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How contamination sources and soil properties can influence the Cd and Pb bioavailability to snails

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Abstract To better understand the fate of metals in the environment, numerous parameters must be studied, such as the soil properties and the different sources of contamination for the organisms. Among bioindicators of soil quality, the garden snail (*Cantareus aspersus*) integrates multiple sources (e.g. soil, plant) and routes (e.g. digestive, cutaneous) of contamination. However, the contribution of each source on metal bioavailability and how soil properties influence these contributions have never been studied when considering the dynamic process of bioavailability. Using accumulation kinetics, this study showed that the main assimilation source of Cd was lettuce (68 %), whereas the main source of Pb was the soil (90 %). The plant contribution increased in response to a 2-unit soil pH decrease. Unexpectedly, an increase in the soil contribution to metal assimilation accompanied an increase in the organic matter (OM) content of the soil. For both metals, no significant excretion and influence of source on excretion have been modelled either during exposure or depuration. This study highlights how the contribution of different sources to metal bioavailability changes based on changes in soil parameters, such as pH and OM, and the complexity of the processes that modulate metal bioavailability.

Keywords Contamination sources · Snails · Kinetics · Soil · Metal · Bioavailability

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Introduction

Due to the increased contamination of the environment with metals since the onset of the industrial revolution, the soil has been monitored with bioindicators (Pérès et al. 2011; Pauget et al. 2013a). Assessing the bioavailability of contaminants is a valuable tool for the decision-making process for contaminated site management, especially when the soil or water is contaminated (Moreno-Jiménez et al. 2011). During an ecological risk assessment (ERA), single-species tests that cover single and identified exposure source (e.g. soil, food) or different routes (e.g. digestive, dermal) associated with a single compartment (e.g. sediment, water) are typically performed (Tarazona and Vega 2002). However, when undertaking passive, i.e. naturally exposed sentinel species, or active, i.e. in situ caging of sentinels species (Beeby 2001), biomonitoring, organisms can be exposed to multiple sources of contamination, such as soil, vegetation and air. Thus, knowing the influence of these exposure sources on how the bioindicator accumulates metal is necessary to accurately characterise the metal bioavailability to this organism (Fairbrother et al. 2007; Burger 2008).

Among soil fauna, the land snail *Cantareus aspersus* has been used for biomonitoring using snails caged in microcosms (Scheifler et al. 2003a; Gimbert et al. 2008a; Pauget et al. 2013b). Active biomonitoring allows all contamination sources to be considered (Scheifler et al. 2003a; Gimbert et al. 2008a). For this purpose, active biomonitoring coupled with accumulation kinetics is relevant to evaluate the metal bioavailability to organisms via the modelling of assimilation fluxes (Gimbert et al. 2008a). After assimilation, snails can sequester metals in various compartments (Gimbert et al. 2008b). Even if sequestration and elimination of metals have been studied (Vijver et al. 2005; Notten et al. 2006; Gimbert et al. 2008b), the influence of the sources of the metals and soil

parameters on excretion remains unknown. Previous studies on the contribution of contamination sources on metal accumulation (Scheifler et al. 2006; Fritsch et al. 2008) considered neither the dynamic processes of bioavailability nor the influence of soil properties on the metal transfer, even though these parameters are key factors. Thus, data on the contribution of each contamination source (e.g. soil, plant) on metal assimilation by snails and on the influence of soil properties on this contribution remain lacking.

Therefore, based on an experimental design using spiked and artificially modified soil, the present study aimed (i) to assess the relative contribution of soil and vegetation on the Cd and Pb assimilation and the influence of these sources on metal excretion by snails and (ii) to evaluate the influence of pH and organic matter (OM) on this contribution using kinetic modelling of accumulation.

Materials and methods

Animals

Juvenile brown garden snails (*C. aspersus* Müller, 1774) were reared under controlled conditions as described previously (Gomot-de Vaufleury 2000; ISO 15952 2006). The individuals used for the test were subadults, reared for 7–9 weeks and weighing 5.11±0.75 g (±SD; n=415) at the beginning of the experiment.

Exposition sources

Soil An uncontaminated agricultural field soil (Chambornay-les-Pin, Eastern France) was used for further soil parameter modifications (Table 1). Sandy loam was taken from the top layer (depth, 0–15 cm) of a maize field and transferred to the laboratory, where it was air-dried and then sieved through a 4-mm mesh.

The soil characteristics (Table 1) were artificially modified by adding to the dried soil of Chambornay-les-Pins: (i) dried ground peat (OECD 1984) to raise the organic matter content (soil P) from 1.4 to 8 % and/or (ii) $CaCO_3$ powder to obtain two nominal pH values (5 and 7) (OECD 1984).

The soils were spiked by spraying Cd ($CdCl_2$, 99.9 % purity, Aldrich Chemical) and Pb ($PbSO_4$, 99.9 % purity, Aldrich Chemical) in aqueous solution to reach nominal concentrations of 20 and 2000 mg kg^{-1} soil DW (dry weight), respectively. Deionised water was added to reach 50 % of the water holding capacities of the different substrates. The soils were left to stabilise in the dark for 1 month (Pauget et al. 2011) before snail exposure.

Lettuce Lettuce was cultivated on polystyrene containers on each soil of the experiment under controlled conditions (20± 2 °C; photoperiod 18 h L/6 h D; relative humidity 80±90 %). Three times per week, the lettuce was watered with uncontaminated water. During the experiment, snails were fed with lettuce that had grown for 2 months on the soil on which snails are exposed. Thus, contaminated lettuce was obtained for each contaminated soil to provide better environmental relevance.

Experimental design

Accumulation kinetics were determined based on a 10-day experiment, in which snails were exposed to (i) contaminated soil with contaminated lettuce (S*L*), (ii) contaminated soil with uncontaminated lettuce (S*L), (iii) uncontaminated soil with contaminated lettuce (SL*), (iv) contaminated soil without lettuce (S*) and (v) contaminated lettuce without soil (L*).

For each treatment, ten snails were housed in each of three replicate polystyrene containers (24×21×8 cm) containing a

Table 1 Main parameters of contamination sources (soils and lettuce)

Source		[Cd] mg/kg	[Pb] mg/kg	pH_w –	OM g/kg	Clay g/kg	CEC cmol+/kg	$[Al]_{ox}$ g/100 g	$[Fe]_{ox}$ g/100 g	Silts g/kg	Sands g/kg
Contaminated soil	7C	18.0	1980	7.29	14.8	140	5.8	0.132	0.909	397	463
	7P	17.9	1980	7.35	74.5	166	11.9	0.133	0.884	430	404
	5P	18.1	2030	4.73	80.9	103	13.1	0.14	0.864	482	415
Uncontaminated soil	7C	0.263	33.1	7.7	15	132	5.75	0.133	0.907	376	492
	7P	0.21	25.6	7.6	76.4	150	13.2	0.127	0.849	406	444
	5P	0.221	27.2	4.93	77.7	163	12.3	0.135	0.867	416	421
Contaminated Lettuce	7C	72.7	144								
	7P	83.7	64.6								
	5P	113	80.4								
Uncontaminated Lettuce	7C	2.28	6.7								
	7P	2.43	2								
	5P	2.19	3.61								

1-cm layer (100 g dry mass) of soil prepared as described above (except for treatment L*, in which no substrate was added). The photoperiod was 18 L/6 D, and the temperature was 20±2 °C. The relative humidity was kept at 80 to 95 %. The soil moisture content was maintained at its initial level by regular spraying with demineralised water. Three times per week, the containers were cleaned, any remaining food was replaced and demineralised water was sprayed to prevent the soil from drying out.

After 2, 4, 6, 8 and 10 days of exposure, one snail per treatment was randomly sampled. After 10 days of exposure, the organisms were transferred onto the corresponding uncontaminated soil and fed with uncontaminated lettuce to assess the metal depuration, and one snail per treatment was randomly sampled after 2, 5, 7 and 10 days of depuration.

Analytical procedures

Soil Total Cd and Pb was measured by inductively coupled plasma atomic emission spectrophotometry (ICP-AES) after digestion of the soil samples (250 mg) with hydrofluoric and perchloric acid, as described in AFNOR (1996). Analyses were performed by the Laboratoire d'Analyse des Sols of Arras (France), which benefits from the COFRAC (French accreditation committee) accreditation n°1-1380 for its analytical quality for metal measurements in soils. Soil characteristics were assessed/described by the same laboratory.

Lettuce Lettuce was freeze-dried until reaching a constant weight (0.08±0.07 mg) and digested in a solution of 6 mL nitric acid and 2 mL hydrogen peroxide (HNO_3 65 %, H_2O_2 30 %, Carlo-Erba analytical quality). After digestion, the samples were diluted by adding ultra-pure water (18.2 MΩ/cm^2) as previously described by Fritsch et al. (2011) and analysed by ICP OES (iCAP 6000, Thermo Fisher Scientific).

Snails Snails for analysis were placed in clean containers, fasted for 48 h (the faeces were removed after 24 h) and then weighed. The snails were sacrificed by freezing at −80 °C. After thawing, the whole soft body was removed from the shell, and the foot and viscera were separated (Gomot-de Vaufleury and Pihan 2002). Only the viscera were studied in this work because they contain the digestive gland (hepatopancreas), which is the main site of metal accumulation and storage in snails (Hopkin 1989). The viscera were oven-dried at 60 °C until reaching a constant weight (0.228±0.049 g, n= 415) and digested in nitric acid (HNO_3 65 %, Carlo-Erba analytical quality), as previously described (Gomot-de Vaufleury and Pihan 2002). After digestion, samples were diluted adding ultra-pure water (18.2 MΩ/cm^2), filtered with ash-free filter paper and analysed by ICP OES (iCAP 6000, Thermo Fisher Scientific).

The analysis reliability was assessed with standard reference materials (ICHTJ-cta-VTL-2, Institute of Nuclear Chemistry and Technology, Poland, for plants and TORT-2 lobster hepatopancreas; National Research Council of Canada–Institute for National Measurement Standard, Ottawa, ON, Canada, for snails) and was within 10 % of the certified values.

Statistical analyses

Accumulation modelling Bioavailability results from a dynamic interaction between the metal concentration in the soil (environmental availability) and the physiology of the target species. To assess bioavailability, a one-compartment model was used to fit the accumulation kinetic data (Gimbert et al. 2006; Pauget et al. 2011). This model expresses the dynamic change of metal concentration in the snail viscera (C_{sn} in $mg_{metal}kg\ DW_{sn}^{-1}$) over time, based on the following equation (Eq. 1):

$$C_{sn}(t) = C_{sn}(0) + \frac{a}{k_2}\left(1-e^{-k_2 t}\right), \quad (1)$$

where a is the assimilation flux constant ($mg_{metal}kg\ DW_{sn}^{-1}\ day^{-1}$), which is considered as an indicator of the metal bioavailability to snails (Gimbert et al. 2008a; Pauget et al. 2011), k_2 is the excretion rate constant (day^{-1}) and t is time (days). $C_{sn}(0)$ is the average metal concentration measured in ten snails at the beginning of the experiment ($mg_{metal}kg\ DW_{sn}^{-1}$).

When negative value estimates of excretion rate k_2 (which do not make biological sense) were modelled by Eq. 1, a linear model was substituted into Eq. 1 according to Eq. 2 to accurately assess the assimilation flux (a) in these particular cases:

$$C_{sn}(t) = C_{sn}(0) + a * t. \quad (2)$$

Depuration modelling During the depuration phase, excretion can occur and lead to variations of metal concentrations in snails (C_{sn}) with time, according to the following equation (Eq. 3):

$$C_{sn}(t) = C_{mu} - C_{mu} * \left(1-e^{-k_{2d}(t-t_c)}\right), \quad (3)$$

where C_{mu} is the metal concentration at the end of the exposure period (mg kg DW_{sn}^{-1} (Gimbert et al. 2006)), k_{2d} is the excretion of metal (day^{-1}), t_c is the time (days) at which animals were transferred to the clean soil and t is the time (days) since the beginning of the experiment. All negative estimates of excretion (k_{2d}) were considered as not biologically relevant and therefore are not presented in the tables of results.

The accumulation and excretion parameters were estimated by fitting the models with a mixed-effect procedure (non-linear mixed-effect (nlme) or linear mixed-effect (lme))

(Lindstrom and Bates 1990), allowing for nested random effects. The within-group errors were allowed to be correlated and/or have unequal variances. The nlme integrated the soil as a fixed factor and the container as a random effect. The lme integrated the metal concentration in snails as the dependent variable and the soil as the explanatory variable. For all models (lme and nlme), variance functions (power and exponential) were applied when residuals were skewed, and the best model was selected according to Akaike's information criterion (AIC, pgirmess package) (Burnham and Anderson 2004). Significant differences in parameter estimates between treatments were judged from the absence of overlap of their 95 % confidence intervals (95 % CI). All statistical analyses were performed with the free statistics software package R (version 2.10.1, R Development Core Team 2011).

Determination of the contribution of soil and lettuce in the Cd and Pb bioavailability

To quantify the relative contribution of soil and lettuce to the metal bioavailability to snails, we compared the mean assimilation flux percentage of snails exposed to one contamination source to the sum of the assimilation fluxes of snails exposed to (i) the soil and (ii) the lettuce according to the following:

$$\text{Contribution of soil} = \text{mean}\left(\frac{a_{S*L}}{a_{S*L}+a_{SL*}}*100+\frac{a_{S*}}{a_{S*}+a_{L*}}*100\right)$$

$$\text{Contribution of lettuce} = \text{mean}\left(\frac{a_{SL*}}{a_{S*L}+a_{SL*}}*100+\frac{a_{L*}}{a_{S*}+a_{L*}}*100\right)$$

Results

Accumulation and depuration kinetics

At the beginning of the exposure, the internal concentrations in snails were 0.73±0.10 mg kg^{-1} for Cd and 0.59± 0.26 mg kg^{-1} for Pb (n=10). After 10 days of exposure, Cd and Pb accumulated in the snails, as indicated by the significant assimilation fluxes, which ranged from 0.604 to 4.34 mg Cd $kg_{sn}^{-1}day^{-1}$ and from 0.954 to 66.7 mg Pb $kg_{sn}^{-1}day^{-1}$. Only 7P S* (p value=0.267) for Cd and 7P SL* (p value=0.169) for Pb yielded positive but non-significant assimilation fluxes, which have been modelled (Table 2).

Overall, the highest assimilation flux values for Cd were observed when the contaminated lettuce was offered, whereas for Pb, snails exposed to the contaminated soil presented with the highest assimilation fluxes.

During the exposure phase, no significant (p value >0.05) excretion rate (k_2) was modelled for Cd. For Pb, few excretion rates were modelled. Only one excretion rate was significant for 5P S*L*.

During the depuration phase, Cd did not appear to be excreted by the snails. Only three positive k_{2d} values were modelled (two are significant and one is not significant) (Table 2). In contrast to Cd, the Pb seems to be excreted, as indicated by the ten modelled positive k_{2d} values (four significant and six not significant).

Influence of soil properties on metal bioavailability to snails

Influence of organic matter content An increase of organic matter content appears to reduce Cd bioavailability to snails when they are exposed to the treatments presenting contaminated lettuce (L*) decreasing from 3.93 to 2.94 $mg_{Cd}kg_{sn}^{-1}day^{-1}$ (S*L*), from 3.15 to 1.36 $mg_{Cd}kg_{sn}^{-1}day^{-1}$ (SL*) and from 3.33 to 1.68 $mg_{Cd}kg_{sn}^{-1}day^{-1}$ (L*) at 1.4 and 8 % of OM content (respectively). In the treatments with contaminated soil (S*), a slight increase is observed (Table 2 and Figs. 1 and 3). For Pb, an increase of OM content seems to increase the Pb bioavailability to snails under the S*L*, S*L and S* treatments, as shown by the assimilation fluxes, which respectively increased from 19.5 to 23.8 $mg_{Pb}kg_{sn}^{-1}day^{-1}$ (S*L*), from 16.3 to 21.3 $mg_{Pb}kg_{sn}^{-1}day^{-1}$ (S*L) and from 7.3 to 20.4 $mg_{Pb}kg_{sn}^{-1}day^{-1}$ (S*) (Table 2 and Figs. 2 and 3).

Influence of pH A decrease of 2.6 units of pH tended to increase Cd bioavailability in all treatments (Table 2 and Figs. 1 and 3) with assimilation fluxes values going from 2.94 to 4.34 $mg_{Cd}kg_{sn}^{-1}day^{-1}$ (S*L*), from 1.07 to 1.95 $mg_{Cd}kg_{sn}^{-1}day^{-1}$ (S*L), from 1.36 to 2.83 $mg_{Cd}kg_{sn}^{-1}day^{-1}$ (SL*) and from 1.68 to 3.69 $mg_{Cd}kg_{sn}^{-1}day^{-1}$ (L*). For Pb, a significant increase of bioavailability was observed (Table 2 and Figs. 2 and 3) for the S*L* treatment at the lower pH, with the assimilation flux going from 23.8 to 66.8 $mg_{Pb}kg_{sn}^{-1}day^{-1}$, and the L* treatment, with the assimilation going from 0.579 to 4.15 $mg_{Pb}kg_{sn}^{-1}day^{-1}$, but no difference was observed between the S*L and S* treatments.

Determination of the contribution of soil and plants to metal bioavailability to snails

We wanted to first determine whether the sum of the assimilation fluxes of snails exposed to (i) soil and (ii) lettuce corresponded to the assimilation flux of snails exposed to the both sources. For Cd, the sum of the assimilation fluxes ($a_{S*L}+a_{SL*}$ and $a_{S*}+a_{L*}$) corresponded to the measured assimilation fluxes on the S*L* treatments for the three soils (Table 3). For Pb, the sum of the assimilation fluxes of the two sources corresponded to the bioavailability to snails of both sources on soils 7P and 7C with the S*L and SL* treatments and on soil 7P with the S* and L* treatment. For the other cases, the assimilation flux with both contamination

Table 2 Estimates of kinetic parameters for Cd and Pb accumulation and depuration in *Cantareus aspersus* exposed to contaminated soils and/or lettuce

Modality	Exposure							Depuration			
	a $mg_{metal}kg_{sn}^{-1}day^{-1}$	95 % CI	p value	k_2 day^{-1}	95 % CI	p value	$C_{sn}(10)$ calc $mg_{metal}kg_{sn}^{-1}$	k_{2d} day^{-1}	95 % CI	p value	$C_{sn}(20)$ calc $mg_{metal}kg_{sn}^{-1}$
Cadmium											
7C S*L*	3.93	2.87/4.97	<0.001	0.216	−0.017/0.449	0.111	16.83				
7C S*L	0.736	0.579/0.893	<0.001				8.09				
7C SL*	3.15	1.23/5.07	0.011	0.17	−0.032/0.372	0.145	15.87				
7C S*	0.604	0.320/0.888	0.002	0.005	−0.021/0.034	0.721	6.62	0.046	0.030/0.062	<0.001	4.18
7C L*	3.33	0.970/5.70	0.024	0.209	−0.046/0.464	0.155	14.69				
7P S*L*	2.94	2.53/3.35	<0.001				30.13	0.052	0.023/0.080	0.007	17.91
7P S*L	1.07	0.507/1.63	0.046	0.181	−0.348/0.710	0.536	5.67				
7P SL*	1.363	0.583/2.14	0.01	0.028	−0.180/0.236	0.809	12.62				
7P S*	1.538	−0.91/3.99	0.267	0.3	−0.380/0.982	0.429	5.60	0.017	−0.001/0.035	0.113	4.73
7P L*	1.68	1.20/2.16	<0.001				17.53				
5P S*L*	4.336	3.34/5.33	<0.001	0.18	−0.018/0.378	0.118	20.84				
5P S*L	1.95	1.08/2.82	0.002	0.074	−0.336/0.484	0.744	14.51				
5P SL*	2.827	0.983/4.67	0.015	0.326	−0.011/0.661	0.098	9.07				
5P S*	1.19	0.788/1.60	<0.001				12.63				
5P L*	3.689	1.78/5.59	0.041	0.288	−0.182/0.758	0.277	12.82				
Lead											
7C S*L*	19.545	8.73/30.4	0.006	0.051	−0.253/0.357	0.76	153.69	0.002	−0.039/0.045	0.913	150.65
7C S*L	16.31	11.3/21.3	<0.001				163.69	0.046	0.011/0.083	0.034	103.33
7C SL*	0.954	0.833/1.07	<0.001				10.13				
7C S*	7.31	4.78/9.82	<0.001				73.69	0.047	0.010/0.082	0.039	46.06
7C L*	1.33	0.922/1.76	<0.001				13.89				
7P S*L*	23.84	17.6/30.0	<0.001				238.99	0.074	0.029/0.119	0.013	114.03
7P S*L	21.34	17.3/25.4	<0.001				213.99	0.031	−0.016/0.080	0.251	156.95
7P SL*	1.349	−0.358/3.06	0.169	0.298	−0.241/0.835	0.325	4.89				
7P S*	20.391	6.32/34.5	0.021	0.23	−0.172/0.632	0.31	80.36				
7P L*	0.579	0.405/0.752	<0.001				6.38	0.038	0.003/0.075	0.083	4.36
5P S*L*	66.75	39.3/94.2	0.001	0.316	0.083/0.549	0.028	202.86				
5P S*L	22.55	19.0/26.1	<0.001				226.09	0.011	−0.015/0.037	0.438	202.54
5P SL*	3.81	2.58/5.01	<0.001				38.69	0.056	0.023/0.089	0.013	22.10
5P S*	20.59	17.9/23.3	<0.001				206.49	0.035	−0.026/0.096	0.306	145.51
5P L*	4.15	1.58/6.70	0.009				42.09	0.05	0.004/0.098	0.071	25.20

a assimilation flux reflecting bioavailability, k_2 excretion rate during the exposure time, k_{2d} excretion rate during the depuration time, $C_{sn}(10)$ modelled snail viscera concentration after 10 days of exposure, $C_{sn}(20)$ modelled snail viscera concentration after 10 days of exposure and 10 days of depuration

sources was higher than the sum of the assimilation fluxes for the separate contamination sources. However, on soil 5P, the Pb bioavailability to snails exposed to the two contamination sources may have been overestimated; the sums of $a_{S*L}+a_{SL*}$ and $a_{S*}+a_{L*}$ were the same, and the internal concentrations of Pb after 10 days of exposure were quite similar among the S*L*, S*L and S* treatments.

The relative contribution of each contamination source is presented in Table 4. Without considering the influence of the soil parameters, the contribution of soil to Pb assimilation by snails is approximately three times higher than for Cd (90 vs 32 %, respectively), whereas the contribution of lettuce was more than six time higher for Cd than for Pb (68 vs 10 %, respectively). When considering the influence of soil characteristics, we found that raising the OM content increased the soil contribution by 29 % for Cd and by 6 % for Pb, thereby decreasing the lettuce contribution. A decrease of two pH units decreased the soil contribution by 13 % for Cd and 11 % for Pb, thereby increasing the lettuce contribution.

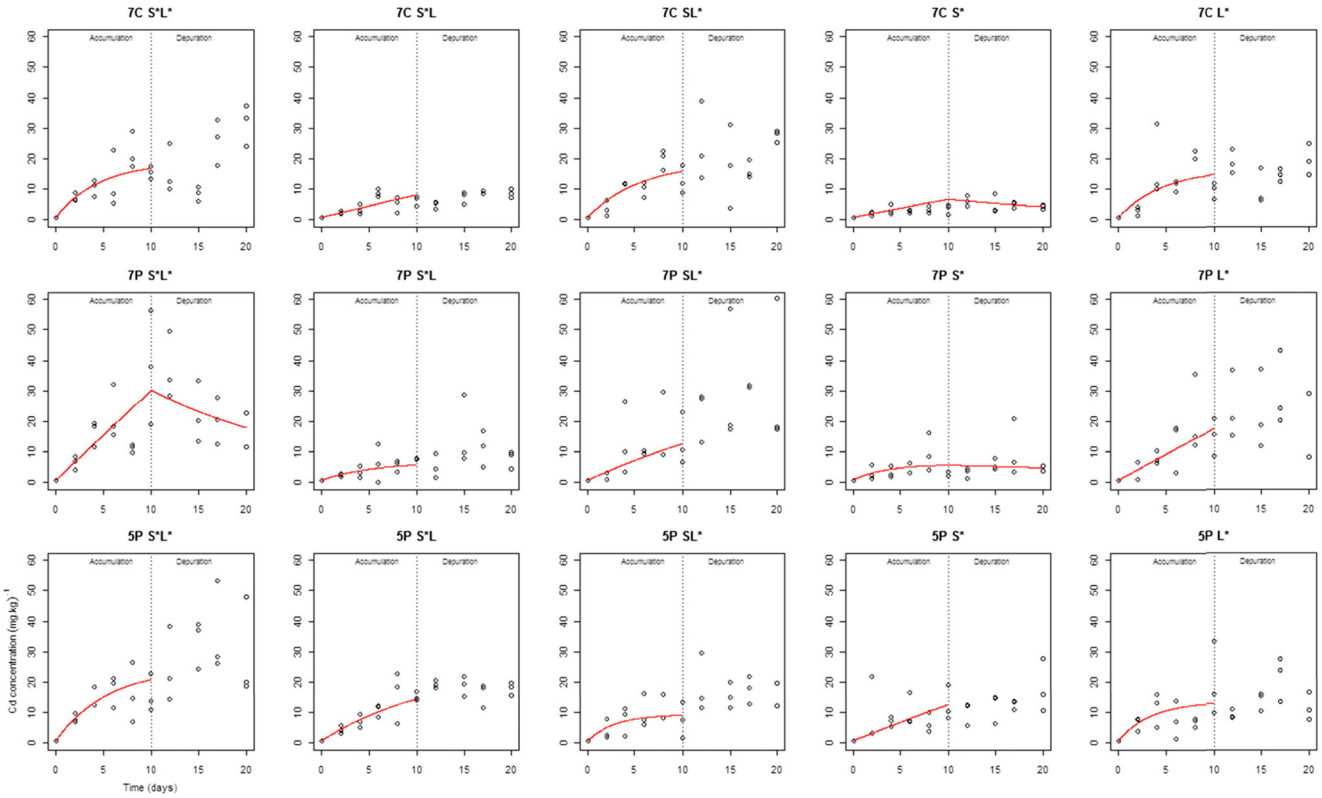

Fig. 1 Modelled accumulation kinetics of Cd in *C. aspersus* exposed to soils C and P (i.e. soil C+peat) with decreasing pH. The Cd concentrations in the snails' viscera are expressed on a dry weight basis. The *curve* represents the accumulation kinetic. *Each data point* represents an individual snail

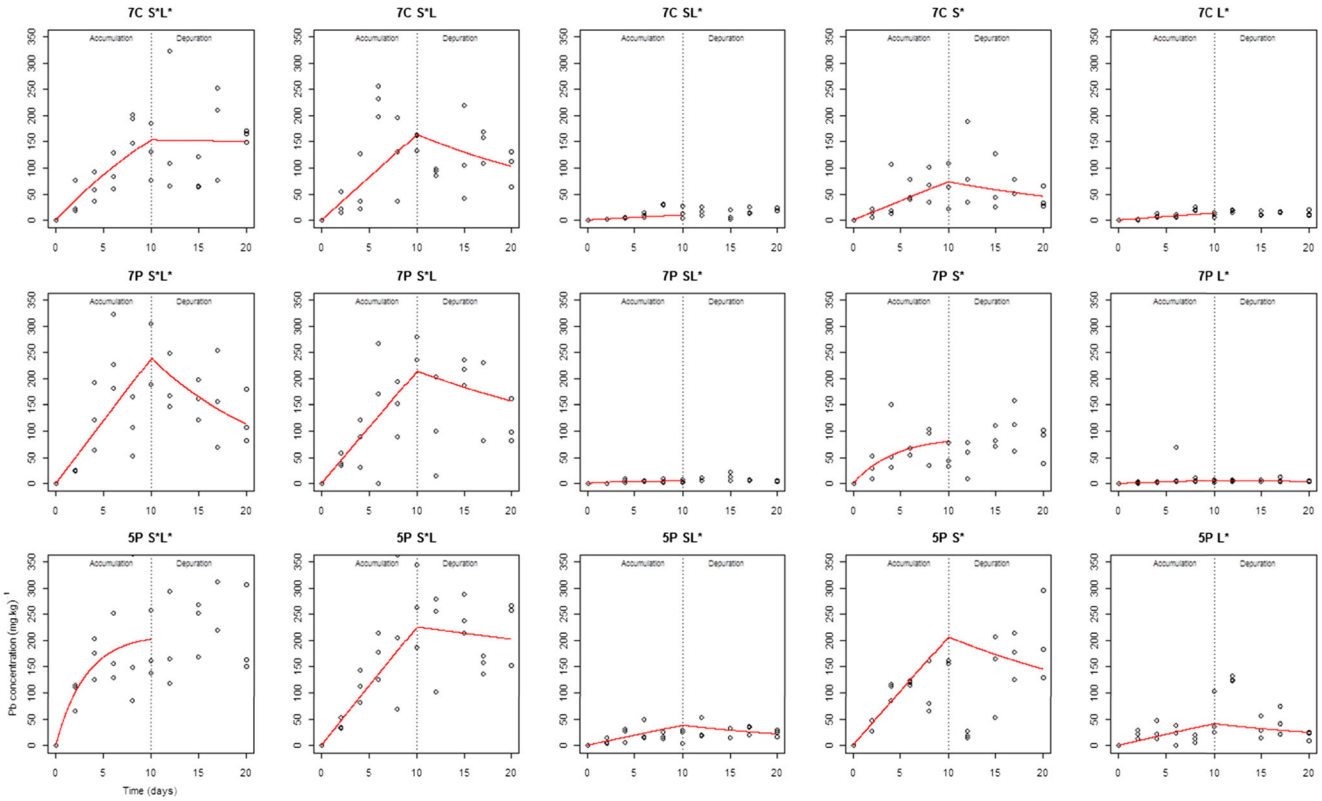

Fig. 2 Modelled accumulation kinetics of Pb in *C. aspersus* exposed to soils C and P (i.e. soil C+peat) with decreasing pH. The Cd concentrations in the snails' viscera are expressed on a dry weight basis. The *curve* represents the accumulation kinetic. *Each data point* represents an individual snail

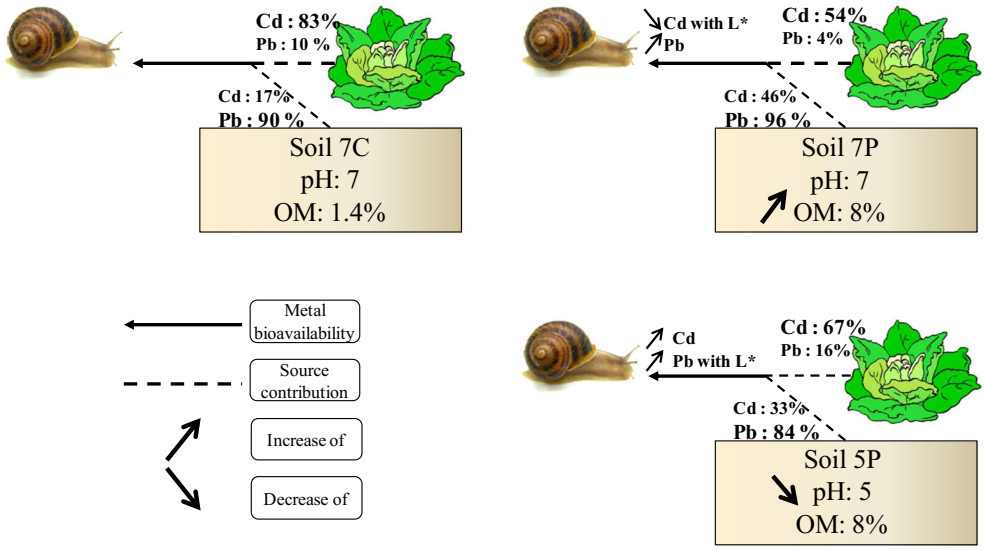

Fig. 3 Overview of the influences of soil properties on Cd and Pb bioavailability and contribution sources

Discussion

Accumulation and excretion of metals

In this 10-day exposure study, we characterised relevant indicators of the bioavailability of Cd and Pb that are present in soil and lettuce to snails by modelling assimilation fluxes for both contamination sources. Soil metal bioavailability to snails appears higher for Cd than for Pb, as shown by the maximum factor of ten between the assimilation fluxes of Cd and Pb, whereas the total concentration in soil is 100 times lower for Cd than for Pb. This high Cd bioavailability can be explained by the ability of the snail to assimilate the non-labile fraction (Scheifler et al. 2003b). Moreover, the use of kinetic studies allows the excretion rates to be modelled, which could be particularly interesting to highlight the internal management of these contaminants (Gimbert et al. 2008a). In our study, significant excretion rates was not reached, probably due to the short exposure duration and inter-individual variability of metal accumulation, the latter of which has already been observed. Moreover, Cd accumulation by snail tends to be linear, with Cd binding to metallothioneins (MT) that are very slowly excreted (Vaufleury 2015). Under our experimental conditions, the sequestration of Cd, which mainly binds to MT in the cytosolic fraction (Vaufleury 2015), may not be efficient enough to be overloaded by the elevated assimilation of Cd (Höckner et al. 2011). The Cd concentration in snails after the exposure duration was greatly lower than that found in snails in the study of Dragos et al. (Nica et al. 2015). For Pb, its sequestration within metal-rich granules allows all assimilated Pb to be stored, limiting its excretion during our short exposure phase (Gimbert et al. 2008b).

The depuration period allowed the identification of positive Cd excretion values in only three treatments and Pb excretion values in eight treatments. In accordance with the literature (Hopkin 1989; Spurgeon and Hopkin 1999; Dallinger et al. 2004), our results highlight the differences in Cd and Pb excretion by snails. The low turnover of Cd-MT may explain the

Table 3 Comparison between assimilation fluxes modelled when snails are exposed to two contamination sources and the sum of assimilation fluxes of snails exposed to a single contamination source

Soil	Parameter	Metal	
		Cd (mg kg^{-1} day^{-1})	Pb (mg kg^{-1} day^{-1})
7C	a_{S*L*}	3.93	19.5
	$a_{S*L}+a_{SL*}$	3.88	17.3
	$a_{S*}+a_{L*}$	3.93	8.64
7P	a_{S*L*}	2.94	23.8
	$a_{S*L}+a_{SL*}$	2.43	22.7
	$a_{S*}+a_{L*}$	3.22	21.0
5P	a_{S*L*}	4.34	66.8
	$a_{S*L}+a_{SL*}$	4.78	26.4
	$a_{S*}+a_{L*}$	4.88	24.7

Table 4 Relative contributions of soil and lettuce to the metal bioavailability to snails

Contribution	Soil	Metal	
		Cd (%)	Pb (%)
Soil	7C	17.1±2.54	89.5±6.98
	7P	45.9±2.70	95.6±2.25
	5P	32.6±11.6	84.4±1.64
	Mean	31.9±14.4	89.9±5.64
Lettuce	7C	82.9±2.54	10.5±6.98
	7P	54.1±2.70	4.4±2.25
	5P	67.4±11.6	15.6±1.64
	Mean	68.1±14.4	10.1±5.64

low excretion, and the internal concentrations remained elevated at the end of the depuration phase, whereas the Pb bounded to granules is more easily excreted (Gimbert et al. 2008b). The absence of modelled Cd excretion values during the depuration phase is due to a slight increase in the Cd concentration in snails even though they were exposed to uncontaminated soil and lettuce. During this study, the metal concentration was not measured in the foot. The slight increase in the Cd concentration during the depuration phase may be due to a metal transfer from the foot to the viscera. Indeed, in the absence of an external metal source during the depuration phase, only the foot can be involved because the shell is not a metal sink (Laskowski and Hopkin 1996). Because the dermal route via the foot is a significant route for metal assimilation (Coeurdassier et al. 2002), Cd may continue to come from the foot during the depuration phase and/or the snails can relocate metal in different parts of their body (Gimbert et al. 2006), thus obscuring metal depuration by a metal flux coming from the foot.

Soil and plant contributions to metal bioavailability to the snails

Our experimental design permitted us to assess the contribution of both soil and lettuce on metal bioavailability to snail. Soil characteristics, such as pH and OM, and plant contributions are important explanatory variables to model Cd and Pb accumulation in snails, as demonstrated in situ (Pauget et al. 2015). However, understanding the complex interactions between soil-plant and soil-plant-snails transfer remains a great challenge. The use of accumulation kinetics allows the influence of soil parameter, such as OM content and pH, on metal fluxes in organisms (assimilation and excretion) to be assessed, whereas these influences are not identified when comparing metal concentrations in the snail viscera after 10 days of exposure.

We observed different influences of contamination sources on the amount and fluxes of metal assimilated by the snails. Cd was mainly assimilated from lettuce (68.1 %), whereas assimilated Pb came mainly from the soil (89.9 %). These results are in accordance with a previous study based on two durations of exposure; this study estimated that the soil contribution to metal bioaccumulation by snails can be higher than 80 % for Pb and from 2 to 40 % for Cd, depending on the stage of development of the plant (Scheifler et al. 2006). The use of kinetic studies to identify the contamination sources allows the fluxes of metal in snails from each source of exposure to be determined and highlights difference even if the same concentrations in metal are measured in the viscera at the end of exposure. The determination of the fluxes, the sources and the speciation of the metal assimilated are important during bioavailability assessments because they condition the adverse effects of metals (Notten et al. 2005; Peijnenburg et al. 2007; Calhôa et al. 2011). The difference between the Cd and Pb in the plant contribution may be due to differences in the metal locations within the plant. Indeed, Cd may be mainly present as free metal ions in the leaves (Leita et al. 1991; Mendoza-Cozatl et al. 2011) and thus be easily bioavailable to snails (Li et al. 2015), whereas Pb is located in the cell walls (Qureshi et al. 1986). The sequestration form of the metal in the contamination source is important because Cd that has bound to metallothioneins is less bioavailable to isopod than Cd bound to heat-denatured proteins (Monteiro et al. 2008).

The similar assimilation fluxes of the snails exposed to both contamination sources and the addition of assimilation fluxes of snails exposed only to one contamination source (S*+L* or S*L+SL*) suggest that the feeding behaviour of snails is not modified by the presence of metal in the food (soil or plant), as previously observed (Noret et al. 2005; Sinnett et al. 2009). When we focus on soil parameters that influence the contribution of the sources on metal bioavailability to snails, we observe an influence of OM content. Unexpectedly, the addition of organic matter increases the contribution of soil for Cd and Pb bioavailability, possibly due to the increase of the OM content in the soil solution, leading to greater metal accessibility (Girard et al. 2005). Another hypothesis is an increase in soil ingestion by the snails. When deriving the uptake rates ($k_1 = a$/metal concentration in soil or lettuce, representing the exposure (Pauget et al. 2011)) for the S*L treatment with soils 7C and 7P, we observed an increase of soil exposure, indicating increased soil ingestion. Snails must eat soil to obtain nutrients (Gomot et al. 1989). Thus, the addition of OM in soil 7P may decrease the concentration of nutrients in soil and may force the snails to eat more soil to satisfy their physiological needs. Moreover, a soil with a high OM content may be more palatable, and the OM consumed by snails contributes to their growth (Elmslie 1998).

When focusing on the influence of a decrease in pH, we observed an increase in the contribution of the lettuce to Cd and Pb assimilation related to the greater lettuce contamination. Alternatively, this increased lettuce contribution may be due to the decreased soil contribution. Even if the soil contribution has decreased, assimilation fluxes from the soil have increased but to a lesser extent than the increased assimilation fluxes in snail exposed to lettuce. This difference may be due to differences in the bioavailability of metal coming from soil and plants. An increase of Cd and Pb bioavailability has already been shown with a 2-pH-unit decrease by Pauget et al. (2011) when soil is the only source of contamination. This increase occurs because at neutral pH, the Cd and Pb adsorption and precipitation increase (Boshoff et al. 2015). This increase is mainly due to the modification of metal mobility in the soil, as observed in previous studies (Sterckeman et al. 2004; Van Gestel and Koolhaas 2004) that have shown that Cd and Pb speciation partially depend on soil acidity. Indeed,

acidic soils might modify colloid effects on proton activity, increasing their solubility and their bioavailability to snails and lettuce. Moreover, Sauvé et al. (2000) reported that the pH explained 47 % of the Pb and Cd partitioning coefficients.

Conclusion

This study provides new information that is necessary to better understand metal bioavailability and transfer to snails. Indeed, the complex evaluation of contamination sources and the respective influences of environmental parameters such as OM and pH on both phyto- and zooavailability is needed to understand metal transfer during in situ metal bioavailability assessment because organisms are exposed to multiple contamination sources. The great contribution of lettuce in Cd assimilation by snails reinforces the fact that only measuring the total soil concentration is insufficient to accurately assess Cd bioavailability to snails. These data on source contributions will improve bioavailability modelling under complex exposure patterns (e.g. active biomonitoring) and biodynamic modelling to assess metal bioavailability to snails for risk-assessment purposes.

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