Impact of platinum group elements on the soil invertebrate *Enchytraeus crypticus*

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Abstract

OBJECTIVES: The platinum group elements (PGE) platinum (Pt), palladium (Pd), and rhodium (Rh) are used in automobile catalytic converters, from which they have been emitted into the environment to an increasing degree over the last 20 years. Despite the bioavailability of these metals to plants and animals, studies determining the effects of PGE on organisms are extremely rare. Enchytraeids are ecologically relevant soil organisms, due to their activity in decomposition and bioturbation in many soil types worldwide.

DESIGN: The experiments were carried out as described in the OECD Guideline 220 [CSN EN ISO 16387 – Soil quality – Effects of pollutants on *Enchytraeidae* (*Enchytraeus sp.*) – Determination of effects on reproduction]. The reproductive effects of platinum ($PtCl_4$), palladium ($PdCl_2$) and rhodium ($RhCl_3$) were examined. The concentrations of PGE tested were as follows: 5, 10, 25, 50 and 100 μmol.L^{-1} $PdCl_2$; 50, 100, 150, 200 and 250 μmol.L^{-1} $PtCl_4$/$RhCl_3$. The EC_{50} (medium effective concentration) was determined after 28 days of exposure. The inhibition of the reproduction of PGE-exposed enchytraeids was compared against controls.

RESULTS: Values of 28dEC_{50} of $PtCl_4$, $PdCl_2$ and $RhCl_3$ amounted to 161.9 μmol.L^{-1}, 70.0 μmol.L^{-1} and 246.6 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 *Enchytraeus crypticus* as an indicator species to assess the risk of soil contamination by platinum, palladium and rhodium. Results of this study contribute important data on the ecotoxicity of a rarely studied elements.

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
PGE - platinum group element
SD - standard deviation
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)
LC_{50} - median lethal concentration – is the concentration of test substance that kills 50% of exposed test organisms within a given time period

ORIGINAL ARTICLE

INTRODUCTION

Ecotoxicity testing is a very useful tool to assess the toxicity of chemicals, contaminated soils and wastes. The ecotoxicity testing is usually a complementary analysis to chemical analysis (Mateju *et al.* 2014; Kobetičová *et al.* 2010). An increase in the ecological relevance of test data is one of the major challenges of soil ecotoxicology (Breitholtz *et al.* 2006; Kuperman *et al.* 2009; van Gestel, 2012; Chelinho *et al.* 2014). Contact bioassays are important for testing the ecotoxicity of solid materials.

Metal pollution in air, water and soil is a global problem that may have damaging effects on ecosystems and human health (He & van Gestel, 2013). The metals platinum (Pt), palladium (Pd), iridium (Ir), rhodium (Rh), ruthenium (Ru) and osmium (Os) are commonly referred to as the platinum group elements (PGE) (Zereini & Wiseman, 2010). Their chemical characteristics make them quite useful as catalysts in a variety of chemical and pharmaceutical processes. In particular, Pt, Pd and Rh are widely used in a number of applications such as in hydration and dehydration reactions in the pharmaceutical industry, in polymer processing and in the production of pesticides and dyestuffs (Wiseman & Zereini, 2009). Automobile catalytic converters have received the most attention as a primary contributor of PGE to the environment. Most PGE appear to be emitted in a metallic form, which is believed to be biologically inert (Jarvis *et al.* 2001). However, small amounts may be released in an oxidized state (Zereini & Wiseman, 2010). Several platinum metal salts are known to be toxic and have a significant potential to elicit hypersensitive reactions in susceptible individuals. The evidence suggests that Pd may be a more reactive species in the environment due to its greater solubility and bioavailability compared with other PGE. Palladium has been demonstrated to be more readily taken up by plants, animals and humans than Pt and Rh (Schäfer *et al.* 1998; Moldovan, 2007). The Pt tends to accumulate in the blood, urine, liver and is primarily excreted in urine, contributing to elevated concentrations in the waste water of hospitals. In Germany, it was estimated that a total of 14.2 kg of Pt was emitted from hospitals in 1996 (Kummerer *et al.* 1999). One recent study investigated the PGE concentrations in soils along a motorway in Germany. The soils had mean concentrations of 83 $\mu g.kg^{-1}$ (20–191 $\mu g.kg^{-1}$) for Pd, 132 $\mu g.kg^{-1}$ (41–254 $\mu g.kg^{-1}$) for Pt and 20 $\mu g.kg^{-1}$ (7–36 $\mu g.kg^{-1}$) for Rh (Zereini *et al.* 2006). The highest concentrations of PGE have been found to occur within 10 m of the roadside. Mihaljevič *et al.* (2013) reported the distribution of PGE in Prague and Ostrava (Czech Republic). Higher PGE contents were determined in Prague; the highest contents were found in the centres of both cities because of the slower, stop-and-go movement of automobiles during which more PGE's are emitted. The maximum PGE contents found were for Pt in Prague (160 $\mu g.kg^{-1}$), with lower contents of Pd (49 $\mu g.kg^{-1}$), followed by Rh (3.9 $\mu g.kg^{-1}$). The bioavailability of PGE in the environment is also discussed in several publications (Shams *et al.* 2014; Kowalska *et al.* 2014; Reith *et al.* 2014).

Soil invertebrates are commonly used to evaluate soil quality (Coleman *et al.* 2004) and ecotoxicity (Marks *et al.* 2014), and emerging soil quality assessments have been based on mesofauna responses because of observations of their sensitivity to unfavourable conditions and toxins, and also due to observed correlations between invertebrate community structure and soil quality parameters (van Straalen, 1998; Verhoef, 2004). In most organisms, the physiological tolerance mechanisms to heavy metals have been attributed, at least in part, to the induction of metal chelating gene products. This leads to the assumption that the genes responsible for this mechanism will be up-regulated or differentially expressed within the heavy metal exposed population. These metals chelating gene products include low molecular weight, cysteine rich proteins, or metallothioneins, known to be involved in heavy metal detoxification and homeostasis (Ardestani *et al.* 2014; Sturzenbaum *et al.* 1998).

Enchytraeids (*Enchytraeidae*, *Clitellata*, *Oligochaeta*) are ecologically relevant soil-dwelling annelids, which play an important role in organic matter decomposition and soil bioturbation (Didden & Römbke, 2001). They are widespread in many soil types and withstand acidic soil, tolerated by a few epigeic earthworm species such as *Dendrobaena octaedra*, *Dendrodrilus rubidus* and *Lumbricus rubellus* (Römbke, 2003; Butt & Lowe, 2004). Enchytraeids live in close contact with the porewater fraction of soil and their routes of exposure are dermal, intestinal and respiratory (Lock & Janssen, 2003; Castro-Ferreira *et al.* 2012). They play an important role in the soil ecosystem and are standard organisms of ecotoxicological studies (Jaensch *et al.* 2005).

The main aim of this study was to assess the reproduction test of *Enchytraeus crypticus* as a tool for the ecotoxicological assessment of platinum, palladium and rhodium applied to or contaminating soils.

MATERIAL & METHODS

Test organisms

The soil worm *Enchytraeus crypticus* originated in 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 220 [CSN ISO EN 16387 – Soil quality – Effects of pollutants on *Enchytraeidae* (*Enchytraeus* sp.) – Determination of effects on reproduction] with minor changes to fit our experimental conditions. This guideline was originally designed for testing with *Enchytraeus albidus*, but other enchytraeid species, like *E. crypticus*, are listed as an alternative. Due to the smaller size and shorter reproductive cycle of the latter species, the test duration was reduced to 28 days. In this case, 20 g of soil was used and the adults were kept in the vessels until the end of the test. For each vessel, ten enchytraeids were placed in the soil. The worms used in the test were adult with eggs in the clitellum region and they were of approximately the same size. Five replicates were used. As food supply, finely ground rolled oats were added. Weekly, when necessary, additional food and water were added. All tests were conducted at 20±2 °C with a 16:8 (light/dark) photoperiod. Both pH and moisture were measured at the beginning and end of the tests (an extra replicate, without organisms, was used for pH and moisture check, in the case of the reproduction tests with *E. crypticus*). 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 platinum ($PtCl_4$ – Sigma-Aldrich, p.a. 99.99%), palladium ($PdCl_2$ – Sigma-Aldrich, p.a. 99.99%) and rhodium ($RhCl_3$ – Sigma-Aldrich, p.a. 98%), in particular, were examined. The soil was contaminated by dissolved $PtCl_4$/PdCl2/$RhCl_3$ in an adequate amount of deionised water to achieve soil moisture equal to 50% of maximum water-holding capacity. The $PtCl_4$/$PdCl_2$/$RhCl_3$ 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} (0.084225 g $PtCl_4$ per 25 ml deionised water; 0.4433 g $PdCl_2$ per 250 ml deionised water; 0.05231 g $RhCl_3$ per 25 ml deionised water). Each solution was mixed with the soil immediately before use, leading to nominal concentrations of 50, 100, 150, 200, 250 µmol.L^{-1} $PtCl_4$/$RhCl_3$ and 5, 10, 25, 50, 100 µmol.L^{-1} $PdCl_2$ 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 220, 2010). 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 25 at the end of the test; the coefficient of variation (CV) calculated for the number of juveniles should be less than 50% at the end of the reproduction 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 corresponded to the normal level. A reference substance suitable for this test is boric acid, which should reduce reproduction by 50% at about 150–170 mg.kg^{-1} dry weight soil (OECD 220, 2010). The concentrations of boric acid tested were as follows: 70, 100, 140, 200 and 280 mg.kg^{-1}.

Statistical analysis

The data analysis software Statistica for Windows® 10 (StatSoft, Inc., Tulsa, OK, USA) was used to compare different PGE 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 *Enchytraeus crypticus* fulfilled the CSN ISO 16387 criteria for validation. The value of 28dEC_{50} (effective concentration) of the boric acid (the reference substance) test was 156.2 mg.kg^{-1} (Figure 1).

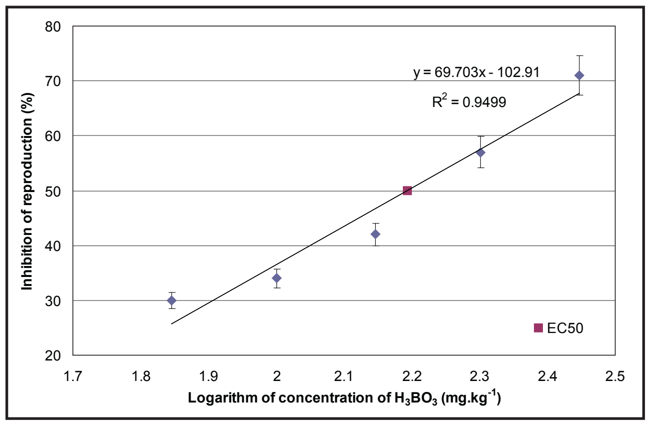

Fig. 1. Effect of boric acid (H_3BO_3) on reproduction in *E. crypticus* after 4 weeks of exposure in artificial soils. The data points are the averages of replicates (n=5). Error bars indicate standard deviations.

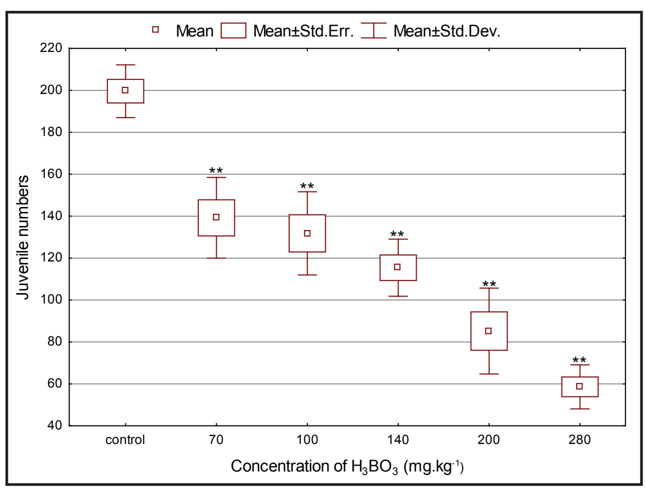

Fig. 2. Juvenile reproduction of *E. crypticus* exposed for 28 days to boric acid (H_3BO_3) (expressed as nominal total boric acid concentrations in the soil). ** = $p<0.01$ compared with the control (n=5 in each group).

In the control, adult survival was 100%, the average number of juveniles per vessel was 199.6 and the CV was 6% in juveniles. The results of the toxicity tests are shown in Figure 2. Mortality was significantly influenced (Figure 3). All CV values were less than 21% for each concentration of boric acid. Mean adult survival rates ranged from 6 to 10 out of 10 enchytraeids per vessel. A summary of H_3BO_3 toxicity in *E. crypticus* is shown in Table 1.

The $28dEC_{50}$ of $PtCl_4$ amounted to 161.9 $\mu mol.L^{-1}$ (Figure 4). The results of the toxicity tests are shown in Figure 5. No significant effect on mortality was found. All CV values were less than 32% for each concentration of platinum. Mean adult survival rates ranged from 8 to 10 out of 10 enchytraeids per vessel. A summary of $PtCl_4$ toxicity on *E. crypticus* is shown in Table 2.

The $28dEC_{50}$ of $PdCl_2$ amounted to 70.0 $\mu mol.L^{-1}$ (Figure 6). The results of the toxicity tests are shown

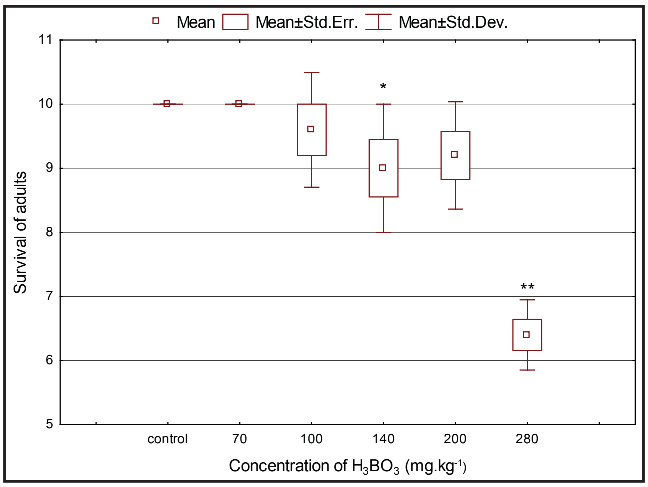

Fig. 3. Effect of boric acid (H_3BO_3) on mortality in *E. crypticus after four weeks* of exposure via artificial soils. The data points are the averages of replicates (n=5).

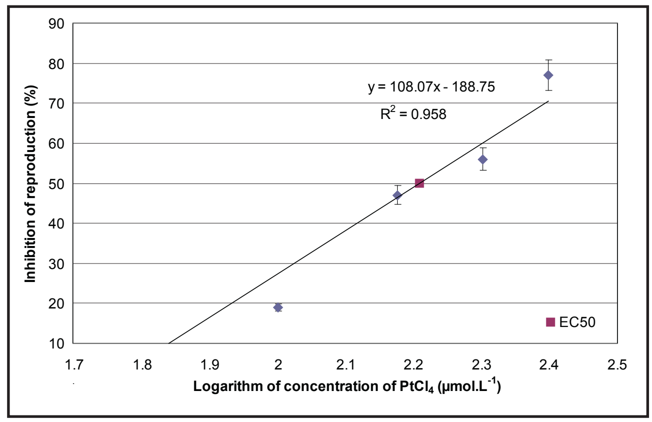

Fig. 4. Effect of platinum ($PtCl_4$) on reproduction in *E. crypticus* after 4 weeks of exposure in artificial soils. The data points are the averages of replicates (n=5). Error bars indicate standard deviations.

Tab. 1. Summary of boric acid (H_3BO_3) toxicity in *E. crypticus*.

Concentrations boric acid (mg.kg^{-1})	No. adult		Mortality (%)	No. juveniles produced (mean ± SD)	CV (%)	Inhibition of reproduction (%)
	at the beginning of the test	at the end of the test				
70	10	10	0	139.2 ± 17.2	12	30
100	10	9.6	4	131.8 ± 17.7	13	34
140	10	9	4	115.4 ± 12.2	11	42
200	10	9.2	8	85.2 ± 18.3	21	57
280	10	6.4	36	58.6 ± 9.4	16	71

CV - coefficient of variation, SD - standard deviation.

Tab. 2. Summary of platinum ($PtCl_4$) toxicity in *E. crypticus*.

Concentrations $PtCl_4$ (μmol.L^{-1})	No. adult		Mortality (%)	No. juveniles produced (mean ± SD)	CV (%)	Inhibition of reproduction (%)
	at the beginning of the test	at the end of the test				
50	10	9.8	2	130.0 ± 11.5	9	0
100	10	9.8	2	105.00 ± 12.4	12	19
150	10	9.4	6	69.2 ± 10.7	15	47
200	10	9.2	8	57.2 ± 12.4	22	56
250	10	8.8	12	30.4 ± 9.6	32	77

CV - coefficient of variation, SD - standard deviation

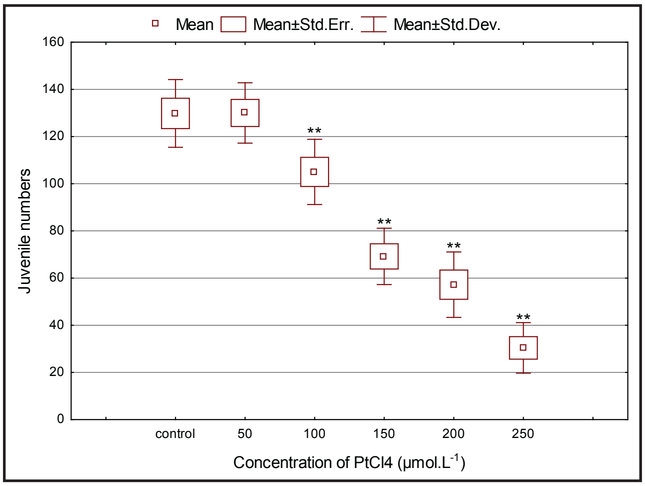

Fig. 5. Juvenile reproduction of *E. crypticus* exposed for 28 days to platinum ($PtCl_4$) (expressed as nominal total rhodium concentrations in the soil). * = p<0.05, ** = p<0.01 compared with the control (n=5 in each group).

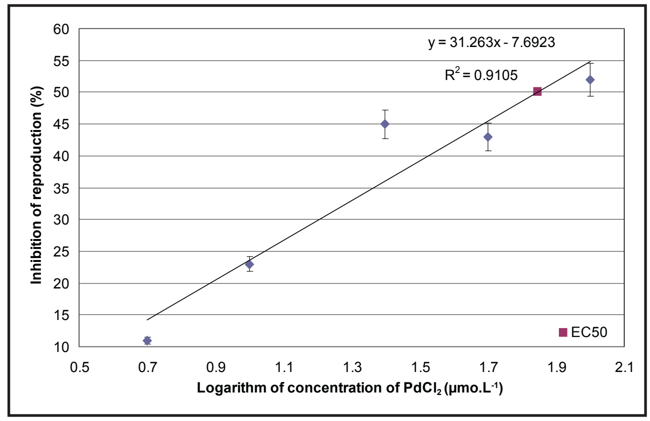

Fig. 6. Effect of palladium ($PdCl_2$) on reproduction in *E. crypticus* after 4 weeks of exposure in artificial soils. The data points are the averages of replicates (n=5). Error bars indicate standard deviations.

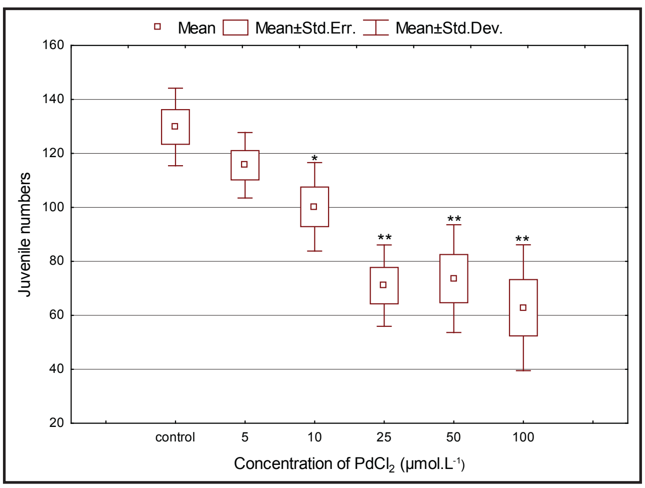

Fig. 7. Juvenile reproduction of *E. crypticus* exposed for 28 days to palladium ($PdCl_2$) (expressed as nominal total rhodium concentrations in the soil). * = p<0.05, ** = p<0.01 compared with the control (n=5 in each group).

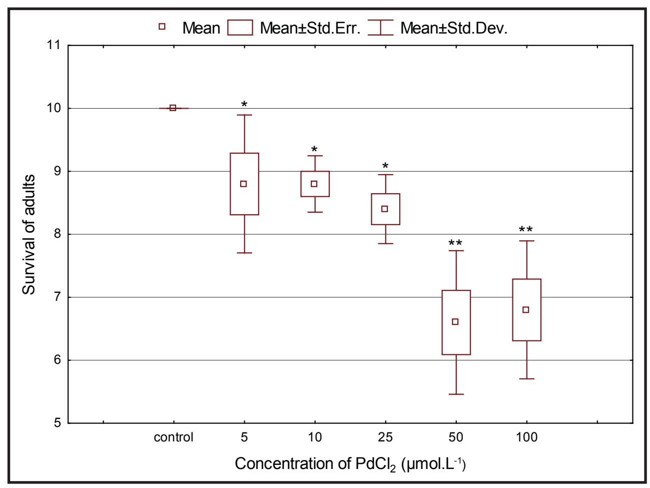

Fig. 8. Effect of palladium ($PdCl_2$) on mortality in *E. crypticus after four weeks* of exposure via artificial soils. The data points are the averages of replicates. * = p<0.05, ** = p<0.01 compared with the control (n=5 in each group).

Tab. 3. Summary of palladium ($PdCl_2$) toxicity in *E. crypticus*.

Concentrations $PdCl_2$ (µmol.L^{-1})	No. adult		Mortality (%)	No. juveniles produced (mean ± SD)	CV (%)	Inhibition of reproduction (%)
	at the beginning of the test	at the end of the test				
5	10	8.8	12	115.6 ± 10.9	9	11
10	10	8.8	12	100.2 ± 14.6	15	23
25	10	8.4	16	71.0 ± 13.5	19	45
50	10	6.6	34	73.6 ± 17.9	24	43
100	10	6.8	32	62.8 ± 20.9	33	52

CV - coefficient of variation, SD - standard deviation

in Figure 7. A significant effect on mortality was found (Figure 8). All CV values were less than 33% for each concentration of palladium. Mean adult survival rates ranged from 5 to 10 out of 10 enchytraeids per vessel. A summary of $PdCl_2$ toxicity on *E. crypticus* is shown in Table 3.

The $28dEC_{50}$ of $RhCl_3$ was 246.6 µmol.L^{-1} (Figure 9). The results of the toxicity tests are shown in Figure 10.

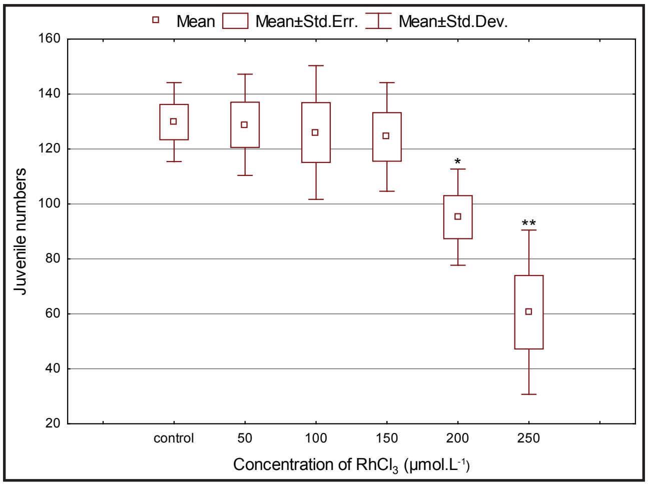

Fig. 9. Effect of rhodium ($RhCl_3$) on reproduction in *E. crypticus* after 4 weeks of exposure in artificial soils. The data points are the averages of replicates (n=5). Error bars indicate standard deviations.

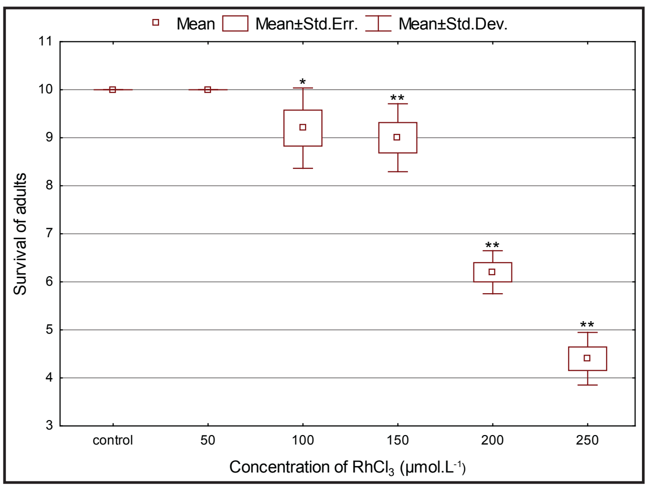

Fig. 10. Juvenile reproduction of *E. crypticus* exposed for 28 days to rhodium ($RhCl_3$) (expressed as nominal total rhodium concentrations in the soil). * = p<0.05, ** = p<0.01 compared with the control (n=5 in each group).

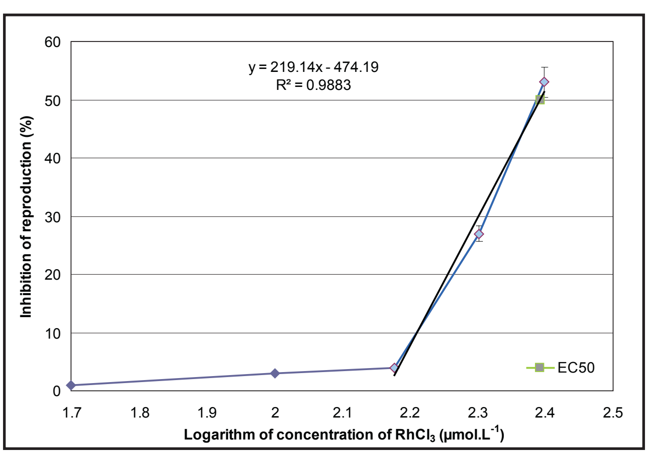

Fig. 11. Effect of rhodium ($RhCl_3$) on mortality in *E. crypticus after four weeks* of exposure via artificial soils. The data points are the averages of replicates (n=5).

There was a significant effect on mortality (Figure 11). The LC_{50} of $RhCl_3$ was 242.7 μmol.L^{-1}. All CV values were less than 44% for each concentration of platinum. Mean adult survival rates ranged from 4 to 10 out of 10 enchytraeids per vessel. A summary of $RhCl_3$ toxicity on *E. crypticus* is shown in Table 4. In the control ($PtCl_4$, $PdCl_2$, $RhCl_3$), adult survival was 100%, the average number of juveniles per vessel was 129.8 and the CV was 10% in juveniles.

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).

The results of this *E. crypticus* 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). There are recently published papers about the ecotoxicity of different PGE, where authors studied the impact e.g., on zebrafish (*Danio rerio*) and ramshorn snail (*Marisa cornuarietis*) (Osterauer *et al.* 2009), European eels (*Anguilla anguilla*) (Sures *et al.* 2001), rats (Artelt *et al.* 1999), zebra mussels (*Dreissena polymorpha*) (Zimmermann *et al.* 2002), green algae (*Pseudokirchneriella subcapitata*) and *Lemna minor (Bednarova et al. 2012).*

In the present study with *E. crypticus*, reproduction showed a continuous decrease as the PGE concentration increased. The value of $28dEC_{50}$ of the boric acid (the reference substance) for reproduction which was determined by this study is compatible with the results published by Mateju *et al.* (2014). It can be assumed that the test was done precisely and can be declared as valid.

After exposure in OECD artificial soil, the toxicity of PGE for *E. crypticus* was compared to the literature data for *Folsomia candida (*Nemcova *et al.* 2012; Nemcova *et al.* 2013*).* As shown in Table 5, the EC_{50} values of $PtCl_4$/$RhCl_3$ obtained for *E. crypticus* were lower than for *F. candida*. However, the EC_{50} values of $PdCl_2$ obtained for *E. crypticus* were higher than for *F. candida*. The results have shown that palladium is more toxic than the others for both of the organisms. Palladium has been demonstrated to be more readily taken up by plants, animals and humans than Pt and Rh (Schäfer *et al.* 1998; Moldovan, 2007). Moreover survival of *E. crypticus* was significantly different from *F. candida*. Survival of *F. candida* adults was not significantly affected. Mean adult survival rates ranged from 8 to 10

Tab. 4. Summary of rhodium ($RhCl_3$) toxicity in *E. crypticus*.

Concentrations $RhCl_3$ (µmol.L^{-1})	No. adult at the beginning of the test	No. adult at the end of the test	Mortality (%)	No. juveniles produced (mean ± SD)	CV (%)	Inhibition of reproduction (%)
50	10	10	0	128.8 ± 16.5	13	1
100	10	9.2	8	126.0 ± 21.8	17	3
150	10	9	10	124.4 ± 17.7	14	4
200	10	6.2	38	95.2 ± 15.7	16	27
250	10	4.4	56	60.6 ± 26.7	44	53

CV - coefficient of variation, SD - standard deviation

out of 10 collembolans per vessel (Nemcova *et al.* 2012; Nemcova *et al.* 2013). Survival was not affected to as great an extent as reproduction. However, a significant effect of $PdCl_2$ and $RhCl_3$ on mortality of *E. crypticus* was found. Mean adult survival rates ranged from 4 to 10 out of 10 enchytraeids per vessel. In comparison to *F. candida*, it was concluded that *E. crypticus* had relatively higher susceptibility to the toxic effects caused by platinum and rhodium, but relatively lower to that by palladium. 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).

Tab. 5. Effect concentration for the toxicity of three tested chemicals to the reproduction (EC_{50}) and survival (LC_{50}) of *Enchytraeus crypticus* and *Folsomia candida* after 4 weeks of exposure in OECD artificial soil.

Test chemical	Species	EC_{50} (µmol.L^{-1})	LC_{50} (µmol.L^{-1})	References
Platinum ($PtCl_4$)	*E. crypticus*	161.9	–	Present study
	F. candida	200.4	–	Nemcova *et al.* (2012)
Palladium ($PdCl_2$)	*E. crypticus*	70	–	Present study
	F. candida	21	–	Nemcova *et al.* (2013)
Rhodium ($RhCl_3$)	*E. crypticus*	246.2	247.2	Present study
	F. candida	266.2	–	Nemcova *et al.* (2013)

CONCLUSION

Combining the literature review (Farago & Parsons, 1994; Nemcova *et al.* 2013) and our new findings, we can confirm that the relative order of toxicities is Pd(II) >Pt(IV) >>Rh(III). Very little is known about the geochemical behaviour of emitted PGE in the biosphere, especially in terms of their mobility and solubility in soils under natural conditions. More toxicity data for various species are needed to evaluate the environmental risks of PGE in soils.

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REFERENCES

1 Amorim MJB, Pereira C, Menezes-Oliveira VB, Campos B, Soares AMVM, Loureiro S (2012). Assessing single and joint effects of chemicals on the survival and reproduction of *Folsomia candida* (Collembola) in soil. Environ Pollut. **160**: 145–152.
2 Ardestani MM, Van Straalen NM, Van Gestel CAM (2014). Uptake and elimination kinetics of metals in soil invertebrates: A review. Environ Pollut. **193**: 277–295.
3 Artelt S, Kock H, Konig HP, Levsen K, Rosner G (1999). Engine dynamometer experiments: platinum emissions from differently aged three-way catalytic converters. Atmos Environ. **33**: 3559–3567.
4 Bednarova I, Haasova V, Mikulaskova H, Nemcova B, Strakova L, Beklova M (2012). Comparison of the effect of platinum on producers in aquatic environment. Neuroendocrinol Lett. **33**: 107–112.
5 Breitholtz M, Rudén C, Hansson SO, Bengtsson B-E (2006). Ten challenges for improved ecotoxicological testing in environmental risk assessment. Ecotox Environ Safe. **63**: 324–335.
6 Butt KR, Lowe CN (2004). Anthropic influences on earthworm distribution, isle of rum national nature reserve. Eur J Soil Biol. **40**: 63–72.
7 Castro-Ferreira MP, Roelofs D, Van Gestel CAM, Verweij RA, Soares AMVM, Amorim MJB (2012). *Enchytraeus crypticus* as model species in soil ecotoxicology. Chemosphere. **87**: 1222–1227.
8 Chelinho S, Domene X, Campana P, Andrés P, Römbke J, Sousa JP (2014). Toxicity of phenmedipham and carbendazim to *Enchytraeus crypticus* and *Eisenia andrei* (Oligochaeta) in Mediterranean soils. J Soils Sediment. **14**: 584–599.
9 Coleman DC, Crossley Jr DA, Hendrix PF (2004). Fundamentals of Soil Ecology. 2nd ed. London: Elsevier. p. 169 – 179.
10 Crouau Y, Gisclard C, Perotti P (2002). The use of *Folsomia candida* (Collembola, Isotomidae) in bioassays of waste. Appl Soil Ecol. **19**: 65–70.
11 CSN EN ISO 16387 (2014). Kvalita půdy – Vliv znečišťujících látek na *Enchytraeidae* (*Enchytraeus* sp.) – Stanovení vlivu na reprodukci. (Soil quality – Effects of pollutants on *Enchytraeidae* (*Enchytraeus* sp.) – Determination of effects on reproduction. 23 p.
12 Didden WAM, Römbke J (2001). Enchytraeids as indicator organisms for chemical stress in terrestrial ecosystems. Ecotox Environ Safe. **50**: 25–43.
13 Farago Me, Parsons PJ (1994). The effects of various platinum metal species on the water-plant *Eichhornia crassipes* (Mart) solms. Chem Spec Bioavailab. **6**: 1–12.

14 He E, Van Gestel CAM (2013). Toxicokinetics and toxicodynamics of nickel in *Enchytraeus crypticus*. Environ Toxicol Chem. **32**: 1835–1841.
15 Jaensch S, Amorim MJ, Roembke J (2005). Identification of the ecological requirements of important terrestrial ecotoxicological test species. Environ Rev. **13**: 51–83.
16 Jarvis KE, Parry SJ, Piper JM (2001). Temporal and spatial studies of autocatalyst-derived platinum, rhodium, and palladium and selected vehicle-derived trace elements in the environment. Environ Sci Technol. **35**: 1031–1036.
17 Kobetičová K, Hofman J, Holoubek I (2010). Ecotoxicity of wastes in avoidance tests with *Enchytraeus albidus*, *Enchytraeus crypticus* and *Eisenia fetida* (Oligochaeta). Waste Manage. **30**: 558–564.
18 Kowalska J, Kińska K, Pałdyna J, Czyżewska M, Boder K, Krasnodębska-Ostręga B (2014). Determination of traces of Pt and Rh in soil and quartz samples contaminated by automobile exhaust after an ion-exchange matrix separation. Talanta. **127**: 250–254.
19 Kummerer K, Helmers E, Hubner P, Mascart G, Milandri M, Reinthaler F, Zwakenberg M (1999). European hospitals as a source for platinum in the environment in comparison with other sources. Sci total environ. **225**: 155–165.
20 Kuperman RG, Checkai RT, Garcia MVB, Römbke J, Stephenson GL, Sousa JP (2009). State of the science and the way forward for the ecotoxicological assessment of contaminated land. Pesqui Agropecu Bras. **44**: 811–824.
21 Lock K, Janssen CR (2003). Influence of aging on metal availability in soils. Rev Environ Contam T. **178:** 1–21.
22 Marks EAN, Mattana S, Alcañiz JM, Domene X (2014). Biochars provoke diverse soil mesofauna reproductive responses in laboratory bioassays. Eur J Soil Biol. **60**: 104–111.
23 Mateju V, Vosahlova S, Kyclt R, Janoch T, Sedivcova G (2014). The reproduction of *Enchytraeus* sp. – technical improvement for the counting of juveniles. Environ Monit Assess. **186**: 711–718.
24 Mihaljevič M, Galušková I, Strnad L, Majer V (2013). Distribution of platinum group elements in urban soils, comparison of historically different large cities Prague and Ostrava, Czech Republic. J Geochem Explor. **124**: 212–217.
25 Moldovan M (2007). Origin and fate of platinum group elements in the environment. Anal Bioanal Chem. **388**: 537–540.
26 Nemcova B, Bednarova I, Mikulaskova H, Beklova M (2012). Impact of platinum on the soil invertebrate *Folsomia candida*. Neuroendocrinol Lett. **33**: 173–178.
27 Nemcova B, Bednarova I, Mikulaskova H, Beklova M (2013). Study of the impact of platinum and palladium on representatives of soil fauna (*Folsomia candida*). Neuroendocrinol Lett. **34**: 5–10.
28 OECD 220 (2004). Guidelines for the testing of chemicals: Enchytraeid reproduction test. 22 p.
29 Osterauer R, Haus N, Sures B, Kohler HR (2009). Uptake of platinum by zebrafish (*Danio rerio*) and ramshorn snail (*Marisa cornuarietis*) and resulting effects on early embryogenesis. Chemosphere. **77**: 975–982.
30 Pedersen MB, Temminghoff EJM, Marinussen MPJC, Elmegaard N, van Gestel CAM (1997). Copper accumulation and fitness of *Folsomia candida* Willem in a copper contaminated sandy soil as affected by pH and soil moisture. Appl Soil Ecol. **6**: 135–146.
31 Reith F, Campbell SG, Ball AS, Pring A, Southam G (2014). Platinum in Earth surface environments. Earth Sci Rev. **131**: 1–21.
32 Römbke J (2003). Ecotoxicological laboratory tests with enchytraeids: a review. Pedobiologia. **47**: 607–616.
33 Schäfer J, Hannker D, Eckhardt JD, Stüben D (1998). Uptake of traffic-related heavy metals and platinum group elements (PGE) by plants. Sci Total Environ. **215**: 59–67.
34 Shams L, Turner A, Millward GE, Brown MT (2014). Extra- and intra-cellular accumulation of platinum group elements by the marine microalga, *Chlorella stigmatophora*. Water Res. **50**: 432-440.
35 Sturzenbaum SR, Kille P, Morgan AJ (1998). The identification, cloning and characterization of earthworm metallothionein. Febs Lett. **431**: 437–442.
36 Sures B, Zimmermann S, Messerschmidt J, Von Bohlen A, Alt F (2001). First report on the uptake of automobile catalyst emitted palladium by European eels (*Anguilla anguilla*) following experimental exposure to road dust. Environ Pollut. **113**: 341–345.
37 Van Gestel CAM (2012). Soil ecotoxicology: state of the art and future directions. ZooKeys. **176**: 275–296.
38 Van Straalen NM (1998). Evaluation of bioindicator systems derived from soil arthropod communities. Appl Soil Ecol. **9**: 429–437.
39 Verhoef HA (2004). Soil biota and activity. In: Doelman P, Eijsackers HJP, editors. Vital soil: function, value and properties. Amsterdam: Elsevier. p. 99 – 126.
40 Wiseman CLS, Zereini F (2009). Airborne particulate matter, platinum group elements and human health: a review of recent evidence. Sci Total Environ. **407**: 2493–2500.
41 Zereini F, Wiseman C, Püttmann W (2006). Changes in palladium, platinum, and rhodium concentrations, and their spatial distribution in soils along a major highway in germany from 1994 to 2004. Environ Sci Technol. **41**: 451–456.
42 Zereini F, Wiseman CLS (2010). Platinum Group Elements. In: Hooda PS, editor. Trace Elements in Soils. Chichester, UK: John Wiley & Sons. p. 567–577.
43 Zimmermann S, Alt F, Messerschmidt J, Von Bohlen A, Taraschewski H, Sures B (2002). Biological availability of traffic-related platinum-group elements (palladium, platinum, and rhodium) and other metals to the zebra mussel (*Dreissena polymorpha*) in water containing road dust. Environ Toxicol Chem. **21**: 2713–2718.