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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 CaCl₂ 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-Repro} expressed on the basis of nominal soil concentration were 182, 111 and 107 µg 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-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 µg mL⁻¹, respectively, for AU, EPC and SV soils.

Keywords:

Ecotoxicity
Soil
Collembola
Cadmium
Reproduction
Mortality
Growth
Bioaccumulation
Critical load
pH

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 LC₅₀ (lethal concentrations), LOEC (lowest efficient concentrations), EC₅₀ and EC₅ (efficient concentration) for reproduction were computed. Cd concentrations in organisms and in soil solution and CaCl₂ 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 EC₅ 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

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 ₂ O)	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

(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(NO₃)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-repro} for Cd after 6 weeks < EC_{50-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 EC₅ and EC₅₀ 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

each Cd test concentration, pools of $17 \pm 9 \mu\text{g}$ 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 $1.6 \times 10^{-3} \mu\text{g L}^{-1}$ 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 $55 \mu\text{g L}^{-1}$). 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 Shimadzu TOC 5000 Carbon Analyser. The detection limit of DOC measurements was $0.1 \mu\text{g L}^{-1}$. Pore water was extracted by soil centrifugation (2000g, 15 min). Exchangeable Cd was obtained by adding $10^{-2} \text{ mol L}^{-1} \text{ CaCl}_2$ (soil/solution=1/10, w/w). Solutions were filtered (with $0.22 \mu\text{m}$ filter) before element and DOC analyses.

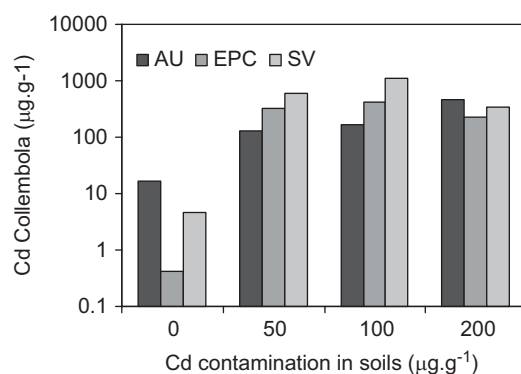


Fig. 1. Cd concentrations in animals for the experimental concentrations (50, 100 and $200 \mu\text{g g}^{-1}$) applied on the three considered soils (AU, EPC and SV), compared to the control Cd content in soils. (y axis: log unit).

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 Nominal ($\mu\text{g g}^{-1}$)	Cd solution ($\mu\text{g mL}^{-1}$)		Cd CaCl_2 ($\mu\text{g mL}^{-1}$)	pH soil solution		pH CaCl ₂	DOC ($\mu\text{g mL}^{-1}$)	
	t_1	t_8		t_1	t_8		t_1	t_8
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.

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 $400 \mu\text{g g}^{-1}$ 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 $200 \mu\text{g g}^{-1}$ condition) with a maximum value of $1100 \mu\text{g g}^{-1}$ dry wt for the $100 \mu\text{g g}^{-1}$ dry soil condition. The lowest concentration was measured in the *Collembola* of the AU soil, except for the $200 \mu\text{g g}^{-1}$ 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 (t_1), 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 t_1 and t_8 for AU, but it decreased slightly (half a unit) for EPC (except at $400 \mu\text{g g}^{-1}$) 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

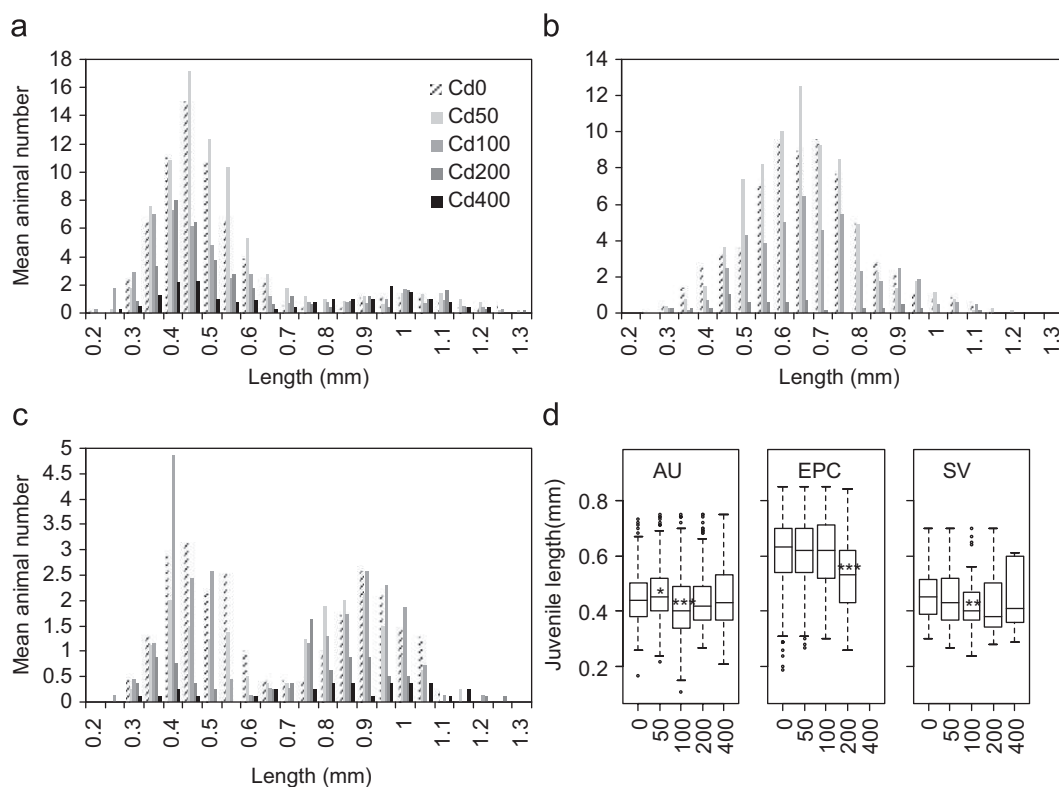


Fig. 2. Number of juveniles and adults for each length class and each Cd soil concentration in AU (a), EPC (b) and SV (c) soils. (d) Medians, quartiles and mode of the lengths of juveniles (significant differences to control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

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, $\mu\text{g g}^{-1}$), in soil solution and to CaCl_2 exchangeable Cd ($\mu\text{g mL}^{-1}$).

	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 $100 \mu\text{g g}^{-1}$ 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 $200 \mu\text{g g}^{-1}$ 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 $100 \mu\text{g g}^{-1}$ dry soil series (Fig. 2d). The lack of significant differences for the highest Cd concentrations (200 and $400 \mu\text{g g}^{-1}$) 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

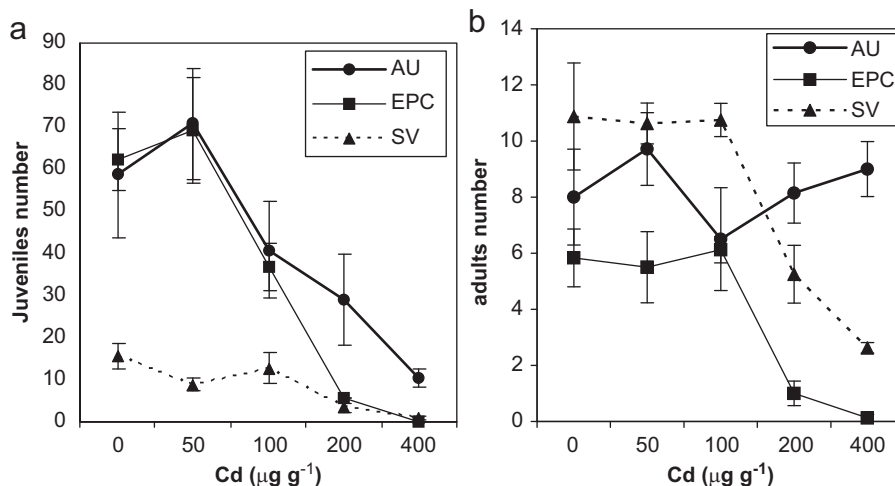


Fig. 3. Dose-effect relationship of Cd (nominal concentrations in soil) on the reproduction ((a) mean number of juveniles \pm S.E.M.) and the mortality ((b) mean number of adults \pm S.E.M.) of *F. candida*.

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

4. Discussion

Juvenile productions were low when compared to literature 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 $400 \mu\text{g g}^{-1}$ 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 $107 \mu\text{g g}^{-1}$ 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 $15 \mu\text{g g}^{-1}$ 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 $200 \mu\text{g g}^{-1}$ series differed significantly from the blank ($p < 0.01$). This effect

on reproduction was partly due to mortality ($LOEC_{\text{mortality}} = 200 \mu\text{g g}^{-1}$; $r = -0.57$; $p < 0.01$). Cd might delay juvenile growth for 200 and $400 \mu\text{g g}^{-1}$ 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 $100 \mu\text{g g}^{-1}$. 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 $200 \mu\text{g g}^{-1}$ nominal Cd were lower than for 50 and $100 \mu\text{g g}^{-1}$ 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 $EPC_{50 \mu\text{g g}^{-1}}$ and $EPC_{100 \mu\text{g g}^{-1}}$ soil solution at t_1 than for $AU_{50 \mu\text{g g}^{-1}}$ and $AU_{100 \mu\text{g g}^{-1}}$ and more than 110 times higher for $EPC_{50 \mu\text{g g}^{-1}}$ and $EPC_{100 \mu\text{g g}^{-1}}$ 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 acidify 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 ($0.009 \mu\text{g g}^{-1}$) but were lower when bulk soil concentrations increased ($0.046 \mu\text{g g}^{-1}$ in algae for $0.56 \mu\text{g g}^{-1}$ 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 $0.22 \mu\text{m}$ filter before analysis. This step eliminates Cd from solution when linked to suspended particles with diameter higher than $0.22 \mu\text{m}$ 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 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.

EC₅₀ and EC₅ 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-repro} for Cd of some soil invertebrates are given in Table 4.

The EC_{50-repro} and LC₅₀ 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-repro} and LC₅₀ 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 $0.3 \mu\text{g mL}^{-1}$. 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} , $5 \times 10^{-4} \mu\text{g mL}^{-1}$, respectively, for AU, EPC and SV. The values computed in this study are close to

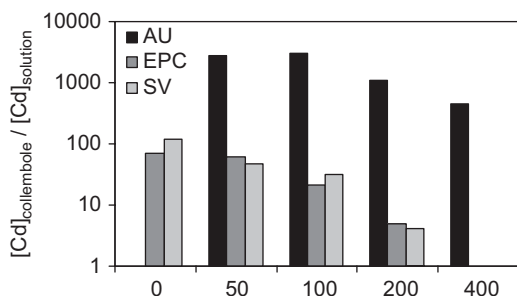


Fig. 4. Cd bioaccumulation factor relatively to pore water concentrations.

Table 4

EC_{50-repro} and LC₅₀ calculated on the basis of Cd soil concentrations for 12 soil invertebrates. (Bold types characters correspond to mean of several data.)

	EC _{50-repro} (Cd soil)	LC ₅₀ (Cd soil)	Reference
<i>Aporrectodea caliginosa</i>	35	540	Khalil et al. (1996)
<i>Caenorhabditis elegans</i>		627	Donkin and Dusenbery (1994)
<i>Enchytraeus albidus</i>	115	364	Lock and Janssen (2001)
<i>Eisenia andrei</i>	33	417	Van Gestel et al. (1993)
<i>Eisenia fetida</i>	116	1095	Spurgeon et al. (1994), Spurgeon and Hopkin (1995), Neuhauser et al. (1985), Fitzpatrick et al. (1996), Lock and Janssen (2001)
<i>Lumbricus terrestris</i>		256	Fitzpatrick et al. (1996)
<i>Folsomia candida</i>	180	1213	Van Gestel and Van Diepen (1997), Crommentuijn et al. (1995, 1997), Sandifer and Hopkin (1996, 1997), Lock and Janssen (2001)
<i>Plectus acuminatus</i>	321		Kammenga et al. (1996)
<i>Paronychiurus kimi</i>	60	90	Son et al. (2007)
<i>Proisotoma minuta</i>	125		Nursita et al. (2005)
<i>Sinella coeca</i>	28	12	Menta et al. (2006)
<i>Sinella communis</i>	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 EC₅₀ 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, EC₅₀ 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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References

- Aldenberg, T., Slob, W., 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. *Ecotoxicol. Environ. Saf.* 25, 48–63.
- Bago, B., Vierheilig, H., Piche, Y., Azcon-Aguilar, C., 1996. Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. *New Phytol.* 133, 273–280.
- Baize, D., 1997. Teneurs totales en éléments traces métalliques dans les sols. INRA Editions.
- Casarin, V., Plassard, C., Souche, G., Arvieu, J., 2003. Quantification of oxalate ions and protons released by ectomycorrhizal fungi in rhizosphere soil. *Agronomie* 23, 461–469.
- Chenon, P., Gisclard, C., Crouau, Y., 1999. The use of *Folsomia candida* (Collembola, Isotomidae) for the bioassay of xenobiotic substances and soil pollutants. *J. Appl. Soil Ecol.* 12, 103–111.
- Crommentuijn, T., Doodeman, C., Vanderpol, J., Doornekamp, A., Rademaker, M., Van Gestel, C., 1995. Sublethal sensitivity index as an ecotoxicity parameter measuring energy allocation under toxicant stress—application to cadmium in soil arthropods. *Ecotoxicol. Environ. Saf.* 31, 192–200.
- Crommentuijn, T., Doornekamp, A., Van Gestel, C.A.M., 1997. Bioavailability and ecological effects of cadmium on *Folsomia candida* (Willem) in an artificial soil substrate as influenced by pH and organic matter. *Appl. Soil Ecol.* 5, 261–271.
- Crouau, Y., Chenon, P., Gisclard, C., 1999. The use of *Folsomia candida* (Collembola, Isotomidae) for the bioassay of xenobiotic substances and soil pollutants. *Appl. Soil Ecol.* 12, 103–111.
- Crouau, Y., Gisclard, C., Perotti, P., 2002. The use of *Folsomia candida* (Collembola, Isotomidae) in bioassays of waste. *Appl. Soil Ecol.* 19, 65–70.
- Crouau, Y., Cazes, L., 2003. What causes variability in the *Folsomia candida* reproduction test. *Appl. Soil Ecol.* 22, 175–180.
- Crouau, Y., Cazes, L., 2005. Unexpected reduction in reproduction of Collembola exposed to an arsenic-contaminated soil. *Environ. Toxicol. Chem.* 24 (7), 1716–1720.
- Crouau, Y., Moia, C., 2006. The relative sensitivity of growth and reproduction in the springtail, *Folsomia candida*, exposed to xenobiotics in the laboratory: an indicator of soil toxicity. *Ecotoxicol. Environ. Saf.* 64, 115–121.
- Crouau, Y., Pinelli, E., 2008. Comparative ecotoxicity of three polluted industrial soils for the Collembola *Folsomia candida*. *Ecotoxicol. Environ. Saf.* 71, 643–649.
- Dambrine E., Probst A., Party J.P., 1993. Détermination des “charges critiques” de polluants atmosphériques pour les écosystèmes naturels, en particulier forestiers. Bases théoriques - Projet d'application au cas des Vosges. *Pollution Atmosphérique, No. spécial Pollution de l'Air et Charges Critiques*, juin, 1993, pp. 21–28.
- De Vries, W., 1988. Critical deposition levels for nitrogen and sulphur on Dutch forest ecosystems. *Water Air Soil Pollut.* 42, 221–239.
- De Vries, W., Reinds, G.J., Posch, M., 1994. Assessment of critical loads and their exceedances on European forests using a one-layer steady-state model. *Water Air Soil Pollut.* 78, 357–394.
- De Vries W., Bakker D.J., 1996. Manual for calculating critical loads of heavy metals for soils and surface waters. S.C-DLO Publishers, report 114, 173 pp.
- De Vries, W., Lofts, S., Tipping, E., Meili, M., Groenenberg, J.E., Schütze, G., 2007. Impact of soil properties on critical concentrations of cadmium, lead, copper, zinc and mercury in soil and soil solution in view of ecotoxicological effects. *Rev. Environ. Contam. Toxicol.* 191, 47–89.
- Donkin, S.G., Dusenbery, D.B., 1994. Using the *Coenorhabditis elegans* soil toxicity test to identify factors affecting toxicity of four metal ions in intact soil. *Water Air Soil Pollut.* 78, 359–373.
- Eom, I.C., Rast, C., Veber, A.M., Vasseur, P., 2007. Ecotoxicity of a polycyclic aromatic hydrocarbon (PAH)-contaminated soil. *Ecotoxicol. Environ. Saf.* 67 (2), 190–205.
- Finlay, R., 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *J. Exp. Bot.* 59, 1115–1126.
- Fitzpatrick, L.C., Muratti-Ortiz, J.F., Venable, B.J., Goven, A.J., 1996. Comparative toxicity in earthworms *Eisenia fetida* and *Lumbricus terrestris* exposed to cadmium nitrate using artificial soil and filter paper protocols. *Bull. Environ. Contam. Toxicol.* 57, 63–68.
- Fountain, M.T., Hopkin, S.P., 2001. Continuous monitoring of *Folsomia candida* (Insecta: Collembola) in a metal exposure test. *Ecotoxicol. Environ. Saf.* 48, 275–286.
- Fountain, M.T., Hopkin, S.P., 2004. Biodiversity of Collembola in urban soils and the use of *Folsomia candida* to assess soil “quality”. *Ecotoxicology* 13 (6), 555–572.
- Gadd, G., 2007. Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycol. Res.* 111, 3–49.
- Greenslade, P., Vaughan, G.T., 2003. A comparison of Collembola species for toxicity testing of Australian soils. *Pedobiologia* 47, 171–179.
- Herbert, I., Svendsen, C., Hankard, P., Spurgeon, D., 2004. Comparison of instantaneous rate of population increase and critical-effect estimates in *Folsomia candida* exposed to four toxicants. *Ecotoxicol. Environ. Saf.* 57, 183–193.
- Hernandez, L., Probst, A., Probst, J.L., Ulrich, E., 2003. Heavy metal distribution in some French forest soils: evidence for atmospheric contamination. *Sci. Total Environ.* 312 (1–3), 195–219.
- Hettelingh, J.P., De Vries, W., Schöpp, W., Downing, R.J., De Smet, P.A.M., 1991. Chap. 3. Methods and data. in : Mapping Critical loads for Europe. In: Hettelingh, J.P., Downing, R.J., De Smet, P.A.M. (Eds.), CEC Technical Report 1. National Institute of Public Health and Environmental Protection, Bilthoven, pp. 1–86.
- Holmstrup, M., 1997. Drought tolerance in *Folsomia candida* Willem (Collembola) after exposure to sublethal concentrations of three soil-polluting chemicals. *Pedobiologia* 41, 361–368.
- Hornung, M., Bull, K.R., Cresser, M., Hall, J., Langan, S.J., Loveland, P., Smith, C., 1995. An empirical map of critical loads of acidity for soils in Great Britain. *Environ. Pollut.* 90, 301–310.
- Humbert, W., 1974. Etude du pH intestinal d'un Collembole (Insecta, Aptérygote). *Rev. Ecol. Biol. Sol.* 11, 89–97.
- ISO 11268-1, 1994. Soil quality—effects of pollutants on earthworms (*Eisenia fetida*). Part 1. Method for the determination of acute toxicity using artificial soil substrate. International Standard Organization, Genève.
- ISO 11268-2, 1997. Soil quality—effects of pollutants on earthworms (*Eisenia fetida*). Part 2. determination of effects on reproduction. International Standard Organization, Genève.
- ISO 11267, 1998. Soil quality—inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. International Standard Organization, Genève.
- ISO 11268-3, 1999. Soil quality—effects of pollutants on earthworms (*Eisenia fetida*). Guidance on the determination of effects in field situations. International Standard Organization, Genève.
- ISO 16387, 2001. Soil quality—effects of pollutants on Enchytraeidae (*Enchytraeus* sp.)—determination of effects on reproduction and survival. International Standard Organization, Genève.

- Ives, M.A., 1996. In: TOXCALC 5.0. Tidepool Scientific Software, McKinleyville, CA, USA 80 pp.
- Kammenga, J.E., Van Koert, P.H.G., Riksen, J.A.G., Korthals, G.W., Bakker, J., 1996. A toxicity test in artificial soil based on the life-history strategy of the nematode *Plectus acuminatus*. *Environ. Toxicol. Chem.* 15, 722–727.
- Khalil, M.A., Abdel-Lateif, H.M., Bayoumi, B.M., Van Straalen, N.M., Van Gestel, C.A.M., 1996. Effects of metals and metal mixtures on survival and cocoon production of the earthworm *Aporectodea caliginosa*. *Pedobiologia* 40, 548–556.
- Lock, K., Janssen, C.R., 2001. Cadmium toxicity for terrestrial invertebrates: taking soil parameters affecting bioavailability into account. *Ecotoxicology* 10, 315–322.
- Lofts, S., Spurgeon, D.J., Svendsen, C., Tipping, E., 2004. Deriving soil critical limits for Cu, Zn, Cd and Pb: a method based on free ion concentrations. *Environ. Sci. Technol.* 38 (13), 3623–3631.
- Lubben, B., 1989. Influence of sewage sludge and heavy metals on the abundance of Collembola on two agricultural soils. In: Third International Seminar on Apterygota. R. Dallai, pp. 419–428.
- Massabuau, J.C., Probst, A., Guerold, F., 1995. Critical loads of acidity to streamwaters in the Vosges mountains (France): biological criteria. In: Landmann, G., Bonneau, M. (Eds.), *Forest Decline and Atmospheric Deposition Effects in the French Mountains*. Springer, Berlin, Heidelberg, New York, pp. 387–393 461p.
- Menta, C., Maggiani, A., Vattuone, Z., 2006. Effects of Cd and Pb on the survival and juvenile production of *Sinella coeca* and *Folsomia candida*. *Eur. J. Soil Biol.* 42, 181–189.
- Moncoulon, D., Probst, A., Party, J.-P., 2004. Weathering, atmospheric deposition and vegetation uptake: role for ecosystem sensitivity to acid deposition and critical load. *C. R. Geosci.* 336, 1417–1426.
- Moncoulon, D., Probst, A., Martinson, L., 2007. Modeling acidification recovery on threatened ecosystems: application to the evaluation of the Gothenburg protocol. *Water Air Soil Pollut.* 7 (1–3), 307–316.
- Nilsson, J., Grennfelt, P., 1988. Critical loads for sulphur and nitrogen. *Miljørapport* 15, 418.
- Neuhauser, E.F., Loehr, R.C., Milligan, D.L., Malecki, D.L., 1985. Toxicity of metals to the earthworm *Eisenia fetida*. *Biol. Fertil. Soils* 1, 149–152.
- Nursita, A., Ayulungit, I., Singh, A., Balwant, A., Lees, E., 2005. The effects of cadmium, copper, lead, and zinc on the growth and reproduction of *Proisotoma minuta* Tullberg (Collembola). *Ecotoxicol. Environ. Saf.* 60, 306–314.
- Party, J.P., Probst, A., Dambrine, E., Thomas, A.L., 1995. Critical loads of acidity to France: sensitivity areas in the north-eastern France. *Water Air Soil Pollut.* 85 (1–4), 2407–2412.
- Party, J.P., Probst, A., Thomas, A.L., Dambrine, E., 2001. Charges critiques d'acidité en polluants atmosphériques en France: conséquences vis à vis des sols et des peuplements forestiers. *Pollution Atmosphérique* 172 (oct-déc), 519–527.
- Ponnette, Q., Ulrich, E., Brethes, A., Bonneau, M., Lanier, M., 1997. RENECOFOR - Chimie des sols dans les 102 Peuplements du réseau. Campagne des mesures 1993/1995. Office National des Forêts Ed., Département des Recherches Techniques, 2-84207-100-X.
- Probst, A., Hernandez, L., Probst, J.L., 2003a. Heavy metals partitioning in three French forest soils by sequential extraction procedure. *J. Phys. IV* 107, 1103–1106.
- Probst, A., Moncoulon, D., Goddérès, Y., Hernandez, L., Party, J.-P., 2003b. Critical loads for lead in France: first results on forest soils. *J. Phys. IV France* 107, 1111–1114.
- Posthuma, L., 1992. Genetic ecology of metal tolerance in Collembola. Thesis, Vrije Universiteit, Amsterdam.
- Riepert, F., 1995. First report of the second international ring test on a method for determining the effects of chemicals or soil contaminants on the reproduction of collembola. Report of the Biologische Bundesanstalt für Land- und Forstwirtschaft Institut für Chemikalienprüfung.
- Sandifer, R., Hopkin, S., 1996. Effects of pH on the toxicity of cadmium, copper, lead and zinc to *Folsomia candida* Willem, 1902 (Collembola) in a standard laboratory test system. *Chemosphere* 33, 2475–2486.
- Sandifer, R., Hopkin, S., 1997. Effects of temperature on the relative toxicities of Cd, Cu, Pb and Zn to *Folsomia candida* (Collembola). *Ecotoxicol. Environ. Saf.* 37, 125–130.
- Scott-Fordsmann, J.J., Krogh, P.H., Weeks, J.M., 1997. Sublethal toxicity of copper to a soil-dwelling springtail *Folsomia fimetaria* (Collembola: Isotomidae). *Environ. Toxicol. Chem.* 16, 2538–2542.
- Slootweg, J., Hettelingh, J., Posch, M., Dutchak, S., Ilyn, I., 2005. Critical loads of cadmium, lead and Mercury in Europe. Working Group on effects of the convention on Long-range Transboundary Air Pollution.
- Smit, C.E., Van Gestel, C.A.M., 1996. Comparison of the toxicity of zinc for the springtail *Folsomia candida* in artificially contaminated and polluted field soils. *Appl. Soil Ecol.* 3, 127–136.
- Smit, C.E., Van Gestel, C.A.M., 1998. Effects of soil type, prepercolation, and ageing on bioaccumulation and toxicity of zinc for the springtail *Folsomia candida*. *Environ. Toxicol. Chem.* 17, 1132–1141.
- Snider, R.M., Butcher, J.W., 1973. The life history of *Folsomia candida* (Willem) (Collembola, Isotomidae) relative to temperature. *Great Lake Entomol.* 6, 97–106.
- Son, J., Mo, H., Kim, J., Ryoo, M., Cho, K., 2007. Effect of soil organic matter content and pH on toxicity of cadmium to *Paronychiurus kimi* (Lee) (Collembola). *J. Asia-Pac. Entomol.* 10 (1), 55–61.
- Son, J., Ryoo, M., Jung, J., Cho, K., 2007. Effects of cadmium, mercury and lead on the survival and instantaneous rate of increase of *Paronychiurus kimi* (Lee) (Collembola). *Appl. Soil Ecol.* 35, 404–411.
- Spurgeon, D.J., Hopkin, S.P., Jones, D.T., 1994. Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. *Environ. Pollut.* 84, 123–130.
- Spurgeon, D.J., Hopkin, S.P., 1995. Extrapolation of the laboratory-based OECD earthworm toxicity test to metal contaminated field sites. *Ecotoxicology* 4, 190–205.
- Syrek, D., Weiner, W.M., Wojtylak, M., Olszowska, G., Kwapis, Z., 2006. Species abundance distribution of collembolan communities in forest soils polluted with heavy metals. *Appl. Soil Ecol.* 31, 239–250.
- Temminghoff, E.J.M., van der Zee, S.E.A.T.M., De Haan, 1995. Speciation and calcium competition effects on cadmium sorption by sandy soil at various pH. *Eur. J. Soil Sci.* 46, 649–655.
- Van Gestel, C.A.M., Dirven-Van Breemen, E.M., Baerselman, R., 1993. Accumulation and elimination of cadmium, chromium and zinc and effects on growth and reproduction in *Eisenia andrei* (Oligochaeta, Annelida). *Sci. Total Environ.*, 585–597.
- Van Gestel, C.A.M., Van Diepen, A.M., 1997. The influence of soil moisture content on the bioavailability and toxicity of cadmium for *Folsomia candida* Willem (Collembola: Isotomidae). *Ecotoxicol. Environ. Saf.* 36, 123–132.
- Van Gestel, C.A.M., Mol, S., 2003. The influence of soil characteristics on cadmium toxicity for *Folsomia candida* (Collembola: Isotomidae). *Pedobiologia* 47, 387–395.
- Van Gestel, C.A.M., Koolhaas, J.E., 2004. Water-extractability, free ion activity, and pH explain cadmium sorption and toxicity to *Folsomia candida* (Collembola) in seven soil-pH combinations. *Environ. Toxicol. Chem.* 23, 1822–1833.
- Van Scholl, L., Kuyper, T., Smits, M., Landeweert, R., Hoffland, E., Van Breemen, N., 2008. Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. *Plant Soil* 303, 35–47.
- Van Straalen, N.M., Schobben, J.H.M., De Goede, R.G.M., 1989. Population consequences of cadmium toxicity in soil microarthropods. *Ecotoxicol. Environ. Saf.* 17, 190–204.
- Warfvinge, P., Sverdrup, H., 1992. Calculating critical loads of acid deposition with profile—A steady-state soil chemistry model. *Water Air Soil Pollut.* 63, 119–143.