Impact of platinum group elements on the soil invertebrate *Folsomia candida*

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Submitted: 2013-06-21 *Accepted: 2013-08-30* *Published online: 2013-11-10*

Key words: **palladium; rhodium; ecotoxicology; Collembola; springtail**

Neuroendocrinol Lett 2013; **34**(Suppl.2):5–10 **PMID:** 24362086 NEL341013A01

Abstract

OBJECTIVES: Trace concentrations of the platinum group elements (PGE; Pt, Pd and Rh) are nowadays an irreplaceable part of environmental analysis and assessment. 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. Effect assessment using ecotoxicological bioassays with organisms at different trophic levels can provide valuable pieces of information on the risk of chemical substances in the ecosystem.

DESIGN: The experiments were carried out as described in the OECD Guideline 232 [CSN ISO 11267 – Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants]. The reproductive effects of palladium ($PdCl_2$) and rhodium ($RhCl_3$) were examined. Concentrations of PGE tested were as follows: 5, 10, 25, 50 and 100 μmol.L^{-1}. The EC_{50} (medium effective concentration) was determined after 28 days of exposure. Inhibition of reproduction of PGE-exposed collembolans was compared against controls.

RESULTS: Values of $28dEC_{50}$ of $PdCl_2$ and $RhCl_3$ amounted to 21.0 μmol.L^{-1} and 266.22 μmol.L^{-1}, respectively. We can confirm that the relative order of toxicities is Pd (II) > Pt(IV) >> Rh(III).

CONCLUSION: To the best of our knowledge, this is the first study to use *Folsomia candida* as an indicator species to assess the risk of soil contamination by palladium and rhodium. However, more toxicity data for various species are needed to evaluate the environmental risks of PGEs 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
- CV - coefficient of variation
- EC_{50} - medium effective concentration – concentration that causes an effect in test organisms amounting to 50% within a given exposure period when compared with the control
- PGE - platinum group element
- SD - standard deviation

ORIGINAL ARTICLE

INTRODUCTION

The hazards of toxic elements such as cadmium, lead and mercury in the environment have long been discussed from a global perspective. Platinum group elements (PGEs) belong to heavy metals and potentially represent a high risk to the environment. The main source of pollution by PGEs is the still increasing transportation. However, they are also used in the chemical, electrical and glass industries (Ravindra *et al.* 2004). While PGEs are used for medical purposes in dental alloys and as anti-cancer drugs (Nemoto *et al.* 2012), the largest proportion of PGE emissions originates from automotive traffic (Schmid *et al.* 2007). In the last decade, a significant increase in the PGE concentration in particular types of environmental samples such as particular matter, soil, road dust, surface water, plants and bottom sediments has been noted (Morton *et al.* 2001).

PGEs can naturally be found only at very low concentrations in the earth's crust (Barbante *et al.* 2001). The first observations of PGE concentrations in soil were reported to be lower than 0.7 $pg.m^{-3}$ near a freeway in California (Alt *et al.* 1997). However, other studies, e.g. those from Germany, have shown that the total PGE concentration in soil along a highway ranged from 9 to 106 $pg.m^{-3}$ (0.6 to 330 $ng.g^{-1}$) (Ravindra *et al.* 2004).

Additional sources of environmental contamination are represented by waste waters from hospitals where antineoplastic drugs such as the platinum compound cisplatin are in use (Supalkova *et al.* 2008).

Trace concentrations of PGEs play an important role in environmental analysis and assessment. The marked increase of PGE emissions coupled with the high allergenic and cytotoxic potential of platinum compounds, even in very low doses, are raising considerable concerns (Hees *et al.* 1998). Detailed pieces of information on PGE emission sources and their characteristics can be found in some original and review papers (Artelt *et al.* 1999; Ravindra *et al.* 2004; Moldovan, 2007; Bednarova *et al.* 2012; Mikulaskova *et al.* 2013).

PGE emissions from car catalysts at current levels are not supposed to threaten human health and life. However, due to the ability of PGEs to bio-accumulate in the 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 from food and drinking water cannot be totally excluded (Merget & Rosner, 2001). Importantly, some PGE species (those containing chloride, in particular) exert toxic, cytotoxic and allergenic effects. For example, signs of acute toxicity of $H_2(PtCl_6)$, $RhCl_3$ and $PdCl_2$ were reported in rats and rabbits (Colombo *et al.* 2008). Immune functions were shown to be deteriorated by rhodium chloride (Iavicoli *et al.* 2012).

Collembola, as one of the most abundant groups in the terrestrial ecosystem, are used to assess soil quality. Collembola are responsible for organic matter decomposition along with many other organisms and play an important role as prey in the soil ecosystem (Nota *et al.* 2011; Youn *et al.* 2013). *Folsomia candida*, recommended as a test species by CSN ISO (2010) and OECD 232 (2009), is the most representative species to assess soil quality (Crouau *et al.* 2002; Fountain & Hopkin, 2005).

Effect assessment using ecotoxicological bioassays with organisms at different trophic levels can provide valuable pieces of information on the risk of chemical substances in the ecosystem. Due to PGEs harmful potential, it is necessary to monitor their content and behaviour in different samples. There are several studies on the uptake of traffic-emitted PGEs. However the most studies on the effects of soil properties on metal bioavailability and bioaccumulation concern earthworms while very little is known about other soil organisms. *Folsomia candida*, as the dominant primary decomposers, makes up a level in the soil environment. As little is known about the responses of collembolans to soil contamination by PGEs hence the main aim of this study was to assess the reproduction of *Folsomia candida* as a tool for the ecotoxicological assessment of palladium and rhodium.

MATERIAL AND METHODS

Test organisms

The collembolans originated from cultures from the Ecotoxicological laboratory of the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic.

Experimental design

The experiments were carried out as described in the OECD Guideline 232 [CSN ISO 11267 – Soil quality – Inhibition of reproduction Collembola (*Folsomia candida*) by soil pollutants] with minor changes to fit our experimental conditions (Nemcova *et al.* 2012). The artificial soil used as a testing substrate was prepared as a mixture of 70% sand, 20% kaolin clay and 10% finely ground Sphagnum peat, pH (6.0±0.5) and was adjusted with $CaCO_3$.

The reproductive effects of palladium ($PdCl_2$ – Sigma-Aldrich, p.a. 99.999%) and rhodium ($RhCl_3$ – Sigma-Aldrich, p.a. 98%), in particular, were examined. The soil was contaminated by dissolved $PdCl_2$/$RhCl_3$ in an adequate amount of deionised water to achieve soil moisture equal to 50% of maximum water-holding capacity. The $PdCl_2$/$RhCl_3$ substances are particularly soluble in water. Concentrations were chosen on the basis of the range finding test. The concentration of the stock solution was 10 $mmol.L^{-1}$ (0.4433 g $PdCl_2$ per 250 ml deionised water; 0.05231 g $RhCl_3$ per 25 ml deionized water). Each solution was mixed with the soil immediately before use, leading to nominal concentrations of 5, 10, 25, 50, 100 $\mu mol.L^{-1}$ $PdCl_2$/$RhCl_3$ per vessel. Five replicates were used per the tested concentration.

The results were evaluated as the inhibition of reproduction and 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 stated in the guideline (OECD 232, 2009). These are as follows: 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 (CV) 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 correspond to the normal level. A reference substance suitable for this test is boric acid, which should reduce reproduction by 50% at about 100 mg.kg^{-1} dry weight soil (OECD 232, 2009). The concentrations of boric acid tested were as follows: 50, 70, 100, 140, and 200 mg.kg^{-1}.

Statistical analysis

The data analysis software Statistica for Windows® 10 (StatSoft, Inc., Tulsa, OK, USA) was used to compare different groups of vultures by one-way analysis of variance (ANOVA) and post-hoc analysis of means by the LSD test. Levene's method was used to test for the homogeneity of variances. Non-homogenous parameters were log-transformed prior to analysis and compared with the non-parametric Kruskal-Wallis test. All tests were considered statistically significant and highly significant when resulting in values of $p<0.05$ and $p<0.01$, respectively.

RESULTS

The test with *Folsomia candida* fulfilled the CSN ISO criteria for validation. The value of $28dEC_{50}$ (effective concentration) of the boric acid (the reference substance) test was 101.5 mg.kg^{-1} (Figure 1). In the control, adult survival was 98%, the average number of juveniles per vessel was 845.8 and the CV was 13% in juveniles. The results of the toxicity tests are shown in Figure 2. Mortality was not significantly influenced. Summary of H_3BO_3 toxicity in *F. candida* is shown in Table 1.

The $28dEC_{50}$ of $PdCl_2$ amounted to 21.0 μmol.L^{-1} (Figure 3). In the control, adult survival was 100%, the

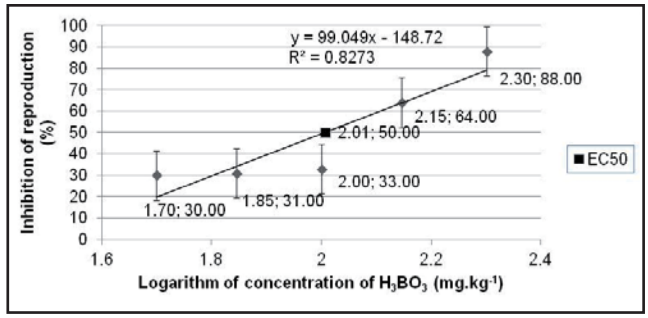

Fig. 1. Effect of boric acid (H_3BO_3) on reproduction in *F. candida* after 4 weeks of exposure in artificial soils. The data points are the averages of replicates (n=5). Error bars indicate standard deviations.

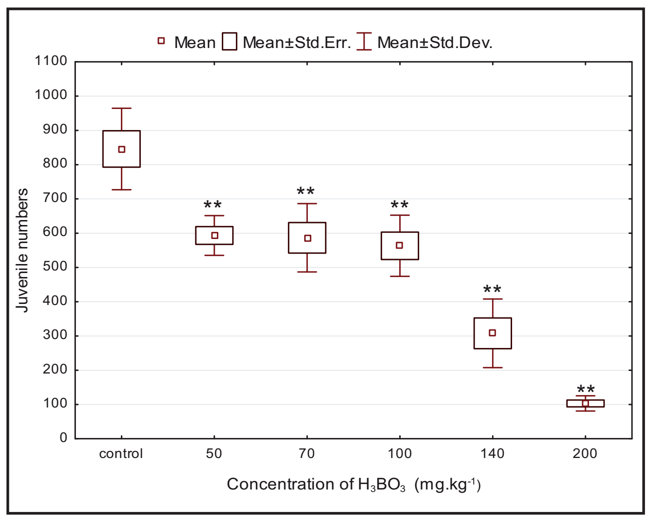

Fig. 2. Juvenile reproduction of *F. candida* exposed for 28 days to boric acid (H_3BO_3) (expressed as nominal total boric acid concentrations in the soil). $^{**}p<0.01$ compared with the control (n=5 in each group).

Tab. 1. Summary of boric acid (H_3BO_3) toxicity in *F. candida*.

Concentrations boric acid (mg.kg^{-1})	No. adult at the beginning of the test	No. adult at the end of the test (mean)	Mortality (%)	No. juveniles produced (mean ± SD)	CV (%)	Inhibition of reproduction (%)
50	10	9.2	8	593.2 ± 51.9	9	30
70	10	9.0	10	586.6 ± 89.2	15	31
100	10	9.4	6	563.2 ± 79.8	14	33
140	10	9.8	2	307.8 ± 89.6	29	64
200	10	9.6	4	103.2 ± 19.9	19	88

CV – coefficient of variation, SD – standard deviation average number of juveniles per vessel was 1280 and the CV was 3% in juveniles. The results of the toxicity tests are shown in Figure 4. No significant effect on mortality was found. Summary of $PdCl_2$ toxicity on *F. candida* is shown in Table 2.

The $28dEC_{50}$ of $RhCl_3$ was 266.22 μmol.L^{-1} (Figure 5). In the control, adult survival was 100%, the average number of juveniles per vessel was 603.6 and the CV was 21% in juveniles. The results of the toxicity tests are shown in Figure 6. There was no significant effect on mortality. Summary of $RhCl_3$ toxicity on *F. candida* is shown in Table 3.

All CV values were less than 21% for each concentration of palladium and rhodium (Tables 1, 2). Mean adult survival rates ranged from 8 to 10 out of 10 collembolans per vessel. Survival was not affected to such an extent as reproduction. Therefore, it is not a good indicator of toxicity.

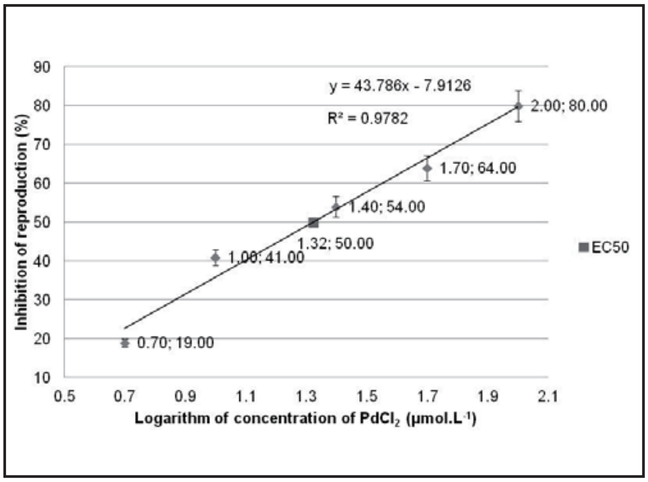

Fig. 3. Effect of palladium ($PdCl_2$) on reproduction in *F. candida* after 4 weeks of exposure in artificial soils. The data points are the averages of replicates (n=5). Error bars indicate standard deviations.

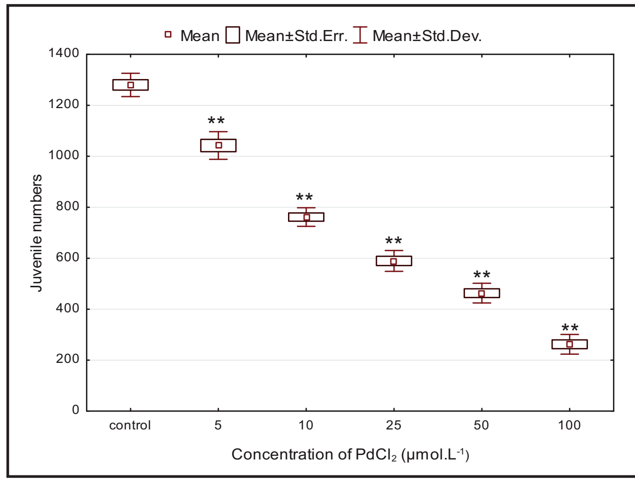

Fig. 4. Juvenile reproduction of *F. candida* exposed for 28 days to palladium ($PdCl_2$) (expressed as nominal total palladium concentrations in the soil). $^{**}p<0.01$ compared with the control (n=5 in each group).

Tab. 2. Summary of palladium ($PdCl_2$) toxicity in *F. candida.*

Concentrations $PdCl_2$ (μmol.L^{-1})	No. adult at the beginning of the test	No. adult at the end of the test (mean)	Mortality (%)	No. juveniles produced (mean ± SD)	CV (%)	Inhibition of reproduction (%)
5	10	9.6	4	1042.4 ± 48.5	5	19
10	10	9.6	4	761.6 ± 32.6	4	41
25	10	9.6	4	589.4 ± 36.5	6	54
50	10	10.0	0	463.0 ± 34.5	7	64
100	10	10.0	0	262.4 ± 34.6	13	80

CV – coefficient of variation, SD – standard deviation

Tab. 3. Summary of rhodium ($RhCl_3$) toxicity on *F. candida.*

Concentrations $RhCl_3$ (μmol.L^{-1})	No. adult at the beginning of the test	No. adult at the end of the test (mean)	Mortality (%)	No. juveniles produced mean ± SD	CV (%)	Inhibition of reproduction (%)
5	10	10.0	0	555.4 ± 41.8	7	8
10	10	9.6	4	542.8 ± 78.8	15	10
25	10	9.4	6	508.6 ± 107.6	21	16
50	10	9.6	4	454.6 ± 48.8	11	25
100	10	9.6	4	326.8 ± 46.6	14	46

CV – coefficient of variation, SD – standard deviation

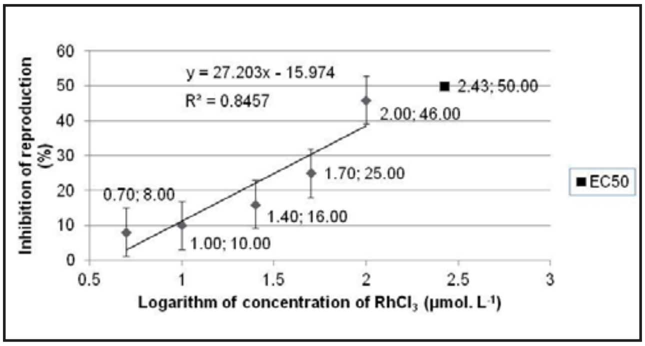

Fig. 5. Effect of rhodium ($RhCl_3$) on reproduction in *F. candida* after 4 weeks of exposure in artificial soils. The data points are the averages of replicates (n=5). Error bars indicate standard deviations.

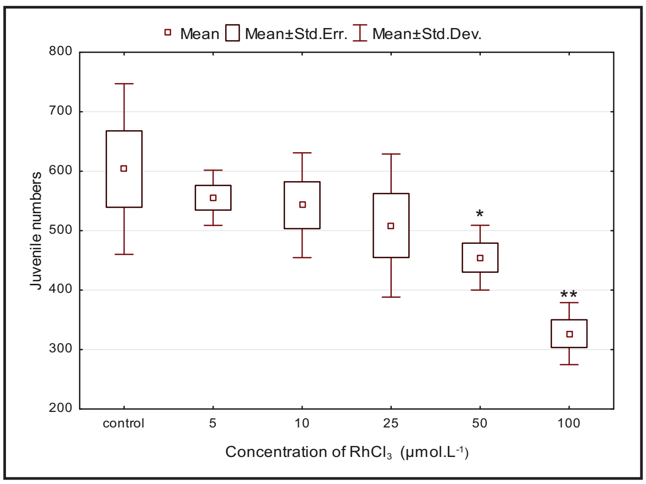

Fig. 6. Juvenile reproduction of *F. candida* exposed for 28 days to rhodium ($RhCl_3$) (expressed as nominal total rhodium concentrations in the soil). *$p<0.05$, **$p<0.01$ compared with the control (n=5 in each group).

DISCUSSION

Levels of contaminants in wastes and soils can be measured by chemical analysis but this technique is often unsuitable as it requires extensive knowledge of the classes of pollutants to be analyzed. It gives little information about the bioavailability of pollutants or their degradation products. Therefore, chemical analysis has to be supported by ecotoxicological tests. These tests provide information on the effects of pollutants on ecologically relevant parameters such as reproduction (Crouau *et al.* 2002).

Very little is known about the uptake of traffic related PGEs by terrestrial components of the biosphere. Several studies on the uptake of traffic-emitted PGEs by animals and some nutrient plants demonstrated the biological availability of Pt, Rh and Pd (Lustig *et al.* 1997; Schafer & Puchelt, 1998; Djingova *et al.* 2003).

In the present study with *F. candida*, survival showed a continuous decrease as the concentrations of metals increased, usually at much higher concentrations than those affecting reproduction. Our subjective impression is that inhibition of body growth was coupled with inhibition of reproduction. However, we are planning a comprehensive study to address this issue. 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. This phenomenon is in agreement with findings of Amorim *et al.* (2012).

The results of this *Folsomia* experiment were difficult to assess because of the lack of published data to compare with. Most studies on the effects of soil properties on metal bioavailability and bioaccumulation concern earthworms, while very little is known about other soil organisms (Pedersen *et al.* 1997). Therefore, a test that is quick, easy, and inexpensive is needed for preliminary evaluation (Peredney & Williams, 2000). *F. candida* seems to fulfil these needs. Crommentuijn *et al.* (1997) reported that the metal toxicity in collembolans is dependent on organic matter and pH in the soil. Despite this shows that the species is relatively insensitive to soil properties and hence highly suitable to be used in ecotoxicological tests with natural soils (Domene *et al.* 2011).

It should be noted that there is genetic variation in parthenogenetic populations of *F. candida* and this variation affects reproduction relative to metal toxicity. This shed new light on the sources of biological variability in test results, even when the test organisms are thought to be genetically homogeneous because of their partenogenetic reproduction (Nota *et al.* 2013).

CONCLUSION

Combining the literature review (Farago & Parsons, 1994; Nemcova *et al.* 2012) and our new findings, we can confirm that the relative order of toxicities is Pd (II) > Pt(IV) >> Rh(III). Additionally, different invertebrate species such as *Folsomia candida*, *Enchytraeus albidus* and *Porcellionides pruinosus* showed different response patterns to metals, confirming that for an adequate ecological risk assessment several groups of organisms and endpoints should be included. No comprehensive studies about PGE behaviour and effects, their distribution in the food chain and bioaccumulation have yet been performed.

ACKNOWLEDGEMENT

The study was supported by grants IGA 25/2012/FVHE and FRVS 965/2013/G4. We would like to thank Charles du Parc for proofreading this paper.

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