlitres of solution R1 (100 mM Tris buffer pH 7.5, 500 mM L-alanine, 1 200 U/l LDH) were pipetted into a plastic cuvette with the subsequent addition of a 15-µl sample. This solution was incubated for 270 seconds. Subsequently, 30 µl of solution R2 (15 mM 2-oxoglutarate, 0.18 mM NADH) was added and the solution was incubated for 90 seconds. Measurement of absorbance then took place, lasting for 180 seconds. The mean decrease in absorbance per minute was calculated (Kleckerova *et al.* 2011).

Statistics

The experimental design of the test with *Lemna minor,* included quintuplicates of tested concentrations. EC_{50} values were calculated using the TOXICITA 3.1 software (VÚV Ostrava, Czech Republic) by means of regression analysis of data with 95% intervals of confidence which were based on squared deviations of experimental values from the selected approximation function using the Student´s coefficients.

RESULTS

The influence of $PtCl_4$ on duckweed manifested as an alteration in plant antioxidant activity. Figure 1A shows a measurable increase from the 2nd day onwards using the FRAP method. The increase in antioxidant activity corresponds well with the increasing concentration of $PtCl_4$. The highest increase in antioxidant activity was observed at the end of the experiment. On the first sampling day (2nd day) 100 µM $PtCl_4$ increased activity by 209%. On the 4th day and 6th day a 355% and 175% increase in activity compared to the control were observed respectively. Plant samples also showed a gradual increase in antioxidant activity measured using the ABTS method (Figure 1B). Thus, cultivation in the 100 µM $PtCl_4$ led to 184% increase in activity after 2 days, 279% increase after 4 days and 109% increase after 6 days, compared to the control. This indicates enhanced antioxidant activity of duckweed plants at the end of the test and under the highest platinum dose. The results of the FRAP and ABTS methods were highly correlated (Pearson´s coefficient 0.962166). This is consistent with the results of Sulc *et al.* (Sulc *et al.* 2007).

Figure 2 shows the impact of platinum on ALT and AST activity. In 100 µM of $PtCl_4$ there was an increase in activity of about 139% after 2 days of treatment; on the 4th day the situation was similar, with a 138% increase. After 6 days of treatment platinum caused a 386% increase in ALT activity (Figure 2A). At 100 µM of $PtCl_4$, there was an approximately 1030% increase in activity in comparison to the control after 2 days of treatment. After 4 days, there was a 929% increase and on the last sampling day (6th day) the increase in activity reached 1363% (Figure 2B).

The microbiotest showed that increasing concentrations of PGE negatively affected the growth of *L. minor*. In the case of $PtCl_4$ and $PdCl_2$, plant growth was considerably inhibited by increasing concentrations of metal (Figure 3A,B). The 168hEC_{50} (50% effective concentration) of $PtCl_4$ was estimated at 12.16 µM (95% confidence interval = 9.88–14.44). The 168hEC_{50} of $PdCl_2$ was 50.39 (95% confidence interval = 23.83–76.96).

The influence of rhodium ($RhCl_3$) on duckweed was difficult to estimate. The lowest concentration (5 µM) caused slight growth stimulation (2%) compared to the control. The highest inhibition effect was found at 25 µM (Figure 3C). Due to the poor solubility of $RhCl_3$ growth inhibition declined in the 50 µM and 100 µM treatment, despite pH adjustment. We also observed insoluble precipitates at the bottom of the test vessels.

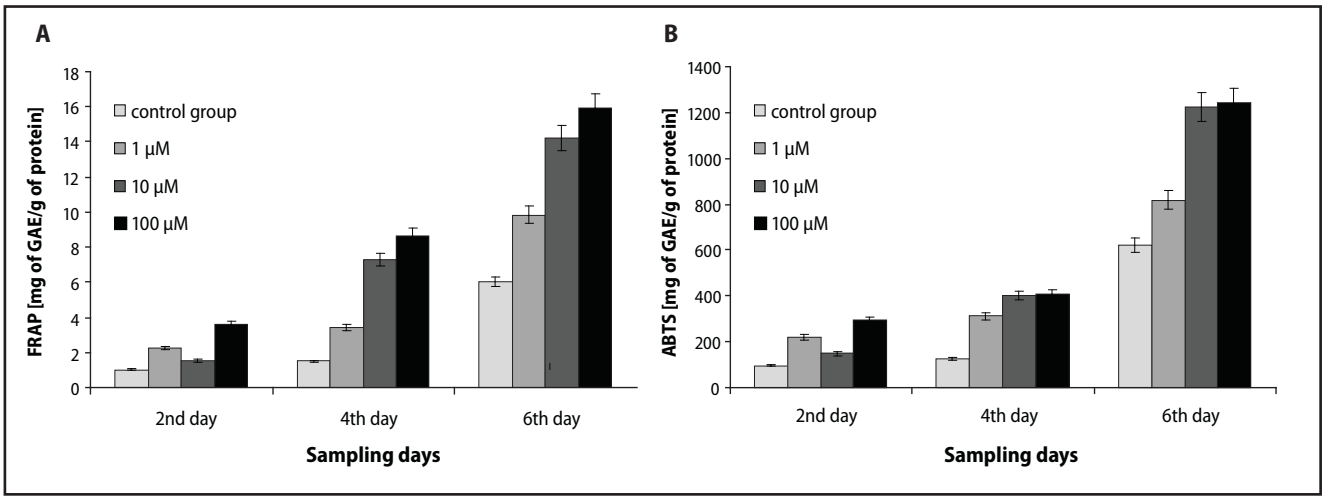

Fig. 1. A: The influence of $PtCl_4$ on antioxidant activity (FRAP method), **B:** The influence of $PtCl_4$ on the antioxidant activity (ABTS method) of duckweed (*Lemna minor*). Values in (mg of GAE/1)/g of protein.

DISCUSSION

Vascular plants can precisely indicate the presence and intensity of different pollutants (heavy metals, chemical substances etc.) in air or soil in natural ecosystems as well as in urban environments (Sijacic-Nikolic *et al.* 2011). Duckweed has many characteristics that make it an appropriate organism for laboratory toxicity testing of various compounds and for the assessment of pollution in the aquatic environment (Supalkova *et al.* 2008), including its small size, high multiplication rate and vegetative propagation by which a population of genetically homogenous plants is produced (Radic *et al.* 2010a; Supalkova *et al.* 2008; Korner *et al.* 2003).

There is little information available regarding the measurement of antioxidant activity in plant samples treated with PGE, although more is known about the phytotoxicity of heavy metals and induction of ROS as a measure of oxidative stress (Halliwell 2006; Verma & Dubey 2003; Jiao *et al.* 2012). Antioxidant activity was monitored in fronds of control and experimental duckweed plants exposed to platinum. We chose two methods, which differ in their sensitivity on the spectrum of molecules with possible antioxidant activity. The obtained results were converted to equivalent of gallic acid (GAE) and protein content, which was determined using the Pyrogallol Red reaction.

Sulc *et al.* (2007) observed the antioxidant activity of different potato varieties, and compared red-, purple- and yellow-fleshed potato varieties using the FRAP, ABTS and DPPH methods. The highest linear correlation was found between FRAP and ABTS arrays (R^2=0.94), and these approaches thus appear to be suitable for the determination of antioxidant activity. Kleckerova *et al.* (2011) determined the antioxidant activity in roots and shoots of control and experimental

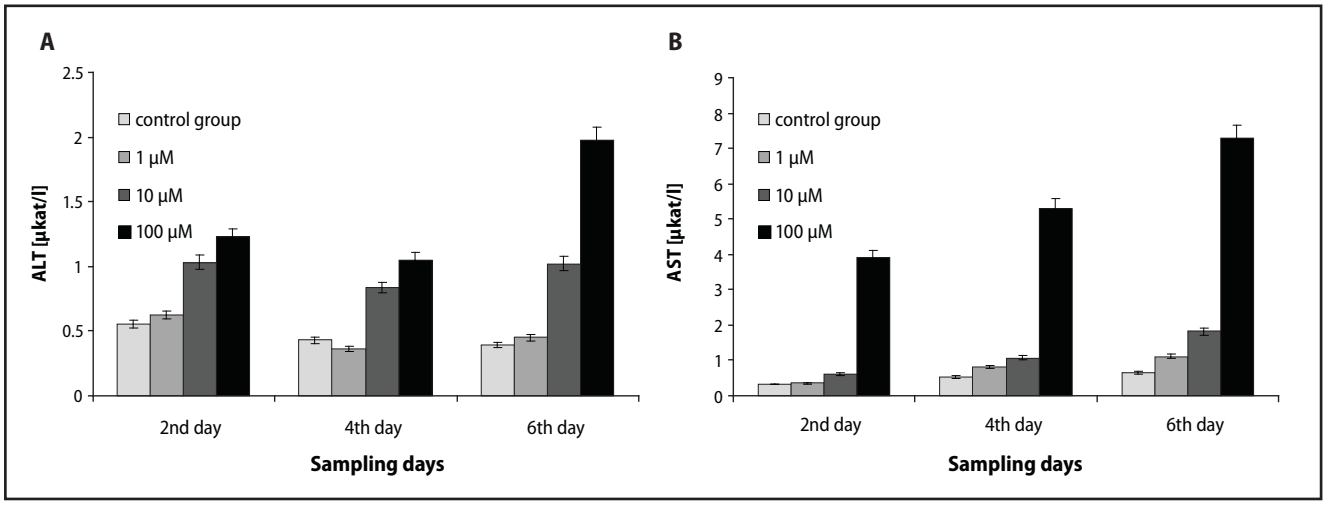

Fig. 2. A: The influence of $PtCl_4$ on the enzymatic activity (ALT) of duckweed, B: The influence of $PtCl_4$ on the enzymatic activity (AST method) of duckweed (*Lemna minor*). Values in (µkat/l.g/g of protein).

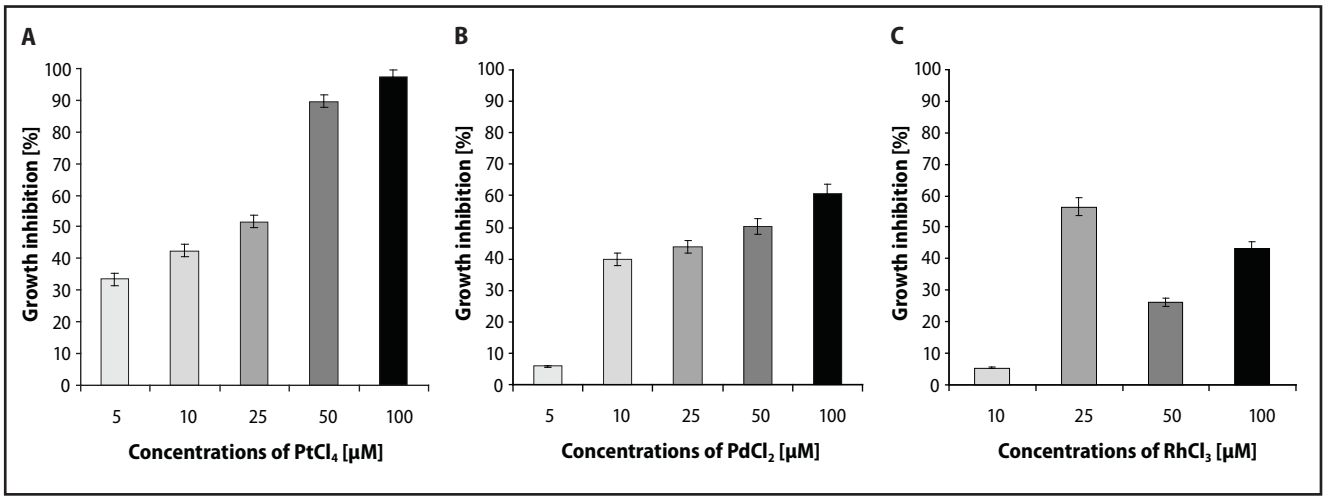

Fig. 3. A: The growth inhibition effect of $PtCl_4$; B: The growth inhibition effect of $PdCl_2$; C: The growth inhibition effect of $RhCl_3$ on duckweed *(Lemna minor)*.

maize plants exposed to cadmium(II) and/or zinc(II) ions. They also used FRAP, ABTS and DPPH. In their experiment the increased concentrations of zinc(II) together with cadmium(II) ions led to increased antioxidant activity using both FRAP and ABTS. The highest antioxidant activity was detected in experimental plants loaded with 100 μM Zn^{2+} and 100 μM Cd^{2+}.

Schmid *et al.* (2007) described the effect of platinum ($Pt(NO_3)_2$ and $PtCl_4$), palladium ($PdSO_4$) and rhodium ($RhCl_3$) on human epithelial lung cells and compared this with the effect of cadmium ($CdCl_2$) and chromium (CrO_3). They found that platinum and palladium had a significant effect but that rhodium had little or no effect. The ability to induce ROS was strongest for $PtCl_4$. It is possible that the most effective Pt(IV) may further induce the expression of the suggested gene programmes leading to antioxidative responses and pro-inflammatory mediator production, via the hierarchical oxidative stress model published by Nel *et al.* (2006). The behaviour of PGE in plants is likely to be at least partially similar.

The significance of some commonly analysed enzymes as markers of stress reactions in plants remains unknown. Several publications have demonstrated that enzymes such as aminotransferases can participate in plant stress reactions (Krizkova *et al.* 2008; Petrek *et al.* 2005; Krystofova *et al.* 2009). We measured transaminases (ALT and AST) as a marker of metabolic activity under the experimental conditions. Firstly we measured total protein content (reaction with red pyrogallol) and then we recalculated our results to obtain more exact information about the amount of transaminases in a certain weight of duckweed sample. The highest enzymatic activity of ALT and AST was detected on the last day of the test in plants treated with 100 μM of $PtCl_4$. This indicates the increased enzymatic effort of duckweed plants at the end of the test treated with the highest platinum concentrations.

Amino transferases, also called transaminases, constitute a group of enzymes that catalyse the interconversion of amino acids in α-ketoacids by transferring amino groups. Alanine and aspartate are precursors for gluconeogenesis, and the initial reaction is catalysed by alanine ALT, and AST. They serve as a strategic link between carbohydrate and protein metabolism in plants and participate in the transformation of nitrogen compounds (Rajamanickam & Muthuswamy 2008). They are also important for crucial biochemical pathways such as the synthesis of amino acids from oxo-acids in the citrate cycle. They also play a major role in the synthesis of secondary metabolites as well as chlorophyll (Krystofova *et al.* 2009). Beyond this, the aminotransferases are good indicators of tissue lesions. They are also known to be altered during various physiological and pathological conditions, making them possible biomarkers (Rajamanickam & Muthuswamy 2008).

Our microbiotest compared the effect of three platinum elements ($PtCl_4$, $PdCl_2$ and $RhCl_3$) on the vegetative growth of colonies of duckweed. Firstly, we monitored changes in plant morphology. During the experiment, we observed a slowdown in growth and a change in the appearance of the fronds. Plants began to turn yellow (chlorosis), and at the end of the experiment, we observed leaves with white or colourless areas (necrosis). In addition, the individual size of colonies declined with increasing PGE concentration.

The use of low volumes (10 ml) in microbiotests is a good tool to include in a battery of tests for phytotoxicity screening of a wide range of chemicals and environmental samples, with the advantage of allowing large numbers of samples to be tested, and generating low volumes of waste (Paixao *et al.* 2008). In microbiotests are commonly fulfilled the validity requirements, and so can be used to assess the toxic effect of chemicals or other hazardous substances and are a suitable alternative to commonly used ecotoxicological biotests .

Stunted growth, chlorosis and necrosis are some of the visible symptoms indicating severe metal phytotoxicity (Rahman *et al.* 2011; Bi *et al.* 2009). Among developmental parameters, the most commonly assessed in ecotoxicological test systems are growth parameters. It has frequently been observed that under toxic stress small buds of duckweed may protrude as individual fronds (Radic *et al.* 2010b; Naumann *et al.* 2007). Radic *et al.* (2010b) observed that frond number was slightly more sensitive parameter than final biomass. Other authors like Mackenzie *et al.* (2003) found that, beside frond area, growth rate based on frond number is the most sensitive endpoint for detecting chronic toxicity (7 days).

The substantial inhibition of the average specific growth rate observed in our experiments suggests bioaccumulation of the PGE present in the cultivation medium. It has been shown that the accumulation of heavy metals disturbs plant water status, which eventually results in osmotic stress and growth reduction (Perfus-Barbeoch *et al.* 2002). A study using cisplatin conducted by Supalkova *et al.* (2008) found that the frond number in control samples at the end of the experiment was much higher than that in treated samples, according to the dose of cisplatin. In addition the fronds differed morphologically and showed chlorosis and necrosis. As the concentration of cisplatin increased, chlorosis also markedly increased. The $96hEC_{50}$, calculated according to growth inhibition with comparison of growth rates, was 6.93 μM (95% confidence interval = 6.51–7.34). The experiment showed that cisplatin (Supalkova *et al.* 2008) and in our case $PtCl_4$ has a toxic effect on *L. minor* and substantially influenced its growth. Our previous study on the representatives of the aquatic producers (*Lemna minor* and *Pseudokirchnerielle subcapitata)* showed comparable results. The value of $168hEC_{50}$, counted on a basis of average specific growth rate was 19.55 μM (95% confidence interval = 14.99–24.11) (Bednarova *et al.* 2012).

For the toxicity test specified in OECD 221 (CSN EN ISO 20079), cultivation of duckweed should be con-

ducted in SIS medium with a balanced pH of 6.5. The plants should be supplied with nutrients throughout cultivation. The use of distilled water only is insufficient and duckweed growth is negatively affected. The addition of PGE at the start of the experiment, especially $RhCl_3$, highly acidified the water. The method requires a pH in the range 5–9. In preparation of stock solutions of $PdCl_2$ and $RhCl_3$ it was already difficult to dissolve.

Another PGE ($RhCl_3$) behaved differently according to our results. This could be due to the migration of PGE in aqueous ecosystems. Migration depends on the chemical forms of heavy metals as well as on the conditions of the environment. The crucial parameter in terms of the mobility of these metal compounds is their solubility. Different pH can be the basis for the greatest mobility of rhodium observed in a strong acidic environment (pH=1) (Zereini *et al.* 1997; Dubiella-Jackowska *et al.* 2009). PGEs can also form complex bonds with organic matter present in water, for example $[RhCl_{6-x}(H_2O)_x]^{x-3}$, where $0 \leq x \leq 6$. Various studies conducted under laboratory conditions have shown that in aqueous conditions PGEs undergo partitioning between the aqueous phase (fraction of diameter less than 0.45 µm) and solid phase (fraction of diameter greater than 0.45 µm, adsorbed onto solid particles of the sediment). With increasing salinity and chloride ion concentration, the rate of palladium and rhodium adsorption onto the surface of sediment samples also increased, unlike that of platinum, whose content in the solid phase decreased (Turner 2007; Dubiella-Jackowska *et al.* 2009).

The phytotoxic influence of platinum ($PtCl_4$) was demonstrated using a standard growth inhibition test and also by modification of this method – the microbiotest. Increasing amounts of platinum in the aquatic environment lead to the activation of detoxification mechanisms in plants. There is a clear relationship between antioxidant and enzymatic activity and the concentrations of metals as well as the duration of the experiment. The expected trend of toxicity found in water hyacinth (*Eichhornia crassipes*) Rh(III) <<Pt(IV) <Pd(II) was not verified (Farago & Parsons 1994).

Using the microbiotest we established the concentrations of $PtCl_4$ and $PdCl_2$ that caused 50% growth inhibition of duckweed compared to the control after 168±2 hours. In the case of $RhCl_3$ we were unable to calculate the $168hEC_{50}$, but the highest growth inhibition was observed at 25 µM. The results obtained suggest that phytotoxicity tests involving *L. minor* should be used in the biomonitoring of the different water samples because of their simplicity, sensitivity and cost-effectiveness.

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