Impact of platinum on the soil invertebrate *Folsomia candida*

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Abstract

OBJECTIVES: Regarding the environmental pollution, platinum group elements (PGE) are in the centre of interest of current research. These rare elements are used as effective substances in automotive catalysts to reduce pollution by emissions originating from fuel combustion. Due to their harmful potential, it is necessary to monitor their content and behaviour in different samples. Comprehensive studies on PGE behaviour and effects are still lacking. Their distribution in the food chain and data on bioaccumulation has not been described so far.
METHODS: We focused on reproductive effects of platinum ($PtCl_4$), in particular. Our study is based on a collembolan laboratory breed, test optimalization and validation according to the OECD 232 standards [CSN ISO 11267 – Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants]. The concentrations of $PtCl_4$ tested were as follows: 5, 10, 25, 50 and 100 μM. The EC_{50} was determined after 28 days of testing.
RESULTS: The results were evaluated using the inhibition of reproduction compared with controls. The EC_{50} was determined after the 28-day test. The value of $28dEC_{50}$ of the boric acid test was estimated at 120 mg/kg and the measured $28dEC_{50}$ of $PtCl_4$ was 200.4 μM.
CONCLUSION: The presented data can be considered as a step forward in the assessment of the potential risk of platinum in the terrestrial environment. However, more toxicity data for various species are needed to evaluate the environmental risk of platinum in soils.

Abbreviations:

$28dEC_{50}$	- 28-day effective concentration, which caused 50% of an effect in test organisms within the given exposure period when compared with the control.
CSN ISO 11267	- Soil quality: Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants
CV	- coefficient of variation.
EC_{50}	- Medium effective concentration – the concentration that causes an effect in test organisms amounting to 50% within a given exposure period when compared with the control.
OECD 232	- OECD Guidelines for testing chemicals: Collembolan reproduction test in soil
PGE	- platinum group elements

ORIGINAL ARTICLE

INTRODUCTION

Effects of toxic substances in different species are dependent on the amount of the toxic substance taken up from the environment. Accumulation depends not only on the characteristics of the organism itself, but also on the characteristics of the substance and the environment. The soil capacity to retain toxic substances is a very important factor determining bioaccumulation of toxicants (Crommentuijn *et al.* 1997). Levels of contaminants in wastes and soils can be measured by chemical analysis but this technique is often unsuitable as it requires extensive knowledge on classes of pollutants to be analyzed. It gives little information about the bioavailability of pollutants or their degradation products. So, chemical analysis has to be complemented with ecotoxicological tests. These tests provide information on the effects of pollutants in ecologically relevant parameters such as reproduction (Crouau *et al.* 2002).

Platinum group elements (PGE) can be naturally found only at very low concentrations in the earth crust (Barbante *et al.* 2001). However, PGE are increasingly emitted into the environment since the introduction of automotive catalytic converters in the 1970s and mid-1980s in the USA and Europe, respectively. A wealth of studies concentrates on the effects of heavy metals such as lead (Pb) or cadmium (Cd). Although PGE are used for medical purposes in dental alloys and as anti-cancer drugs (Nemoto *et al.* 2012), the largest part of PGE emissions originates from automotive traffic (Schmid *et al.* 2007).

In the last decade, a significant increment in the PGE concentration in particular types of environmental samples has been noted, such as: particular matter, soil, road dust, surface water, plants and bottom sediments (Morton *et al.* 2001). This situation results first of all from the widespread use of PGE in different industrial branches. The main emission sources of PGE to the environment are automotive catalysts, industrial ones and hospital wastes. More detailed pieces of information on PGE emission sources and their characteristics can be found in the following original and review papers (Ranvidra *et al.* 2004; Moldovan, 2007; Artelt *et al.* 1999). It is supposed that PGE emissions from car catalysts at current levels do not threaten human health and life. However, due to the PGE ability to undergo accumulation in tissues of living organisms, the negative impact of these metals as a result of direct contact with road dust, inhaling solid particles (that constitute approximately 30% of all particles emitted from vehicle catalysts) as well as by food and drinking water cannot be totally excluded (Merget & Rosner, 2001).

The collembolan (springtail) *Folsomia candida* is frequently used as an indicator species in soil bioassays and is representative for soil-living arthropods (Nota *et al.* 2011). The parthenogenetic *Folsomia candida* and sexually reproducing *Folsomia fimetaria* are two of the most accessible species of Collembola; they are suitable for culture and commercially available (OECD 232, 2009). *F. candida* is a cosmopolitan species found almost all over the globe and is considered a tramp species. However, only few outdoor records exist for *F. candida* that prefers high organic matter like in compost, greenhouses, flower pots or manure (Fountain & Hopkin, 2005). Although not common in most natural soils, it often occurs in very high numbers at humus-rich sites (OECD 232, 2009).

For standard laboratory tests used *F. candida* has been suggested as a reference soil animal in ecotoxicological studies. Subsequently, ample toxicity data for *F. candida* has been generated. *F. candida* has several advantages in ecotoxicological studies (Fountain & Hopkin, 2005), but it is unknown as a representative of other Collembola. Low population density of *F. candida* in natural habitats, especially outside Europe, poses a disadvantage in terms of ecological relevance (Nursita *et al.* 2005). To compensate these disadvantages, the parallel use of *F. candida* with common, ecologically relevant species is recommended if data on *F. candida* are to be generalised to other species (Menta *et al.* 2006).

The main aim of this study was to assess the reproduction test of *Folsomia candida* as a tool for the ecotoxicological assessment of platinum ($PtCl_4$) applied or contaminating soils.

MATERIAL AND METHODS

Test organisms

The collembolans originated from cultures of the Ecotoxicological laboratory of the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, and were reared on Petri dishes filled with a plaster of Paris/charcoal mixture (9:1). Individuals were kept at 20±1 °C in the dark and fed twice a week with dried baker's yeast. Only young adults (10–12 days of age) were used. Collembolans were fasted for 24 h before introduction into the experimental system.

Experimental design

The experiments were carried out on the basis of OECD 232 [CSN ISO 11267 – Soil quality – Inhibition of reproduction Collembola (*Folsomia candida*) by soil pollutants]. In the chronic toxicity tests, a total of 10 synchronized springtails of 10–12 days of age were exposed in each glass vessel. Per vessel, 30 g wet weight of soil was added. Five replicates were used per the tested concentration. Tests were carried out in the standard artificial soil. The artificial soil used as test substrate was composed as prescribed by the OECD Guideline 232 (2009): 70% sand, 20% kaolin clay and 10% finely ground Sphagnum peat, pH was adjusted with $CaCO_3$. Calcium carbonate was used to adjust the pH (1 M KCl) to 6.0±0.5. For the determination of pH a mixture of soil and 1 M potassium chloride (KCl) solution in a 1:5 ratio is used. If the soil is more acidic than the required range, it can be adjusted by addition of an appropriate amount of $CaCO_3$. Tests were carried out at soil moisture of 50% of the water holding capacity. The test vessels were capped with a polyethylene lid and the edge of the lid was sealed with Parafilm. There was a small hole between the Parafilm and lid allowing airflow which did not allow Collembola to escape.

The soil was contaminated by dissolved $PtCl_4$ in the correct amount of deionised water to achieve soil moisture equal to 50% of maximum water-holding capacity. The $PtCl_4$ substance is very soluble in water. Concentrations were chosen on the basis of the range finding test. The concentration of the stock solution was 10 mM (0.3369 g $PtCl_4$ per 100 ml deionised water). Each solution was mixed with the soil immediately before use, leading to nominal concentrations of 5, 10, 25, 50, 100 μM $PtCl_4$ per vessel. Growth rate, time to first batch of eggs, quantity of food consumed and the presence of graphite in the gut were all affected by metal contaminated diets. Non-contaminated control soil was prepared as described above without the addition of the test chemicals.

The reproduction test with *F. candida* (Figure 1) took 4 weeks to complete. Granulated dry yeast (2 mg) was added on the first day and then after 14 days on the soil surface as food. At the end of the test, 20 ml of deionised water were added to each container and stirred thoroughly, but carefully, allowing all the animals present in the soil to float to the surface. The water surface was coloured by ink (Figure 2). After that the whole Petri dish was photographed and then adults and juveniles were counted (Figure 3). The endpoint is the total number of juveniles per test vessel. During exposure, all test vessels were kept at 20°C and a light: dark cycle of 16:8 at 400–800 lx. Soil moisture content was adjusted three times a week by replenishing weight loss with the appropriate amount of deionised water. More than 2% mass loss was recovered by adding water.

The results were evaluated as the inhibition of reproduction compared to the control. The EC_{50} (median effective concentration) was determined after 28 days. For the test to be valid, we followed the criteria mentioned in the guideline (OECD 232, 2009): mean adult mortality should not exceed 20% at the end of the test; the mean number of juveniles per vessel should be at least 100 at the end of the test; the coefficient of variation calculated for the number of juveniles should be less than 30% at the end of the definitive test.

A reference substance was tested at its EC_{50} concentration for the chosen test soil type at regular intervals to verify that the response of the test organisms in the system are responding within the normal level. A suitable reference substance is boric acid, which should reduce reproduction by 50% at about 100 mg/kg dry weight soil (OECD 232, 2009).

Fig. 1. *Folsomia candida* - a very common soil springtail. Photo by Jeff Hahn.

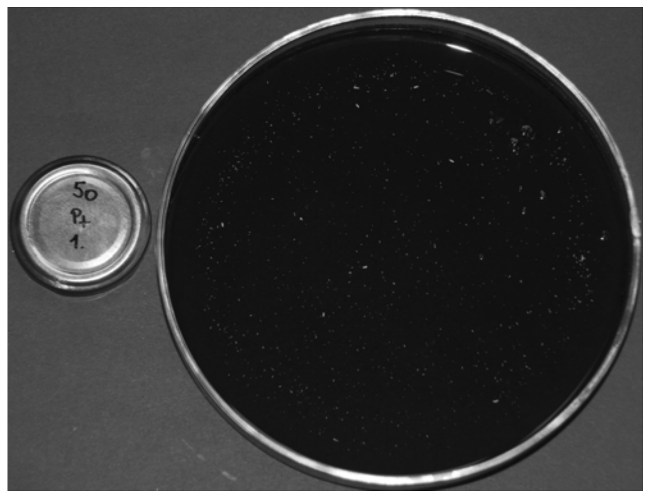

Fig. 2. Water surface coloured by ink.

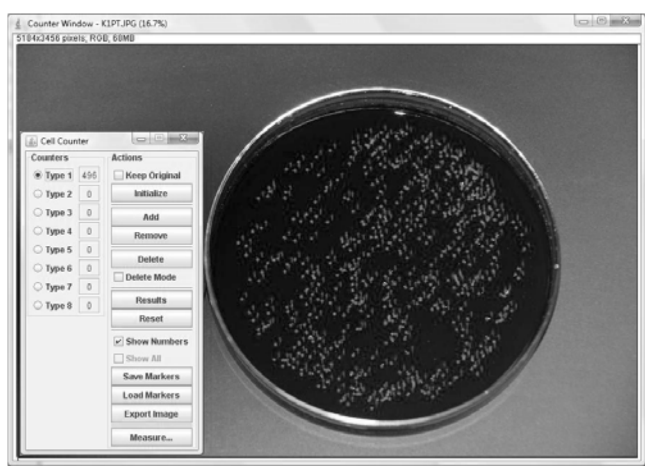

Fig. 3. Counting adults and juveniles using the ImageJ.

RESULTS

The test with *Folsomia candida* fulfilled the CSN ISO criteria for validation. In the control, adult survival was 100%, the average number of juveniles per vessel was 1043.8 and the coefficient of variation (CV) was 3% in

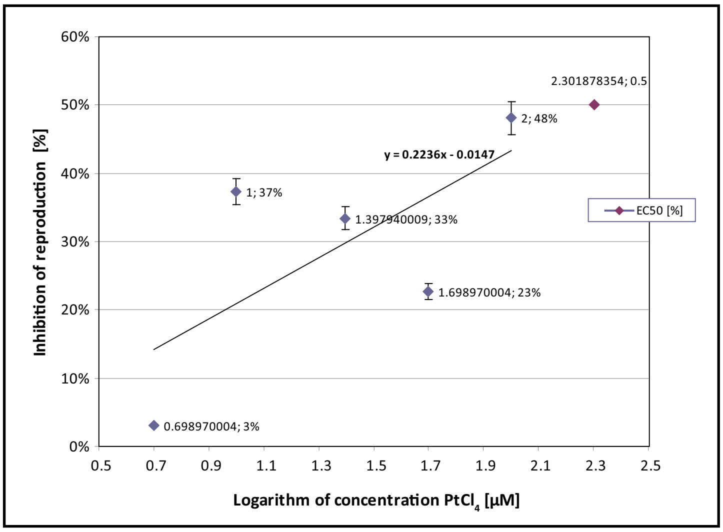

Fig. 4. Effect of $PtCl_4$ on reproduction in *F. candida* after 4 weeks. Error bars indicate standard deviations.

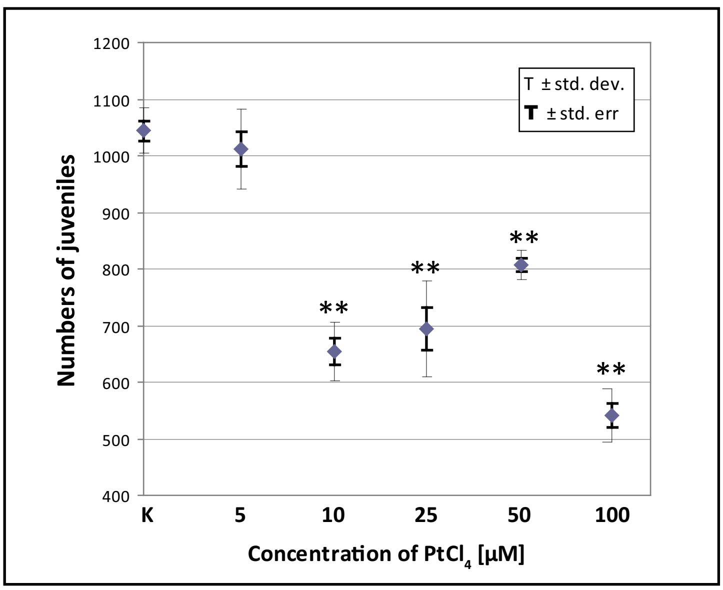

Fig. 5. Juvenile reproduction of *F. candida* exposed for 28 days to platinum (expressed as nominal total platinum concentrations in the soil). Error bars indicate standard deviation. ** = $p<0.01$ compared with the control (n=5 in each group).

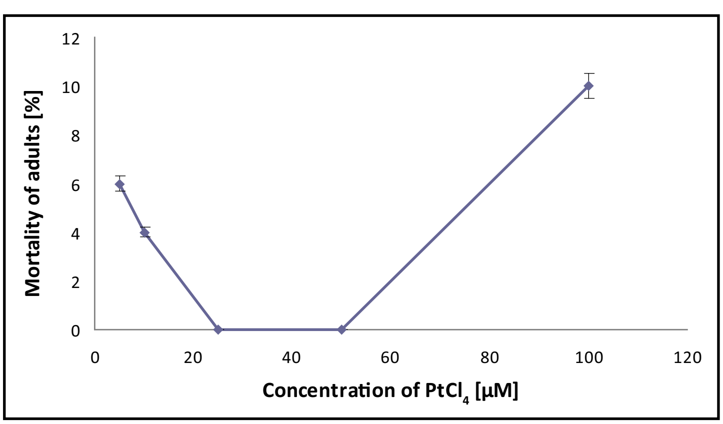

Fig. 6. Effect of platinum on mortality in *Folsomia candida* after four weeks of exposure via artificial soils. The data points are the averages of replicates (n=5).

adults. The value of $28dEC_{50}$ of the boric acid (the reference substance) test was estimated at 120 mg/kg. Survival was not so greatly affected as reproduction; therefore, it is not as good an indicator of toxicity (Figure 6).

The results of the toxicity tests are shown in Figures 4 and 5. Survival of adults was not significantly affected however reproduction decreased with increasing metal concentrations. It is also noteworthy that reproduction usually showed a higher variability between replicates than survival. On the other hand, body growth was either not affected by the metal concentration or even slightly stimulated. Body growth increased in parallel with the decrease in reproduction. Juveniles' growth in various concentrations was compared to control. These results were recorded only subjectively because the selected methodology was not properly set for the measurement of juveniles' body. The $28hEC_{50}$ (effective concentration) $PtCl_4$ was estimated at 200.4 μM. All CV values were less than 11% for each concentration of platinum (Figure 4). Mean adult survival ranged from 9 to 10 out of 10 per vessel, and reproduction was between 541.6 and 1043.8 per vessel. In the Table 1 there is summary of platinum toxicity on *F. candida*. No significant effect on mortality was found.

DISCUSSION

Platinum concentrations up to 303±40 ng/g in the street dust are measured along highways in Germany (Djingova *et al.* 2003), indicating that the platinum concentrations causing reproductive effects in *F. candida*, are still environmentally relevant.

Very little is known on the uptake of traffic related PGE by terrestrial components of the biosphere. In field studies, elevated and continuously increasing Pt and Pd levels were described in roadside grass samples (Helmers, 1997). Djingova *et al.* (2003) reported the accumulation of Pt, Pd, Rh, Ru and Ir in dandelion (*Taraxacum officinale*), plantain (*Plantago lanceolata*), annual ryegrass (*Lolium multiforum*), moss (*Rhytidiadelphus squarrosus*) and mushroom (*Vascellum pratense*) along highways in Germany with a very diverse extent. Lustig *et al.* (1997) also provided a proof of the Pt uptake under natural conditions by some nutriet plants, i.e. onion (*Allium cepa* L.), radish (*Raphanus sativus* L.), broad bean (*Vicia faba* L.), maize (*Zea mays* L.), and potato (*Solanum tuberosum* L.). Several studies on the uptake of traffic-emitted

Tab. 1. Summary of platinum toxicity on *F. candida*.

Concentrations $PtCl_4$ [μM]	No. adult at the beginning of the test	No. adult at the end of the test	Mortality [%]	No. juveniles produced	CV [%]	SD	Inhibition of reproduction [%]
5	10	9.4	6	1011.8	6	63	3
10	10	9.6	4	654.6	7	46	37
25	10	10	0	694.8	11	76	33
50	10	10	0	806.8	3	23	23
100	10	9	10	541.6	8	41	48

PGE by animals demonstrated the biological availability of Pt, Rh and Pd; i.e. for earthworms (Schafer *et al.* 1998).

In the present study with *F. candida*, the survival showed a continuous decrease as the platinum concentration increased, usually at much higher concentrations than those affecting reproduction. Stimulation of body growth was coupled with inhibition in reproduction. This may be explained by the previously demonstrated negative trade-off between reproduction and growth in other insects (Domene *et al.* 2007).

Several factors must be considered to compare the potential of soil toxicity testing procedures for the evaluation of potentially contaminated sites. The first factor to be considered is the representativeness of the test organism. *F. candida* is a natural soil species that inhabits the soil matrix (Fountain & Hopkin, 2005). The second issue is the sensitivity of the organism to the toxicants in question. The third is the difficulty of the testing procedure and the time required to perform the test. The final factor is the difficulty of culturing or obtaining the organism. Due to the large variability in soil types between sites as well as microvariability within the site, a large number of tests would be necessary to fully characterize the condition of the site. Therefore, a test that is quick, easy, and inexpensive is needed for preliminary evaluation (Peredney & Williams, 2000). *F. candida* seems to fulfil these needs.

The health of soils must be maintained not just for several years but indefinitely. To sustain life and agriculture, the ecological function of soils must not be compromised. As far as regulating the input of heavy metals into soils, many factors must be considered, such as background concentration, physicochemical properties, and sustainability. *F. candida* appears to be an ideal organism for such testing. The relative ease of the tests, the rapid turnaround period of samples, and the low cost are factors to be considered for frequent testing.

Most studies on effects of soil properties on metal bioavailability and bioaccumulation concern earthworms; while very little is known concerning other soil organisms (Pedersen *et al.* 1997).

CONCLUSION

There has not been done yet any comprehensive study about PGE behaviour and effects, their distribution in the food chain and bioaccumulation have not been described so far. The presented data can be considered as a step forward in the assessment of the potential risk of platinum in the terrestrial environment; more toxicity data for different species are needed to evaluate the environmental risk of platinum in soil.

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Potential Conflicts of Interest: *None disclosed.*

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