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Avoidance tests with the oribatid mite *Oppia nitens* (Acari: Oribatida) in cadmium-spiked natural soils

Masoud M. Ardestani[1,2] · Maryam Keshavarz-Jamshidian[3] · Cornelis A. M. van Gestel[4] · Nico M. van Straalen[4]

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Abstract

Avoidance behavior can be a useful parameter for assessing the ability of organisms to escape from pollutants in their environment. For soil evaluation, a variety of invertebrates is used including the oribatid mite *Oppia nitens*. Here, we tested the avoidance behavior of *O. nitens* using a two-chamber test and an escape test with exposures to different cadmium concentrations of up to 800 mg kg^{-1} dry LUFA 2.2 soil for 2, 4, and 6 days, and up to 7 weeks. With the two-chamber method, the oribatid mites had the choice between clean and polluted soils, whereas they were allowed to escape from a box with polluted soil to clean containers without soil with the escape method. Avoidance of cadmium was observed after 2 days in both tests and the net response of the mites in the two-chamber test increased with increasing cadmium exposure concentrations. Mite responses varied through time, especially with the escape method; with the avoidance behavior becoming more variable and overall non-significant with longer test durations. This is the first study investigating the escape test simultaneously with long-term avoidance of cadmium by *O. nitens*. This mite species is a promising species for avoidance testing in soil ecotoxicology, but more experiments are needed to evaluate the factors that influence its responses in laboratory tests and the consequences for its distribution in contaminated ecosystems.

Keywords Avoidance · Escape test · Exposure time · Metal · Oribatida · Two-chamber test

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✉ Masoud M. Ardestani
mortazavi_m2000@yahoo.com

1 Institute for Environmental Studies, Charles University in Prague, Benátská 2, 12801 Prague, Czech Republic

2 Institute of Soil Biology and SoWa Research Infrastructure, Biology Centre, Czech Academy of Sciences, Na Sádkách 7, 37005 České Budějovice, Czech Republic

3 Department of Plant Protection, University of Tehran, Karaj, Iran

4 Department of Ecological Science, Faculty of Science, Vrije Universiteit, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

Introduction

Pollution of the environment due to anthropogenic activities may pose serious risks to human and environmental health (Vitousek et al. 1997). Contamination of soil, air, and water caused by metals as well as other organic and inorganic compounds is one of the factors contributing to the disturbance of natural habitats (e.g., Fountain and Hopkin 2004; Venter et al. 2016). As a result, pollution influences the biodiversity of organisms by changing their distribution and abundance in a particular area (Van Straalen 2004; Austruy et al. 2016). For example, a decrease in collembolan species richness and abundance in human-disturbed urban areas compared to natural sites has been linked to elevated concentrations of pollutants (Santorufo et al. 2012). Adverse effects of chemicals on the presence and activities of an organism therefore need to be considered in environmental conservation programs.

Avoidance tests demonstrate the ability of organisms to escape from pollutants they encounter in their habitats, which, in the case of soil, is very relevant due to its high heterogeneity. From an ecological point of view, this avoidance behavior can alter the distribution of organisms in their natural environment and diminish their presence in parts of the habitat, with possible consequences for ecosystem functions (i.e., soil quality) due to pockets of polluted soil being insufficiently inhabited by soil invertebrates (e.g., Gillet and Ponge 2003). For a proper risk assessment of chemicals, it is recommended to combine this type of avoidance tests with standard toxicity tests (Aldaya et al. 2006; Natal da Luz et al. 2008; Owojori et al. 2011; Gainer et al. 2019). Another practical advantage of avoidance tests is the short time required to perform them; i.e., generally 2 days (for example, earthworms or collembolans) compared to the longer-term reproduction and survival tests (OECD 2004; Lukkari et al. 2005; ISO 2008, 2011). However, it should be noted that soil invertebrates do not always detect and avoid contaminants, and this behavior appears to be contaminant- and species-specific; for example, enchytraeids did not show clear avoidance behavior to boric acid (Amorim et al. 2008) and phenanthrene (Gainer et al. 2019). Moreover, Owojori at al. (2011) showed that oribatid mites avoided boric acid only at 150 mg kg^{-1} dry soil.

Orbatid mites are among the most abundant soil organisms, found in different soil types and regions (Krantz and Walter 2009; Fajana et al. 2019). They are classified as organisms contributing to the decomposition of soil organic matter and as a consequence, enhancement of soil fertility (Coleman et al. 2004). Although Lebrun and van Straalen (1995) prospected their usage several years ago, oribatid mites have only recently been proposed for the ecotoxicological assessment of contaminated soils. Studies exist on the survival and reproduction of the mite *Oppia nitens* Koch (Acari: Oribatida, Oppiidae) exposed to field and artificial soils contaminated with metals, pesticides, and organic chemicals (Princz et al. 2010; Owojori et al. 2012; Princz et al. 2012; de Lima e Silva et al. 2017; Keshavarz-Jamshidian et al. 2017; Li et al. 2018; Princz et al. 2018). This mite species is easy to maintain and to culture under laboratory conditions, and has recently been adopted as test organism for a standard toxicity test (ISO 2018; ECCC 2019). Cadmium showed to be toxic to *O. nitens* and toxicity increased with time of exposure (Keshavarz-Jamshidian et al. 2017).

There are many studies in the literature in which the avoidance of metals (especially cadmium and copper) and other pollutants by soil organisms has been investigated; e.g., earthworms, collembolans, enchytraeids, and isopods (Natal da Luz et al, 2004, 2008; Amorim et al. 2008; Kobetičová et al. 2009; Boiteau et al. 2011; Niemeyer, et al. 2018;

Mariyadas et al. 2018). However, only two studies have assessed the avoidance behavior of oribatid mites (Owojori et al. 2011; Gainer et al. 2019). Owojori et al. (2011) investigated the avoidance behavior of *O. nitens* to metals (cadmium, copper, zinc, and lead) and organic pollutants (phenanthrene, benzo[a]pyrene, geraniol, and boric acid) using a one-day avoidance test at their first attempt. Their results showed mite avoidance at high concentrations of copper and lead, to phenanthrene, geraniol, and boric acid. These authors did not find significant effects of time of exposure on the avoidance of the mites to copper and phenanthrene; but, a significant difference was found between low and high concentrations of copper at day 1 and day 2 (not for phenanthrene). The authors concluded that one day was enough to assess the avoidance of copper and phenanthrene. No other soil invertebrate avoidance study has assessed long-term avoidance. Moreover, the latter authors assessed the effect of soil properties (organic matter, clay content, moisture content, and pH) on avoidance behavior of *O. nitens* by changing soil characteristics (one at a time) in one side of the two-chamber test while control standard OECD artificial soil was used in the other side. They reported that changing soil properties had little effect on mite behavior in a one-day test. In another study, Gainer et al. (2019) showed that *O. nitens* avoided petroleum hydrocarbons while some other invertebrates, such as enchytraeids, did not. They recommended the inclusion of avoidance tests, as a first step, in toxicity test batteries for assessing soils contaminated with these hydrocarbons. Moreover, invertebrate avoidance occurred at concentration levels that caused chronic toxicity to sensitive plant species.

The aim of the present study was to assess the avoidance behavior of *O. nitens* when exposed to different cadmium concentrations using two different avoidance test methods and different test durations. The avoidance methods used were a two-chamber test and an escape test (see details in 'Materials and methods'). Therefore, this study is the first soil ecotoxicology test to investigate escape behavior simultaneously with a conventional two-choice avoidance test and assessing long-term avoidance behavior.

Materials and methods

Test soils

Standard LUFA 2.2 natural soil (Speyer, Germany), was used for the experiment. The physico-chemical characteristics of the soil were: $pH_{CaCl_2} = 5.5 \pm 0.1$, water holding capacity $= 45.2 \pm 5.0\%$, cation exchange capacity $= 10.0 \pm 0.8$ $cmol_c$ kg^{-1}, and total organic carbon content $= 1.93 \pm 0.20\%$. Soil samples were air-dried, sieved (2 mm mesh), and homogenized prior to the experiments.

Test animals

Oppia nitens had been cultured at the Vrije Universiteit Amsterdam, The Netherlands, for more than 4 years. The original stock was kindly provided by Dr. J.I. Princz, Environment and Climate Change Canada, and was originally sampled from soils at the Central Experimental Farm, Ottawa, Canada, by Dr. V.M. Behan-Pelletier. Animals were cultured on a plaster of Paris substrate base, which was moistened every week, and baker's yeast (Algist Bruggeman) was used as food source. The animals were kept in a climate chamber at 20 °C, 75% relative humidity and 8:16 h dark:light regime. For the avoidance tests, newly emerged age-synchronized adults identified by having a pink-brown color were used.

Spiking soils

Test soils were spiked with an aqueous solution of cadmium nitrate [$Cd(NO_3)_2 \times 4H_2O$; Sigma-Aldrich; 99% pure] dissolved in deionized water in a concentration such that the desired final concentration of cadmium in soil was obtained by adding sufficient deionized water to moisten the soil to 50% of its water holding capacity. Nominal cadmium concentrations of 50, 100, 200, 400, and 800 mg kg^{-1} dry soil were used for both avoidance tests (two-chamber and escape, see below). Non-spiked LUFA 2.2 soil was used as a control (also moistened with deionized water to 50% of its water holding capacity). The selected test concentrations were within the range of cadmium concentrations in field-contaminated soils and based on a previous toxicity test where LC50 values of 265–490 mg kg^{-1} dry soil were estimated when exposing *O. nitens* to cadmium for up to 7 weeks in LUFA 2.2 soil (Keshavarz-Jamshidian et al. 2017). Spiked soil was kept for two weeks prior to starting the experiments. Then, soils were distributed over the experimental containers. Every week, the containers were checked and deionized water was added to compensate for moisture loss, assessed by weighing.

Experimental procedures

Mite avoidance was evaluated using two methods:

(1) Two-chamber avoidance test
Circular plastic containers (diameter 4 cm, height 3 cm) were divided into two sections with a metallic separator (Fig. S1, Supporting Information). One section was filled with approximately 20 g spiked soil, the other section with the same amount of clean soil. The test also had a clean/clean treatment in which both sections were filled with the non-spiked soil. For each cadmium concentration/time, five replicates were prepared. After adding the soils, the divider was removed. For each experimental container, ten mites were placed on the line of contact between the soils (spiked/clean). After 2, 4, 6, and 49 days, mites were extracted (Tullgren) from five replicate containers per treatment (Van Straalen and Rijninks 1982) with the cadmium-spiked soil and clean soil sections from each container being placed in separate containers. The number of mites collected was counted after 48-h extraction. The collecting containers (diameter 8 cm, height 10 cm) in the Tullgren extractor were positioned so as to prevent escaping of the mites (see Fig. S1).

(2) Escape avoidance test
Circular plastic containers (diameter 4 cm, height 3 cm) with a gauze bottom (mesh 1 mm) were placed on top of another plastic cylinder (diameter 4 cm, height 3 cm), both were then placed into a small Petri dish (Fig. S2, Supporting Information). Spiked or clean soils were placed in the top containers, which were closed with a plastic lid to prevent the mites leaving the boxes. To each experimental container, ten mites were added. Mites were allowed to move freely in the soil, incubated at normal lab conditions. The collecting Petri dishes were checked every day for mites. After 2, 4, and 6 days, and 2, 4, 6, and 7 weeks, the number of mites escaped up to that time was evaluated. Due to the experimental design and the height of both containers (total 6 cm), mites could not return to the test containers with soil after they had escaped to the clean Petri dish. For each cadmium concentration/time, five replicates were used. Additional replicates

were included for pH and cadmium analyses. For long-term treatments (2, 4, 6, and 7 weeks), the animals were fed every week by adding a few grains of dry baker's yeast on the top of the soil; the animals were not fed in the shorter tests (i.e., up to 6 days).

Soil analyses

At the beginning of the experiment, the soils were analyzed for pH_{CaCl_2} and total and 0.01 M $CaCl_2$-extractable cadmium concentrations, using analytical methods described earlier (Ardestani and van Gestel 2013; Ardestani et al. 2014a; Keshavarz-Jamshidian et al. 2017). Cadmium concentrations were measured using a flame atomic absorption spectrophotometer (F-AAS, Perkin Elmer, AAnalyst 100). The reference material for soil analysis was ISE 989 River clay (Wageningen, The Netherlands). Measured cadmium concentrations for the reference material were always within 15% of the certified values. Blanks were always used for checking the accuracy of the analyses. At the end of the experiment (after 7 weeks), pH_{H_2O}, pH_{CaCl_2}, and total cadmium concentrations in soil and in water- and 0.01 M $CaCl_2$-extracts were measured. In the extracts, pH was measured using a WTW Inolab 7110 instrument and cadmium by F-AAS.

Data analysis

The avoidance behavior in the two-chamber method was expressed as follows:

$$A = \frac{N - 2T}{N}, \quad (1)$$

where A (avoidance score) is the net response of the mites, N is total number of oribatid mites added to each replicate, and T is number of mites observed in the cadmium-spiked soil. From the formula, it may be seen that if the mites show no preference or avoidance, $T = (1/2)N$ and $A=0$; if all the mites avoid the contaminated soil, $T=0$ and $A=+100$; if all the mites prefer the contaminated soil, $T=N$ and $A = -100$. So, A is a measure of the avoidance response on a scale between -100 and $+100$ (Hund-Rinke and Wiechering 2001). In the two-chamber avoidance test, we used the avoidance score as defined above, for each replicate.

For the escape method, we used the number of mites collected in the dishes underneath the test and clean soils. A Levene's test was conducted for checking the homogeneity of variances. Then, a generalized linear model was used to establish any effect of cadmium concentration on the two-chamber avoidance with a binomial error distribution (BGLM) and on the escape tests with a poisson error distribution (PGLM). One-way analysis of variance (ANOVA) was also used to compare pH values at the beginning and the end of the experiment. All analyses were run in Statistica 13.3.

Results

Soil pH and measured cadmium concentrations

At the beginning of the test, pH_{CaCl_2} slightly increased with increasing cadmium concentration, except for the treatment with 800 mg Cd kg^{-1} dry soil (Table 1; $F_{5,6} = 34.1$,

Table 1 The pH of the LUFA 2.2 test soil (Speyer, Germany) used in avoidance tests with *Oppia nitens* exposed to nominal cadmium concentrations of 0 (control) to 800 mg kg^{-1} dry soil

pH	Nominal Cd (mg kg^{-1} dry soil)					
	0	50	100	200	400	800
pH_{H_2O}-end	5.54 ± 0.04[bc]	5.46 ± 0.02[c]	5.39 ± 0.01[c]	5.39 ± 0.00[c]	5.86 ± 0.09[a]	5.76 ± 0.03[ab]
pH_{CaCl_2}-start	5.39 ± 0.03[c]	5.43 ± 0.005[bc]	5.47 ± 0.00[b]	5.55 ± 0.00[a]	5.60 ± 0.005[a]	5.42 ± 0.02[bc]
pH_{CaCl_2}-end	4.96 ± 0.005[b]	4.93 ± 0.01[b]	4.91 ± 0.005[b]	4.92 ± 0.04[b]	5.44 ± 0.01[a]	5.52 ± 0.04[a]

Shown are the averages of two replicates (± SE; n = 2). The end values were measured after 7 weeks. Means within a row followed by the same letter do not differ significantly (one-way ANOVA followed by Tukey HSD post hoc test: $p < 0.05$)

$p < 0.001$). The same pattern was observed for pH_{CaCl_2} at the end of the test ($F_{5,6} = 161.3$, $p < 0.001$). Soil pH_{CaCl_2} was lower at the end than at the start of the test. pH_{H_2O} was measured only at the end of the test and slightly increased with increasing cadmium concentrations (Table 1; $F_{5,6} = 24.8$, $p < 0.001$).

The measured total concentrations of cadmium in the LUFA 2.2 soil were in the range of nominal values, except for the two highest concentrations at the end of the test (Table 2). Extractable cadmium concentrations increased with increasing total cadmium concentrations in the test soils and were always higher in 0.01 M $CaCl_2$- than in water-extracts (Table 2; see also Fig. S3, Supporting Information).

Two-chamber avoidance test

The average mite recovery (± SE) was 89 ± 3.2% after 2 days. Except for 49 days, homogeneity of variances was confirmed for 2, 4, and 6 days of the test (Levene's test; $F_{4,20}$ = 0.90; $p < 0.05$, for 2 days, $F_{4,20}$ = 1.91; $p < 0.05$, for 4 days, and $F_{4,20}$ = 0.16; $p < 0.05$, for 6 days). At 100 mg Cd kg^{-1} dry soil, a relative preference response (attraction, negative A) to the contaminated soil was observed compared to other cadmium concentrations. The net response of the mites increased at higher cadmium concentrations and was generally above

Table 2 Mean (± SE; n = 2) total and water- and 0.01 M $CaCl_2$-extractable cadmium concentrations (mg kg^{-1}) in LUFA 2.2 soil used in avoidance tests with *Oppia nitens*

Nominal Cd (mg kg^{-1} dry soil)	Start-soil	End-soil	End-water	Start-0.01 M $CaCl_2$	End-0.01 M $CaCl_2$
0	0.001 (± < 0.001)	0.001 (± < 0.001)	0.002 (± < 0.001)	0.001 (± < 0.001)	0.001 (± < 0.001)
50	53.9 (± 2.06)	49.2 (± 1.62)	0.04 (± < 0.001)	0.34 (± 0.005)	0.49 (± 0.009)
100	127 (± 1.57)	105 (± 1.06)	0.10 (± 0.002)	0.82 (± 0.07)	1.16 (± 0.003)
200	324 (± 27.1)	217 (± 0.30)	0.20 (± 0.004)	1.58 (± 0.04)	2.32 (± 0.004)
400	423 (± 1.04)	411 (± 26.9)	0.31 (± 0.002)	3.78 (± 0.16)	4.04 (± 0.005)
800	826 (± 33.7)	377 (± 55.2)	1.32 (± 0.02)	10.1 (± 0.46)	9.99 (± 0.03)

Animals were exposed to cadmium for up to 7 weeks at nominal concentrations of 0-800 mg kg^{-1} dry LUFA 2.2 soil zero (avoidance) in the two-chamber test after 2 days exposure, except for one concentration at which the response on average was below zero (BGLM, Wald's $\chi^2 = 1.91$, df = 1, $p > 0.05$; Fig. 1a). The overall net response of *O. nitens* to cadmium was statistically significant across cadmium concentrations (BGLM, Wald's $\chi^2 = 6.78$, df = 1, $p < 0.01$).

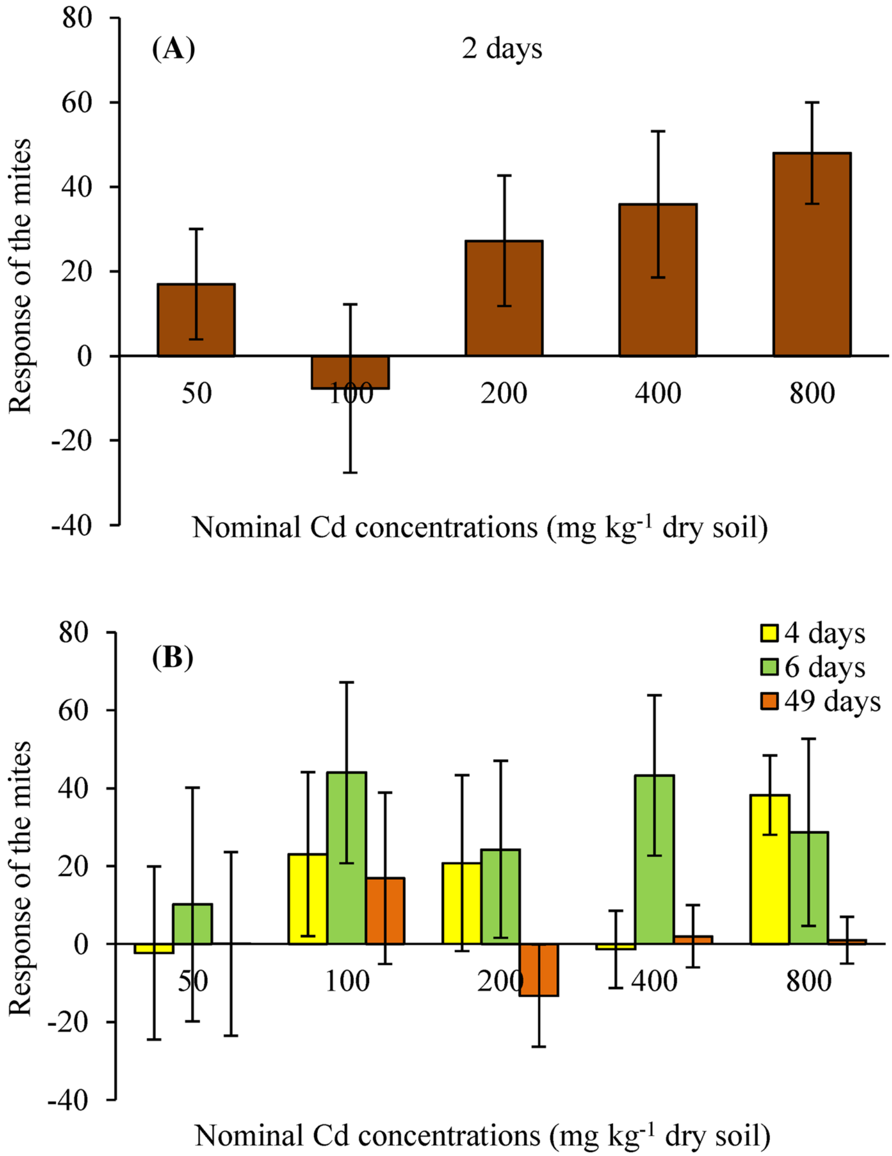

Fig. 1 Mean (± SE; n = 5) net response of *Oppia nitens* after 2 days (**a**) and 4, 6, and 49 days (**b**) of exposure to cadmium concentrations of 50 to 800 mg kg^{-1} dry LUFA 2.2 soil in the two-chamber avoidance test. Positive net response indicates avoidance and negative net response indicates attraction (preference)

The effect of time was quite variable in the two-chamber test; in some cases, a clear pattern for the effect of time on the net response of the mites was found when cadmium concentrations increased (after 2 days and relatively after 4 days, but not in other cases). Variation in the data did not allow generalizing this pattern. Overall, no significant differences were found when considering the effect of time across different cadmium concentrations (BGLM, Wald's $\chi^2=0.45$, df = 1, $p>0.05$; Fig. 1b). Except for a few cases (200 mg Cd kg^{-1} dry soil) in which attraction to the contaminated soil was observed, avoidance was the dominant behavior of the mites. Mite recoveries were 92 ± 2.1 and 89 ± 1.9% after 4 and 6 days, respectively. The avoidance behavior of the mites was higher after 6 days of test compared to other time intervals. After 7 weeks of exposure, mite avoidance was quite low, especially at the cadmium concentrations of 400 and 800 mg kg^{-1} dry soil.

Escape avoidance test

In the escape avoidance test, the effect of exposure concentrations on the average number of escaped *O. nitens* was not statistically significant across different cadmium-spiked soils using all data from 2, 4, and 6 days and 2, 4, 6, and 7 weeks (PGLM, Wald's $\chi^2=1.10$, df = 1, $p>0.05$; Fig. 2a, b). However, the effect of time was significant (PGLM, Wald's $\chi^2=60.1$, df = 1, $p<0.001$). In a 2-day escape test, the average number of mites escaped from contaminated soil was the same across all cadmium concentrations (Fig. 2a; PGLM, Wald's $\chi^2=0.70$, df = 1, $p>0.05$). The only exception was the cadmium concentration of 800 mg kg^{-1} dry soil where the lowest number of mites escaped from the experimental containers. An increasing trend for the number of mites escaping cadmium-contaminated soils was observed with increasing cadmium concentrations for the long-term treatments (4, 6, and 7 weeks). A 7-week duration for the escape test resulted in the highest number of escaped mites, except for the cadmium concentration of 800 mg kg^{-1} dry soil (Fig. 2b).

Discussion

Soil pH was only slightly affected (up to 0.5 units) by different cadmium concentrations (Table 1); therefore, pH was likely not an influencing factor for the avoidance behavior. This result is consistent with Owojori et al. (2011) who did not observe a significant effect of pH on the net response of the mite *O. nitens* at pH levels of 3.1–8.1 in OECD artificial soil. This could actually be an advantage of using this mite species for testing different soils with a variety of pH ranges. Princz et al. (2010) also demonstrated the tolerance of *O. nitens* to different field soils with pH ranging from 3.9 to 7.5. In the two-chamber tests, mite recoveries varied between 89 ± 3.2 and 92 ± 2.1%, indicating the validity of the test as specified in the ISO guidelines (ISO 2008, 2011, 2018).

The results of the present study showed an avoidance behavior of *O. nitens* after 2 days in the two-chamber test, with a general increasing trend with increasing cadmium concentrations (except for 100 mg kg^{-1} dry soil, Fig. 1a). It should be noted that we did not observe a significant difference among avoidance of the mites when comparing different cadmium concentrations together at all time points. However, the net response of *O. nitens* to cadmium was consistent with a previous study on metals (Owojori et al. 2011), but not actually as strong as for other contaminants (phenanthrene and geraniol). Contrary to our results, other studies could not demonstrate such clear avoidance of oribatid mites and collembolans exposed to cadmium at concentrations of up to 1000–1200 mg kg^{-1} dry

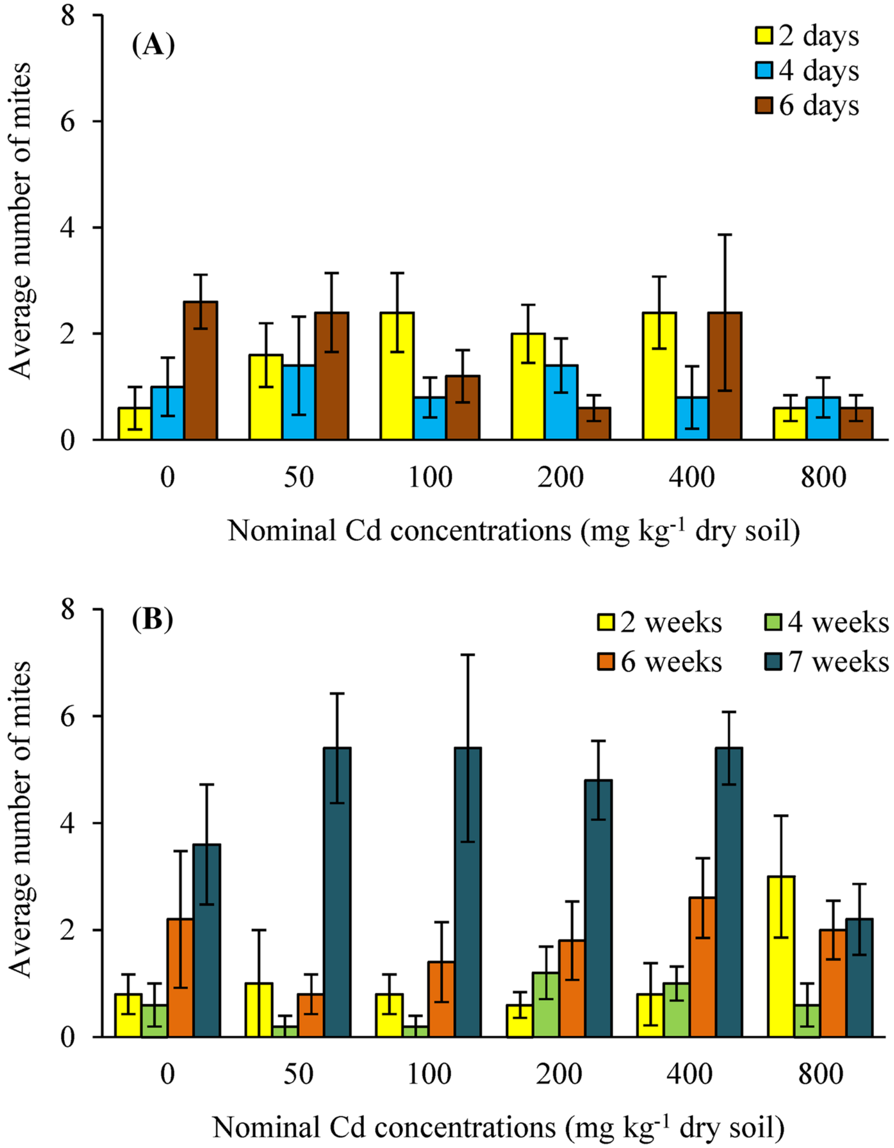

Fig. 2 Mean (± SE; n = 5) number of *Oppia nitens* escaped after 2, 4, and 6 days (**a**) and 2, 4, 6, and 7 weeks (**b**) of exposure to cadmium concentrations of 50 to 800 mg kg^{-1} dry LUFA 2.2 soil in the escape avoidance test

soil (Greenslade and Vaughan 2003; Owojori et al. 2011). The net response in the latter studies varied across different concentrations, with the mites showing attraction (preference), instead of avoidance, to half of the cadmium concentrations tested. Studies on earthworms and potworms exposed to cadmium in different soils, however, did show avoidance and confirmed that 2 days could be a suitable assessment time for these tests (Stephenson et al. 1998; Natal da Luz et al. 2008; Amorim et al. 2008). Van Gestel (2012) also recognized avoidance behavior as a valuable endpoint for pollution assessment which may be as sensitive as reproduction. The avoidance of mites in the present study was highest at concentrations close to and above the reported EC50 value of 345 mg kg^{-1} dry soil reported by Keshavarz-Jamshidian et al. (2017) for *O. nitens* after 7 weeks of exposure to cadmium. It should be noted that the low response of the mites could be due to cadmium toxicity at the higher concentrations (see below).

Time was an important factor in our avoidance experiments. We used long-term two-chamber avoidance tests for the first time to assess this behavior in a soil invertebrate species. Although *O. nitens* showed avoidance in longer time intervals, up to 49 days, the variation in the data did not allow us to establish a clear trend (Fig. 1b). In few cases, attraction (preference) of mites to contaminated soils was found. After 49 days, we observed quite low avoidance at higher cadmium concentrations which may be attributed to mortality of the mites. These (high) concentrations are in the range of the LC50s of up to 490 mg kg^{-1} dry soil, as reported by Keshavarz-Jamshidian et al. (2017) after 3 weeks of exposure. After 7 weeks of exposure, LC50 was 265 mg kg^{-1} dry soil showing the high toxicity of cadmium for the mites (Keshavarz-Jamshidian et al. 2017). We observed mite avoidance for the cadmium concentration of 50 up to 800 mg kg^{-1} dry soil after 2 days of the test (Fig. 1a) which is contradictory to Owojori et al. (2011) who did not observe a difference in the net response of mites to cadmium in a short-term test. Our results for 4 and 6 days are similar to Owojori et al. (2011). However, for copper and lead, the short-term avoidance test did result in clear net response of *O. nitens* (Owojori et al. 2011). Since there is still no consensus on the level of variability of the results that can be accepted as a standard level (or validity criterion) among different studies, the comparison of different studies should be considered with caution.

For different soil organisms, short (one day) or longer durations of exposure (2 days to 14 days) may be needed for their actual response to different metal (and/or other chemical) concentrations. This might depend on the test species and their sensitivities (Table S1, Supporting Information). Different soil invertebrates have different chemical and mechanical sensory organs allowing them to escape from harmful conditions and/or move to more favorable places (see below; see also Slifer and Sekhon 1978; Edwards and Bohlen 1992; Lukkari et al. 2005; Curry and Schmidt 2007). However, this needs further investigation in different species. For example, Gainer et al. (2019) showed that a 2-day test is suitable to get similar test results for four soil invertebrates exposed to petroleum hydrocarbons-contaminated soils, except for *Enchytraeus crypticus* which turned out to be less exposed to these hydrocarbons due to the very low solubility of these chemicals.

The difference in the avoidance response of the oribatid mite species *O. nitens* compared to other soil organisms may be related to the chemoreceptor characteristics (as shown by Stephenson et al. 1998 for earthworms), physiological and morphological characteristics, and behavioral and ecological characteristics of the test species, as shown before for earthworm species (Edwards and Bohlen 1996; Lukkari and Haimi 2005). The response of organisms to metals and other pollutants may be species-specific and contaminant-specific (Ardestani et al. 2014b). In previous studies, it was shown that oribatid mites can continue to accumulate cadmium for more than 7 weeks without reaching equilibrium in the animal or that they were able to survive at 1000 mg kg^{-1} dry soil up to 36 weeks of exposure (Ludwig et al. 1991, 1993; Keshavarz-Jamshidan et al. 2017). This might indicate a high ability of oribatid mites to compartmentalize metals (Ludwig et al. 1991), i.e., detoxifying them and storing in a form shut off from metabolism.

In the escape test, we observed the moving out of *O. nitens* from contaminated soils after 2 days and a relative increase in the number of mites with increasing cadmium concentrations, except at 800 mg kg^{-1} dry soil (Fig. 2a). This again confirms that a 2-day escape test could be an indicator of avoidance behavior. It should be noted that the low number of mites escaping at higher concentrations is likely due to potential toxic effects influencing their behavior. However, the highest number of escaped mites from the experimental containers was observed after 6 and 7 weeks of exposure (long-term test, Fig. 2b). This was probably related to the high number of mites produced during the test, forming the new generation of emerged adults at sub-lethal cadmium concentrations. Sengbusch and Sengbusch (1970) reported that 40–45 days was needed for the mites to reach the adult stage from eggs at 20 °C. *Oppia nitens* laid eggs at the rate of one egg/female/day in laboratory condition (Fajana et al. 2019) and the reproduction rate of this mite was 2.7 juveniles per adult up to 28 days of its life cycle (Princz et al. 2010). However, a reproduction cycle of 28 days (for having age-synchronized deutonymphs, in a substrate of plaster of Paris, fed with grains of baker's yeast in the laboratory at 21 °C) has been recommended as a full life cycle for soil toxicity tests with *O. nitens* (Princz et al. 2010). Therefore, the increasing number of mites after 4–5 weeks in the present study is in agreement with current knowledge on the life cycle of *O. nitens*. Moreover, low mite avoidance was observed for 800 mg kg^{-1} dry soil after 7 weeks of exposure in the escape test. This is due to the fact that mites could be dead, since this concentration is higher than LC50 values reported for cadmium in LUFA 2.2 natural soil (Keshavarz-Jamshidian et al. 2017). The latter authors reported increasing toxicity of cadmium to *O. nitens* from 3 to 7 weeks of exposure with the LC50 values decreasing from 490 to 265 mg kg^{-1} dry soil.

In conclusion, the oribatid mite *O. nitens* only avoided cadmium at concentrations that did already affect its survival, and more obviously after 2 days of exposure. Extending the test duration did no longer show clear avoidance responses in the mites, especially for the two-chamber test. However, the results of the escape test suggested avoidance from cadmium-spiked soils by *O. nitens*. This finding needs further confirmation by performing additional tests for metals and other contaminants.

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References

Aldaya MM, Lors CL, Salmon S, Ponge JF (2006) Avoidance bio-assays may help to test the ecological significance of soil pollution. Environ Pollut 140:173–180

Amorim MJB, Novais S, Römbke J, Soares AMVM (2008) Avoidance test with *Enchytraeus albidus* (Enchytraeidae): effects of different exposure time and soil properties. Environ Pollut 155:112–116

Ardestani MM, van Gestel CAM (2013) Using a toxicokinetics approach to explain the effect of soil pH on cadmium bioavailability to *Folsomia candida*. Environ Pollut 180:122–130

Ardestani MM, Oduber F, van Gestel CAM (2014a) A combined toxicokinetics and toxicodynamics approach to assess the effect of porewater composition on cadmium bioavailability to *Folsomia candida*. Environ Toxicol Chem 33:1570–1577

Ardestani MM, van Straalen NM, van Gestel CAM (2014b) Uptake and elimination kinetics of metals in soil invertebrates: a review. Environ Pollut 193:277–295

Austruy A, Laplanche C, Mombo S, Dumat S, Deola F, Gers C (2016) Ecological changes in historically polluted soils: meta(loid) bioaccumulation in microarthropods and their impact on community structure. Geoderma 271:181–190
Boiteau G, Lynch DH, MacKinley P (2011) Avoidance tests with *Folsomia candida* for the assessment of copper contamination in agricultural soils. Environ Pollut 159:903–906
Coleman DC, Crossley Jr DA, Hendrix PF (2004) Fundamentals of soil ecology, 2nd edn. Elsevier Academic Press, Burlington, MA
Curry JP, Schmidt O (2007) The feeding ecology of earthworms—a review. Pedobiologia 50:463–477
de Lima e Silva C, Brennan N, Brouwer JM, Commandeur D, Verweij RA, van Gestel CAM (2017) Comparative toxicity of imidacloprid and thiacloprid to different species of soil invertebrates. Ecotoxicology 26:555–564
Environment and Climate Change Canada (ECCC) (2019) Biological test method: test for measuring reproduction of oribatid mites exposed to contaminants in soil. Environmental Protection Series, EPS 1/RM/61, Ottawa, ON, Canada
Edwards CA, Bohlen PJ (1992) The effects of toxic chemicals to earthworms. Rev Environ Contam Toxicol 125:23–99
Edwards CA, Bohlen PJ (1996) Biology and ecology of earthworms. Chapman and Hall, London, p 426
Fajana HO, Gainer A, Jegede OO, Awuah KF, Princz JI, Owojori OJ, Siciliano SD (2019) *Oppia nitens* C.L. Koch, 1836 (Acari: Oribatida): current status of its bionomics and relevance as a model invertebrate in soil ecotoxicology. Environ Toxicol Chem 38:2593–2613
Fountain MT, Hopkin SP (2004) A comparative study of the effects of metal contamination on Collembola in the field and in the laboratory. Ecotoxicology 13:573–587
Gainer A, Hogan N, Siciliano SD (2019) Soil invertebrate avoidance behavior identifies petroleum hydrocarbon contaminated soils toxic to sensitive plant species. J Hazard Mater 361:338–347
Gillet S, Ponge JF (2003) Changes in species assemblages and diets of Collembola along a gradient of metal pollution. Appl Soil Ecol 22:127–138
Greenslade P, Vaughan G (2003) A comparison of Collembola species for toxicity testing of Australian soils. Pedobiologia 47:171–179
Hund-Rinke K, Wiechering H (2001) Earthworm avoidance test for soil assessment. J Soils Sediments 1:15–20
International Organization for Standardization (ISO) (2008) Soil quality–Avoidance test for determining the quality of soils and effects of chemicals on behavior-No. 17512, Part 1: test with earthworms (*Eisenia fetida* and *Eisenia andrei*). Geneva, Switzerland
International Organization for Standardization (ISO) (2011) Soil quality–Avoidance test for determining the quality of soils and effects of chemicals on behavior-No. 17512, Part 2: test with Collembola (*Folsomia candida*). Geneva, Switzerland
International Organization for Standardization (ISO) (2018) Soil quality—Test for measuring the inhibition of reproduction of oribatid mites (*Oppia nitens*) exposed to contaminants in soil. No. 23266, Geneva, Switzerland
Keshavarz-Jamshidian M, Verweij RA, van Gestel CAM, van Straalen NM (2017) Toxicokinetics and time-variable toxicity of cadmium in *Oppia nitens* Koch (Acari: Oribatida). Environ Toxicol Chem 36:408–413
Kobetičová K, Hofman J, Holoubek I (2009) Avoidance response of *Enchytraeus albidus* in relation to carbendazim ageing. Environ Pollut 157:704–706
Krantz GW, Walter DE (2009) A manual on acarology, 3rd edn. Texas Technical University Press, Lubbock, p 807
Lebrun P, van Straalen NM (1995) Oribatid mites: prospects for their use in ecotoxicology. Exp Appl Acarol 19:361–379
Li J, Verweij RA, van Gestel CAM (2018) Lanthanum toxicity to five different species of soil invertebrates in relation to availability in soil. Chemosphere 193:412–420
Ludwig M, Kratzmann M, Alberti G (1991) Accumulation of heavy metals in two oribatid mites. In: Dusbábek F, Bukva V (eds) Modern acarology. Academia and SPB Academic, The Hague, pp 431–437
Ludwig M, Kratzmann M, Alberti G (1993) The influence of some heavy metals on *Steganacarus magnus* (Acari: Oribatida). Z Angew Zool 4:455–467
Lukkari T, Aatsinki M, Väisänen A, Haimi J (2005) Toxicity of copper and zinc assessed with three different earthworm tests. Appl Soil Ecol 30:133–146
Lukkari T, Haimi J (2005) Avoidance of Cu- and Zn-contaminated soil by three ecologically different earthworm species. Ecotoxicol Environ Saf 62:35–41

Mariyadas J, Amorim MJB, Jensen J, Scott-Fordsmand JJ (2018) Earthworm avoidance of silver nanomaterials over time. Environ Pollut 239:751–756

Natal da Luz T, Ribeiro R, Sousa JP (2004) Avoidance tests with Collembola and earthworms as early screening tools for site-specific assessment of polluted soils. Environ Toxicol Chem 23:2188–2193

Natal da Luz T, Amorim MJB, Römbke J, Sousa JP (2008) Avoidance tests with earthworms and springtails: defining the minimum exposure time to observe a significant response. Ecotoxicol Environ Saf 71:545–551

Niemeyer JC, Carniel LSC, Pech TM, Crescencio LP, Klauberg-Filho O (2018) Boric acid as a reference substance in avoidance behaviour tests with *Porcellio dilatatus* (Crustacea: Isopoda). Ecotoxicol Environ Saf 161:392–396

Organization for Economic Cooperation and Development (OECD) (2004) Guideline for Testing of Chemicals No. 222, Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*), Paris, France

Owojori OJ, Healey J, Princz J, Siciliano SD (2011) Can avoidance behavior of the mite *Oppia nitens* be used as a rapid toxicity test for soils contaminated with metals or organic chemicals? Environ Toxicol Chem 30:2594–2601

Owojori OJ, Siciliano SD (2012) Accumulation and toxicity of metals (copper, zinc, cadmium, and lead) and organic compounds (geraniol and benzo [a] pyrene) in the oribatid mite *Oppia nitens*. Environ Toxicol Chem 31:1639–1648

Princz JI, Behan-Pelletier VM, Scroggins RP, Siciliano SD (2010) Oribatid mites in soil toxicity testing—the use of *Oppia nitens* (C.L. Koch) as a new test species. Environ Toxicol Chem 29:971–979

Princz JI, Moody M, Fraser C, van der Vliet L, Lemieux H, Scroggins RP, Siciliano SD (2012) Evaluation of a new battery of toxicity tests for boreal forest soils: assessment of the impact of hydrocarbons and salts. Environ Toxicol Chem 31:766–777

Princz JI, Jatar M, Lemieux H, Scroggins RP (2018) Perfluorooctane sulfonate in surface soils: effects on reproduction in the collembolan, *Folsomia candida*, and the oribatid mite, *Oppia nitens*. Chemosphere 208:757–763

Santorufo L, van Gestel CAM, Rocco A, Maisto G (2012) Soil invertebrates as bioindicators of urban soil quality. Environ Pollut 161:57–63

Sengbusch HG, Sengbusch CH (1970) Post-embryonic development of *Oppia nitens* (Acarina: Oribatei). J NY Entomol Soc 78:207–214

Slifer EH, Sekhon SS (1978) Sense organs on the antennae of two species of Collembola (insecta). J Morphol 157:1–19

Stephenson GL, Kaushik A, Kaushik NK, Solomon KR, Steele T, Scroggins RP (1998) Use of an avoidance–response test to assess toxicity of contaminated soils to earthworms. In: Sheppard SC, Bembridge JD, Holmstrup M, Posthuma L (eds) Advances in earthworm ecotoxicology. SETAC, Pensacola, pp 67–81

Van Gestel CAM (2012) Soil ecotoxicology: state of the art and future directions. Zookeys 176:275–296

Van Straalen NM (2004) The use of soil invertebrates in ecological survey of contaminated soils. In: Doelman P, Eijsackers HJP (eds) Vital soil function, value and properties. Elsevier, New York, pp 159–194

Van Straalen NM, Rijninks PC (1982) The efficiency of Tullgren apparatus with respect to interpreting seasonal changes in age structure of soil arthropod populations. Pedobiologia 24:197–209

Venter O, Sanderson EW, Magrach A, Allan JR, Beher J, Jones KR, Possingham HP, Laurance WF, Wood P, Fekete BM, Levy MA, Watson JEM (2016) Sixteen years of change in the global terrestrial human footprint and implications for biodiversity conservation. Nat Commun 7:12558

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of earth's ecosystems. Science 277:494–499