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Dose-dependent effects of lead and cadmium and the influence of soil properties on their uptake by *Helix aspersa*: an ecotoxicity test approach

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Abstract

Three soil types with different physicochemical properties were selected to evaluate their effect on lead and cadmium bioavailability and toxicity in the land snail *Helix aspersa*. In 28-day ecotoxicity tests, *H. aspersa* juveniles were exposed to increasing concentrations of Pb or Cd. EC50s, concentrations reducing snail growth by 50%, differed between the soils and so did Cd and Pb uptake in the snails. For lead, EC50s were 2397–6357 mg Pb/kg dry soil, while they ranged between 327 and 910 mg Cd/kg dry soil for cadmium. Toxicity and metal uptake were highest on the soil with the lowest pH, organic matter content and Cation Exchange Capacity (CEC). Growth reduction was correlated with metal accumulation levels in the snails' soft body, and differences in toxicity between the soils decreased when EC50s were expressed on the basis of internal metal concentrations in the snails. These results confirm the effect of soil properties; pH, CEC, OM content, on the uptake and growth effect of Pb and Cd in *H. aspersa*, indicating the importance of properly characterizing soils when assessing the environmental risk of metal contaminated sites.

Keywords *Helix aspersa* · Cadmium · Lead · Bioavailability · Soil properties · Ecotoxicity test

Introduction

Nowadays the world suffers from various kinds of pollution caused by the expanding human activities and associated use and emission of chemicals (Commission of the European communities 2006). This triggers questions like whether the Earth is capable of coping with such a burden of anthropogenically-released chemicals (Gavrilescu 2010). Pollutants can be organic compounds that are not naturally found in the environment or inorganic compounds present

at concentrations exceeding background levels, with heavy metals representing the latter ones (Hamers et al. 2006; Boyd 2010; Sahli et al. 2012; Rieuwerts 2017; Savorelli et al. 2017; Masindi and Muedi 2018; Burgos-Aceves et al. 2018; Capillo et al. 2018). Indeed, unlike organic pollutants, heavy metals once introduced into the environment cannot be broken down by chemical or biological processes (Ayangbenro and Babalola 2017). They persist indefinitely because of their non-degradable nature, and cause pollution of air, water, and soil. This kind of pollution not only negatively affects the quality of ecosystems, but also threatens the health and well-being of all forms of life. They can accumulate in living organisms and inflict toxic effects and damage to these organisms at different levels in the food chain (Mani and Kumar 2014; Yu et al. 2017).

Recently, a lot of attention has been paid to the impact of metals on human and environmental animal health (Prasad et al. 2006; Sharpley 2007; Hindersmann and Mansfeldt 2014). Metals occur naturally in soils and other ecosystems, but their levels and bioavailability can increase through numerous anthropogenic activities such as metallurgical industry, mining and smelting, vehicle exhaust, energy and

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fuel production, soil fertilizers and pesticides, and the application of sewage sludge (Dias and Edwards 2003; Davis et al. 2009; Alloway 2013; El-Hadef El-Okki et al. 2016; Dhaliwal et al. 2020).

The accumulation of metals in soils can directly affect the life cycle of organisms such as land snails, earthworms, springtails, nematodes, and enchytraeids, causing mortality, inhibiting growth, reducing metabolic activities, and hampering their reproduction. This may decrease their density and abundance provoking imbalance in trophic levels, and affect their role in the functioning of the soil ecosystem (Chassin et al. 1996; Edwards 2002; Hodson 2013; Sivakumar 2015; Belhouioui et al. 2019). Lead and cadmium are two trace elements naturally present in the environment due to rock degradation, but known as soil contaminants by their release due to human activities (Marín et al. 2016). Cd is released from combustion, traffic, metal industry, landfills, sewage sludge, and mining (Merkel and Sperling 1998; Sprynskyy et al. 2011; Bigalke et al. 2017; Fiorino et al. 2018). The presence of Cd in phosphate fertilizers as an impurity is the main reason of increasing Cd levels in soil and groundwater. Cd can enter the human body and be highly accumulated in tissues (Pan et al. 2010; Hajeb et al. 2014). The similar ionic radius of Cd and calcium and their identical charge make them behave the same which means that Cd may interfere with calcium balance in living organisms, changing the uptake rate of both elements (Thornton 1986; Kiewiet and Ma 1991; Schoenmakers et al. 1992; Gonçalves et al. 2015; Gomes et al. 2018). Lead is one the most widely used metals in industry due to its exceptional properties. As a consequence about 1.9 million tons of lead were used in the production of glass pigments and painting in the United States and another 1.6 million for the gasoline production in the past 40 years (Kelly et al. 2005). Like Cd, Pb has no proven physiological role in living organisms but its high toxicity is well known. Pb may cause mortality, reproduction and behavioural effects in soil invertebrates (Langdon et al. 2005; Zhang and Van Gestel 2017).

For the risk assessment, total metal concentration usually is not the most relevant measure of exposure as it represents all the chemical forms of the element. More relevant is the fraction or concentration that is available for uptake by organisms or leaching to ground water (Alloway 2013). To what extent the available fraction in the pore water is bioavailable for organisms depends on metal speciation and on competition with other ions for binding and uptake. The properties of soils, especially pH, clay content, organic matter content, cation exchange capacity, and redox conditions, play a major role in determining metal bioavailability in the soil (Ardestani et al. 2014; Kim et al. 2015; Zhang et al. 2019).

The terrestrial mollusc *Helix aspersa* (syn. *Cantareus aspersus* or *Cornu aspersum* O.F. Muller 1774) is a

pulmonary gastropod considered as one of the most abundant land snails (Barker 2001). Their ecological position (herbivorous and detritivore organisms) in the trophic chain can play an important role in metal transfer through terrestrial ecosystems (Beeby 1985; Notten et al. 2005). *H. aspersa* may be exposed to pollutants via air, the digestive tract and by contact with the soil. They may accumulate pollutants in their shell, in the foot and mostly in the viscera. Like other soil invertebrates (e.g. Collembola, earthworms, enchytraeids, nematodes, and isopods), snails can be used as model for soil monitoring and ecotoxicity tests (Gomot-De Vaufléury et al. 1989; Gomot-De Vaufléury 2000; Scheifler et al. 2002; Regoli et al. 2006; Fritsch et al. 2011; Gomot-De Vaufléury et al. 2015; Louzon et al. 2020a, b) to determine the toxicity of pollutants (Van Gestel 2012). Gastropods of the genus *Helix* are macro concentrators for several metals (Dallinger 1993). For their capacity to resist and accumulate trace metals, in 2006 the International Standards Organisation (ISO) has developed a standardized ecotoxicity test using *H. aspersa*. So far, this test was largely used in the biomonitoring of contaminated soils (Gomot-De Vaufléury 1997; Swaileh, Ezzughayyar A 2000; Coeurdassier et al. 2002; Gomot-De Vaufléury et al. 2006; Gimbert et al. 2006, 2008) or in studies employing food exposure (Russell et al. 1981; Laskowski and Hopkin 1996a, b; Gomot-De Vaufléury 2000). Only a few studies have assessed the effect of soil properties on metal bioavailability to *H. aspersa* (Pauget et al. 2011, 2012, 2013).

The aim of this study is to assess the influence of physicochemical properties on the uptake and toxicity of cadmium and lead using the land snail *H. aspersa* as a biological model. In addition, by quantifying Cd and Pb concentrations in snails exposed in different types of soils, useful information is obtained to improve the use of *H. aspersa* in environmental monitoring studies.

Material and methods

Ecotoxicity tests

Ecotoxicity tests were performed following the ISO standard 15952 (ISO 2006), which involved 28-day exposures of juvenile *Helix aspersa* to a natural soil spiked with increasing concentrations of Cd or Pb. The animals used in this experiment came from a control breeding in big transparent plastic boxes (40 × 28 × 17 cm) under the following conditions: 5 cm layer of soil (soil moisture content was 50–60% of its Water Holding Capacity (WHC)), relative humidity inside the boxes = 80–95%, temperature = 20 ± 2 °C, photoperiod = 18/6 h light/dark. For the tests, homogeneous groups of 10 snails were selected. In order to follow individual growth, each animal was marked with a

Table 1 Physico-chemical properties of the soils used for assessing the bioavailability of Cd and Pb to *Helix aspersa*

Soil	Organic carbon (%)	WHC (%)	pH (0.01 M CaCl ₂)	CEC (cmolc/kg)	Clay (%)
LUFA 2.2	1.9	45.2	5.5	10.0	8.60
Garden soil	3.4	51.0	5.9	18.8	4.80
Soccer field soil	9.6	38.6	6.9	20.0	5.10

WHC water holding capacity, CEC cation exchange capacity (data taken from Romero-Freire et al. 2015)

numbered and coloured small sticker. The snails used had an average ($\pm$ SD; $n = 420$) fresh weight of 1.0 ± 0.13 g, and 15 ± 0.34 mm as shell diameter. The young snails were in aestivation under dry conditions for a week, after which they were fed and woken up by spraying with deionized water three days before starting the toxicity tests.

Test soils

Three soils were used with different properties (Table 1): the standard soil LUFA 2.2 obtained from the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) at Speyer, Germany, Garden soil from the Netherlands (a non-contaminated garden in Bilthoven), and a Grassland soil collected from a soccer field at De Kwakel, the Netherlands. Before use in the experiments, the soils were homogenized, 4 mm sieved and air dried at 40 °C.

Lead nitrate ($\text{Pb}(\text{NO}_3)_2$; purity 99.99%; Sigma-Aldrich: USA) was spiked into the soils to obtain concentrations of 13.6 - 40.7 - 122 - 366 - 1100 - 3300 mg Pb/kg dry soil. Cadmium nitrate ($\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ purity 99%; Sigma-Aldrich:USA) was spiked into the soils at concentrations of 8.2 - 24.7 - 74 - 222 - 667 - 2000 mg Cd/kg dry soil. The concentration ranges for both elements were chosen basing on the results of preliminary tests. Soils were spiked with the metals in aqueous solutions, at the same time bringing water content to 50% of their WHC. The soils were carefully mixed manually until homogeneity. Controls soils received only deionized water. Before use in tests, the spiked soils were equilibrated for 10 days in a climate room at 20 °C.

Experimental plan and tests conditions

All tests were carried out in transparent plastic containers ($20 \times 15 \times 10$ cm) containing 100 g of moist soil spiked with increasing concentrations of Pb or Cd. Five snails were placed in each container, using two replicate containers for each concentration and for the control as recommended in ISO15952 (2006). The young snails were fed *ad libitum* with uncontaminated fresh lettuce and special snail food as

described by Lodi and Koene (2015). Food was provided every three days during the entire test period to satisfy the energy needs of the snails and to eliminate the effect of other stress factors. To provide a source of calcium for the snails, CaCO_3 was mixed in with the food at a concentration of 18% (w/w). The humidity inside the test containers was maintained sufficiently high by spraying deionized water on the walls and directly on the soil after the daily removal of faeces and food waste to avoid mould formation.

The tests were kept in a climate room under low lightning (50–100 lux) with artificial light, at 18 h light/6 h dark, and a temperature of 20 ± 2 °C. Every week, snail weights were measured using an analytical scale having a precision of 0.01 g and the shell diameters were measured with a calliper rule with a precision of 0.01 mm in order to assess the effect of the metals on snail growth. Mortality rates (%) were also noted for each concentration during the test period.

Chemical analysis

At the end of test (28 days of exposure), the surviving snails were left without food for two days in empty boxes and under dry conditions to let them empty their intestinal tract. Individual snails were sacrificed by freezing at -18 °C. After thawing the frozen snails, the soft bodies were removed from the shell before being dried for 48 h in a stove (50 °C), and digested in a 4:1 mixture of HNO_3 (65%, Sigma-Aldrich, USA) and HCl (37%, Sigma-Aldrich, USA), in tightly closed Teflon containers heated in an oven (Binder FD) at 140 °C for 7 h. The total Pb and Cd concentrations were measured using flame atomic absorption spectrometry (AAS; AAnalyst 100, Perkin Elmer, Germany). The whole soft bodies of four snails were analyzed separately for each treatment level and test soil. The detection limit for these analyses was 0.03 mg/L for lead and 0.003 mg/L for cadmium, corresponding with a range of 1.43–4.74 mg Pb/kg dry tissue and 0.10–0.59 mg Cd/kg dry tissue, respectively. The reference material used for quality control was DOLT 4 (Dogfish liver, LGC standards); Cd recovery from this material was 99.5% of the certified level, but Pb concentration was too low (0.16 mg/kg) to allow for detection by flame AAS.

Total lead and cadmium concentrations in the three test soils were measured at the end of test using the same method, but by digesting 130 mg dry soil samples. ISE 989 (international Soil-Analytical Exchange) was used as a reference material, with recoveries of 99.3% of the certified concentration for cadmium and 95.9% for lead.

Data analysis

After 28 days of exposure the GC_w (weight growth coefficient) and GC_d (diameter growth coefficient) were

Table 2 The actual total lead and cadmium concentrations in Lufa 2.2, Garden and Soccer field soils measured at the end of the toxicity tests with *Helix aspersa*

Lead test				Cadmium test			
Nominal concentrations (mg Pb/kg)	Measured concentrations (mg Pb/kg dry soil)			Nominal concentrations (mg Cd/kg)	Measured concentrations (mg Cd/kg dry soil)		
	Lufa 2.2 soil	Garden soil	Soccer field soil		Lufa 2.2 soil	Garden soil	Soccer field soil
Control (0)	19.5	76.2	37.1	Control (0)	0.07	0.18	0.18
13.6	31.8	75.3	51.6	8.2	7.3	7.9	8.8
40.7	51.7	91.6	75.9	24.7	23.8	23.5	22.3
122	125	176	170	74	71.5	66.7	69.2
366	338	400	382	222	217	206	202
1100	1051	1181	1165	666	601	564	635
3300	3011	2895	3237	2000	1726	1863	1899

calculated for the growth of the snails in the three soils and at the different metal concentrations according to the formulas (ISO 2006):

$$GC_w = \left(\frac{mn - mt0}{mt0} \right) \times 100$$

and

$$GC_d = \left(\frac{dn - dt0}{dt0} \right) \times 100$$

where mn is the average weight of the surviving snails at the end of test, $mt0$ is the initial average weight of the snails, dn is the mean shell diameter of the surviving snails at the end of test and $dt0$ is the initial mean shell diameter of the snails.

The concentrations causing 50% of growth inhibition (EC50s) expressed based on GC_w and GC_d data were estimated using a logistic model:

$$Y = \frac{a}{1 + \left(\frac{C}{EC50} \right)^b}$$

Where Y is the mean of growth coefficient (GC_w or GC_d) of the surviving snails per concentration, a the mean of growth coefficient in the control, C the concentration tested, and b the parameter which characterizes the slope of the curve.

The effect concentrations causing $\times$ % growth inhibition (ECx) were estimated using a modification of this model:

$$Y = \frac{a}{1 + \left(\frac{x}{100-x} \right) \left(\frac{C}{ECx} \right)^b}$$

ECxs were estimated based on the total concentrations of lead and cadmium measured at the end of test and also on the internal concentrations in the surviving snails.

The bioaccumulation factor BAF was calculated as the ratio of the average lead or cadmium concentrations in *H. aspersa* measured at the end of test and the average total soil concentrations.

Growth and bioaccumulation data were analysed by two-way ANOVA considering the test concentrations and soil type as the two factors. In order to compare for each soil the significance of effects (growth and uptake effects) at the different test concentrations with the control, we performed Dunnett's test multiple comparisons ($P < 0.05$) for the growth data and Tukey's multiple comparisons test ($P < 0.05$) for the bioaccumulation data.

Results

The measured total soil concentrations overall showed good recoveries for Cd, but slightly more variation was observed for lead: Table 2.

Lead test

Mortality was low in the lead test, with all the snails surviving till the end of tests in the control and at the lowest concentrations in all three test soils. Only 10% mortality was observed for the Soccer field soil at 40.7, 122 and 3300 mg Pb/kg, and in the Lufa 2.2 soil at 366 and 1100 mg Pb/kg. At the highest concentration (3300 mg Pb/kg), 20% mortality was recorded in the Lufa 2.2 and Garden soils.

At the highest Pb concentrations and during the first two weeks, the snails were avoiding direct contact with the soil and the amounts of food consumed and faeces produced were visually reduced compared to the control and lower test concentrations.

A weak dose-related effect on snail growth was seen for Pb (Fig. 1). In all soils both weight increase and shell diameter growth of the tested snails were slightly decreased with increasing soil Pb concentrations. The two-way ANOVA test on growth coefficients showed a significant effect only at the two highest concentrations (1100 and 3300 mg Pb/kg) in Lufa 2.2 soil for GC_w and GC_d ($P = 0.0001$). For GC_w in Garden soil a significant effect was

Fig. 1 Effect of lead (left) and cadmium (right) on the Weight (top) and Diameter (bottom) growth % of *Helix aspersa* after 28 days exposure in Lufa 2.2, Garden and Soccer field soils. Lines show the fit of a logistic dose-response model to the data

Table 3 EC50s and EC20s (in mg Pb/kg dry weight) for the effect of Pb on the growth (mass gain: GCw; shell diameter increase: GCd) of *Helix aspersa* after 28 days exposure in three test soils, expressed on the basis of measured soil concentrations and internal concentrations in the surviving snails

	Lufa 2.2 soil	Garden soil	Soccer field soil
GCw			
EC20soil	332 (–)	468 (–)	1506 (273, 2739)
EC50soil	2397 (820, 3973)	3390 (1015, 5766)	4804 (1361, 8248)
EC20body	55 (–)	37 (–)	146 (40, 251)
EC50body	431 (128, 734)	437 (50, 824)	428 (135, 722)
GCd			
EC20soil	390 (–)	832 (–)	1667 (293, 3041)
EC50soil	3129 (814, 5444)	3526 (1509, 5542)	6357 (286, 12429)
EC20body	65 (–)	64 (–)	166 (54, 278)
EC50body	579 (103, 1055)	457 (113, 801)	540 (68, 1012)

Values between brackets represent 95% confidence intervals. (–) very wide confidence interval

seen at 1100 mg Pb/kg ($P = 0.0116$) and 3300 mg Pb/kg ($P = 0.0008$). GCd in Garden soil was also significantly lower at 1100 and 3300 mg Pb/kg with $P = 0.0077$ and $P = 0.0001$, respectively. For the Soccer field soil, a significant effect was found at the highest concentration only for both GCw ($P = 0.0003$) and GCd ($P = 0.0004$).

EC50s for the effect of Pb on GCw,d, related to measured concentrations in the test soils and internal concentrations in the snails, are shown in Table 3; Table S1 in the Supporting Information shows all parameters calculated when analysing the dose-response relationships.

The EC50s for the effect of Pb on GCw based on total Pb concentrations showed a clear difference between the test soils, and ranged between 2397 and 4804 mg Pb/kg dry soil. The same trend was noted for the effect on GCd, with EC50s ranging between 3129 and 6357 mg/kg dry soil.

There were almost no differences in the estimated EC50s based on internal lead concentrations in the surviving snails, with values ranging between 428 and 437 mg Pb/kg dry body weight for GCw and between 457 and 579 mg Pb/kg dry body weight for GCd.

The lead concentrations in *H. aspersa* (Fig. 2) showed a clear increase with increasing lead exposure concentrations in all test soils. The highest accumulation was observed in Lufa 2.2 soil with 545 mg Pb/kg dry body weight, compared with 375 mg Pb/kg dry body weight in the Garden soil and 294 mg Pb/kg dry body weight in the Soccer field soil.

Two-way ANOVA on *H. aspersa* lead bioaccumulation data showed no significant differences between the soils for all the concentrations tested except for the highest one where Pb levels were significantly higher in animals exposed to Lufa 2.2 soil compared to the other two soils ($P = 0.0001$). At the highest concentration, internal Pb concentrations were also significantly higher in animals kept on the Garden soil compared to the Soccer field soil ($P = 0.0489$).

The results in Fig. 3 shows clear differences in BAF values between the different soils with the highest BAF in Lufa 2.2 soil compared to Garden and Soccer field soils. BAFs did not show a concentration effect.

Cadmium test

At the end of cadmium tests there was almost no snail mortality in the controls, except in the Soccer field soil

Fig. 2 Average ($\pm$ SD; $n = 4$) internal Pb (left) and Cd (right) concentrations (mg Pb/kg dry body weight) in *Helix aspersa* exposed for 28 days to different Pb and Cd concentrations in Lufa 2.2, Garden and Soccer field soils

Fig. 3 Bioaccumulation factors (BAF) for the uptake of Pb (left) and Cd (right) in *Helix aspersa* exposed for 28 days to different Pb and Cd concentrations in three different soils

where 10% mortality was seen. Also at the lower Cd concentrations survival was very high except at 2000 mg Cd/kg in the Lufa 2.2 soil where 40% of the snails died. At this concentration, mortality was 30% in the Soccer field soil and 20% in the Garden soil. Soil avoidance was observed at most concentrations, with the snails becoming less active and showing less mobility compared with the control and the lowest test concentrations.

Two-way ANOVA showed a significant effect ($P = 0.0001$) on weight gain for snails exposed to 666 and 2000 mg Cd/kg compared with the controls in all three tested soils. At 222 mg Cd/kg, a highly significant effect was observed for snails exposed in Lufa 2.2 ($P = 0.0016$) compared with Soccer field soils ($P = 0.0422$). The shell diameter increase was significantly affected in Lufa 2.2 soil at 222, 666 and 2000 mg Cd/kg ($P = 0.0001$) and weakly significant at 74 mg Cd/kg ($P = 0.0449$). The effect of Cd on the shell diameter increase in Garden soil was highly significant at the two highest concentrations (666 and 2000 mg Cd/kg) ($P = 0.0001$) and significant at 222 mg Cd/kg ($P = 0.0052$). In the Soccer field soil, weakly significant effects were seen on shell diameter for snails exposed to 222 mg Cd/kg ($P = 0.0054$) and highly significant effects at 666 and 2000 mg Cd/kg ($P = 0.0001$).

Table 4 EC50s and EC20s (in mg Cd/kg dry weight) for the effect of Cd on the growth (mass gain: GCw; shell diameter increase: GCd) of *Helix aspersa* after 28 days exposure in three test soils, expressed on the basis of measured soil concentrations and internal concentrations in the surviving snails

	Lufa soil 2.2	Garden soil	Soccer field
GCw			
EC20soil	120 (58, 183)	203 (119, 286)	229 (95, 363)
EC50soil	327 (230, 424)	478 (362, 593)	591 (392, 791)
EC20body	129 (86, 172)	185 (133, 236)	125 (93, 156)
EC50body	257 (205, 310)	326 (273, 378)	194 (162, 226)
GCd			
EC20soil	100 (26, 173)	169 (67, 272)	177 (42, 312)
EC50soil	470 (285, 654)	654 (437, 872)	910 (544, 1275)
EC20body	117 (60, 175)	169 (98, 240)	106 (63, 149)
EC50body	333 (245, 421)	396 (312, 480)	261 (202, 319)

Values between brackets represent 95% confidence intervals

EC50s and EC20s for effects of Cd on GCw and GCd are reported in Table 4, while corresponding dose-response relationships are shown in Fig. 1. Table S2 in the Supporting Information shows the details of all parameters calculated when analysing the dose-response relationships.

The EC50s for effects on both GC_w and GC_d showed clear differences between the soils, and ranged between 327 and 591 mg Cd/kg dry soil for GC_w, and between 470 and 910 mg Cd/kg dry soil for GC_d. The EC50s based on internal Cd concentrations in the snails ranged between 194 and 326 mg/kg dry body weight for GC_w, and between 261 and 396 mg Cd/kg dry body weight for GC_d. The lowest EC50s based on total measured concentrations in soils were noted in Lufa 2.2 soil, but EC50s based on internal Cd concentrations in the snails were lowest in Soccer field soil.

After 28 days of exposure, the average internal Cd concentrations in *H. aspersa* (Fig. 2) were dose-related increased with increasing Cd concentrations in the three test soils. The highest values recorded in the snails were 752, 706 and 407 mg Cd/kg dry body weight for Lufa 2.2, Garden soil Soccer field and, respectively.

Applying a two-way ANOVA, no significant differences were found in internal Cd concentrations between the three soils at the lowest exposure concentrations. At 666 mg Cd/kg dry soil, Cd concentrations in animals exposed to the Soccer field soil were significantly lower compared to the Lufa 2.2 ($P = 0.0001$) and the Garden soils ($P = 0.0020$). Cadmium bioaccumulation in *H. aspersa* exposed to 2000 mg Cd/kg was significantly lower in animals kept on the Soccer field soil compared to the other two soils ($P = 0.0001$), but did not differ between the Lufa 2.2 and Garden soils.

BAF values for the uptake of Cd in the snails (Fig. 3) decreased with increasing soil concentrations in all three test soils. The highest BAF values were noted in Lufa 2.2 ranging from 2.53 to 0.43; BAF values ranged from 2.38 to 0.37 for garden soil and from 1.47 to 0.21 in soccer field soil.

Discussion

This study focused on the effect of soil properties on the availability of lead and cadmium as measured from its toxicity and bioaccumulation in the land snail *Helix aspersa*. The results show that soil properties can affect the availability of lead and cadmium to *H. aspersa*, with the highest toxicity (lowest EC50s) and highest bioaccumulation on Lufa 2.2 soil and lowest toxicity and bioaccumulation on Garden and Soccer field soils. This difference in the toxicity and bioaccumulation of lead and cadmium can be explained from soils properties affecting their bioavailability.

Lead and cadmium effects on *Helix aspersa* growth

The significant effect of lead on *H. aspersa* growth with EC50s of 2397–6357 mg Pb/kg dry soil disagrees with the

finding of Gomot-De Vaufleury (2000) who reported for growth effects an EC50 higher than 30,000 mg Pb/kg in snail food. Laskowski and Hopkin (1996b) found an EC50 of 13,900 mg Pb/kg in snail food for the effect on food consumption. Our results suggest that for lead exposure, soil is a more important route than food, with dermal uptake by diffusion through the foot epithelium (Gomot-De Vaufleury and Pihan 2002) and the ingestion of soil particles (Gomot-De Vaufleury et al. 1989; Scheifler et al. 2006) being the main routes of uptake.

The growth effect of Cd in this study confirms the results of previous studies for soil exposure (Coeurdassier et al. 2002; Gomot-De Vaufleury et al. 2006). Growth EC50s values estimated by Coeurdassier et al. (2002) ranged between 500 and 1000 mg Cd/kg for direct contact with polluted soil. Gomot-De Vaufleury et al. (2006) also reported EC50s for shell diameter and weight gain effects with a range of 373 to 781 mg Cd/kg dry soil in semi-static tests and 526 to 1060 mg Cd/kg dry soil in static tests. These values are similar to our EC50s ranging between 327 and 910 mg Cd/kg dry soil. On the other hand, the toxicity of Cd to *H. aspersa* growth for food exposure was approximately close to our finding (Russell et al. 1981; Laskowski and Hopkin 1996b; Gomot-De Vaufleury 1997). An EC50 of 140 mg Cd/kg dry food was reported by Gomot-De Vaufleury (1997) for the effect of Cd on juvenile snail growth, while Laskowski and Hopkin (1996b) found an EC50 of 206 mg Cd/kg dry food for the effect on food consumption.

Scheifler et al. (2006) concluded that the soil can contribute up to 80% to lead bioaccumulation by *H. aspersa*, compared to 30–60% for Zn and 2–40% for Cd. The fact that our EC50s for soil exposure were much lower than literature values for food exposure in case of Pb, but in agreement or even slightly higher in case of Cd, confirms this conclusion.

This study reports a dose-dependent relation between *H. aspersa* growth inhibition and Cd concentration in the test soils. The cutaneous pathway by the direct contact of the snail's foot with Cd in soil was shown to explain for the effect on *H. aspersa* individuals (Coeurdassier et al. 2002). Like Pb, Cd is taken up into the animal's body through the epithelium located on the foot and then accumulated mostly in the hepatopancreas (Coughtrey and Martin 1976; Gomot-De Vaufleury et al. 1989; Menta and Parisi 2001; Gomot-De Vaufleury and Pihan 2002).

Weight and shell growth can vary in response to environmental factors, including metal pollution (Ebenso and Ologhobo 2008) and calcium bioavailability (Ireland 1991). The body weight inhibition is more sensitive than shell size of sub-adult snails exposed to soil Cd (Locher and Baur 2000). Growth inhibition may be due to the lack of calcium uptake as found by Schoenmakers et al. (1992) for fish intestines. Indeed, for terrestrial gastropods, calcium is first

stored in the hepatopancreatic calcareous cells, then dissolved and transported into body parts in need of calcium carbonate, especially the shell during the growth period (Barker 2001). Cadmium and calcium are competitive antagonists (Massaro 1997). So, one potential explanation is that Cd reduces Ca transport from the hepatopancreas into the shell via competitive antagonism. Furthermore, it should be noted that the exact mechanism of the impact of Cd on snail shells is currently not known despite the evidence for metal-induced abnormal shell growth and shell damages in response even to short-term Cd exposure (Sunila, Lindström 1985; Fritsch et al. 2011).

The growth inhibition effects on the snails were directly related to the metal accumulation levels. This result is coherent with unpublished results of Gomot-De Vaufléury presented by Coeurdassier et al. (2002), who suggested that *H. aspersa* internal Cd concentration can be used to characterize its toxic effect on growth, independent of the uptake source. Also in our study, expressing toxicity on the basis of body concentrations reduced the difference in EC50 values between soils for Pb and to a lesser extent also for Cd, confirming that body concentration is a better indicator of metal toxicity in soil than soil concentration.

Compared to other soil invertebrates snails are less sensitive to lead. For *Enchytraeus crypticus* EC50s reported by Zhang and Van Gestel (2017) were as low as 13–189 mg Pb/kg dry soil, while an EC50 of 993 mg Pb/kg dry soil was reported for the earthworm *Eisenia fetida* (Davies et al. 2003) and of 428 mg Pb/kg dry soil for the springtail *Paronychiurus kimi* (lee) (Son et al. 2007). Also EC50s for the effect of cadmium on *H. aspersa* were higher (less sensitive) than the values for *E. crypticus* of 35 mg Cd/kg dry soil (Castro-Ferreira et al. 2012) and of 53.7–193 mg Cd/kg dry soil for the collembolan *Folsomia candida* (Van Gestel and Mol 2003).

***Helix aspersa* lead and cadmium bioaccumulation and soil properties**

BAF values were higher for Cd than for Pb, and were higher than 1.0 at low Cd levels. This suggests a high risk of Cd accumulation in the terrestrial food chain, especially for organisms feeding on snails. Risk for Pb accumulation in the food chain is much smaller considering the low BAFs for uptake in the snails. Terrestrial gastropods are considered as macro-concentrator species of Cd, but as micro-concentrators of Pb (Dallinger et al. 2001). BAF values decreased with increasing Cd concentrations but were independent of Pb exposure levels. The decrease with increasing soil concentrations is in agreement with findings for metal uptake in other soil and aquatic organisms (e.g. Coeurdassier et al. 2002; McGeer et al. 2003; Díez-Ortiz et al. 2010; Ardestani et al. 2014; Lanno et al. 2019).

Soil physico-chemical properties (e.g. pH, organic matter, redox conditions, clay and hydrous oxide content) control metal behaviour and bioavailability for soil organisms (Van Gestel et al. 1995; Kabata-Pendias 2004; Young 2013). In the present study, the variation in the bioaccumulation of lead and cadmium in *H. aspersa* was related to the differences in soil properties. The snails accumulated more lead and cadmium in Lufa 2.2 soil than in the other soils, suggesting that metals were more available in this soil. The soil physico-chemical characteristics, particularly the pH and organic matter content, are known to control soil heavy metals bioavailability especially Cd to land snails (Pauget et al. 2011). Compared with the other soils, Lufa 2.2 had the lowest pH and OM content and also the lowest CEC, making the metals more available (Lock and Janssen 2001; Bradham et al. 2006; Smolders et al. 2009; Romero-Freire et al. 2015; Zhang et al. 2019). The effect of soil pH on metal speciation and solubility, and its consequent effects on metal availability in soil, was also shown by other authors (Sandifer and Hopkin 1996; Sauvé et al. 2000). According to Kiewiet and Ma (1991), in case of earthworms the effect of soil pH on cadmium was not the same as for lead: cadmium uptake was independent of soil pH while for lead the internal body concentrations increasing were pH-dependent. A similar result was found in many other studies (Sandifer and Hopkin 1996; Van Gestel and Mol 2003; Ardestani and Van Gestel 2013). Thus, cadmium availability can be related to other soils properties than that of Pb. CEC can also change the sorption behaviour of metals in soils (Pardo 2000), pH and CEC are major soil properties influencing the ecotoxicity of Cd and Zn (Lock and Janssen 2001). This is supported by the findings of our study.

The BAF values of the current study for both Cd and Pb were similar to many previous findings (Laskowski and Hopkin 1996a; Gomot-De Vaufléury and Pihan 2002; Coeurdassier et al. 2002; Pauget et al. 2013), showing the bioaccumulation capacity of *H. aspersa* but also the effect of soil properties on the bioavailability of Cd and Pb. According to our findings, lead and cadmium were more available for the snails in the Lufa 2.2 soil compared with the Garden and Soccer field soils. This may again be explained by the lower pH, organic matter content and CEC of the Lufa 2.2 soil.

Numerous studies have confirmed the influence of soil properties on the mobility and availability of the metals (Bradham et al. 2006; Pauget et al. 2013; Zhang et al. 2019), but it should also be noted that the effects of soil properties on metal uptake and elimination rates were also species-dependent (Nahmani et al. 2009; Gomot-De Vaufléury et al. 2015). Ardestani and Van Gestel 2013 reported that proton competition with free Cd ions to bind with soil particles resulted in an increased cadmium availability at lower pH, but this did not affect the bioaccumulation in *F.*

candida (probably because of protons also competing with cadmium for uptake in the animals). This is contrary with our study in which the snails accumulated more Cd at the lowest pH. The bioaccumulation in *H. aspersa* in the present study was clearly higher for Cd compared to Pb in the different soils tested. Pauget et al. (2011) explained the difference in the accumulation rate between lead and cadmium by the fact that snails use different sequestration strategies for different metals. According to the literature, pH is the most dominant parameter controlling Pb availability in soil. As a consequence, pH plays a major role in metal adsorption and speciation processes (Bur et al. 2012; Van Gestel and Mol 2003; Sauvé et al. 2000).

Former studies have revealed a high affinity of metals to bind to soil organic matter, like Cd and Pb associating with –OH groups (Hooda and Alloway 1998; Pauget et al. 2011). This may explain our findings of the low cadmium and lead bioaccumulation in the snails exposed in the Garden and Soccer field soils with the highest OM contents compared with the low organic Lufa 2.2 soil. Emmanuel, Erel (2002) reported that 23 to 47% of anthropogenic Pb was bound to organic matter in forest soils. The amount of sorption sites for (metallic) cations on the soil (CEC) is related to organic matter content, but also Al and Fe oxyhydroxide, and clay content, which therefore directly determine metal availability in soils (Janssen et al. 1997).

While the effect of soil properties on metal availability, adsorption, and speciation is fairly straightforward, their effect on the toxicity and bioavailability of metals to soil organisms also depends on several other factors such as physiology of the species, exposure duration, environmental conditions, exposure pathway, and metal sources (Gomot-De Vaufléury et al. 2015). Smolders et al. (2009) concluded that metal toxicity cannot always be related to their availability, making it harder to link bioavailability to chemical availability.

Since only three soils were tested, our findings do not allow drafting regressions relating toxicity of bioaccumulation of Cd or Pb in the snails to soil properties. Further studies are needed to arrive at such relationships. The principal advantage of these laboratory tests is that they provide direct evidence of the role of soil exposure as the cause of metal toxicity for *H. aspersa*. The relation between the observed responses and soil concentrations can be used as a first indication of the risk of field-contaminated soil. It should, however, be noted that our tests were performed with freshly spiked soils, so probably overestimating metal availability in aged field-contaminated soils. Gomot-De Vaufléury et al. (2015) when studying in situ and *ex situ* methods for the use of land snails *H. aspersa* to characterize the fate and effects of metals and other chemicals compounds in soils, concluded that both toxicity and bioaccumulation results for land snails *H. aspersa* in laboratory studies can help improving the ecological risk assessments

of field-contaminated soils. The relationship between effects and body concentrations in the snails may be more indicative of metal bioavailability in soil and may therefore be used as a relevant endpoint for assessing field-contaminated soils. The bioaccumulation potential of the snails may also be indicative of the potential risk for the food chain of metal-contaminated soils.

Conclusion

The present study confirms the feasibility of using the land snail *Helix aspersa* as a biological model in laboratory tests to assess the toxicity of metals in soil. We confirmed that for lead more than for cadmium the soil is an important exposure route for the bioaccumulation in the snails. Internal concentrations in the snails were the best indicator of metal toxicity compared to soil concentrations. The soil with the lowest pH, organic matter content and CEC showed the highest toxicity and metal uptake in the snails, but since we tested only three soils it is hard to define which soil parameter had the greatest influence on Cd and Pb bioavailability to *H. aspersa*. This can be the subject of future work in order to determine which soil properties affect metal bioaccumulation in snails using a larger number of soils.

Also, it will be interesting to complete this study with an in situ exposure. This approach provides generally unique assessment information that is complementary to traditional laboratory-based toxicity and bioaccumulation testing. Results can reduce the uncertainties of extrapolating from the laboratory to field responses.

Data availability

The authors declare the availability of data and material.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants, Clinical Trials Registration or Plant Reproducibility performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Informed consent All the related authors have consented to participate and publish this article.

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