Determining cadmium critical concentrations in natural soils by assessing Collembola mortality, reproduction and growth ☆

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A B S T R A C T

The toxicity of cadmium for the Collembola *Folsomia candida* was studied by determining the effects of increasing Cd concentrations on growth, survival and reproduction in three cultivated and forested soils with different pH (4.5–8.2) and organic matter content (1.6–16.5%). The Cd concentration in soil $CaCl_2$ exchangeable fraction, in soil solution and in Collembola body was determined. At similar total soil concentrations, the Cd concentration in soil solutions strongly decreased with increasing pH. Reproduction was the most sensitive parameter. Low organic matter content was a limiting factor for reproduction. Effect of Cd on reproduction was better described by soil or body concentrations than by soil solution concentration. Values of $EC_{50\text{-Repro}}$ expressed on the basis of nominal soil concentration were 182, 111 and 107 $\mu g\ g^{-1}$, respectively, for a carbonated cultivated soil (AU), an acid forested soil with high organic matter (EPC) and a circumneutral cultivated soil with low organic content (SV). Sensitivity to Cd was enhanced for low OM content and acidic pH. The effect of Cd on reproduction is not directly related to Cd concentration in soil solution for carbonated soil: a very low value is found for $EC_{50\text{-Repro}}$ (0.17) based on soil solution for the soil with the highest pH (AU; pH=8.2). Chronic toxicity cannot be predicted on the basis of soluble fractions. Critical concentrations were 8×10^{-5}, 1.1, 0.3 $\mu g\ mL^{-1}$, respectively, for AU, EPC and SV soils.

1. Introduction

Critical load (CL) was defined as "the quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to the present knowledge" (Nilsson and Grennfelt, 1988). CL has first been developed to evaluate and mitigate the effect of acid rain on forest ecosystems in Europe (De Vries, 1988; De Vries et al., 1994; Warfvinge and Sverdrup, 1992; Hornung et al., 1995) and particularly in France (Dambrine et al., 1993; Massabuau et al., 1995; Party et al., 1995, 2001; Moncoulon et al., 2004, 2007). Since 1991, CL maps are used for international negotiations on air pollution abatement strategies (Hettelingh et al., 1991). It is now well admitted as a powerful tool to estimate ecosystem sensitivity to pollutant inputs.

As a first attempt, CL for heavy metals (HM) in soils (De Vries and Bakker, 1996) were estimated in a similar way (De Vries and Bakker, 1996; Probst et al., 2003a, b; Slootweg et al., 2005). CL are based on the estimate of the highest concentration in soil solution (critical concentration, CC, De Vries et al., 2007) supposed to have no effect on a soil function, community or population. Indeed this CC remains difficult to determine. Actually, models based on free ions concentrations are often used for that purpose (Lofts et al., 2004). But calibrations are still needed regarding toxicological impact on various soil organisms under different soil properties (De Vries et al., 2007). Bioassays using soil organisms might be useful tools to determine CC if they refer to ecologically relevant parameters like mortality, growth or reproduction. Molecular parameters (for example enzymatic activities) do not allow to determine the ecological impact of a specific chemical. Five standardized tests using soil animal models are used in Europe: *Eisenia fetida* (Annelide) mortality and reproduction tests (International Standard Organisation, ISO 11268-1, 1994, ISO 11268-2, 1997), *Folsomia candida* (Collembola) reproduction test (ISO 11267, 1998), Enchytraeids reproduction test (ISO 16387, 2001), and the assay for field testing with earthworm (ISO 11268-3, 1999). These assays usually concern normalised substrate rather than field soils.

Collembola are relevant target organisms to determine CC of HM in soils since (i) they are detritivorous, contribute to the

☆The experiments were conducted in accordance with national and institutional guidelines for the protection of human subjects and animal welfare.

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nutrient cycle in soils and are widespread in most natural soils. Moreover, they have been extensively studied, particularly *F. candida*, which is commonly used as a biological model in laboratory test for pollutant toxicity assessment. The *F. candida* reproduction test is increasingly used because it is sensitive and because the breeding of *F. candida* is easy (Riepert, 1995). It was mainly used to assess the toxicity of pure metals (Van Straalen et al., 1989; Crommentuijn et al., 1995, 1997; Sandifer and Hopkin, 1996; Scott-Fordsmann et al., 1997; Smit and Van Gestel, 1998; Crouau et al., 1999; Fountain and Hopkin, 2001) or of organic chemicals (Herbert et al., 2004; Eom et al., 2007). Application to complex mixtures such as polluted soils or wastes are less numerous (Smit and Van Gestel, 1996; Fountain and Hopkin, 2004; Crouau et al., 2002; Crouau and Cazes, 2005; Crouau and Pinelli, 2008). The effects of temperature (Snider and Butcher, 1973), pH and soil moisture (Holmstrup, 1997; Van Gestel and Van Diepen, 1997; Van Gestel and Mol, 2003) as well as the variability between strains on test sensitivity have been studied (Crommentuijn et al., 1995; Chenon et al., 1999; Crouau and Moia, 2006).

Organic matter and pH have often been identified as key factors, which control the complexation and availability of metals, and thus influence metal toxicity, noticeably for Cd (see as example, Crommentuijn et al., 1997; Son et al., 2007).

To ensure their protection regarding agriculture and atmospheric inputs, CCs of HM must be determined for different kinds of soils with emphasis on Collembola as target living organisms. With that aim, processes and key factors of metal toxicity in "natural" soils must be better investigated. French soils present a large variety of conditions and show pollution by HM, noticeably Cd and Pb (Hernandez et al., 2003; Probst et al., 2003a, b).

In this paper, we used the reproduction of *F. candida* as an indicator of soil ecosystem sensitivity to cadmium and to determine CCs in soil solution. Moreover, we studied the mortality and the growth of *F. candida* to improve the understanding of Cd effects. To be as representative as possible of natural conditions, three spiked typical natural French soils with different pH and OM characteristics were used to perform toxicity tests. In these particular conditions, Cd LC_{50} (lethal concentrations), LOEC (lowest efficient concentrations), EC_{50} and EC_5 (efficient concentration) for reproduction were computed. Cd concentrations in organisms and in soil solution and $CaCl_2$ exchangeable Cd, were determined. The influence of the main soil parameters (pH, OM) on toxicity, was assessed. Finally, a method for estimating critical concentration from EC_5 values is presented.

2. Materials and methods

2.1. Soil characteristics

Experiments were performed using three soils : two cultivated soils from the South-West area of France (Auradé, AU and Saint-Victor, SV) and a forest soil (EPC, under spruce cover) from the centre part of France were chosen (Table 1). These soils were selected given their varying pH and OM contents (Table 1), to investigate the influence of these two parameters on *F. candida* reproduction. The two cultivated soils have a very low OM content and are circumneutral to basic (particularly the carbonated soil, AU) compared to the acidic forested soil (EPC). Soil moisture were set up to 25%, 30% and 20%, respectively, for AU, EPC and SV (50% water holding capacity, WHC), to enhance development of Collembola by creating an adequate crumbly structure. Cd concentrations were in the range of low contaminated soils (Baize, 1997).

The aim of the study was to compare the responses to Cd toxicity of various soils with different physico-chemical characteristics. Consequently, to match field conditions as closely as possible, the ISO 11267 guidelines were not appropriate.

2.2. Collembola cultures

A culture of *F. candida* was reared in the laboratory at 20 ± 1 °C in darkness, in glass containers with a base of plaster of Paris/charcoal powder mixture (ratio 4/1). Distilled water and a small amount of dried Baker's yeast (as a food source) were added weekly. *F. candida* juveniles were collected two times a week with a suction apparatus in order to select synchronized populations.

2.3. Toxicity tests

The test consists in exposing juveniles to field soils contaminated by Cd and in comparing reproduction, growth and mortality with those of animals placed in non-contaminated control soil. For toxicity tests, plastic containers of approximately 100 mL were used. The natural soils were spiked with $Cd(NO_3)_2$ (0, 50, 100, 200, 400 µg Cd g^{-1} dry soil) and were equilibrated for a week before starting the toxicity tests. Cd was dissolved in distilled water, which was used for soil moistening. For each soil and each spiking concentration, 8 test containers (12 for the control) were filled with 45 g of moist soil. Fifteen *F. candida* juveniles (8–12 days old juveniles) were introduced into each container. Indeed, we used 8–12 days old animals rather than 10–12 days (as recommended by the ISO guideline no. 11267). Young animals are more sensitive to metal toxicity and thus we think that animals as young as possible must be tested. The containers were aerated twice a week. Exogenous yeast was not added during testing in order to be closer to field conditions. The duration of exposure was 50 days, instead of the 28 days recommended in the ISO guideline (ISO no. 11267, 1998) in order to counterbalance the lack of added food during the assay. Indeed, this might lead to a lower reproduction rate than those of normalised assays with food addition. Moreover, a longer exposure duration increases the sensitivity of the assay ($EC_{50\text{-repro}}$ for Cd after 6 weeks $< EC_{50\text{-repro}}$ after 4 weeks; Van Gestel and Mol, 2003), decreases the variability of test results, and therefore increases the efficiency of the assay (Crouau and Cazes, 2003). Lastly, a longer exposure duration was more realistic because in field conditions, Collembola are exposed to pollutants for longer than 28 days. Moreover, increasing the assay duration allows to increase the number of individuals at the end of the assay and, on account of this, counterbalances the effect of none food addition, which would have decreased the number of individuals.

At the end of the exposure time (20 ± 1 °C, in darkness), containers were flooded with deionised water and gently stirred in order to make all living animals to float at the water surface. The water surface of each container was photographed. Adults and juveniles were counted and their lengths (from the end of the posterior abdominal segment to the anterior margin of the head) were measured. All the animals were measured, but the animal number depended on the series and was the lowest for highest Cd concentration series. The limit length between juvenile and adult populations was defined as the length class presenting the lowest individual abundance (sum of the five concentration abundances), around the third quartile value.

Wilcoxon's two-sample test was used to assess significant differences of reproduction, mortality and length between exposed and control series. Non-parametric methods were used given the non-normal distribution of the data and the heterogeneity of variances. The EC_5 and EC_{50} values were calculated by using the maximum likelihood-probit procedure (Toxcalc 5.0 software, Ives, 1996; EPA methods).

2.4. Chemical analyses

For each spiked concentration, Collembola were extracted (alive) and placed during 3 days in fasting containers with only a humid filter paper to control hygrometry. Collembola were then killed by freezing (−80 °C). For each soil and

Table 1
Physico-chemical properties of soils used for toxicity tests on reproduction of *F. candida* (OM: organic matter content; RMQS: French national network for soil quality measurement; RENECOFOR: French national network for forest ecosystems long-term survey, Ponette et al., 1997, data from Hernandez et al., 2003).

Soils	Origin	Soil cover	Clay (%)	pH (H_2O)	OM (%)	Cd (µg g^{-1})
AU	Experimental catchment (SW France)	Wheat/sunflower	37.2	8.2	2.0	0.29
EPC	RENECOFOR (W France)	Forest	19.4	4.5	16.5	0.10
SV	RMQS (SW France)	Corn	24.8	6.1	1.6	0.17

each Cd test concentration, pools of 17 ± 9 μg dry weight (about 20 animals) were digested (ultrapure HNO_3, 90 °C) together with blank and standard samples in a clean room. Cd concentration measurements in Collembola were controlled by using the international reference material TORT-2 (Lobster Hepatopancreas).

After dissolution procedure, a single analytical measurement was done in a series including blank and reference material. Cd concentrations were analysed in Collembola with a Perkin-Elmer inductively coupled plasma–mass spectrometer (ICP–MS). In soil solutions and in exchangeable $CaCl_2$ solutions, Cd was analysed by inductively coupled plasma-optical emission spectrometry (ICP-OES). The main Cd isotope (^{114}Cd) was measured as it presented the lowest deviation during ICP–MS measurements. The detection limit of Cd measurements was 1.6×10^{-3} μg L^{-1} for body concentration and 1 mg L^{-1} for water concentration. The significance of analytical interference was negligible (e.g. As and Co levels were lower with several orders of magnitude than Cd in spiked bodies and soil solutions).

ICP–MS and ICP-OES measurements were calibrated using a set of gradually concentrated external standards. For body concentration measurements, 3 calibration lines were performed for a 14 sample set analysis, using 4 standards for each (with Cd content from 0 to 55 μg L^{-1}). The correlation coefficient of the calibration line for ^{114}Cd was very elevated (r=0.9999), which guaranties the quality of Cd measurement in bodies. During ICP–MS measurements, as a routine procedure, an In/Re mix was used as an internal standard for body concentration measurements.

Dissolved organic carbon (DOC) in soil solutions was analysed using a Shimatzu TOC 5000 Carbon Analyser. The detection limit of DOC measurements was 0.1 μg L^{-1}. Pore water was extracted by soil centrifugation (2000g, 15 min). Exchangeable Cd was obtained by adding 10^{-2} mol L^{-1} $CaCl_2$ (soil/solution=1/10, w/w). Solutions were filtered (with 0.22 μm filter) before element and DOC analyses.

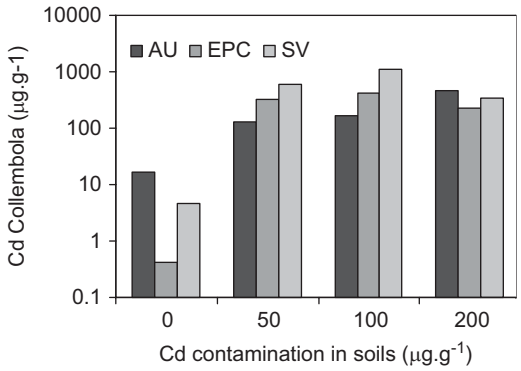

Fig. 1. Cd concentrations in animals for the experimental concentrations (50, 100 and 200 μg g^{-1}) applied on the three considered soils (AU, EPC and SV), compared to the control Cd content in soils. (y axis: log unit).

3. Results

3.1. Collembola and soil analyses

3.1.1. Cd concentrations in *F. candida*

Cd concentrations in body have been measured for all the experiment concentrations, except for 400 μg g^{-1} condition because not enough animals were available for analytical determination (Fig. 1). Cd concentrations were high in all exposed animals. The highest Cd concentrations were found in Collembola of the SV soil (except for the 200 μg g^{-1} condition) with a maximum value of 1100 μg g^{-1} dry wt for the 100 μg g^{-1} dry soil condition. The lowest concentration was measured in the Collembola of the AU soil, except for the 200 μg g^{-1} condition for which the organisms concentrations in EPC and SV decreased.

3.1.2. Soil solution and exchangeable Cd concentrations, pH and DOC

Cd concentrations in the soil solutions and in the exchangeable fractions are summarised in Table 2. At the beginning of the assay (t1), soil solution concentrations increased with Cd nominal soil concentrations for the three soils. But, concentrations in the soil solutions and in the exchangeable fractions were much higher in the EPC and SV soils than in the AU soil. Cd in soil solution increased with time for SV and decreased for EPC.

Soil solution pH was circumneutral for AU and acidic for EPC and SV; pH was similar at t1 and t8 for AU, but it decreased slightly (half a unit) for EPC (except at 400 μg g^{-1}) and very much for SV. EPC showed DOC concentrations higher than those of AU and SV. This difference can be explained by the higher OM content of EPC (16.5% for EPC, 2% and 1.6%, respectively, for AU and SV). DOC did not change very much during the assay in AU, whereas it increased in EPC and decreased in SV for the highest Cd concentrations.

3.2. Collembola length

AU soil: Two groups of distinct lengths were observed for each soil concentration. The individuals added to the test containers at the beginning of the assay are the longest animals. The shortest group was composed of juveniles, which were born during the

Table 2
Cd concentrations in the three soils (nominal value, dry wt. basis), in corresponding soil solution (at t_0+1 week and t_0+8 weeks) and in $CaCl_2$ exchangeable fraction. pH($CaCl_2$) and pH(H_2O) and DOC (at t_0+1 week and t_0+8 weeks) are also indicated.

Cd soil	**Cd solution** (μg mL^{-1})		**Cd $CaCl_2$**(μg mL^{-1})	**pH soil solution**		**pH**	**DOC** (μg mL^{-1})	
Nominal (μg g^{-1})	t1	t8		t1	t8	$CaCl_2$	t1	t8
AU								
0	< DL	< DL	0.05	7.11	7.72	6.9	26	23
50	0.003	< DL	0.24	7.25	7.65	7.04	25.6	20.2
100	0.009	< DL	0.58	7.56	7.57	6.98	27.1	20.3
200	0.47	0.43	2.07	7.45	7.45	7.03	31.4	30.1
400	1.69	2.93	9.95	7.09	7.4	6.94	31.7	33
EPC								
0	0.02	0.01	0.11	4.09	3.73	2.98	14.3	21.2
50	10.60	5.21	29.85	3.85	3.58	2.96	24.5	24.9
100	30.02	19.80	67.11	3.89	3.73	3.01	25.8	43.7
200	90.54	45.28	112.65	3.4	3.1	3.05	26.3	66.2
400	280.29	195.06	284.59	3.4	3.49	2.98	40	94.2
SV								
0	< DL	0.04	0.67	4.16	2.57	4.44	30.2	28.8
50	6.68	12.91	27.28	4.2	3.53	4.44	32.3	49.3
100	18.45	35.04	47.33	4.05	3.3	4.47	43.2	26.1
200	51.89	82.33	112.97	3.99	3.36	4.57	66.4	48.6
400	143.13	185.23	264.47	3.91	3.19	4.64	72.7	59

< DL: inferior to detection limit.

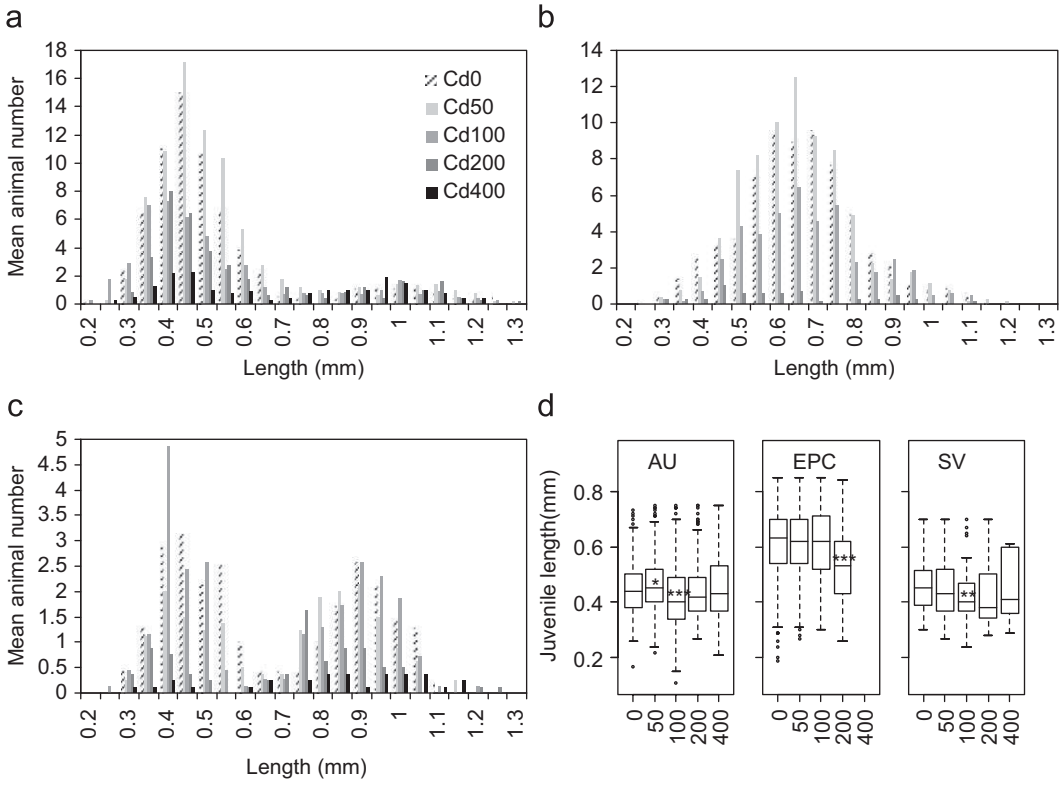

Fig. 2. Number of juveniles and adults for each length class and each Cd soil concentration in AU (a), EPC (b) and SV (c) soils. (d) Medians, quartiles and mode of the lengths of juveniles (significant differences to control: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$)

Table 3
Mean and range (in brackets) EC_5, EC_{50} and LOEC for reproduction (juveniles number) and mortality (adults number) for the three soils (AU, EPC, SV) relatively to Cd concentrations in soil (nominal concentration, $\mu g\ g^{-1}$), in soil solution and to $CaCl_2$ exchangeable Cd($\mu g\ mL^{-1}$).

	EC_5	EC_{50}	LOEC
Juveniles			
AU			
Soil	43.5 *(11–77)*	182 *(134–254)*	400 *($p < 0.05$)*
Soil solution	7×10^{-4} *(5×10^{-6}–5×10^{-3})*	0.17 *(0.05–0.6)*	1.7 *($p < 0.05$)*
Exchangea.	0.13 *(0.012–0.32)*	1.9 *(1.1–3.8)*	9.9 *($p < 0.05$)*
EPC			
Soil	56 *(26–72)*	111 *(96–133)*	100 *($p < 0.05$)*
Soil solution	9 *(2.6–13.4)*	22 *(18–28)*	30 *($p < 0.05$)*
Exchangea.	42 *(19–52)*	72 *(63–83)*	67 *($p < 0.05$)*
SV			
Soil	15	107	200 *($p < 0.01$)*
Soil solution	2.6	36	52 *($p < 0.01$)*
Exchangea.	8	57	113 *($p < 0.01$)*
Adults			
AU			
Soil	–	–	–
Soil solution	–	–	–
Exchangea.	–	–	–
EPC			
Soil	110 *(47–137)*	16 *(118–177)*	200 *($p < 0.05$)*
Soil solution	22 *(8–29)*	35 *(24–39)*	45 *($p < 0.05$)*
Exchangea.	72 *(38–85)*	95 *(76–103)*	77 *($p < 0.05$)*
SV			
Soil	78 *(29–113)*	223 *(178–280)*	200 *($p < 0.05$)*
Soil solution	26 *(8–42)*	93 *(71–121)*	82 *($p < 0.05$)*
Exchangea.	35 *(12–55)*	129 *(98–170)*	113*($p < 0.05$)*

assay (Fig. 2a). The mean lengths of the two groups (juveniles and adults) were 0.45 and 1 mm, respectively. The limit point between the two populations was 0.75 mm. There is no significant difference between the lengths of the blank series and of the other series (adults and juveniles groups) except for the 100 $\mu g\ g^{-1}$ series (Fig. 2d).

EPC soil: The maximum number of individuals was observed for the 0.65 mm length. Contrary to what was observed for the AU and SV soils, Collembola length in EPC soil showed a single population distribution for all tested Cd concentrations (Fig. 2b). Applying the same method as for the other two soils, the limit between juveniles and adults was 0.85 mm. The increase of the soil Cd concentration did not change significantly the median of juvenile length, except for the 200 $\mu g\ g^{-1}$ series.

SV soil: Collembola of the SV soil showed two populations with different length ranges (Fig. 2c) for all series. The limit between juveniles and adults was 0.7 mm. Significant length differences of the blank series were only detected for the 100 $\mu g\ g^{-1}$ dry soil series (Fig. 2d). The lack of significant differences for the highest Cd concentrations (200 and 400 $\mu g\ g^{-1}$) could be due to the higher variability and the less numerous data for these two series.

3.3. Collembola reproduction and mortality

The EC_5, EC_{50} and LOEC for reproduction and mortality tests are given in Table 3. Comparison of the $EC_{50\text{-repro}}$ of the three soils on the basis of soil concentrations indicated that Cd has the same toxicity for the SV and EPC soils and a lower toxicity for the AU soil. It was the opposite for the $EC_{50\text{-repro}}$ calculated on the basis of soil solutions. A decrease in juveniles (effect on reproduction—Fig. 3a) and in adults (effect on mortality—Fig. 3b) populations with increasing Cd concentrations was observed for the EPC and SV soils. The AU soil showed a decrease in the juvenile number, but not in the adult number. A small increase in adults and juveniles was often observed for the lowest Cd concentrations, except in SV; the EPC and AU soils showed an

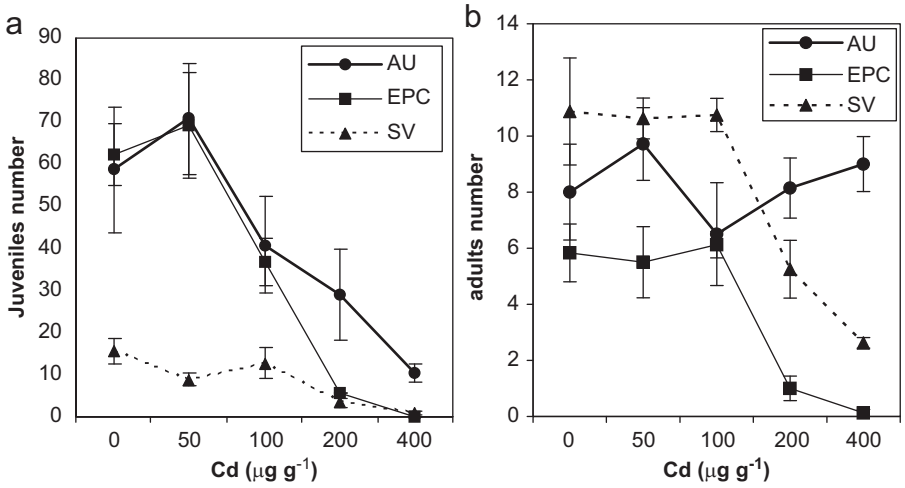

Fig. 3. Dose–effect relationship of Cd (nominal concentrations in soil) on the reproduction ((a) mean number of juveniles ± S.E.M.) and the mortality ((b) mean number of adults ± S.E.M.) of *F. candida*.

increase in juvenile number for the 50 μg g^{-1} series, followed by a regular decrease for the three highest concentrations.

4. Discussion

Juvenile productions were low when compared to litterature references (Van Gestel and Mol, 2003; Greenslade and Vaughan, 2003) and the results of the assays did not fulfil validity criteria of the norm, probably due to differences in experimental conditions (no food addition, natural vs. artificial soil). Indeed, the objectives were not to strictly follow the norm but to fit as much as possible the field conditions. The lowest juvenile production in control series was observed for the SV soil (average of 14 ± 3 juveniles), which was nearly neutral. The two other soils are either calcareous (AU) or have a high organic matter content (EPC), which seems to increase the reproduction rate. The decrease in juveniles numbers in the SV and EPC soils (200 and 400 μg g^{-1} series) could be partly due to the effect of Cd on mortality of adults (Fig. 3).

Values of EC_{50} based on reproduction ($EC_{50\text{-repro}}$) were 182, 111 and 107 μg g^{-1} dry soil for AU, EPC and SV, respectively. These values were consistent with some studies (Crommentuijn et al., 1997;Crouau et al., 1999; Herbert et al., 2004), but two studies (Van Gestel and Van Diepen, 1997; Van Gestel and Mol, 2003) reported values which are about two times lower than the range of this study. High pH value (AU) increased EC_{50} values based on nominal concentrations. The $EC_{5\text{-repro}}$ were 43, 56 and 15 μg g^{-1} dry soil for AU, EPC and SV, respectively. The 95% confidence interval for AU overlapped that of EPC, indicating that the two values are almost equivalent.

For the AU soil, juvenile numbers decreased strongly with the increase in Cd concentration (Fig. 3b). Mortality of adults was low whatever the soil Cd concentrations. We did not observe a significant shift towards smaller lengths of juveniles with Cd concentration increase; therefore, in AU soil, Cd affected reproduction rather than mortality and growth of *F. candida*. Such a higher sensitivity of the reproduction parameter by comparison to mortality and growth parameters was previously observed (Van Straalen et al., 1989; Scott-Fordsmann et al., 1997; Fountain and Hopkin, 2001).

For the SV soil, only the number of juveniles in the 200 μg g^{-1} series differed significantly from the blank ($p < 0.01$). This effect on reproduction was partly due to mortality ($LOEC_{mortality}$ = 200 μg g^{-1};$r = -0.57$; $p < 0.01$). Cd might delay juvenile growth for 200 and 400 μg g^{-1} series (Fig. 2c). In contrast to the AU soil, for which juveniles were much more abundant than adults, animal numbers were quite similar between juvenile and adult groups for the SV soil. A low juvenile number was also found in control containers indicating that the SV soil did not favour *F. candida* reproduction. The low reproduction observed for the SV soil was not related to an indirect effect on mortality since more adults were counted in control containers for SV among the three soils. The low reproduction rate can be explained by the low OM content of the SV soil, which inhibits microbial activity and food production for *F. candida*.

Contrary to the AU and SV soils, juvenile and adult groups cannot be distinguished on length histogram of the EPC soil. The right part of the juvenile peaks (corresponding to the longest juveniles) was confounded with the left part of the adult peaks (shortest adults). Nevertheless, a separation between juvenile and adult populations was defined at 0.85 mm according to the procedure previously used for AU and SV. The overlap of juvenile and adult populations can be attributed to the high OM content in this soil: it enhanced growth rate of initially introduced individuals and caused consequently an early laying and a quick growth of produced juveniles.

Cd concentrations in organisms were lower for the AU soil than for the other soils at nominal Cd soil concentrations of 50 and 100 μg g^{-1}. AU also had the lowest $EC_{50\text{-repro}}$ on the basis of nominal concentrations. These results were consistent with the lowest soluble and exchangeable Cd concentrations found for the AU soil, if we consider that Cd measured in solution is representative of bioavailable Cd (Van Gestel and Koolhaas, 2004). However, several results did not corroborate this hypothesis: (1) body concentrations for EPC and SV with 200 μg g^{-1} nominal Cd were lower than for 50 and 100 μg g^{-1} exposures, whereas Cd in solution was 2 or 3 times higher; (2) it is noteworthy that $EC_{50\text{-repro}}$ values based on total soil concentrations were very close among the three soils whereas exchangeable Cd and Cd in solution were very different (more than 3500 times higher for $EPC_{50\,g\mu g^{-1}}$ and $EPC_{100\,g\mu g^{-1}}$ soil solution at t_1 than for $AU_{50\,g\mu g^{-1}}$ and $AU_{100\,g\mu g^{-1}}$ and more than 110 times higher for $EPC_{50\,g\mu g^{-1}}$ and $EPC_{100\,g\mu g^{-1}}$ exchangeable Cd than for the equivalent with AU soil). These last differences can probably be attributed to the effect of pH since it is well known that Cd availability in solution decreases with increasing pH (Van

Gestel and Mol, 2003). The results showed that Cd concentrations in soil solution and exchangeable fraction were not the only determining parameters for Cd toxicity on *F. candida*. Sources other than Cd contained in pore water must be considered to explain the results: (1) soil fungi are able to acidificate their close environment and to increase nutrient absorption, causing at the same time the solubilisation and absorption of trace elements (Bago et al., 1996; Casarin et al., 2003; Gadd, 2007; Finlay, 2008; Van Scholl et al., 2008). When these organisms are consumed by Collembola, they represent a privileged route for trace metal absorption. This phenomenon should be more important for a carbonated soil as AU and would balance the very low Cd concentrations in pore water. (2) Microorganisms consumed by *F. candida* constitute a first step in metal internalisation which leads to Cd ingested concentrations that are different from pore water Cd concentrations. As an example, Posthuma (1992) found that Cd concentrations in soil algae were equivalent to Cd concentrations in bulk soil for low values (0.009 $\mu g\, g^{-1}$) but were lower when bulk soil concentrations increased (0.046 $\mu g\, g^{-1}$ in algae for 0.56 $\mu g\, g^{-1}$ in soil). The Cd accumulation rate of these organisms would be more important for the AU soil than for the two other soils, relatively to pore water concentrations. (3) Soil and exchangeable solutions were sieved with a 0.22 μm filter before analysis. This step eliminates Cd from solution when linked to suspended particles with diameter higher than 0.22 μm but which could be absorbed by Collembola (organic particles or clays). When such particles are consumed, Cd would be partly desorbed from particles in Collembola digestive system due to lowest pH (pH 6; Humbert, 1974) and consequently it would be absorbed through intestinal epithelium. A decrease of one pH unit reduces Cd sorption by about 75% (Temminghoff et al., 1995). Cd adsorbed onto these particles was not measured as Cd in soil solution. The process described above can only be observed when soil pH is very elevated (as in AU soil), but cannot occur in the EPC and SV soils with a lower pH.

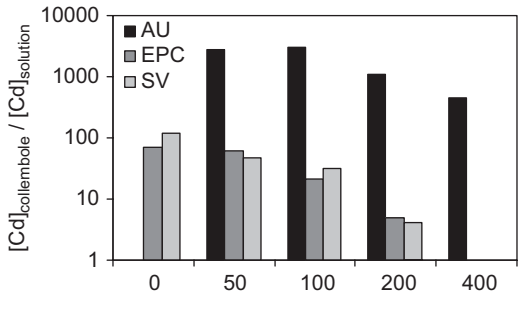

Fig. 4. Cd bioaccumulation factor relatively to pore water concentrations.

Fig. 4 shows the ratio of Cd concentrations in Collembola on Cd concentrations in pore water for the different nominal soil concentrations. Cd accumulation by *F. candida* relative to pore water concentration decreases with nominal concentration but is always higher for AU soil.

EC_{50} and EC_5 give informations on Cd effects on *F. candida*. However, the final object of that study is the evaluation of the contamination level compatible with the protection of almost all the soil invertebrates community. We have kept the reproduction parameter because it is the most sensitive and ecologically relevant one. (Crommentuijn et al., 1995). Ideally, it would be necessary to test Cd effect on almost all the soil arthropods species. It is clearly impossible and we must try to correct the ECx that we get for *F. candida* on the basis of the relative sensitivities of *F. candida* and of the other soil invertebrates for which tests have been done. With this aim in mind, we tried to evaluate the Cd concentration preserving 95% of the reproduction of 95% of soil invertebrates (critical concentrations (CC)). Several authors consider that *F. candida* is less sensitive to trace metal than other species of the pedofauna (Lubben, 1989; Greenslade and Vaughan, 2003; Son et al., 2007). $EC_{50\text{-repro}}$ for Cd of some soil invertebrates are given in Table 4.

The $EC_{50\text{-repro}}$ and LC_{50} of soil invertebrates were approximated with the log-normal distribution applied to the data of Table 4. It allowed to quantify the sensitivity of *F. candida* in comparison with other soil invertebrates. This model has previously been used to study species distribution of Collembola in natural communities (Syrek et al., 2006) and NOEC distribution (Aldenberg and Slob, 1993). We get 20 and 37, respectively, for the $EC_{50\text{-repro}}$ and LC_{50} of the 5% more sensitive species. On this basis, *F. candida* would be about 9 times less sensitive for reproduction and 33 times for lethality than the 5% most sensitive group. So, the difference is larger for lethality than for reproduction. This observation is in agreement with the high sublethal sensitivity index (ratio between the lethal effect concentration and the sublethal effect concentration—SSI) previously found for *F. candida* (Crommentuijn et al., 1995). *F. candida* put a higher priority on survival than on reproduction. Applying the correction factors dealing with reproduction, the CC of the AU, EPC and SV soils would be, respectively, 8×10^{-5}, 1.1 and 0.3 $\mu g\, mL^{-1}$. These values can be compared to critical concentrations obtained according to the method exposed in De Vries et al. (2007), which is based on modelling, using pH and MO content as prediction parameters: 1×10^{-4}, 1.7×10^{-3}, 5×10^{-4} $\mu g\, mL^{-1}$, respectively, for AU, EPC and SV. The values computed in this study are close to

Table 4
$EC_{50\text{-repro}}$ and LC_{50} calculated on the basis of Cd soil concentrations for 12 soil invertebrates. (Bold types characters correspond to mean of several data.)

	$EC_{50\text{-repro}}$ (Cd soil)	LC_{50} (Cd soil)	Reference
Aporrectodea caliginosa	35	540	Khalil et al. (1996)
Caenorhabditis elegans		627	Donkin and Dusenbery (1994)
Enchytraeus albidus	115	364	Lock and Janssen (2001)
Eisenia andrei	33	417	Van Gestel et al. (1993)
Eisenia fetida	116	1095	Spurgeon et al. (1994), Spurgeon and Hopkin (1995), Neuhauser et al. (1985), Fitzpatrick et al. (1996), Lock and Janssen (2001)
Lumbricus terrestris		256	Fitzpatrick et al. (1996)
Folsomia candida	180	1213	Van Gestel and Van Diepen (1997), Crommentuijn et al. (1995, 1997), Sandifer and Hopkin (1996, 1997), Lock and Janssen (2001)
Plectus acuminatus	321		Kammenga et al. (1996)
Paronychiurus kimi	60	90	Son et al. (2007)
Proisotoma minuta	125		Nursita et al. (2005)
Sinella coeca	28	12	Menta et al. (2006)
Sinella communis	50	374	Greenslade and Vaughan (2003)

the values determined by the mean of de Vries et al. method for AU but greatly higher for EPC and SV. Indeed, it means that for soils with a significant proton and organic matter content (EPC and SV), the model from De Vries et al. based on these parameters does not give suitable results. Thus, the apparent adequacy for critical limits obtained for carbonate soil (AU) might be coincidental.

Our results state that comparing critical limits determined by direct toxicological effect on soil organisms with those predicted by models based on soil parameter remain still difficult. Further investigations are needed.

5. Conclusion

This study confirms that Collembola reproduction is a more sensitive parameter than mortality and growth regarding Cd toxicity. It also shows that low organic matter content is a limiting factor for reproduction. Sensitivity to Cd was enhanced for low OM content and acidic pH. We succeed in determining EC_{50} for Cd with three different natural soils and we show that effect of Cd on reproduction is better described by soil or body concentrations than by soil solution concentration. In spite of different Cd concentrations in soil solutions, EC_{50} are rather similar. The critical limits determined in this study do not really fit those given by soil parameter modelling, which implies further investigations on coupled model particularly considering the influence of particulate matter and microorganisms.

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