Comparison of the effect of platinum on producers in aquatic environment

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Submitted: 2012-09-01 Accepted: 2012-11-15 Published online: 2012-12-26

Key words: **platinum; *Pseudokichneriella subcapitata*; *Lemna minor*; ecotoxicology biotests**

Neuroendocrinol Lett 2012; **33**(Suppl.3):107–112 **PMID:** 23353852 NEL330912A15

Abstract

OBJECTIVES: An enhanced worldwide application of platinum group elements (PGE), in particular platinum, has been observed during recent decades. An increased concentration of PGE was determined in collected samples of great amount of aqueous ecosystems.The aim was to compare phytotoxic effect of platinum ($PtCl_4$) by performing two different bioassays on green algae *Pseudokirchneriella subcapitata* and macrophyte duckweed, *Lemna minor*.

MEDTHODS: The algal experiment (*Pseudokichneriella subcapitata*) followed OECD 201, the concentration row for $PtCl_4$ was: 0.05; 0.01; 0.25; 0.5; 1 µM. The duckweed (*Lemna minor*) experiment was conducted according to OECD 221, employed $PtCl_4$ concentrations were: 5; 10; 25; 50; 100 µM. Plants were cultivated as a microbiotest, using micro-volumes.

RESULTS: The results of the algal test showed significant growth inhibition of the final biomass. The values of $72hEC_{5(\mu)}$, $72hEC_{10(\mu)}$, $72hEC_{20(\mu)}$ counted on a basis of average specific growth rate (µ) were 0.31 µM, 0.58 µM and 1.12 µM of $PtCl_4$, respectively. The values, obtained on a basis of the area under the growth curves (A), were 0.04 µM ($72hEC_{5(A)}$), 0.24 µM ($72hEC_{10(A)}$) and 0.64 µM ($72hEC_{20(A)}$). The experiment with duckweed showed 50% of growth inhibition and the values of $168hEC_{50(\mu)}$ were 19.55 µM and $168hEC_{50(A)}$ 13.63 µM of $PtCl_4$.

CONCLUSION: The fronds of duckweed showed strong adverse effect of platinum influence (chlorosis, necrosis). The algal test and the estimation of $72hEC_{5(A)}$ appears to be the most sensitive.

ORIGINAL ARTICLE

Abbreviations

PGE - platinum group elements
OECD 201 - Fresh water alga and cyanobateria, Growth inhibition test
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
CSN EN ISO 8692 - Water quality - Fresh water algal growth inhibition test with unicellular green algae
$72hEC_{5(\mu)}$ - 72 hours effective concentration, which caused 5% of growth inhibition, calculated with the assessment of average specific growth rate
$72hEC_{10(\mu)}$ - 72 hours effective concentration, which caused 10% of growth inhibition, calculated with the assessment of average specific growth rate
$72hEC_{20(\mu)}$ - 72 hours effective concentration, which caused 20% of growth inhibition, calculated with the assessment of average specific growth rate
$72hEC_{5(A)}$ - 72 hours effective concentration, which caused 5% of growth inhibition, calculated with the assessment of area under the growth curve
$72hEC_{10(A)}$ - 72 hours effective concentration, which caused 10% of growth inhibition, calculated with the assessment of area under the growth curve
$72hEC_{20(A)}$ - 72 hours effective concentration, which caused 20% of growth inhibition, calculated with the assessment of area under the growth curve
$168hEC_{50(\mu)}$ - 168 hours effective concentration, which caused 50% of growth inhibition, calculated with the assessment average specific growth rate
$168hEC_{50(A)}$ - 168 hours effective concentration, which caused 50% of growth inhibition, calculated with the assessment of area under the growth curve
$96hEC_{50}$ - 96 hours effective concentration, which caused 50% of growth inhibition, calculated with the assessment of average specific growth rate

INTRODUCTION

Scientific discussions concerning the global exposition of the environment to toxic elements (e.g. cadmium, lead, mercury)from various sources have been studied for a long period of time. Since the worldwide introduction of the catalytic converters of the exhaust gases from motor cars, platinum was included into the main interest of the investigations. Anthropogenic pollutions of platinum group elements (PGE), namely the employment of platinum containing products and the immission by exhaust gases from motor cars equipped with catalytic converters, are the major sources (Alt *et al.* 1997; Djingova *et al.* 2003; Ravindra *et al.* 2004). The first observations about the Pt concentration in air, was reported to be lower than 0.05 pg m^{-3}near a freeway in California. However, other studies e.g. in Germany have shown that the total Pt concentration in air along a highway ranged from 0.02 to 5.1 pg m^{-3} (0.6 to 130 ng g^{-1}) (Ravindra *et al.* 2004).

The additional source of environmental contamination comes from waste waters from hospitals, where the treatment of patients with cancer take place and platinum compound (cisplatin) is very frequently used as an antineoplastic drug (Supalkova *et al.* 2008; Nemoto *et al.* 2012).

Trace concentrations of the PGE, especially platinum, play an important role in environmental analysis and assessment. The strong increase of PGE emissions and the high allergenic and cytotoxic potential of platinum compounds, even in very low doses are in the main concern (Hees *et al.* 1998).

These metals reaching the environment as a consequence of road traffic undergo deposition in particulate matter, road dust, soil, water, sediments and biota. The most concerning transport pathways are runoff waters and rain waters, analysis of water that drains from roads and other sites contaminated with PGE, it is possible to obtain very interesting information on the bioavailability of these precious metals (Artelt *et al.* 1998; Ravindra *et al.* 2004; Dubiella-Jackowska *et al.* 2009).

The sustainability of the environment is dependent on the effective prognosis and prevention of the impact of pollutants and consequently on the preservation of the biological chain. Planktonic algae, as the dominant primary producers, represent an essential level in the aquatic environment. They are the basic element in aquatic food chains and are a crucial functional group of organisms which has the fundamental importance in true structure and function of the whole ecosystem (Burkiewicz *et al.* 2005). *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum* and *Raphidicelis subcapitata*) was stated as a one of the most sensitive algae species. A great amount of environmental samples like leachates, sewage samples, surface waters and soil elutriates as well as chemicals and mixtures are ecotoxicologically characterized using algal growth-inhibition assays (Rojickova-Padrtova & Marsalek 1999; Paixao *et al.* 2008). Vascular plants of *Lemnaceae* family play an important role as a bioindicators of ecotoxicological changes as well. A number of investigations based on the ability to accumulate metals have been conducted, and this property makes duckweed an appropriate organism for water quality monitoring (Jenner & Jansenmommen 1993; Devare & Bahadir 1994; Wang & Freemark 1995; Fenske *et al.* 2006; Oporto *et al.* 2006; Naumann & Appenroth 2007; Supalkova *et al.* 2008). Disruptions in production level would probably result in adverse effects at higher trophic levels (Geis *et al.* 2000; Paixao *et al.* 2008).

The aim of our study was to compare phytotoxic effect of platinum ($PtCl_4$) by performing two bioassays on two different representatives of aquatic producers, green algae *Pseudokirchneriella subcapitata* and macrophyte duckweed, *Lemna minor*.

MATERIAL & METHODS

Experimental organisms

A culture of green algae, *Pseudokirchneriella subcapitata* (Korshikov) Hindak, was obtained from the collection of autotrophic organisms of Botanical Institute of the

Academy of Sciencein Trebon, Czech Republic. Plant material, *Lemna minor*, was obtained from a culture collection of the Ecotoxicological laboratory of the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic.

Experimental design

Algal growth inhibition test

Experimental design was based on guideline OECD 201 – Fresh water alga and cyanobateria, growth inhibition test (CSN EN ISO 8692). The purpose of our test was to determine the effect of $PtCl_4$ on growth of fresh water alga (*Pseudokirchneriella subcapitata*). Exponentially growing algae were exposed to various concentrations of $PtCl_4$ (Sigma-Aldrich, USA): 0.05; 0.1; 0.25; 0.5 and 1 μM over a period of 72 ± 2 hours. The concentration span was chosen with the help of the study by Supalkova *et al.* (2008) and for the algae, because of the expected metal sensitivity, we used multiple of 10^{-3}. The test was performed in three replicates at each test concentration and controls. The response of this test substance was evaluated as a function of exposure concentration by comparison with the average growth of replicate, unexposed control cultures. For full expression of the system response to toxic effect of platinum, the cultures were allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for sufficient period of time (3 days) to measure reduction of the specific growth rate. Growth and growth inhibition were quantified from measurements of algal biomass density as a function of time.

Test medium was prepared as CSN EN ISO 8692 describes. The inoculum culture in the test medium was prepared 3 days before start of the experiment and was incubated under the same conditions as the test cultures. The initial cell concentration for whole platinum concentration row and the controls was 25×10^3cells mL^{-1}.

For the test to be valid, we followed the criteria mentioned in guideline. As the test vessels were used sterile 250 ml conical glass Erlenmeyer flasks and the sample volume was 100 ml, as a cap we used air-permeable foil stoppers. Whole 72 hours were maintained recommended conditions 21–24 °C ± 2 °C under continuous lightning (6 000–10 000 lux) in a cultivation chamber. Apparatus employed to determine algal biomass was microscope with Bürker counting chamber.

The test endpoint was inhibition of growth (average growth rate – μ), expressed as logarithmic algal biomass increase during the exposure period. From the average growth rates recorded in series of test solutions and the concentration which brought 5%, 10% and 20% was determined and expressed as the $72hEC_{5(\mu)}$, $72hEC_{10(\mu)}$ and $72hEC_{20(\mu)}$. As an additional endpoint, was determined the log-biomass integral (area under the growth curve – A) due to the irregular growth in inhibited culture and calculated values of $72hEC_{5(A)}$, $72hEC_{10(A)}$ and $72hEC_{20(A)}$ (OECD 201, 2002).

***Lemna* sp. growth inhibition test**

The experiments were performed under the conditions specified in OECD 221 – *Lemna* sp. growth inhibition test (CSN EN ISO 20079) in our modification, using microvolumes (Soukupova & Beklova 2010). Plants of the genus *Lemna* – *Lemna minor*, were allowed to grow as monocultures in different concentration of $PtCl_4$ over a period of seven days. Employed concentrations were: 5; 10; 25; 50; 100 μM diluted in recommended SIS medium. As test vessels we used polystyren macroplates (macrotitration plates) of maximum sample volume 15 ml, the tested volume was 10 ml and the initial number of fronds was 5. Each plate is equipped with plastic cover with special design, which provides free access of oxygen to the plants. During the whole duration of the test we maintained 24±2 °C and the continuous lightning (6 000–10 000 lux) in a cultivation chamber. The test design included static test within five replicates, and also the validity criteriain our microbiotest were fulfilled.

All visible fronds were counted each 24 hours and observed changes in plant development (e.g. frond size, appearance, chlorosis, necrosis, gibbosity). Significant features of the test medium (e.g. presence of undissolved material, growth of algae in the vessel) was also noted. The objective of the test was to quantify substance-related effects on vegetative growth over this period based on assessment of average specific growth rate (μ) and area under the growth curve (A). The average specific growth rate was calculated on the basis of changes in frond number determined during the exposure period in controls an each treatment group. The area under the growth curve was also assessed on the basis of frond number determined during the test in controls and each treatment group but integrated the logarithmic value of frond number over the exposure period. To observe of the effect caused by platinum, growth in the test solutions is compared with that the controls and the concentration which brought specified 50% inhibition of growth was determined and expressed as the $168hEC_{50(\mu)}$ and $168hEC_{50(A)}$(OECD 221; 2002).

Statistics

The experiment with green algae *Pseudokichneriella subcapitata*, was conducted as a pilot study to reveal the phytotoxicity of $PtCl_4$. The EC_{50} has not been stated. 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

In the first experiment we focused on the impact of platinum ($PtCl_4$) *P. subcapitata*. We monitored the algal biomass density under 0; 0.05; 0.1; 0.25; 0.5 and 1 µM $PtCl_4$ concentration treatment, during three days. The results showed substantial growth inhibition of the final algal biomass (Figure 1). The decrease in growth was observable form the beginning of the experiment, it varied from 2% at 0.05 µM to about 30% at 1 µM of $PtCl_4$. The values of $72hEC_{5(\mu)}$, $72hEC_{10(\mu)}$, $72hEC_{20(\mu)}$ counted on a basis ofaverage specific growth rate (µ) were 0.31 µM, 0.58 µM and 1.12 µM of $PtCl_4$, respectively. The values, obtained on a basis of the area under the growth curves (A), were 0.04 µM ($72hEC_{5(A)}$), 0.24 µM ($72hEC_{10(A)}$) and 0.64 µM ($72hEC_{20(A)}$).

Also we attempted to investigate the effect of platinum ($PtCl_4$) on *L. minor*. The duckweed was treated by 0;5; 10; 25; 50; 100 µM of $PtCl_4$ for seven days. The elementary characteristic, as growth curve, number of leaves, and observable macroscopic changes have been studied during the test treatment. The increase of growth inhibition varied from about 30% at 5 µM to almost 100% at 100 µM of $PtCl_4$ in comparison to control during the week of cultivation (Figure 2). The experiment with duckweed showed 50% of growth inhibition and the values of $168hEC_{50(\mu)}$ were 19.55 µM (95% confidence interval = 14.99–24.11 µM) and $168hEC_{50(A)}$ 13.63 µM(95% confidence interval = 11.35–15.91 µM) of $PtCl_4$. The inhibition of the vegetative growth was evident immediately in 24 hours but the morphology was changed generally from the half of the duration of the experiment (3rd day). The size of fronds decreased and showed yellow discoloration areas (chlorosis), and dead, white and also water-soaked frond tissues in samples with the highest platinum concentrations (Figure 3).

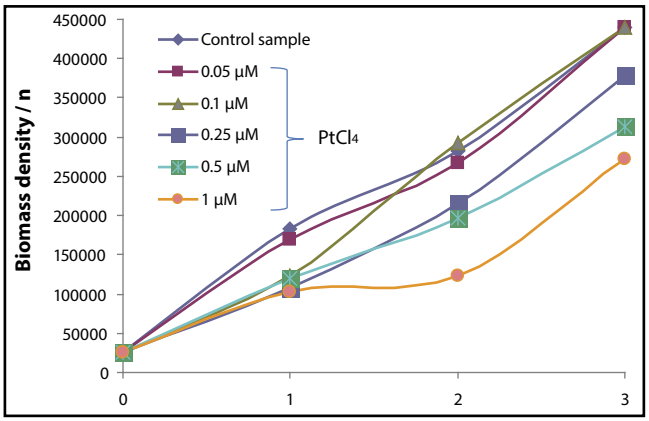

Fig. 1. Growth curve of *Pseudokirchneriella subcapitata* treated with different doses of $PtCl_4$ expressed as a biomass density.

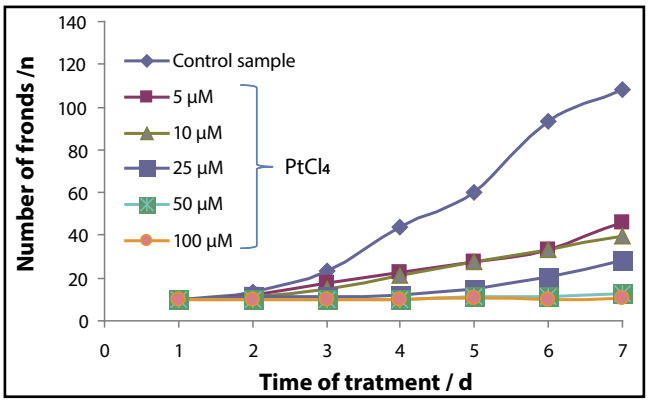

Fig. 2. Growth curve of *Lemna minor* treated with different doses of $PtCl_4$, expressed as number of fronds.

DISCUSSION

To predict the possible negative consequences of different hazardous substances from effluents on water ecosystems effective tools for estimation of the impact on living organisms are needed (Blinova 2000). There are recent published papers about phytotoxicity of different PGE, like antineoplastic pharmaceutical cisplatin (Zounkova *et al.* 2007) and palladium (Easton *et al.* 2011; Vannini *et al.* 2011) where authors studied the impact on *Pseudokirchneriella subcapitata* and marine algae, *Ulva lactuca*.

Metal phytotoxicity on *Lemna minor* and *Pseudokirchneriella subcapitata* was also studied by Blinova (2004), discussed results of the study with Cr(III), Pb(II), Cu(II), Cd(II) and pyrene, indicated that the sensitivity of animal and plant species to individual contaminants and mixtures was unpredictable and considered that *L. minor* is more sensitive than the algae. But it is generally expected that *L. minor* might be less sensitive then algae because duckweeds are used to remove toxicants and nutrients from wastewater (Lewis 1995) and also some authors mentioned that duckweed plants mightbe tolerant to environmental toxicity (Verdissson *et al.* 2001). Other authors in their studies suggested that duckweed plants (*L. minor*) are as senstitive to toxicity as algae *P. subcapitata* (Fairchild *et al.* 1997) or more sensitive (Blinova 2004; Mohammad *et al.* 2006) according to the chemical or environmental sample tested. Duckweed and algae represent independent levels of complexity in the plant kingdom (Paixao *et al.* 2008).

In our study we attempt to compare the sensitivity of these two different representatives of aquatic producers on the impact of platinum, represented as $PtCl_4$ by monitoring of the algal biomass density and the vegetative propagation and morphology of duckweed colonies. Growth inhibition in both experiments confirmed the assumptions about the adverse effect of PGE (platinum) and well correlated with the increasing platinum concentration and the morphological changes responded the results pointed out in study by Stejskal *et al.* (2007) and Supalkova *et al.* (2008) with cisplatin. For the comparison with our results the value of $96hEC_{50}$

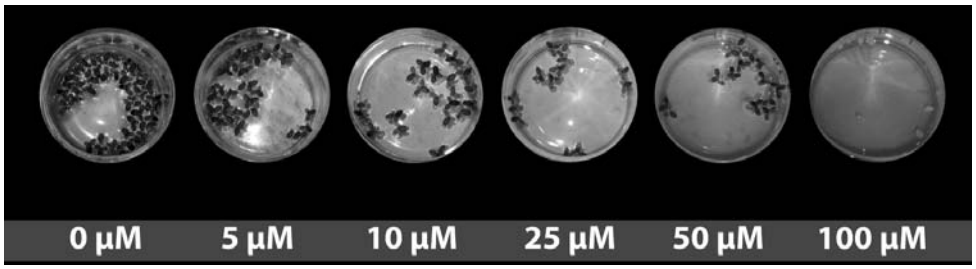

Fig. 3. Colonies of *Lemna minor* fronds after 7 days of treatment with different doses of $PtCl_4$.

of cisplatin, calculated from growth inhibition based on the comparison of growth rates, was 6.93 µM (95% confidence interval = 6.51–7.34 µM). And the value of $96hEC_{50}$, calculates from growth inhibition with comparison of areas under growth curves, was <5 µM (Supalkova *et al.* 2008). It has frequently been observed that under toxic stress the duckweed colonies protrude in small buds, which we also observed (Radic *et al.* 2010).

The results of the algal experiment were difficult to discuss because of the missing data to compare from the other studies. The biomass density under 0.1 µM $PtCl_4$ in the algal experiment caused slight growth stimulation (the tested biomass density was higher than in control sample). The concentration span for the algal test has not provided the value of 50% growth inhibition ($72hEC_{50}$), but from the calculated values of $72hEC_{5(\mu)}$, $72hEC_{10(\mu)}$, $72hEC_{20(\mu)}$ and $72hEC_{5(A)}$, $72hEC_{10(A)}$, $72hEC_{20(A)}$ could be stated, that the estimation of $72hEC_{5(\mu)}$ was the most sensitive parameter. $PtCl_4$ was more toxic for green microalga *P. subcapitata* than for *L. minor*.

The comparison of sensitivity and the estimation of effective concentrations, which caused the growth inhibition assessed as a percentage with the connection of platinum compounds ($PtCl_4$), have not been investigated yet. As a conclusion we can say that our experiments with two representatives of aquatic producers showed significant phytotoxicity of $PtCl_4$ described as a growth inhibition, changes in plant size and morphology and the more sensitive was found the algal species – *Pseudokirchneriella subcapitata*.

ACKNOWLEDGEMENTS

The study was supported by IGA 82/2011/FVHE.

Potential Conflicts of Interest: *None disclosed.*

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