

Investigating the toxicity and bioaccumulation of palladium black and palladium chloride in earthworms (*Eisenia fetida*)

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Research Article

Keywords: Platinum group element, Terrestrial, Soil, Metal, Pollution, Vehicle emissions

Posted Date: September 30th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11043901/v1>

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Additional Declarations: No competing interests reported.

Abstract

Palladium (Pd) emissions to the soil are increasing due to the weathering of automotive catalytic converters. However, the toxicity and bioavailability of different Pd speciation in soil organisms remains poorly understood. This study is the first to investigate the toxicity of Pd in *Eisenia fetida* (earthworm) and to compare the toxicity and bioaccumulation of soluble PdCl₂ and particulate Pd black in soil. A 28-day soil exposure experiment was conducted to evaluate the effects of PdCl₂ and Pd black on the survival, body weight, and Pd bioaccumulation of *E. fetida*. Exposure to 50 mg/kg PdCl₂ resulted in a 17% greater weight loss than control ($P = 0.007$), whereas Pd black at the same concentration caused no significant effect on body weight. Furthermore, Pd bioaccumulation in *E. fetida* was significantly higher following exposure to PdCl₂ than to Pd black at both 10 mg kg⁻¹ ($P = 0.014$) and 50 mg kg⁻¹ ($P < 0.001$) treatments. These findings suggest that particulate Pd species, such as Pd black, are less readily taken up by earthworms unless they are transformed into more soluble forms in soil or within the earthworm digestive system. The results highlight the importance of Pd speciation in determining its environmental fate and ecological risk, suggesting that the transformation of metallic Pd into soluble Pd species may increase Pd bioavailability and toxicity in terrestrial environments.

1. Introduction

Platinum-group elements (PGEs), including palladium (Pd), platinum (Pt), and rhodium (Rh), are used in vehicle catalytic converters to reduce emissions of hydrocarbons, carbon monoxide, and nitrogen oxides (NO_x) (Aarzo et al., 2022). However, catalyst wear during vehicle operation can release PGE particles into the environment (Aarzo et al., 2022) with the accumulation of PGEs for vehicle emissions detected in soil, water, air and organisms (Savignan et al., 2021).

Since the 1990s, Pd-Rh catalytic converters have increasingly replaced Pt-Rh catalytic converters in gasoline vehicles (Aarzo et al., 2022). Consequently, environmental Pd concentrations in roadside soil have increased over recent decades. For example, Pd concentrations in highway soil in Germany increased from 8.9 µg kg⁻¹ in 1994 to 210 µg kg⁻¹ in 2016 (Zereini et al., 2016). More recently, Pd has been found in roadside soil and dust at concentrations between 100 and 2000 µg kg⁻¹, with vehicle catalytic converters being the primary anthropogenic source of Pd in the environment (Savignan et al., 2021).

After entering soils, Pd particles can undergo chemical transformation into different Pd species (Šebek et al., 2011; Suchá et al., 2016). Palladium emitted from automotive catalytic converters occurs predominantly as particulate metals or oxides, although a small proportion may be present in relatively soluble forms (Moldovan et al., 2002; Palacios et al., 2000). Environmental weathering and dissolution may further produce hydroxyl- and chloride-associated Pd species with greater mobility than metallic Pd (Šebek et al., 2011; Suchá et al., 2016). Consequently, both particulate and relatively more soluble Pd species may coexist in contaminated soils. Therefore, ecotoxicity assessments should consider

environmentally relevant Pd species, as differences in chemical speciation are likely to influence Pd bioavailability

As different Pd forms occur in soil, the bioaccumulation and toxicity of Pd to soil organisms may differ based on the species of Pd they are exposed to. Studies with other metals have shown that toxicity and bioaccumulation in soil invertebrates varies depending on chemical species (Singh et al., 2024; Velicogna et al., 2021). For example, differences in bioaccumulation among Pb species, including PbCl_2 , PbCO_3 , PbO , and PbS , have also been reported in earthworms (*Eisenia fetida*) (Žaltauskaitė et al., 2020). However, there are no published comparable studies for toxicity and bioaccumulation of different Pd species in soil invertebrate.

Previous studies have shown that Pd may be more toxic than other PGEs to aquatic organisms (Havelkova et al., 2014; Schertzinger et al., 2017). For instance, Schertzinger et al. (2017) reported that the 96-hr 50% effect concentration (EC_{50}) for reproduction in the nematode *Caenorhabditis elegans* ranged from 10 to 100 $\mu\text{g L}^{-1}$ for Pd. In comparison, Pt showed a substantially higher EC_{50} value (497 $\mu\text{g L}^{-1}$), and Rh showed no observable effects on reproduction at concentrations up to 10,000 $\mu\text{g L}^{-1}$. Similar trends were observed in midge larvae *Chironomus riparius*, where Pd showed greater chronic toxicity than Pt (Luderwald et al., 2016).

While several studies have investigated the toxicity of Pd to aquatic organisms, including nematodes (Schertzinger et al., 2017), fish (Anila et al., 2021), and midge larvae (Luderwald et al., 2016), studies in terrestrial environments remain limited. To date, only two studies have examined Pd toxicity in soil invertebrates (Nemcova et al., 2013; Havelkova et al., 2014) with no published earthworm studies despite earthworms being key soil ecosystem engineers whose activities influence soil structure, organic matter decomposition, nutrient cycling, microbial processes and plant productivity. Therefore, in this study, we investigated the toxicity and bioaccumulation of two environmentally relevant Pd species, particulate Pd black and soluble PdCl_2 , to the earthworm, *Eisenia fetida*.

2. Materials and Methods

The toxicity and bioaccumulation of Pd black and PdCl_2 in soil were examined via an exposure experiment with *Eisenia fetida* (earthworm) that assessed earthworm survival, weight loss and Pd bioaccumulation.

2.1 Soil preparation

The soil was collected from the Burnley campus of the University of Melbourne, Victoria, Australia (-37° 49' 51.71" S, 145° 1' 30.54" E). The collected soil was air dried for 7 days followed by sieving to < 2 mm. Sieved soil was confirmed as free from Pd (< 0.016 $\mu\text{g/kg}$) by measurement on an Agilent 8900 Inductively Coupled Plasma Triple Quadrupole Mass Spectrometry (ICP-QQQ) after acid digestion with aqua regia (Rayment and Lyons, 2010). Soil characteristics are summarized in Table 1.

Table 1
Physicochemical characteristics of soil collected from the Burnley campus of the University of Melbourne, Victoria, Australia

Soil property	Value	Method
Soil texture (%)	Clay: 0.46% Silt: 18% Sand: 81.5%	(Gee and Bauder, 1986)
Water holding capacity (%)	55%	(OECD, 2016)
Effective Cation Exchange Capacity (ECEC) (cmol/kg)	6.5	15E-1 (Ammonium Acetate) (Rayment and Lyons, 2010)
Calcium (%)	73.3	
Magnesium (%)	16.1	
Potassium (%)	10.1	
Sodium (%)	0.5	
pH	6.2	1:5 soil/water extract (Rayment and Lyons, 2010)
Electrical conductivity (EC) (µS/cm)	92	1:5 soil/water extract (Rayment and Lyons, 2010)
Total Carbon (%)	1.76	LECO CNS TruMAC Analyser (Rayment and Lyons, 2010)
Total Nitrogen (%)	0.17	
Carbon: Nitrogen ratio	10.35	
Metal(oid) concentration		
Palladium (mg/kg)	< 0.016 µg/kg	APHA 3125 ICPMS (Rayment and Lyons, 2010)
Arsenic (mg/kg)	1.93	
Cadmium (mg/kg)	< 0.00	
Chromium (mg/kg)	8.05	
Copper (mg/kg)	9.22	
Lead (mg/kg)	1.42	
Manganese (mg/kg)	175	
Nickel (mg/kg)	5.79	

Soil property	Value	Method
Selenium (mg/kg)	0.26	
Silver (mg/kg)	< 0.00	
Zinc (mg/kg)	3.30	

2.2 Palladium solution preparation

Palladium chloride (PdCl_2) and palladium black were purchased from Novachem Pty Ltd (Heidelberg West, VIC, Australia). A 200 mg Pd L^{-1} stock solution of PdCl_2 was prepared by dissolving PdCl_2 in ultrapure water ($\geq 18.2 \text{ M}\Omega\cdot\text{cm}$) and gradually adding 1 M HNO_3 to support the dissolution. The acidity of the PdCl_2 stock solution was then reduced using 1 M NaOH to reach $\text{pH } 5.0 \pm 0.2$. A separate batch test confirmed that adding PdCl_2 stock solution at pH 5 to the test soil did not alter the test soil pH (data not presented). The Pd concentration of the PdCl_2 stock solution was $200 \pm 5 \text{ mg L}^{-1}$, confirmed by ICP-QQQ analysis after appropriate dilution, using an external Pd calibration curve over the range of $1\text{--}10 \text{ mg L}^{-1}$ ($R^2 = 0.999$) before adding to the test soil. For the Pd black stock solution, Pd black was added to ultrapure water to reach 200 mg L^{-1} . Before adding Pd black solution into the test soil, the Pd black solution was shaken to ensure that Pd black particles were uniformly suspended in water. To verify the Pd concentration of the Pd black stock solution, a separate Pd black suspension was prepared using the same amount of Pd black and subjected to the same pH-adjustment procedure used for the PdCl_2 stock solution to facilitate Pd dissolution. The resulting solution was analysed by ICP-QQQ, and the Pd concentration was $200 \pm 5 \text{ mg L}^{-1}$.

2.3 Exposure experiment for *Eisenia fetida* with Pd black and PdCl_2

The exposure experiment was based on the OECD Earthworm Reproduction Test (OECD, 2016). *Eisenia fetida* (250–600 mg wet mass) were selected from established laboratory cultures and transferred to untreated Burnley soil with cow manure added (50 g per 500 g soil) as food source in a polystyrene box for 7 days acclimatisation. Earthworms were then removed from the soil, washed with ultrapure water and placed on Whatman 90 mm filter paper (Cytiva, UK) to remove excess water before being reweighed.

The effects of Pd black and PdCl_2 were each tested at five Pd concentrations ($0, 0.1, 1.0, 10, 50 \text{ mg kg}^{-1}$) with each treatment replicated three times. For each treatment, 500 g of air-dried soil were added to 1 L polypropylene (PP) containers. The soil was then moistened by adding ultrapure water to reach 25% of the maximum water holding capacity, and Pd black or PdCl_2 was then spiked to the soil and stirred for homogenization. Sequentially, the Pd-spiked soil was further moistened by adding ultrapure water to reach 50% of the maximum water holding capacity.

Fifteen acclimatised earthworms were then added to each Pd spiked soil container. Five grams of cow manure, as a food source, were spread on the soil surface of each container and moistened with an extra

0.5 mL ultrapure water before the containers were sealed with a polypropylene lid.

The experimental containers were placed in an incubation chamber set at $20 \pm 2^\circ\text{C}$ and daily light-dark cycles (light: dark = 16 hr: 8 hr) with illumination of 570 ± 30 lux. An extra 5 g of cow manure was provided once a week as a food source. The water content in the sample was maintained throughout the experiment by weighing the containers twice a week, and any losses were replenished with ultrapure water to 25% of water holding capacity.

After 28 days, the soil from each experimental container was poured into a separate clean tray. Living earthworms were collected from each tray, washed with ultrapure water and placed on moistened filter paper in a clean plastic Petri dish with lid for 12 hours to depurate. No dead earthworms were found so earthworms were recorded as dead if they were not found in the soil. After depuration, live earthworms were weighed and stored in a 50 mL polypropylene tube for further analysis.

1. Bioaccumulation assessment of Pd black and PdCl₂ through measuring Pd concentration in earthworm after exposure experiment

For the bioaccumulation assessment, earthworms in 50 mL polypropylene tube were freeze-dried for 24 hours before being ground into a powder in a mortar and pestle. For each sample, a 500 mg aliquot of powdered earthworm tissue was weighed into a glass digestion tube with a 5 mL of aliquot of aqua regia (3:1 37% HCl: 70% HNO₃) added. The mixture was left to cold digest overnight before heating the digestion block to 110°C for 2 hours. After cooling, the digest was diluted to 25 mL in a volumetric flask with ultrapure water before being filtered through a $0.45\ \mu\text{m}$ cellulose acetate syringe filter (WS6-04525N, Hawach, China) into a polypropylene tube. Samples were then stored in the refrigerator at 4°C before being analyzed using ICP-QQQ within 3 days.

The Pd digestion and quantification method was validated using pre- and post-digestion Pd-spiked earthworm samples (SI Table S2). Recovery was 101% at $0.1\ \text{mg Pd kg}^{-1}$ earthworm, although lower recovery (73%) was observed at $0.015\ \text{mg Pd kg}^{-1}$ earthworm.

2.5 Statistical analysis

Earthworm survival was calculated as the number of living earthworms at the end of the experiment (day 28) divided by the number of earthworms in each container at the start of experiment (i.e. 15). The weight change was calculated as the difference between the mean weight of earthworms in a container at the start of the experiment minus the mean weight at the end of the experiment divided by the mean weight of earthworms at the start of the experiment. The survival rate and weight change between treatments were each compared by Kruskal Wallis test by ranks. Where the Kruskal Wallis test was significant ($P < 0.05$), Dunn's test was then used to differentiate which treatments were significantly different.

The bioaccumulation of Pd in earthworms was normalized by dividing the measured Pd concentration in the earthworm by the final weight of the earthworms in a given container. The bioaccumulation factor

(BAF) was calculated as the Pd concentration in earthworm divided by the spiked Pd concentration in the soil. Two-way ANOVA was used to compare the effects of Pd species and concentrations on Pd bioaccumulation. To meet the assumptions of normality and homogeneity of variance, a natural log transformation was applied to Pd concentration in earthworms data (Shapiro–Wilk test, $W = 0.955$; $P = 0.281$) before analysis by ANOVA. Post-hoc pairwise comparisons of estimated marginal means were applied when $P < 0.05$ for two-way ANOVA.

All statistical analysis, including descriptive analysis (e.g. means, standard deviation), Kruskal-Wallis test, Dunn's test, two-way ANOVA, post-hoc pairwise comparisons of estimated marginal means, and visualization of data, were conducted in R environments (version 4.0.5) with the “ggpubr”, “car”, “emmeans”, and “FSA” packages (Fox and Weisberg, 2019; Kassambara, 2020; Ogle et al., 2022; Lenth, 2025). Statistical tests were considered significant when $P < 0.05$.

3. Results

3.1 Survival and Weight loss

The survival of *Eisenia fetida* was higher than 90% in all treatments, and there was no significant difference in survival between the soil control and exposure to Pd black or PdCl₂ at all treatment concentrations (Kruskal-Wallis $X^2 = 6.324$; d.f. = 8; $P = 0.611$; Fig. 1). However, weight loss of *E. fetida* was significantly increased by exposure to PdCl₂ (Kruskal-Wallis $X^2 = 16.36$; d.f. = 8; $P = 0.038$; Fig. 1). The PdCl₂ treatments had ~ 17% higher weight loss in the 50 mg kg⁻¹ treatment compared to the soil control ($P = 0.007$). In addition, there was no significant difference in the weight loss of *E. fetida* between the control soil and the 10 mg kg⁻¹ PdCl₂ treatment ($P = 0.681$), while *E. fetida* exposed to 50 mg kg⁻¹ showed a ~ 16% higher weight loss than earthworms exposed to 10 mg kg⁻¹ ($P = 0.002$). Therefore, the no observed effect concentration (NOEC) of PdCl₂ on *E. fetida* weight was 10 mg kg⁻¹ in this study.

3.2 Bioaccumulation

The concentration of Pd in *Eisenia fetida* increased with increasing exposure to Pd in the soil ($F = 354.39$; d.f. = 3; $P < 0.001$). The Pd species affected the bioaccumulation of Pd in earthworms ($F = 116.42$; d.f. = 2; $P < 0.001$; Fig. 2). Thus, *E. fetida* had higher bioaccumulation of Pd when exposed to PdCl₂ than Pd black in the 10 mg kg⁻¹ ($P = 0.014$) and 50 mg kg⁻¹ ($P < 0.001$) treatments (Fig. 2). The bioaccumulation factor (BAF) of Pd was greater than 1 at exposure concentrations of 0.1 and 1 mg kg⁻¹ for both Pd treatments (Fig. 2). However, the BAF gradually decreased with increasing exposure concentration for both PdCl₂ and Pd black.

4. Discussion

To date, only two studies investigate Pd toxicity in soil organisms (Table 2). Nemcova et al. (2013) and Havelkova et al. (2014) reported reproductive effects and increased mortality following PdCl₂ exposure to *Enchytraeus crypticus* and *Folsomia candida*, respectively. However, these studies only reported concentrations of PdCl₂ solution added to the soil and not final soil exposure concentrations which makes it difficult to ascertain soil concentration of Pd the organisms were exposed to. Consequently, the present study on earthworms provides the first standardised soil concentration datasets for Pd toxicity in a soil invertebrate.

Table 2
Ecotoxicity studies of Pd for different soil species

Reference	Species	Matrix	Material	Concentration	Notes
This study	Earthworm (<i>Eisenia fetida</i>)	Soil	Pd black and PdCl ₂	0.1–50 mg/kg	NOEC of 10 mg/kg for weight loss; No effect on survival
Havelkova et al. (2014)	Annelida (<i>Enchytraeus crypticus</i>)	Soil	PdCl ₂	5-100 μ mole/L	28-d EC ₅₀ of 70 μ mol/L for reproduction 32% Mortality at 100 μ mol/L
Nemcova et al. (2013)	Collembola (<i>Folsomia candida</i>)	Soil	PdCl ₂	5-100 μ mole/L	28-d EC ₅₀ : 21 μ mol/L for reproduction No effect on survival

Our results suggest that the risk of Pd toxicity to earthworms is low at environmentally relevant concentrations. Palladium (Pd) has been detected in roadside soils and dust at concentrations ranging from 100 to 2,000 μ g kg⁻¹ (Savignan et al., 2021). In the present study, a significant reduction in body weight was observed only at 50 mg kg⁻¹ PdCl₂, which is substantially higher than currently reported environmental concentrations. However, Pd concentrations in roadside soils have increased over recent decades, and continued accumulation due to increasing emissions may elevate the ecological risk in the future.

In the research presented here, PdCl₂ showed greater toxicity to *Eisenia fetida* than particulate Pd black. Thus there was significant weight loss observed at 50 mg kg⁻¹ (P = 0.007) for PdCl₂ but Pd black caused no significant effects on survival (P = 0.611) or weight (P = 0.258) even at the highest Pd concentration tested (50 mg/kg) (Fig. 1). In addition, Pd accumulation in *E. fetida* was significantly higher in PdCl₂ treatments than in the Pd black treatments (P < 0.001). The combination of higher toxicity and bioaccumulation suggests that the chemical form of Pd strongly affects Pd bioavailability in soil. As Pd black and PdCl₂ differ in oxidation state and solubility, these findings further imply that transformation of metallic Pd into soluble Pd species in soil may increase Pd toxicity in terrestrial environments.

The higher bioaccumulation observed for PdCl₂ relative to Pd black in earthworms may be related to the greater bioavailability of more soluble Pd species in soil. Dissolved metals in soil pore water can be absorbed by earthworms through dermal contact as well as through ingestion of soil particles (Vijver et al., 2003; Diez-Ortiz et al., 2015; Laycock et al., 2016). In contrast, particulate Pd species like Pd black are likely to be less readily bioavailable for uptake unless transformed into more soluble forms in soil or the earthworm digestive system (Zereini et al., 2016). Thus, these differences in bioavailability and uptake pathways are likely to explain the higher bioaccumulation and toxicity observed in earthworms exposed to PdCl₂.

Bioaccumulation factors greater than 1 were observed at environmentally relevant concentrations (0.1–1 mg kg⁻¹) for both Pd forms, indicating that even low Pd exposure may result in bioaccumulation in earthworms in the absence of significant toxic effects. Given the ecological role of earthworms in soil food webs, such accumulation may facilitate trophic transfer of Pd to organisms higher up the food chain like earthworm eating birds.

Compared with PdCl₂, Pd black showed lower toxicity and bioaccumulation in *Eisenia fetida*. This is likely related to the low solubility of particulate Pd species and their slower transformation into soluble forms in soil. Zereini et al. (2016) reported that the dissolution of Pd black was a slow process, with approximately 90% of the total metallic Pd becoming soluble after 251 days in the presence of 0.1 M L-methionine and 0.1 M NaClO₄. Thus, longer-term weathering and transformation of metallic Pd in soil than occurred in the experiment presented here may increase the bioavailability of Pd in soil.

In conclusion, this study is the first to investigate Pd toxicity in a soil invertebrate under soil exposure conditions and the first to directly compare the toxicity of soluble and particulate Pd species in soil. Our results demonstrate that Pd toxicity and bioaccumulation are strongly dependent on chemical speciation, with soluble PdCl₂ exhibiting substantially greater bioavailability and toxicity than Pd black. These findings highlight that environmental risk assessments of Pd-contaminated soils should consider Pd speciation as well rather than relying solely on total Pd concentrations. As environmental Pd concentrations continue to increase due to anthropogenic activities, understanding the speciation-dependent bioavailability of Pd will be essential for improving ecological risk assessment and management of contaminated soils.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval

Ethical approval was not required for this study because the experiments involved the invertebrate *Eisenia fetida* and did not fall within the scope of institutional animal ethics approval requirements.

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by P-H. C.. Supervision was performed by S.R. and M.A.. Project administration, and funding acquisition were performed by S.R.. The first draft of the manuscript was written by P-H.C and all authors reviewed and edited all versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgement

We gratefully acknowledge the support from all members of the SoilTox Lab, University of Melbourne, for their guidance and assistance throughout this project. We also thank The University of Melbourne TrACEEs platform for providing ICP-QQQ training and analytical support.

Data Availability

All data supporting the findings of this study are available within the paper, its Supplementary Information and on GitHub (<https://github.com/SoilTox-Lab/Toxicity-and-bioaccumulation-data-for-Pd-in-earthworm>)

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Figures

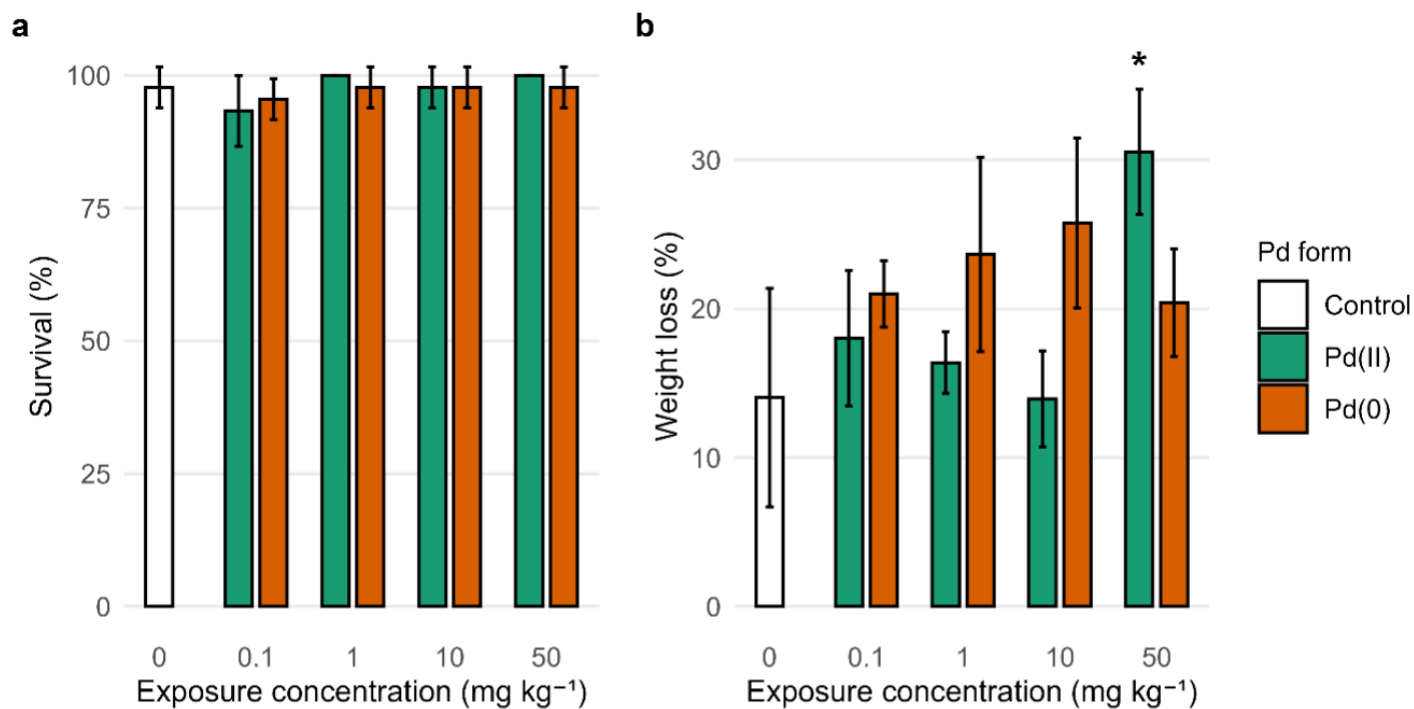


Figure 1

Eisenia fetida (a) survival and (b) weight loss after 28 days of exposure to Pd black (Pd(0)) and PdCl₂ (Pd(II)) spiked soil. Values are the mean $\pm$ standard deviation (n = 3). * represents P < 0.05.

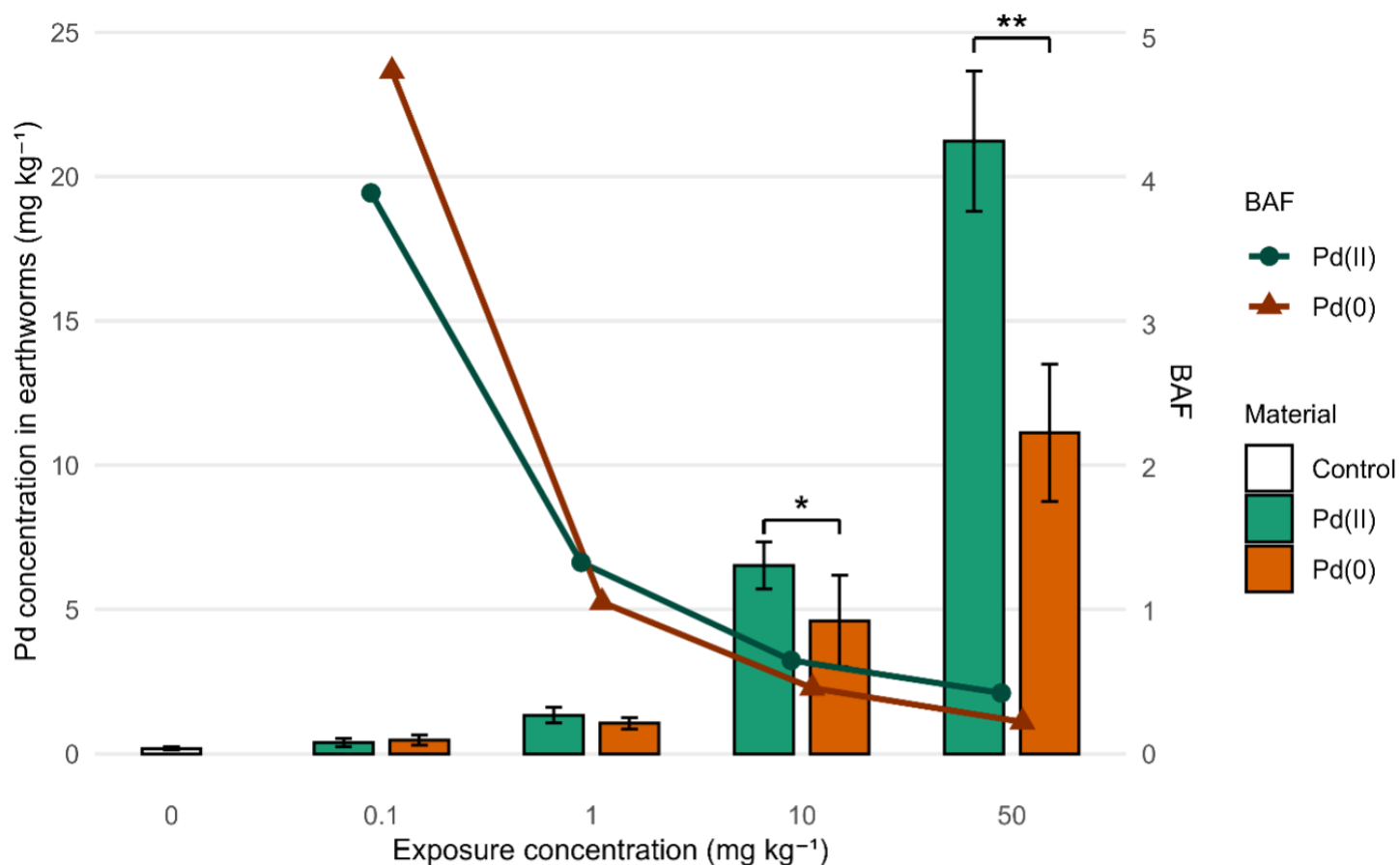


Figure 2

Bioaccumulation of Pd for *Eisenia fetida* after 28 days of exposure to Pd black (Pd(0)) and PdCl₂ (Pd(II)) spiked soil. Values are mean $\pm$ standard deviation (n = 3). The bioaccumulation factor (BAF) was calculated as the Pd concentration in earthworms divided into spiked concentrations in the soil. * and ** represent statistical significance of P < 0.05 and P < 0.001, respectively.

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