To link to this article: DOI:10.1016/j.scitotenv.2011.10.029
http://dx.doi.org/10.1016/j.scitotenv.2011.10.029

Toxicity of Pb and of Pb/Cd combination on the springtail *Folsomia candida* in natural soils: Reproduction, growth and bioaccumulation as indicators

T. Bur, Y. Crouau, A. Bianco, L. Gandois, A. Probst *

Université de Toulouse; INP, UPS; EcoLab (Laboratoire Ecologie Fonctionnelle et Environnement); ENSAT, Avenue de l'Agrobiopole, 31326 Castanet Tolosan, France
CNRS; EcoLab; 31326 Castanet Tolosan, France

A R T I C L E I N F O

Keywords:
Collembola
Ecotoxicity
Metal combination
Soil solution
pH
Organic matter

A B S T R A C T

The toxicity of Pb and Cd + Pb was assessed on the Collembola *F. candida* in two cultivated soils (SV and AU) with low organic matter (OM) content and circumneutral to basic pH, and an acid forested soil (EPC) with high OM content. Collembola reproduction and growth as well as metal content in Collembola body, in soil, exchangeable fraction and soil solutions, pH and DOC were investigated. Pb and Cd + Pb were the highest in exchangeable fraction and soil solution of the acidic soils. Soil solution pH decreased after metal spiking in every soil due to metal adsorption, which was similar for Cd and the highest in AU for Pb. With increasing Pb and Cd + Pb, the most important reproduction decrease was in EPC soil. The LOEC for reproduction after metal addition was 2400 (Pb) and 200/2400 (Cd/Pb), 1200 and 100/1200, 300 and 100/1200 μg g^{-1} for AU, SV and EPC, respectively. The highest and the lowest Pb toxicity was observed for EPC and AU bulk soil, respectively. The metal in Collembola increased with increasing soil concentration, except in AU, but the decreasing $BF_{solution}$ with increasing concentrations indicates a limited metal transfer to Collembola or an increased metal removal. Loading high Pb concentrations decreases Cd absorption by the Collembola, but the reverse was not true. The highest Pb toxicity in EPC can be explained by pH and OM content. Because of metal complexation, OM might have a protective role but its ingestion by Collembola lead to higher toxicity. Metal bioavailability in Collembola differs from soil solution indicating that soil solution is not sufficient to evaluate toxicity in soil organisms. The toxicity as a whole decreased when metals were combined, except for Pb in AU, due to adsorption competition between Cd and Pb on clay particles and OM sites in AU and EPC soils, respectively.

1. Introduction

Soil is a living interface layer between atmosphere and lithosphere. Its physical and chemical properties are always changing due to environmental and inherent processes but also under the influence of anthropogenic pressure. Soils have a natural bioremediation capacity assumed by living organisms: micro-, meso- and macro-fauna. Soil ecosystem can thus contribute to reduce pollutant's transfer to surface and deep waters. But, soil remediation capacity for trace metal remains weak (Horckmans et al., 2007), and consequently soil is a major compartment in the ecosystem where past and current inputs of metal contaminants accumulate (Hernandez et al., 2003).

In sites where soil metal contamination is high (like mining, industrial sites or towns, etc.…), important modifications of soil fauna are widely described in the literature (ISO, 11267, 1999; Crouau et al., 1999; Cole et al., 2001; Migliorini et al., 2005). Although these sites constitute a very severe danger for the environment, they generally concern limited areas. On the opposite, cultivated soils cover large areas and they receive permanent but relatively low metallic inputs. In France, agricultural areas cover more than half of the territory (Agreste Midi-Pyrénées, 2008). Several trace metal sources can be identified as potential inputs to cultivated soils: sewage sludge spreading (Gerritse et al., 1982; Lubben, 1989; McBride, 1995; Cole et al., 2001; Gavalda et al., 2005; Collin and Doelsch, 2008), atmospheric deposition (Hernandez et al., 2003; Gandois et al., 2010b), inorganic fertilisers and pesticide applications (Nicholson et al., 2003). The long-term impact of these metal fluxes remains difficult to determine. The importance of atmospheric metal deposits was initially determined under forest cover in France and Europe (Bergkvist et al., 1989; Probst et al., 2003). Sustainable agriculture development might take into account the sensitivity of soil fauna to metal contamination. This parameter can be used to determine critical loads, i.e. the maximum metal flux inputs to soil that will not cause any irremediable effect on fauna. Cadmium and lead are non essential toxic metals widely added to soils via past or current anthropogenic inputs (Nursita et al., 2005; Bur et al., 2010). Reduction of inputs and

* Corresponding author at: Université de Toulouse; INP, UPS; EcoLab (Laboratoire Ecologie Fonctionnelle et Environnement); ENSAT, Avenue de l'Agrobiopole, 31326 Castanet Tolosan, France. Tel.: +33 5 34 32 39 42; fax: +33 5 34 32 39 01.
E-mail address: anne.probst@ensat.fr (A. Probst).

remediation of these elements are of main concern because of their increasing occurrence in ecosystems.

Collembola are small soil invertebrates which play an important role for litter decomposition. They are usually used as bioindicators for pollution (Van Straalen et al., 1989). They feed with fungi and altered organic matter residues and are thus able to give indications of soil ecosystem health. Among Collembola, *Folsomia candida* (Isotomidae) is an anophtalm, unpigmented and parthenogenetic species. It was selected as a biological model for normalized tests to determine the lethal properties of a contaminant (ISO 11267, van Gestel and Koolhaas, 2004; Vijver et al., 2004). Krogh and Petersen (1995) have shown that the change in reproduction to the input of a contaminant is a more sensitive parameter than mortality. This was shown in both experimental and field conditions and on various kinds of soils. As a consequence, the reproduction is more appropriate than mortality to determine critical limits for metals, i.e. the maximum threshold concentration in soil that will not cause any irremediable effect on fauna.

Ecotoxicological tests are a key way of estimating soil pollution impact. They are used as a complementary tool to soil chemical analysis in order to assess chemical toxic effects on living organisms. Only five ecotoxicological tests using soil animals (Collembola and earthworms) are normalized. Although numerous studies concern the effects of metals on *F. candida* (Crommentuijn et al., 1994; Sandifer and Hopkin, 1996; Smit and Van Gestel, 1998; Crouau et al., 1999; Fountain and Hopkin, 2001; Herbert et al., 2004; Van Gestel and Koolhaas, 2004; Bur et al., 2010), very few used a combination of metals (Fountain and Hopkin, 2004). It is indeed a major gap in toxic effect knowledge since in field conditions the organisms are always under the influence of a combination of several toxic compounds.

This study aims to investigate: (1) the effects of metal (Pb and a combination of Cd and Pb) addition on soil parameters; (2) the effects of metal addition on Collembola reproduction and growth; (3) the interactions between Cd and Pb when spiked in combination; (4) the mechanisms involved in soil response regarding to Collembola toxicity.

For that purpose, the Collembola *F. candida* was used as a target model in three spiked soils (cultivated and forested) combining a range of organic matter content and pH.

2. Materials and methods

2.1. Soil characteristics and pre-treatment

Experiments were performed using three sampled soils in order to fit as closely as possible to field conditions. Two cultivated soils from the South-West 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 for their varying pH and organic matter content (OM) (Table 1), to investigate the influence of these two parameters on the effect of metals on *F. candida* reproduction and growth. 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). After sampling, soils were dried at 40 °C and sieved. The <2 mm fraction was used for experiments. Soil moisture was set up to 25, 30 and 20%, respectively for AU, EPC and SV, to enhance the development of Collembola by creating an adequate crumbly structure. Pb and Cd concentrations before spiking are in the range of low contaminated soils (Baize, 1997).

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 bakker'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

In a previous study (Bur et al., 2010), the ecotoxicological effect of Cd on *F. candida,* was investigated. The present study aims in an analogous way to investigate the addition of Pb and a combination of Cd and Pb to the same soils. The test consists in exposing juveniles to field soils spiked with Pb or Cd + Pb in various proportions, and in comparing reproduction and growth 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 $Cd(NO_3)_2$ and $Pb(NO_3)_2$ to reach the concentrations indicated in Table 2. These concentrations were chosen on a large range of values, which indeed exceed the current levels found in moderately polluted soils (0 Cd/0 Pb; 50 μg g^{-1} Cd/600 μg g^{-1} Pb; 100 μg g^{-1} Cd/1200 μg g^{-1} Pb; 200 μg g^{-1} Cd/2400 μg g^{-1} Pb; 400 μg g^{-1} Cd/4800 μg g^{-1} Pb). Nevertheless, they were selected on the basis of literature results of ecotoxicological tests of Cd and Pb effects on *F. candida* reproduction (Crommentuijn et al., 1994; Crouau et al., 1999; Herbert et al., 2004). Afterwards, the soils were equilibrated for a week (t8 in Table 3) before starting the toxicity tests. Metal nitrate was dissolved in distilled water, which was used for soil moistening. For each soil and each spiked concentration, 8 test containers (12 for the control) were filled with 45 g of moisted soil. Fifteen *F. candida* juveniles (8–12 days old juveniles) were introduced into each container. The containers were aerated twice a week. Exogenous yeast was not added during testing in order to prevent Collembola from feeding non contaminated yeast and to keep closer to field soil conditions. The duration of exposure was 50 days, instead of the 28 days recommended in the ISO guideline (ISO, 11267, 1999) in order to counterbalance the lack of added food during the assay. Indeed, this might have led to a lower reproduction rate than those of normalised assays with food addition. Consequently, the apparition of a second generation of juveniles is unlikely and was not observed here. Moreover, a longer exposure duration might increase the sensitivity of the assay (Van Gestel and Mol, 2003), decrease the variability of the test results, and therefore increase the efficiency of the assay (Crouau and Cazes, 2003). Lastly, a longer exposure duration was evaluated to be more realistic because in field conditions Collembola are exposed to pollutants for longer than 28 days. As explained in the paper by Bur et al. (2010), the results of the assays do not strictly aim to respond to a risk assessment according to criteria of the norm.

At the end of the exposure period (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

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, Ponnette et al., 1997, data from Hernandez et al., 2003).

Soils	Origin	Soil cover	Clay (%)	pH (H_2O)	OM (%)	Cd (μg.g^{-1})
AU	Cultivated 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

Table 2
Range of concentrations used for toxicity tests on reproduction and expected $EC50_{repro}$ according to literature data; (−) not of concerned.

Metal	Range of spiking concentrations (μg.g^{-1} of dry soil)						$EC50_{repro}$ from literature[a] (μg.g^{-1} of dry soil)		
	C0	C1	C2	C3	C4	C5	Min	Moy	Max
Cd[b]	0	50	100	200	400	–	43	321	780
Pb	0	300	600	1200	2400	4800	60	1407	3160
Cd/Pb	0/0	50/600	100/1200	200/2400	400/4800	–	–	–	–

[a] See Bur, 2008.
[b] According to Bur et al., 2010.

juveniles were counted and their lengths (from the end of the posterior abdominal segment to the anterior margin of the head) were measured and classified (one class each 0.5 mm). The threshold length between juveniles and adult populations was defined as the length class presenting the lowest individual abundance (sum of the five concentration abundances), i.e. around the first quartile value.

2.4. Statistical analysis

For each comparison modality, the distribution normality of the data was assessed. Then, a Student's or Wilcoxon's two-sample test was used (respectively for normal and non normal data) to assess the significance of the differences of reproduction and length between exposed and control series. The test was considered as significant when the data length were higher than 3 and when the corresponding P-value was below 0.05, 0.01 or 0.001 and the corresponding threshold was indicated. The comparison tests were computed using R software (R development Core Team, 2010). The EC_5 and EC_{50} values were calculated by using the maximum likelihood-probit procedure (Toxcalc 5.0 software, Ives, 1996; EPA methods).

2.5. Chemical analyses

For each spiked concentration, Collembola were extracted (alive) and placed during 3 days in fasting containers with a humid filter paper to control hygrometry and to decrease soil content in Collembola stomach. Collembola were then killed by freezing (−80 °C). For each soil and each metal test concentration, pools of 17 ± 9 μg dry weight (about 20 animals) were digested (ultrapure HNO_3, 90 °C) in a clean room. Cadmium and Pb concentrations were analysed in Collembola with a Perkin Elmer inductively coupled plasma–mass spectrometer (ICP-MS), and in soil solutions and in exchangeable $CaCl_2$ solutions (see below) by ICP-OES. The accuracy of Collembola dissolution procedure for metal concentration measurements was assessed using the international reference material TORT-2 (Lobster Hepatopancreas). The detection limit of Cd and Pb

Table 3
Concentrations of Cd* (data in italic, from Bur et al., 2010), Pb, COD, and pH of soil solutions (pore water) and $CaCl_2$ exchangeable fractions for the three soils, and concentrations of the different treatments (two first columns). All measures concerning the soil solution were done at t1 (one week) and t8 (eight weeks). DL: detection limit; (−) not of concerned.

		Cd soil	Pb soil	DOC (μg mL^{-1})		Cd solution (μg mL^{-1})		Pb solution (μg mL^{-1})		Cd $CaCl_2$	Pb $CaCl_2$	pH solution		pH $CaCl_2$
		(μg g^{-1})	(μg g^{-1})	t1	t8	t1	t8	t1	t8	(μg.mL^{-1})	(μg.mL^{-1})	t1	t8	
Treatment Pb (and Cd*)	AU	*0*	0	57.01	19.38	*<DL*	*<DL*	0.02	0.01	*0.05*	0.03	7.34	7.61	7.24
		50	300	16.39	19.85	*0.003*	*<DL*	0.09	<DL	*0.24*	<DL	7.48	7.06	7.20
		100	600	19.48	17.63	*0.009*	*<DL*	0.14	<DL	*0.58*	<DL	7.27	7.18	7.11
		200	1200	19.32	18.15	*0.47*	*0.43*	0.31	<DL	*2.07*	<DL	7.13	6.89	6.93
		400	2400	60.09	35.72	*1.69*	*2.93*	2.28	0.10	*9.95*	0.30	6.4	6.96	6.94
		–	4800	22.15	57.11	–	–	29.89	3.87	–	3.22	5.98	6.45	6.93
	EPC	*0*	0	121.55	135.40	*0.02*	*0.01*	<DL	<DL	*0.11*	<DL	3.68	3.58	3.02
		50	300	160.33	190.52	*10.60*	*5.21*	1.50	0.81	*29.85*	9.31	3.55	3.36	3.08
		100	600	202.63	468.72	*30.02*	*19.80*	7.91	3.97	*67.11*	23.55	3.4	3.35	2.99
		200	1200	254.28	608.01	*90.54*	*45.28*	27.97	25.50	*112.65*	60.40	3.29	2.67	3.00
		400	2400	255.83	586.29	*280.29*	*195.06*	122.57	94.66	*284.59*	164.90	3.05	3.03	2.99
		–	4800	372.48	357.75	–	–	678.20	171.80	–	773.00	2.95	–	3.05
	SV	*0*	0	78.20	22.42	*<DL*	*0.04*	<DL	<DL	*0.67*	<DL	4.35	3.43	4.32
		50	300	43.71	37.82	*6.68*	*12.91*	1.51	1.78	*27.28*	3.21	4.39	3.32	4.26
		100	600	63.82	35.22	*18.45*	*35.04*	9.45	9.46	*47.33*	29.01	4.23	4.12	4.29
		200	1200	66.57	96.28	*51.89*	*82.33*	60.49	60.49	*112.97*	108.99	4.03	3.72	4.26
		400	2400	53.06	65.58	*143.13*	*185.23*	599.97	498.15	*264.47*	478.15	3.72	3.63	4.14
		–	4800	66.91	80.78	–	–	4073.01	3794.91	–	2273.37	3.48	3.44	3.96
Treatment Cd + Pb	AU	0	0	24.65	17.61	0.06	4.92	0.05	<DL	0.41	0.46	7.26	7.35	7.14
		50	600	19.55	18.33	0.16	0.18	0.09	0.003	0.23	<DL	7.34	7.21	7.26
		100	1200	19.59	18.39	0.82	0.42	0.16	0.02	0.82	<DL	7.2	6.92	7.31
		200	2400	28.44	38.84	5.59	3.09	1.80	0.75	2.94	0.20	6.75	5.57	6.89
		400	4800	35.79	50.62	55.39	26.91	30.01	5.46	23.78	2.84	6.04	5.45	6.86
	EPC	0	0	17.32	23.85	<DL	0.11	<DL	<DL	0.09	0.43	3.62	3.56	3.97
		50	600	38.87	28.11	41.74	20.17	36.01	10.45	35.93	62.98	3.47	3.48	3.92
		100	1200	62.19	118.71	123.63	76.14	152.79	51.50	67.27	131.24	3.45	3.43	3.90
		200	2400	77.06	108.56	283.69	227.39	473.35	286.51	155.26	374.31	3.46	3.35	3.84
		400	4800	299.59	177.86	623.25	672.02	2126.23	1233.94	337.41	1346.18	3.41	3.36	3.79
	SV	0	0	25.35	18.06	<DL	0.07	<DL	0.02	0.03	<DL	3.22	3.93	4.42
		50	600	37.48	24.54	18.24	24.15	10.44	14.79	33.65	23.22	3.57	2.97	4.27
		100	1200	49.57	53.51	87.26	80.80	108.92	72.78	78.03	122.47	3.27	3.43	4.29
		200	2400	53.60	63.36	286.78	305.10	753.11	547.07	157.28	494.84	3.35	3.29	4.12
		400	4800	68.95	93.60	988.28	956.93	4649.36	3642.54	374.60	2175.17	3.09	3.27	3.99

measurements was $1.6 \cdot 10^{-3}$ µg L^{-1} and $4.2 \cdot 10^{-3}$ µg L^{-1}, respectively for body concentration, and 1 mg L^{-1} and 3 mg L^{-1}, respectively, for water concentration. 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 (2000 *g*, 15 min). Exchangeable Cd and Pb were 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 elemental and DOC analyses.

3. Results

3.1. Metal and DOC in soil solutions and extractable fraction after soil spiking

Metal partitioning between soil and soil solution or $CaCl_2$ extract was different in the 3 soils (Table 3). Following Pb spiking and soil equilibrium, a large range of concentrations was measured in the soluble fraction. As already observed for Cd (Bur et al., 2010), Pb concentrations in AU soil solutions and extractable fraction were significantly lower (about 10 to 150 times) than in the same fractions of the EPC and SV soils. In SV soil solution, Pb concentration was up to 22 times higher than in EPC. This fraction is generally considered as the available fraction for soil organisms.

In the same way to what was observed for Pb alone, Cd and Pb concentrations in soil solutions and in the exchangeable fractions were up to several hundred times higher for EPC and SV than for AU soil, when these metals were spiked in combination (Table 3). They were also higher for SV than for EPC. In EPC soil, Pb concentrations in soil solution and in the exchangeable fraction were higher than when Pb was spiked alone.

DOC concentrations (Table 3) were very variable and no trend could be observed between t1 and t8. Because DOC concentration was measured in soils after rewetting process, this may have lead to significant differences in activation of the biological activity, and consequently to the observed differences in DOC concentrations. The DOC concentrations were higher in EPC soil than in AU and SV soils, in relation to the higher OM content in EPC soil compared to the other soils (16.5% OM for EPC, 2% and 1.6%, respectively, for AU and SV, see Table 1).

3.2. Metal partitioning between soil and soil solution

Metal addition decreased the pH in almost all soil solutions (Table 3; Fig. 1). This decrease was more important for AU soil (Δ from −0.02 to −1.9 pH units) than for the two other soils (Δ from 0 to −0.87 pH units). The decrease of soil solution pH in relation to increasing concentrations of metal inputs could be converted to an amount of protons released to the solution ($[H^+]$ (mol.L^{-1}) = $10^{-\Delta pH}$) by comparing to the reference untreated soil. The gain of protons in the solution was related to the total amount (in moles) of Cd and/or Pb added to the soil after the treatment (Fig. 1). On the opposite, pH of the $CaCl_2$ extractable solution remained constant whatever the metal treatment in every soil. In the first case, adsorbed protons were exchanged with the metallic cations in relation to the quantities and the soil solution pH was modified accordingly. In the second case ($CaCl_2$ extraction), all the protons hold on the soil exchange complex were exchanged with Ca and pH was then buffered, whatever the metal input.

In order to quantify metal partitioning between soil and soil solution, adsorption diagram was drawn (Fig. 2). Adsorption could be predicted using Langmuir adsorption isotherm (Sposito, 1989; Sumner, 2000; van Gestel and Mol, 2003; Sparks, 2003; van Gestel and Koolhaas, 2004) as follows:

$$C_{sol} = \frac{C_{max} K_L C_{solution}}{1 + K_L C_{solution}}. \quad (1)$$

Where C_{sol}, is the metal concentration in soil (in mol.kg^{-1}), $C_{solution}$, the metal concentration in soil solution (mol.L^{-1}), K_L (L.mol^{-1}), the Langmuir constant (i .e. the inverse of the concentration in solution for which 50% of the sorption sites are filled up (Sposito, 1989; van Gestel and Koolhaas, 2004), and C_{max}, the maximal soil sorption capacity (mol.kg^{-1}). These two last parameters were calculated for the three soils and for each type of spiking assay, by referring to the metal content in soil solution and to $CaCl_2$ exchangeable fraction (Tables 3 and 4).

For the three soils, C_{max} for Cd is similar (for the assay mixture of Cd and Pb), indicating that the total adsorption capacity of soils is similar for this metal, which is consistent with results found in other kinds of soils (Pokrovsky et al., 2012). Whatever the spike assay (metal alone or in combination), Cd and Pb concentrations in solution for which 50% of the sorption sites are filled up (C50), were similar for soils EPC and SV but were very much lower for the soil AU (Table 4).

3.3. Reproduction of F. candida

3.3.1. Exposure to lead

In the reference soils, the reproduction was lower for SV than for the two other soils (Fig. 3), since less juveniles were observed. A significant decrease of the number of juveniles was observed in the three soils, for the highest Pb concentrations. Nevertheless, the

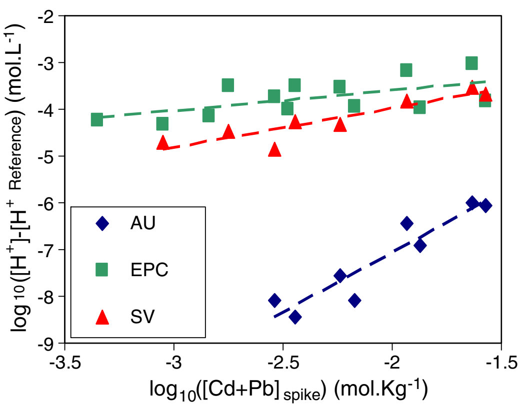

Fig. 1. Relationships between the concentrations of protons in spiked soil solutions minus those of the reference untreated soils and the total concentration (in mol. kg^{-1}) of spiked Cd + Pb in the considered soils AU, EPC and SV.

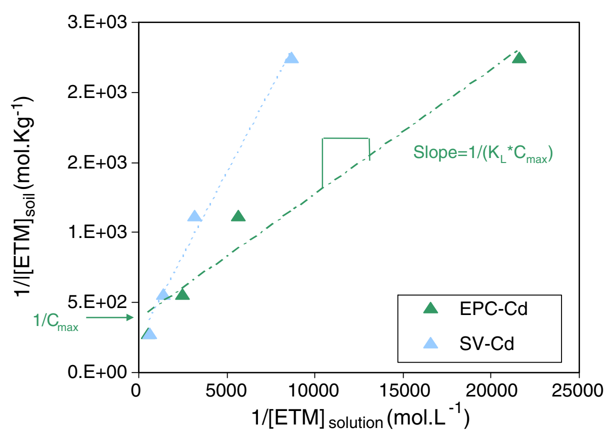

Fig. 2. Relationship between the inverse of Cd concentration in soil (mol.kg^{-1}) and in soil solution (mol.L^{-1}) for soil EPC and SV considering elemental spiking. Example of Langmuir constant determination for EPC–Cd; K_L was calculated as the inverse of the concentration in solution for which 50% of the sorption sites are occupied; C_{max} is the maximal soil sorption capacity.

Table 4
Langmuir constant for Cd and Pb sorption in the three soils for Cd spike alone, Pb spike alone and Cd/Pb in combination. Values of Log_{10} 1/K_L (mol^{-1}.L) and $Log_{10}C_{max}$ (mol. kg^{-1}) were calculated by using the 1/[Metal] in soil (mol kg^{-1}) v.s. [Metal] in soil solution (at t8).

Soil	Spiking	Log_{10} 1/K_L	C50	$Log_{10}C_{max}$	r²
			Cd solution		
AU	Cd	−5.65	$10^{-5.65}$	−2.46	0.888
EPC	Cd	−3.63	$10^{-3.63}$	−2.58	0.981
SV	Cd	−2.99	$10^{-2.99}$	−2.37	0.994
AU	Cd/Pb	−5.07	$10^{-5.07}$	−2.54	0.996
EPC	Cd/Pb	−3.06	$10^{-3.06}$	−2.59	0.982
SV	Cd/Pb	−2.97	$10^{-2.97}$	−2.58	0.988
			Pb solution		
AU	Pb	−6.29	$10^{-6.29}$	−1.62	–[a]
EPC	Pb	−4.72	$10^{-4.72}$	−2.08	0.962
SV	Pb	−4.39	$10^{-4.39}$	−2.09	0.960
AU	Cd/Pb	−7.32	$10^{-7.32}$	−1.92	0.965
EPC	Cd/Pb	−3.73	$10^{-3.73}$	−1.88	0.972
SV	Cd/Pb	−3.59	$10^{-3.59}$	−1.88	0.973

K_L = Langmuir constant; C_{max} = Maximal sorption capacity.
C50 = Csolution so that 50% of the soil adsorption capacity is reached.
[a] Only two available values, the other ones being inferior to the detection limit.

Table 5
Pb concentration in soil, soil solution (at t1 for AU and t8 for EPC and SV) and exchangeable fraction, leading to a decrease of 5% (EC05) and 50% (EC50) of *F. candida* reproduction. CI: [Confidence Interval 95%]. The CI in SV soil could not be accurately calculated using the software ToxCalc because of few individuals.

Pb	Soil (μg g^{-1})			
	EC_{05}	CI	EC_{50}	CI
AU	1077.7	[545.3–1441.5]	2573.8	[2177.8–3038.9]
EPC	5.5	[0–48.7]	181.0	[0.9–387.6]
SV	259.8	–	1110.4	–
	Solution (μg mL^{-1})			
	EC_{05}		EC_{50}	
AU	0.2	[0–0.4]	3.1	[1.9–5.7]
EPC	1.E-04	[0–0.01]	0.2	[0–1.4]
SV	0.9	–	53.5	–
	Exchangeable (μg mL^{-1})			
	EC_{05}		EC_{50}	
AU	–	–	–	–
EPC	0.03	[0–0.7]	4.4	[0–13.1]
SV	3.9	–	95.0	–

influence of Pb addition was stronger for EPC: a significant decrease was observed even for the lowest spiked Pb concentration (300 μg. g^{-1}, Fig. 3a). The LOEC for reproduction was 2400, 1200 and 300 μg g^{-1}, for AU, SV and EPC, respectively. The $EC_{05repro}$ and $EC_{50repro}$ calculated using total Pb concentration, Pb in solution or exchangeable Pb, are mentioned in Table 5. The highest Pb toxicity was observed for EPC soil regarding to bulk soil, soil solution or exchangeable concentrations. The lowest toxicity regarding to bulk soil Pb was observed for AU whereas the lowest toxicity regarding to soil solution or exchangeable concentrations was observed for SV.

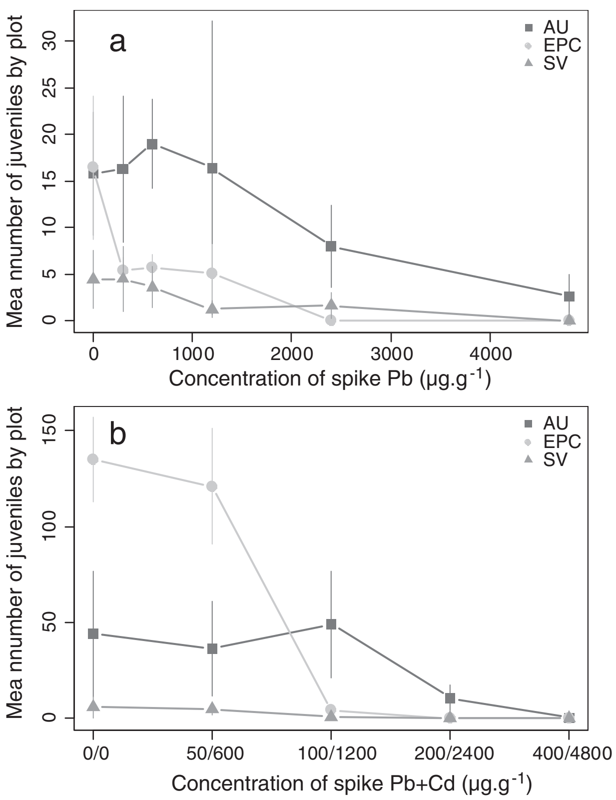

Fig. 3. Number of juveniles in relation to Pb (a) and Pb/Cd (b) spiked concentrations (abscissa : 0 Cd/0 Pb; 50 μg g^{-1} Cd/600 μg g^{-1} Pb; 100 μg g^{-1} Cd/1200 μg g^{-1} Pb; 200 μg g^{-1} Cd/2400 μg g^{-1} Pb; 400 μg g^{-1} Cd/4800 μg g^{-1} Pb).

3.3.2. Exposure to a combination of lead and cadmium

The reproduction rates decreased with the increasing concentrations of metals (Fig. 3b) and this decrease was faster and more significant for the EPC soil. The reproduction was very weak for SV. The LOEC for the number of juveniles were 200/2400, 100/1200 and 100/1200 μg g^{-1} Cd/Pb, for AU, SV and EPC, respectively (Fig. 3b). The $EC_{05repro}$ and $EC_{50repro}$ calculated using total Cd and Pb concentrations in soil, soluble Pb and Cd, and exchangeable Pb and Cd, are shown in Table 6.

The highest Cd toxicity regarding to bulk soil concentrations was observed for EPC and SV soils with almost equivalent EC values. Cd toxicity was the lowest for AU regarding to bulk soil but the highest regarding to soil solution and exchangeable concentrations.

The highest Pb toxicity was observed for EPC soil regarding to bulk soil concentrations and for AU soil regarding to soil solution or exchangeable concentrations. The lowest toxicity regarding to bulk soil Pb was observed for AU. The lowest toxicity regarding to soil solution or exchangeable concentrations was observed for SV.

3.4. Growth of F. candida

3.4.1. Exposure to lead

The distributions of the number of Collembola by length classes were very different for the three soils (Fig. 4a,b,c). For AU soil, two populations of Collembola characterised by two distinct length modes, were observed: the first one (the shortest animals) corresponds to the juveniles, which were born during the exposure period; the second one (the longest animals) corresponds to adult individuals introduced at the beginning of the experiment and which had survived to metal toxicity. A limit (0.65 mm) was identified between the two populations in order to accurately take into account Pb effect on the amount and the length of the juveniles (see Section 2.3). The distinction between juvenile and adult populations was less clear for the two other soils. Nevertheless, a limit was set up to 0.75 and 0.55 mm for EPC and SV, respectively, by considering the length class presenting the lowest individual abundance (sum of the five or six concentration abundances), around the third quartile value. For AU and SV soils, the median length remained almost stable, between 0.4 and 0.35 mm. A significant decrease in Collembola length ($P<0.05$) was observed in AU soil for the series of 300 and 2400 μg Pb g^{-1}. The median length for the juveniles in EPC soil, was superior to 0.4 mm in the case of untreated soils and of the two first spiked concentrations ($P<0.001$). Their lengths decreased significantly for

Table 6
Concentrations of Cd and Pb (used in combination) in the soil, in the soil solution (at t1 for AU and at t8 for EPC et SV) and in the exchangeable fraction, which have led to a 5% (EC05) or 50% (EC50) decrease of *F. candida* reproduction. In brackets (Confidence Interval 95%) when possible to calculate; (−) not of concerned.

Cd	Soil (μg g^{-1})				Pb	Soil (μg g^{-1})			
	EC_{05}		EC_{50}			EC_{05}		EC_{50}	
AU	146.1	–	182.0	–	AU	1753.6	–	2183.5	–
EPC	46.1	[41.4–49.6]	66.2	[61.3–74]	EPC	552.7	[496.9–595.2]	794.2	[736.2–887.5]
SV	40.3	–	68.6	–	SV	483.3	–	823.4	–
	Soil solution (μg mL^{-1})					Soil solution (μg mL^{-1})			
	EC_{05}		EC_{50}			EC_{05}		EC_{50}	
AU	1.4	–	2.5	–	AU	0.1	–	0.4	–
EPC	17.2	[14.1–19.9]	34.5	[29.9–42.7]	EPC	8.6	[6.8–10.3]	19.9	[16.7–25.7]
SV	16.6	–	41.9	–	SV	9.0	–	30.6	–
	Exchangeable fraction (μg mL^{-1})					Exchangeable fraction (μg mL^{-1})			
	EC_{05}		EC_{50}			EC_{05}		EC_{50}	
AU	–	–	–	–	AU	–	–	–	–
EPC	33.4	[30.3–35.7]	46.3	[43.2–51.2]	EPC	57.7	[51.6–62.4]	84.8	[78.2–95.3]
SV	25.9	–	49.4	–	SV	13.8	–	49.6	–

the highest spiked concentrations. For this soil, the mean length increased (but not significantly) for 300 and 600 μg Pb/g soil addition.

3.4.2. Exposure to a combination of cadmium and lead

The limit values for length between juvenile and adult populations were determined as 0.85, 0.9 and 0.7 mm, respectively for AU, EPC and SV soils. Compared to the reference condition, the mean juvenile length was considerably reduced for the combined concentrations 1, 2 and 3 in AU soil (Fig. 4d), for the highest combined Cd/Pb concentrations in EPC soil (Fig. 4e), and for the combination 3 for SV soil (Fig. 4f). The juvenile lengths are thus reduced significantly in the case of an exposure to a combination of Cd + Pb compared to each metal taken alone (Bur et al., 2010, this study).

3.5. Metal content in F. candida

As a whole, Cd and Pb concentrations in *F. candida* increased with higher metal concentrations in soils, except for Cd (Bur et al., 2010) and the mixture of Cd/Pb in AU soil noticeably for the highest concentrations (Table 7). A wide range of Cd concentrations in Collembola

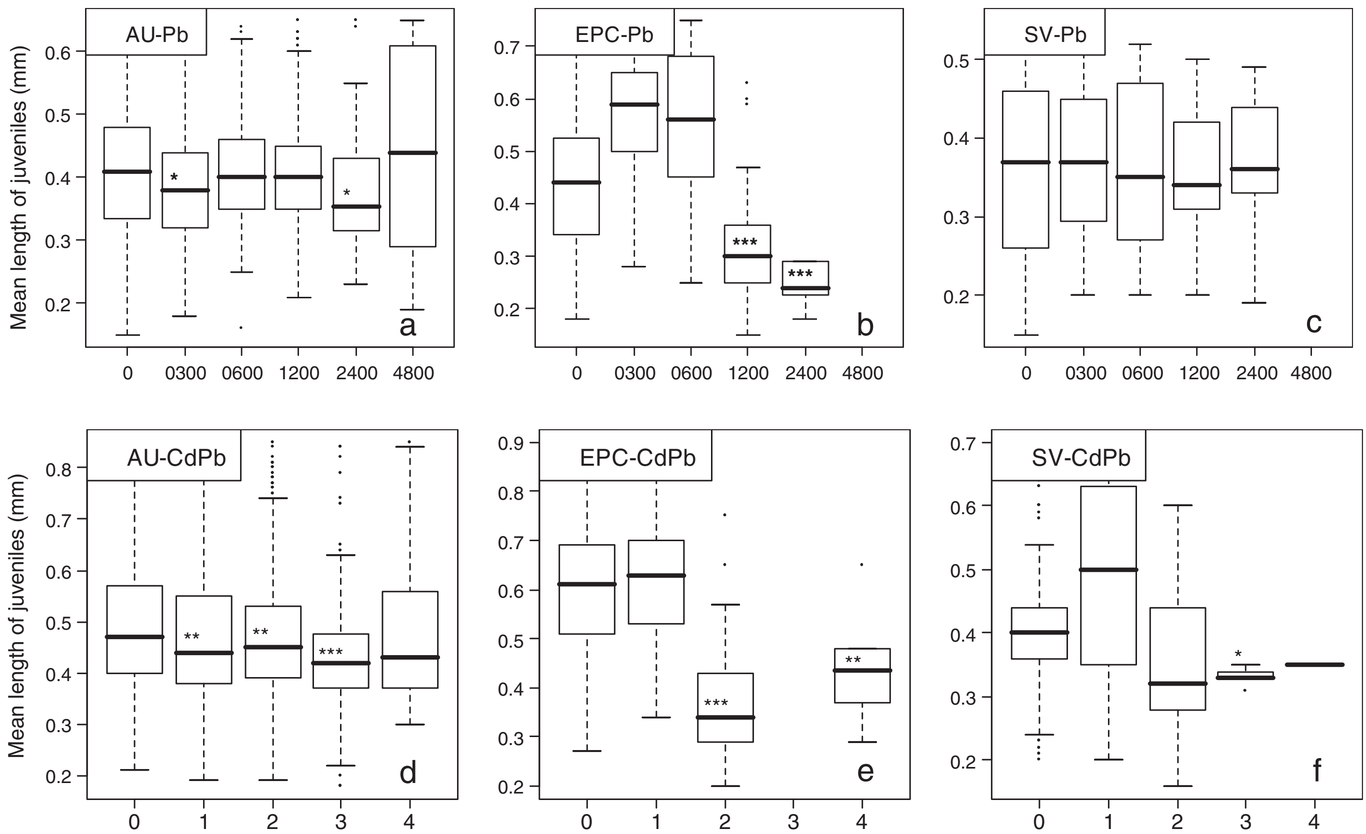

Fig. 4. Influence of Pb and of Cd + Pb spiked concentrations on the length of juveniles for soils AU (a,d), EPC (b,e) and SV (c,f), respectively. The spiked conditions 0, 1, 2, 3, 4 (in (d), (e) and (f)) correspond to an addition of 0/0, 50/600, 100/1200, 200/2400 and 400/4800 μg.g^{-1} of Cd/Pb, respectively. In the mentioned boxes, medians, quartiles and mode of the lengths are mentioned. *: P<0.05; **P<0.01; ***: P<0.001 means that the median length is significantly lower than that of the reference soil.

Table 7
Cd and Pb concentrations in Collembola body for Pb spiked alone and for Cd and Pb spiked in combination for each soil. (−) indicates that the number of Collembola was not sufficient to measure body metal concentrations due to high concentration toxicity.

	Soil reference	Cd soil (μg.g^{-1})	Pb soil (μg.g^{-1})	Cd Collembola (μg.g^{-1})	Pb Collembola (μg.g^{-1})
Cd or Pb spike	AU	–	0	< DL	< DL
		–	300	4.7	–
		–	600	1.6	27
		–	1200	11.4	75
		–	2400	1.1	333
		–	4800	0.2	973
	EPC	–	0	2.3	7.8
		–	300	1.1	48.3
		–	600	6.7	138
		–	1200	–	–
		–	2400	–	–
		–	4800	–	–
	SV	–	0	<DL	44
		–	300	1.9	95
		–	600	0.46	152
		–	1200	–	–
		–	2400	–	–
		–	4800	–	–
Cd/Pb spike	AU	0	0	3.9	16.2
		50	600	79.8	71.6
		100	1200	196	219
		200	2400	428	705
		400	4800	270	516
	EPC	0	0	0.48	7.3
		50	600	472	125
		100	1200	–	–
		200	2400	–	–
		400	4800	–	–
	SV	0	0	5.8	59.5
		50	600	367	431
		100	1200	556	1928
		200	2400	–	–
		400	4800	–	–

was observed from less than 1 μg g^{-1} to more than 500 μg g^{-1} for the reference soils and the highest spiked concentrations (for which measurements on Collembola could be done), respectively. Body concentrations for Pb were comprised between 7 and 60 μg g^{-1} in the reference soils and could reach up close to 1000 μg g^{-1} for AU soil (in case of 4800 μg g^{-1}with Pb spiking only) and close to 2000 μg g^{-1} for SV soil (in case of 1200 μg g^{-1} with combined Cd + Pb spiking).

Bioaccumulation factor ($BF_{solution}$) decreased with increasing spiked concentration for the three soils and for the two types of spiking conditions, Pb alone or in combination with Cd (Fig. 5). The values of $BF_{solution}$ were similar for EPC and SV soils but were much higher for AU soil whatever the metal concentration added to soil, alone or in combination.

4. Discussion

4.1. Soil/solution equilibrium of metal in soils, in relation to soil properties

Metal ageing in soils was assessed by comparing the concentrations in soil solutions at t1 and t8 (Table 3). Concentrations had generally decreased between t1 and t8. This was already observed in the literature and related to the affinity of some metals to the particulate phase. This affinity is more important for Pb than for Cd (McBride, 1995; Sumner, 2000). Cations are adsorbed by the way of mechanisms prevailing on the long term, such as diffusion processes within solid particules (Morioka, 1980) or formation of complexes, which need a significant activation energy (Sumner, 2000).

The decrease of soil pH, observed after metal addition noticeably in AU soil compared to the other soils, is the consequence of the higher total number of available protons in soils for EPC and SV soils (pH_{soil} 4.5 and 6.1, respectively) compared to AU (pH_{soil} = 8.2). Cd^{2+} and Pb^{2+} ions, which were added, were partly adsorbed on exchangeable cation sites initially occupied by protons before spiking (Stumm, 1992; Sparks, 2003; van Gestel and Mol, 2003). While EPC and SV soils follow a similar pattern, the gain of protons in AU soil solution is much lower. The number of available protons in this latter soil is weak because of the high pH. Nevertheless, it is worth noticing that as indicated by the higher slope observed for AU soil (Fig. 2), relatively to the initial stage the gain of protons was higher in this soil compared to the other two ones.

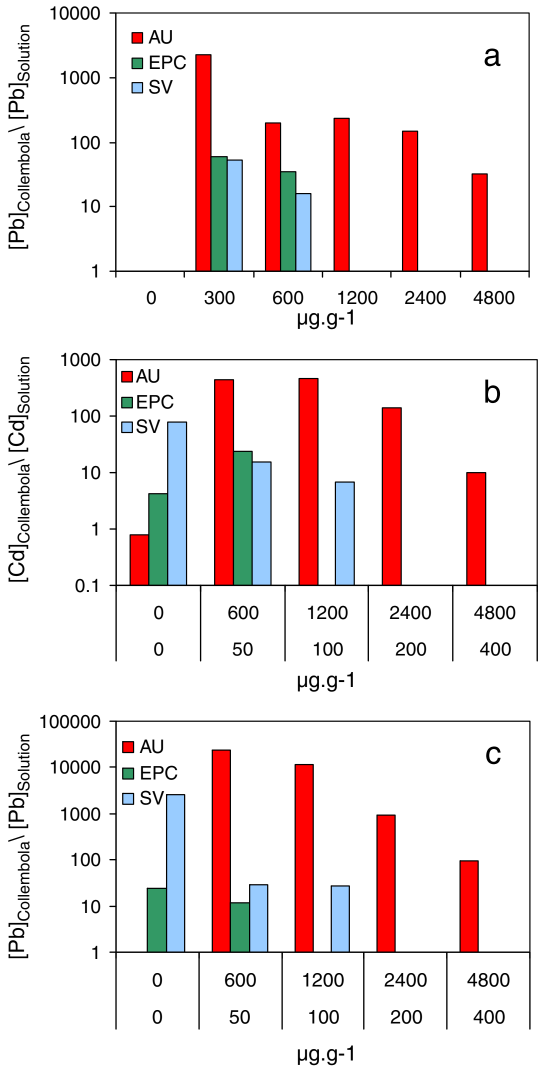

Fig. 5. Bioaccumulation factor in the three considered soils ($BF_{solution}$: concentration in Collembola over concentration in soil solution) for spiked Pb alone (a) or spiked Cd and Pb in combination (b and c). Spiked concentrations are indicated on abscissa (upper line for Pb in case of the combined spiked concentration with Cd (b,c)).

The important difference of soil pH between AU on one way and EPC and SV on the other way, partly rules adsorption processes, and leads to very different behaviours for Cd and Pb. Indeed, pH is one of the major parameters, and controls the amount of adsorbed metal at the equilibrium (Schindler et al., 1976; Ainsworth et al., 1994; van Gestel and Koolhaas, 2004; Mustafa et al., 2006; Gandois et al., 2010a).

In addition to pH, other soil properties, like clay content can influence metal partitioning in soils (Hernandez et al., 2003). For example, for Pb, the total adsorption capacity in solution and as exchangeable form is equivalent for EPC and SV soils, but the soil AU shows a higher C_{max}. This can be related to the high percentage of clay (smectite type) in AU soil and consequently an elevated CEC for this soil (20 cmol+.kg^{-1} for AU against 9 cmol+.kg^{-1} for EPC and SV). In AU soil, C_{max} tends to decrease for Pb, when Pb is applied in association to Cd. This indicates the occurrence of a competition between Cd and Pb for the sorption sites, whereas this was not observed for EPC and SV acidic soils.

Whatever the spike assay (metal alone or in combination), Cd and Pb concentrations in solution for which 50% of the sorption sites are filled up (C50), were similar for soils EPC and SV but were very much lower for the soil AU (Table 4). Thus, considering a similar spiked concentration, metals were more significantly adsorbed on the particulate phase in the basic AU soil compared to the two other acidic soils. This trend was already observed in other case studies and was again related to high pH (Gerritse et al., 1982; Anderson and Christensen, 1988; Lee et al., 1996; van Gestel and Koolhaas, 2004). On the opposite, the amount of metals in solution or exchangeable was weaker for AU soil compared to EPC and SV soils (Table 3). The mixed spiked Cd + Pb concentration led to an increase of C50 for Cd in soils EPC and AU, indicating a competition with Pb for sorption on particules. The same trend was observed for Pb and the mixed spike Cd + Pb conditions for SV and EPC soils.

Consequently, since they greatly influence metal partitioning in soil (Sauvé et al., 2000; Gandois et al., 2010a), soil properties should be carefully addressed when considering metal toxicity in soils (Bur et al., 2010). Acid pH increases Cd and Pb concentrations in solutions (Gandois et al., 2010a) and might thus increase their availability for soil organisms (van Gestel and Mol, 2003; van Gestel and Koolhaas, 2004; Bur et al., 2010). On the contrary, high content of adsorbent phases, like clays decreased the available fraction of metal in soils.

4.2. Impact of metal on reproduction and growth of F. candida

As a former point, it is important to notice that the reproduction levels obtained in our assays, particularly for the SV soil, were very low (even for the reference soils) if compared to literature data. One hypothesis to explain the highest reproduction level found for the untreated reference samples of the EPC soil (the forest soil enriched in organic matter), compared to the two other ones, could be the presence of a higher pool of microorganisms and consequently of food resources for the Collembola. Since the objective of the study was to be as close as possible to field conditions, we decided not to bring external food during the experiment, and this can be a major explanation. Smit and van Gestel (1998) have already observed a decrease of reproduction for some assays without food addition. Nevertheless, food adding had only a weak influence on EC_{50} reproduction in soil following a contamination by Zn. In the present study, EC_{50} in the SV reference soil is two to three folds lower than that of AU soil and this can be explained by the significant differences of soil pH (4.3 and 7.2, respectively, Table 1), which mean less metal in soil solution for AU soil.

For Pb spike alone, the three soils could be ranked according to decreasing EC_{50} data (based on total content in soils), i.e. by the increasing toxicity impact of Pb on Collembola: AU > SV > EPC. With reference to soil solution the order became: SV > AU > EPC and SV > EPC with reference to exchangeable concentrations (the $EC_{50repro}$ for AU could not be determined).

Contrary to what was observed for Cd (Bur et al., 2010), on the basis of the bulk soil, SV and EPC soil do not have the same sensitivity regarding Pb, but AU still led to the lowest toxicity. For soil solution and exchangeable fraction, SV became the less sensitive.

The $EC_{50repro}$ determined in this study on the basis of nominal concentrations was in the range of those found in some studies related to Collembola sensitivity to Pb (Sandifer and Hopkin, 1996; Menta et al., 2006) for AU and SV, but are much lower for EPC. Soil pH can explain the difference observed between EPC and AU but not that observed between EPC and SV, which are both in the range of acid soils. The organic matter content is the other main soil parameter, which discriminates these two soils. It is two to three times higher for EPC, and Pb has a high affinity to organic matter (Sauvé et al., 2000; Hernandez et al., 2003). Indeed it is difficult to identify its role to explain such differences of EC50, but it might have a protection role regarding metal complexation. If ingested the toxicity may be higher for the Collembola.

The order of EC50 for the three soils was almost the same when considering Pb spiked alone as well as when Pb was combined with Cd. The $EC_{50repro}$ order for Cd in the case of the mixture Cd/Pb spike, was AU > EPC ≈ SV when considering the total soil. This order became SV ≈ EPC > AU if considering soil solution concentrations and SV ≈ EPC for exchangeable concentrations (the EC_{50} for AU could not be determined for this latter).

The assays with Pb spiking only indicated that Pb has a much higher negative effect on reproduction (if one considers nominal concentrations) for soil EPC compared to the two other soils. This trend was not observed for Cd alone for the lowest spiked concentrations, but occurred by the 100 μg g^{-1} series (Bur et al., 2010). EPC has always the lowest $EC_{50repro}$ when referring to nominal concentrations as well as to soil solution concentrations. The $EC_{50repro}$ was lower for EPC than for AU and this could be explained by the lowest concentrations in soil solution and exchangeable $CaCl_2$ fraction in AU soil. On the other hand, Pb concentrations in soil solution of SV were higher than in EPC, and this did not allow to explain why the corresponding $EC_{50repro}$ was about the same for these two soils. The only identified explanation was that the bioavailabity of metals in soils differed from that of the "intra body" in the Collembola. Indeed, the bowel tract determines its own pH (Humbert, 1974). Consequently, it erases the initial difference of soil solution pH of the soils, and the bioavalability of metals in the final stage of assimilation by the body is probably modified. Similar observations have been done on previous assays related to spiking experiments with Cd alone on these three soils (Bur et al., 2010). Consequently, if concentrations of soil solutions are more accurate to assess metal toxicity for soil organisms than nominal concentrations in soils, they are still not completely satisfactory. A difference in Cd uptake by *Proisotoma minuta* was observed in relation to Cd distribution on soil components (Nursita et al., 2009).

The body concentrations of metals are often used to calculate the concentrations which have an effect on reproduction, as a complementary approach to the estimation of ECx based on total concentration in soils or in soil solutions (Crommentuijn et al., 1994; Smit and van Gestel, 1998; van Gestel and Mol, 2003; van Gestel and Koolhaas, 2004; Vijver et al., 2004; Bur et al., 2010). Nevertheless, in our case study, this calculation could not be done since measured body concentrations did not increase continuously with increasing spike concentrations, and consequently, it did not allow applying the EC estimation model. This large variability range of body concentrations could be explained by the heterogeneity of the population of Collembola, which was composed of a mixture of adults and juveniles.

The bioaccumulation factor decreased slightly with the increase of metal concentration in solution. $BF_{solution}$ is almost always weaker for EPC and SV than for AU; moreover for one soil type, BF decreased with increasing spiking concentrations whatever the soil and the concentration. These two statements show that $BF_{solution}$ decreased with increasing metal concentrations in soil solutions; it could be explained by the occurrence of some mechanisms involving limitation for metal transfer to Collembola body or to an increase of their removal as soon as the concentrations increase. Van Straalen et al. (1989) have shown that Pb and Cd excretion by the Collembola *Orchesella cincta* was higher in the case of the most polluted conditions. Moreover, in the case of high Pb contamination (Joosse and Verrhoef, 1983), the intermolt duration decreases and this favours the efficiency of metal excretion by bowel cell walls, where metal mostly accumulated.

$BF_{solution}$ was similar for EPC and SV soils, and they were much higher in AU soils compared to the two other ones. This indicates again that soil solution concentration cannot be the only efficient tool to predict metal bioavailability and toxicity levels in soils. Humbert (1977) has shown that when metal concentration in food is high, then it cannot be stored in gowel granules (midgut concretions) and go inside the blood (hemolymph) leading to a negative effect. If Collembola are regularly fed with such a food highly contaminated with Pb, their metabolism is reduced. It was not possible to conclude if this is related to a direct effect of lead on enzymes of the breath or a decrease in the level of nutrition.

Moreover, a competition between Cd and Pb for adsorption by the Collembola could be evidenced by comparing the $BF_{solution}$ for Cd for spike alone or in combination with Pb (Bur et al., 2010 and Fig. 5). A decrease of $BF_{solution}$ was obvious for Cd when spiked with Pb compared to Cd load alone. This indicated that loading high concentrations of Pb leads to a decrease of Cd absorption by the Collembola. This trend was not observed for Pb and thus absorption by the Collembola is weakly influenced by the addition of Cd in combination with Pb. Indeed, not the same concentration of Pb and Cd was applied (10 times more concentrated Pb than Cd), and this might explain the higher chemical pressure of Pb compared to Cd. (Fig. 5c).

Concerning Collembola growth, a significant impact was observed particularly for the EPC soil. One explanation was that the absence of food addition leads to a decrease of mean growth even for the three reference soils. This probably avoided detecting a significant influence of metal addition on growth. Indeed, for EPC this should be less obvious since the organic matter content in this soil was much higher than for the two other soils, and as a consequence on the food resource for microorganisms and microalgae, which constitutes the food basis of Collembola. Nevertheless, the parameter growth is known to be less sensitive than reproduction (Crouau and Moia, 2006; Bur et al., 2010). These authors have shown a significant effect of Cd on *F. candida* growth (however less significant than for reproduction). Joosse and Verrhoef (1983) did not notice any significant effect of Pb on the growth of the Collembola *O. cincta.* Soil arthropods do not have the same sensitivity to metals and some important differences can even be observed in the Collembola group: for example, reproduction of *Sinella coeca* decreases by a concentration of 10 $\mu g\ g^{-1}$ Cd and 500 $\mu g\ g^{-1}$ Pb (Menta et al., 2006). Greenslade and Vaughan (2003) have determined a $EC50_{repro}$ of 50 $\mu g.g^{-1}$ for Cd in soil for *Sinella communis,* whereas it reached about 130 $\mu g\ g^{-1}$ for *F. candida.*

4.3. Interactions between Cd and Pb and toxicity of metal combination on F. candida

The evolution of EC05 and EC50 allowed evaluating the change of toxicity of Cd and Pb when they were spiked alone or in combination. The ratio between EC for a mixture of Cd and Pb and each metal considered alone, were very variable (Fig. 6): the difference between the mixture of metal and the metal alone conditions could reach several orders of magnitude. Moreover, a general trend of values higher than one, was observed. This indicated that unexpectedly the toxicity as a whole decreased when the metals were combined. Similar results were observed for reproduction of *F. candida* for a combination of Cd and Zn (Van Gestel and Hensbergen, 1997), as well as for other arthropods (Weltje, 1998). A similar trend was also noticed for the worm *Aporrectodea caliginosa* (Khalil et al., 1996).

For Cd, the ratio $EC_{mixture}/EC_{element}$ for the soil is about 1 (Fig. 6), indicating that considering the nominal concentrations, the mixture Cd + Pb did not really modify Cd toxicity. Nevertheless, as mentioned above, we did observe a competition between Cd and Pb for adsorption on particulate phase in soils AU and EPC. This led to an increase of Cd concentration in the soil solution when considering the metal mixture condition. Consequently, if we consider that no other processes have occurred, the ratio $EC_{mixture}/EC_{element}$ based on soil total concentration should be below 1. But this was not the case, indicating that a competition process between Cd and Pb found in the solution could be evoked for *F. candida* toxicity. This was indeed confirmed by the ratio $EC_{mixture}/EC_{element}$ based on the concentration of Cd in the soil solution (Fig. 6), since Cd toxicity seemed reduced when Cd was applied in combination with Pb (high ratios).

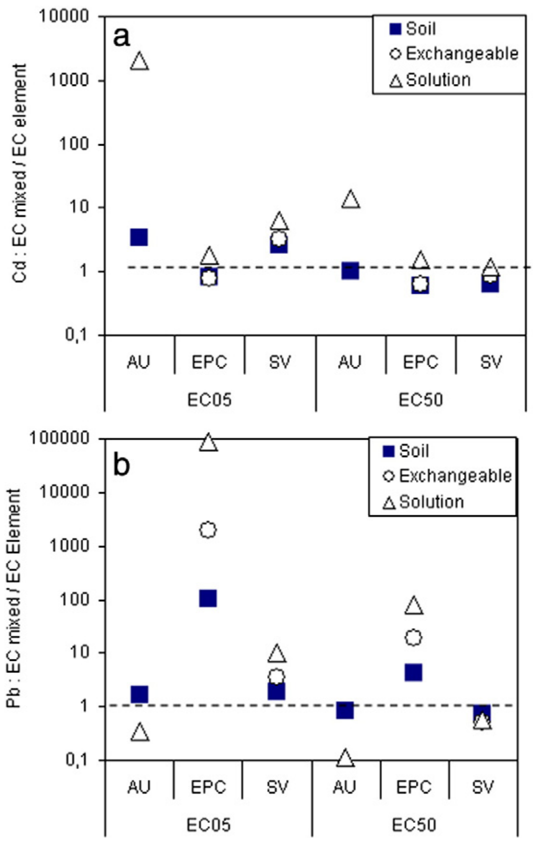

Fig. 6. Influence of the combined spike of Cd/Pb on the EC05 and EC50 relatively to each metal spiked alone (Cd (a) and Pb (b)) for the three soils. The horizontal line corresponds to the ratio ECmixed/ECelement = 1. Values above the line indicate a reduce toxicity (and reversely), particularly obvious for Pb in EPC soil when spiked in combination with Cd.

The ratios $EC50_{mixture}$/EC50 Pb for EPC soil were 4.4 and 18 for soil concentration and soil solution concentration, respectively. This is a main difference, which is never observed for Cd. The greater affinity of Pb for organic matter compared to Cd is well documented in the literature (Hernandez et al., 2003; Gandois et al., 2010a,b). Indeed the organic matter content in EPC soil was 8 and 10 times higher than in AU and SV, respectively. The organic matter content might be the key parameter controlling the reduction of Pb toxicity when Pb was combined to Cd. EPC was the most sensitive soil to Pb, but the observed competition with Cd to adsorption sites led to this toxicity reduction.

5. Conclusion

This study has addressed the role of soil properties in evaluating metal toxicity to collembola in soils. Metal addition (Pb or the mixture Cd + Pb) to the three studied soils, has lead to a pH decrease in soil solution. Acidic soils have about the same maximum sorption capacity, which is much lower than that of the high pH soil. These soils are thus less protective regarding metal toxicity. High pH plays a more efficient protective role compared to organic matter content for Collembola reproduction. Nevertheless, organic matter was found to control more efficiently Pb compared to Cd in the solution. A decrease of metal concentration in soil solution was detected particularly for Pb, as a result of metal affinity to particulate phase and as a consequence of an ageing effect. The total adsorption capacity of the three soils was found to be similar and a competition for the sorption sites between Cd and Pb was observed mainly in the basic soil.

Metal concentration in Collembola generally increased with increasing soil spiking, but the accumulation in body with reference to soil solution decreased whatever the soil, probably as a result of regulation mechanism. Pb was less accumulated in body than Cd. The Collembola accumulated more Pb and Cd + Pb in acidic soils, where soluble or exchangeable metals are higher, but $EC50_{repro}$ was similar in spite of different metal concentration in solution. More accurate than nominal concentrations in soils, concentrations of soil solutions are indeed still not completely adequate to assess metal toxicity for soil organisms. Whatever the soil, Cd and Pb spike, noticeably in combination, decreased collembola reproduction, but the influence on Collembola length could not be clearly quantified.

A competition between Cd and Pb for adsorption by the Collembola was also evidenced, but absorption by the Collembola was weakly influenced by the addition of Cd in combination with Pb. Finally, one striking point was that the toxicity was as whole decreased when metals were combined rather when applied alone, except for Pb in carbonate soil. The high organic matter content of EPC soil plays a key protective role for Collembola toxicity in relation to the competition between Cd and Pb for adsorption sites.

Mechanisms of metal assimilation by soil organism still remain to be studied in a large range of soil pH conditions. Our results have emitted some hypothesis on possible ways of metal contamination: the role of particle ingestion and dissolution in the collembola body, the ingestion of soil microfauna, pore transfer or any other mechanism leading to toxicity. This would allow to better constraint critical limits in natural soil conditions.

Acknowledgements

This study benefited of financial aids from ADEME, CNRS, INRA and Toulouse University. Our acknowledgements also go to A. Canut, A. Magro, F. Candaudap and A. Alric, for their help in field sampling, sample preparation, or analysis.

T. Bur benefited from a joined fellowship ADEME (Agence de l'Environnement et de la Maîtrise de l'Energie) — INRA (Institut National de la Recherche Agronomique)/RMQS (Réseau de Mesure de la Qualité des Sols). L. Gandois benefited a ADEME fellowship.

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

We thank anonymous reviewers for their helpful comments.

References

Agreste Midi-Pyrénées. L'agriculture, l'agro-alimentaire et la forêt. Mémento édition 2008; 2008.
Ainsworth C, Pilon J, Gassman P, Vandersluys W. Cobalt, cadmium, and lead sorption to hydrous iron-oxyde — residence time effect. Soil Sci Soc Am J 1994;58:1615–23.
Anderson P, Christensen T. Distribution coefficients of Cd, Co, Ni and Zn in soils. J Soil Sci 1988;39:15–22.
Baize D. Teneurs totales en éléments traces métalliques dans les sols français. Références et Stratégies d'interprétation. Paris: INRA Editions; 1997. 410p.
Bergkvist B, Folkeson L, Berggren D. Fluxes of Cu, Zn, Pb,Cd, Cr and Ni in temperate forest ecosystems. A literature review. Water Air Soil Pollut 1989;47:217–86.
Bur T. Impact anthropique sur les éléments traces métalliques dans les sols agricoles de Midi-Pyrénées. Implication en termes de limites et de charges critiques. Thèse Université, Toulouse, 2008. 367 pp.
Bur T, Probst A, Bianco A, Gandois L, Crouau Y. Determining cadmium critical concentrations in natural soils by assessing Collembola mortality, reproduction and growth. Ecotoxicol Environ Saf 2010;73:415–22.
Cole LJ, McCracken DI, Foster GN, Aitken MN. Using Collembola to assess the risks of applying metal-rich sewage sludge to agricultural land in western Scotland. Agric Ecosyst Environ 2001;83:177–89.
Collin B, Doelsch E. Evaluation de la mobilité et de la phytodisponibilité des éléments traces métalliques des sols. Etude réalisée sur les sols de la Communauté Intercommunale Réunion Est (CIREST), en perspective d'une demande de dérogation à la réglementation sur l'épandage des boues de stations d'épuration. CIRAD; 2008. 62 pp.
Crommentuijn T, Doodeman C, Doornekamp A, van der Pol J, Bedaux J, van Gestel C. Lethal body concentrations and accumulation patterns determine time-dependant toxicity of cadmium in soil arthropods. Environ Toxicol Chem 1994;13:1781–9.
Crouau Y, Cazes L. What causes variability in the *Folsomia candida* reproduction test. Appl Soil Ecol 2003;22:175–80.
Crouau Y, Moia C. 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 2006;64:115–21.
Crouau Y, Chenon P, Gisclard C. The use of Folsomia candida (Collembola, Isotomidae) for the bioassay of xenobiotic substances and soil pollutants. Appl Soil Ecol 1999;12:103–11.
Fountain M, Hopkin SP. Continuous monitoring of *Folsomia candida* (Insecta: Collembola) in a metal exposure test. Ecotoxicol Environ Saf 2001;48:275–86.
Fountain MT, Hopkin SP. Comparative study of the effects of metal contamination on Collembola in the field and in the laboratory. Ecotoxicology 2004;13(6):573–87.
Gandois L, Probst A, Dumat C. Modelling Trace metal extractability and solubility in French forest soils by using soil properties. Eur J Soil Sci 2010a;61:271–86.
Gandois L, Tipping E, Dumat C, Probst A. Canopy influence on trace metal atmospheric inputs on forest ecosystems: speciation in throughfall. Atmos Environ 2010b;44:824–33.
Gavalda D, Scheiner J, Revel J, Merlina G, Kaemmerer M, Pinelli E, et al. Agronomic and environmental impacts of a single application of heat-dried sludge on an Alfisol. Sci Total Environ 2005;343:97-109.
Gerritse R, Vriesema R, Dalenberg J, de Roos H. Effect of sewage sludge on trace element mobility in soils. J Environ Qual 1982;11:359–64.
Greenslade P, Vaughan GT. A comparison of Collembola species for toxicity testing of Australian soils. Pedobiologia 2003;47:171–9.
Herbert I, Svendsen C, Hankard P, Spurgeon D. Comparison of instantaneous rate of population increase and critical-effect estimates in Folsomia candida exposed to four toxicants. Ecotoxicol Environ Saf 2004;57:183–93.
Hernandez L, Probst A, Probst JL, Ulrich E. Heavy metal distribution in some French forest soils: evidence for atmospheric contamination. Sci Total Environ 2003;312:195–219.
Horckmans L, Swennen R, Deckers J. Retention and release of Zn and Cd in spodic horizons as determined by pH_{stat} analysis and single extractions. Sci Total Environ 2007;376(1–3):86–99.
Humbert W. Etude du pH intestinal d'un Collembole (Insecta, Aptérygote). Rev Ecol Biol Sol 1974;11:89–97.
Humbert W. The mineral concretions in the midgut of *Tomocerus minor* (Collembola): microprobe analysis and physioecological significance. Rev Ecol Biol Sol 1977;14:71–80.
ISO 11267. Soil quality — Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants — Inter Stand Org Ed. Genève; 1999.
Ives MA. *TOXCALC 5.0*, Tidepool Scientific Software, McKinleyville, CA, USA; 1996. 80 pp.
Joosse NG, Verrhoef SC. Lead tolerance in Collembola. Pedobiologia 1983;25:11–8.
Khalil MA, Abdel-Lateif HM, Bayoumi BM, Van Straalen NM, Van Gestel CAM. Effects of metals and metal mixtures on survival and cocoon production of the earthworm *Aporectodeacaliginosa*. Pedobiologia 1996;40:548–56.
Krogh PH, Petersen B. Laboratory toxicity testing with Collembola. In: Løkke H, editor. Effects of pesticides on Meso- and Microfaune in Soil. Danish Environment Protection Agency; 1995, pp. 39–58.
Lee S, Allen H, Huang C, Sparks D, Sanders P, Peijnenburg W. Predicting soil–water partition coefficients for cadmium. Environ Sci Technol 1996;30:3418–24.
Lubben B. Influence of sewage sludge and heavy metals on the abundance of Collembola on two agricultural soils. 3rd international seminar on Apterygota; 1989.
McBride M. Toxic metal accumulation from agricultural use of sludge: are USEPA regulations protective? J Environ Qual 1995;24:5-18.
Menta C, Maggiani A, Vattuone Z. Effects of Cd and Pb on the survival and juvenile production of *Sinella coeca* and *Folsomia candida*. Eur J Soil Biol 2006;42:181–9.
Migliorini M, Pigino G, Caruso T, Fanciulli PP, Leonzio C, Bernini F. Soil communities (Acari Oribatida; Hexapoda, Collembola) in a clay pigeon shooting range. Pedobiologia 2005:1-13.
Morioka M. Cation diffusion in olivine. 1. cobalt and magnesium. Geochim Cosmochim Acta 1980;44(5):759–62.
Mustafa G, Kookana R, Singh B. Desorption of cadmium from goethite: Effects of pH, temperature and aging. Chemosphere 2006;64:856–65.
Nicholson FA, Smith SR, Alloway BJ, Carlton-Smith C, Chambersa BJ. An inventory of heavy metals inputs to agricultural soils in England and Wales. Sci Total Environ 2003;311:205–19.
Nursita AI, Singh B, Lees E. The effects of cadmium, copper, lead, and zinc on the growth and reproduction of *Proisotoma minuta* Tullberg (Collembola). Ecotoxicol Environ Saf 2005;60:306–14.
Nursita AI, Singh B, Lees E. Cadmium bioaccumulation in *Proisotoma minuta* in relation to bioavailability in soils. Ecotoxicol Environ Saf 2009;72(6):1767–73.
Pokrovsky O, Probst A, Leviel E, Liao B. Interaction of cadmium and lead with acid soils: Experimental evidences of similar adsorption pattern in a wide range of metal concentration and implications for metals migration. J Hazard Mater 2012;199–200:358–66.
Ponnette Q, Ulrich E, Brethes A, Bonneau M, Lanier M. RENECOFOR- Chimie des sols dans les 102 peuplements du réseau. Campagne des mesures 1993/1995. Office National des Forêts Ed., Département des Recherches Techniques; 1997. ISBN 2-84207-100-X.
Probst A, Moncoulon D, Goddéris Y, Hernandez L, Party JP. Critical loads for lead in France: first results on forest soils. J Phys IV France 2003;107:1111–4.
R Development Core Team. R: A language and environment for statistical computing. R foundation for statistical computing. Vienna, Austria3-900051-07-0; 2010http://www.R-project.org. URL.

Sandifer RD, Hopkin SP. 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 1996;33(12):2475–86.

Sauvé S, Hendershot W, Allen H. Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. Environ Sci Technol 2000;34:291–6.

Schindler P, Fürst B, Dick B, Wolf P. Ligand properties of surface silanol groups. I. Surface complex formation with Fe^{3+}, Cu^{2+}, Cd^{2+} and Pb^{2+}. J Colloid Interface Sci 1976;55:469–75.

Smit CE, van Gestel CAM. Effects of soil type, prepercolation, and ageing on bioaccumulation and toxicity of zinc for the springtail *Folsomia candida*. Environ Toxicol Chem 1998;17:1132–41.

Sparks D. Environmental soil chemistry Second Edition. San Diego; 2003. 352 pp.

Sposito G. The chemistry of soils. New York; 1989. 277 pp.

Stumm W. Chemistry of the solid–water interface. New York: John Wiley & Sons; 1992. 428 pp.

Sumner M. Handbook of soil science. CRC Press; 2000. 2148 pp.

Van Gestel CAM, Hensbergen PJ. Interaction of Cd and Zn toxicity for Folsomia candida Willem (Collembola: Isotomidae) in relation to bioavailability in soil. Environ Toxicol Chem 1997;16:1177–86.

Van Gestel CAM, Koolhaas JE. Water-extractability, free ion activity, and pH explain cadmium sorption and toxicity to *Folsomia candida*. (Collembola) in seven soil-pH combinations. Environ Toxicol Chem 2004;23:1822–33.

Van Gestel CAM, Mol S. The influence of soil characteristics on cadmium toxicity for *Folsomia candida* (Collembola: Isotomidae). Pedobiologia 2003;47:387–95.

Van Straalen NM, Schobben JHM, De Goede RGM. Population consequences of cadmium toxicity in soil microarthropods. Ecotoxicol Environ Saf 1989;17: 190–204.

Vijver M, Van Gestel C, Lanno R, Van Straalen N, Peijnenburg W. Internal metal sequestration and its ecotoxicological relevance: a review. Environ Sci Technol 2004;38: 4705–12.

Weltje L. Mixture toxicity and tissue interactions of Cd, Cu, Pb and Zn in earthworms (Oligochaeta) in laboratory and field soils: a critical evaluation of data. Chemosphere 1998;36(12):2643–60.