Comparative ecotoxicity of three polluted industrial soils for the Collembola *Folsomia candida* ☆

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Abstract

We tried to quantify the ecotoxicity of three industrial soil samples contaminated by different metals, using the Collembolan *Folsomia candida* as a biological model and mortality, growth and reproduction as parameters. The observed ecotoxicities are rather normal for the first such soil sample (aluminum factory) but are high for the second sample (ore treatment) and relatively low for the third one (zinc factory) considering its high metal concentrations. For these last two soil samples, an unusual ecotoxicity plotting is observed: two high ecotoxicity recordings fit with a low and high percentage of polluted soil to non-polluted soil and noticeably lower ecotoxicity recordings are observed between them. Chemical analyses of metals in pore waters show that arsenic probably explains part of such an unusual ecotoxicity curve. Otherwise, mortality and growth of the animals are less sensitive parameters than reproduction. Our experiments show that the results of the ecotoxicological assays of polluted soils are complex and difficult to interpret.

Keywords: Toxicity testing; Soil pollution; Collembolan; Mortality; Growth; Reproduction

1. Introduction

In France, thousands of industrial soils are polluted by organic and metallic xenobiotics. This pollution is due to waste deposits, chemical leakages or to fall-outs of atmospheric emissions and is often quantified by means of chemical analyses. However, that mode of investigation gives little information on the contaminated soils' ecotoxicity and does not take into account their bioavailability; the investigation must be completed by ecotoxicological assays with biological models. The Collembolan reproduction assay ISO 11267 (1998) was developed to quantify the ecotoxicity of pure chemicals. Therefore, more experience in using this test for the assessment of contaminated soils is needed. To check whether it was suitable to test polluted soil, we applied it to three industrial contaminated soils, using mortality, growth and reproduction as parameters. Collembolan (springtails), apterygote primitive hexapods, are common in litter and topsoil. *Folsomia candida* is blind and reproduces parthenogenetically.

The Collembolan reproduction assay is increasingly used because it is very often sensitive and the breeding of this species is easy (Riepert, 1995). It has mainly been used to assess the toxicity of pure metals (Van Straalen et al., 1989; Scott-Fordsmann et al., 1997, 1999; Smit and van Gestel, 1998; Fountain and Hopkin, 2001; Lock and Janssen, 2002; Herbert et al., 2004; Smit et al., 2004) or organic chemicals (Crommentuijn et al., 1995; Crouau et al., 1999; Herbert et al., 2004; Campiche et al., 2006; Idinger et al., 2006; Eom et al., 2007). Application to complex mixtures such as polluted soils or wastes are less numerous (Crouau et al., 2002; Fountain and Hopkin, 2004; Smit and van Gestel, 1996). The effects of several different physical parameters such as temperature (Grégoire-Wibo and Snider, 1983), pH and soil moisture (Holmstrup, 1997), feeding (Smit et al., 1998), ageing of soils (Smit and van Gestel, 1998) and the variability between strains have been studied (Crommentuijn et al., 1995; Chenon et al., 2000).

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Thus we attempted to assess the toxicity of three metal contaminated soils by monitoring the reproduction, the growth and the mortality of *F. candida* juveniles in series with increasing proportions of polluted soils diluted in non-polluted ISO 11268-1 (1994) soil (Riepert and Kula, 1996). Three different contaminated soils were tested. They came from an aluminum factory, an ore treatment plant and a zinc factory. We studied the evolution of mortality, growth and reproduction after 35 days of exposure to the blend of polluted soil and ISO soil. We compared the evolution of these parameters with the metal concentrations in pore water of the different series. Finally, we studied the effects of pH on polluted soils ecotoxicities. Some studies show that a lowering of pH increases the soil's ecotoxicity for the soil organisms (Crommentuijn, 1994; Spurgeon and Hopkin, 1996). Other studies do not show such an effect (Crommentuijn et al., 1997; Pedersen et al., 1997).

2. Materials and methods

2.1. Experimental soils

We tested three soil samples taken near three factories situated in South West France.

A/Lannemezan: It is an aluminum factory (produced by the electrolysis of aluminum bauxite) located in the foothills of the Pyrenees. The relevant soil sample was taken near the Eastern boundary of the factory. The soil is an humiferous soil lying above an argilaceous mud.

B/Salsigne: that soil came from the vicinity of a gold mine in southern France located between the hercynian area of the Montagne Noire and the tertiary and quaternary area of the Lauragais plain. It is an auriferous and arseniferous mineralization trapped in hydrothermal fluids.

C/Viviez: It is an old zinc plant located in Aveyron and the relevant soil sample was taken adjacent to this factory. The subsoil at this location is made of modern deposits of the tertiary valley.

We used ISO soil (corresponding to the ISO standard 11268-1, 70% quartz sand, 20% kaolinite clay, 10% peat ground, dried and sieved to 0.5 mm and $CaCO_3$ for the pH (5.5 ± 0.5)) as a dilution soil to control if it is well-adapted for testing ecotoxicity of polluted soils; it is very difficult or impossible (particularly for the Salsigne and Viviez soils) to find non-polluted dilution soil with physico-chemical characteristics similar to the polluted soil. The polluted soils were mixed with the ISO soil in various proportions. The mixtures were moistened to 53% of their water-holding-capacity and homogenized to obtain a crumbly structure.

2.2. Test organisms

Before the experiments, the *F. candida* clones were reared in darkness at 20 °C in plastic boxes containing a regularly dampened charcoal/plaster of Paris mixture. They were fed on baker's yeast.

2.3. Experimental design

The experimental conditions were the same for the three soil samples tested except for the proportions of the polluted soil in the ISO dilution soil.

The *F. candida* reproduction test was carried out following the recommendations of ISO 11267 with minor modifications: in order for the results to be more meaningful, we preferred to extend the total duration of the trials from 28 (as recommended by the ISO 11267 norm) to 35 days (Crouau et al., 1999). Ten juveniles, 10–12-days old, were placed in tightly closed glass containers (100 mL capacity) containing the soils at different concentrations (32 g wet weight of the test substrate; 6–10 pots/concentration). The temperature was 20 ± 1 °C. The glass containers were opened twice a week for aeration and every two weeks for feeding with yeast. At the end of the experiment, the water content and pH of the soils were determined, water was added and the floating animals were counted. Digital photographs were taken and the length of the animals (adults; from the end of the posterior abdominal segment to the anterior margin of the head) was measured by means of the ImageTool software. The Lowest Observed Effect Concentration (LOEC), No Observed Effect Concentration (NOEC) and 50% Effective Concentration (EC_{50}) were obtained using Toxcalc 5.0 (Tidepool Scientific Software, Mc Kinleyville, CA, USA, US Environment Protection Agency methods; Wilcoxon, maximum likelihood probit, trimmed Spearman-Karber). For growth measurements, digital photographs of the animals were taken (Nikon Coolpix 990) at the end of each assay. Then the length of the animals, from the end of the posterior abdominal segment to the anterior margin of the head, was measured by on-screen viewing by means of the ImageTool software. A small plastic rule was placed near the animal as a calibration scale. Effect of soil pH was studied modifying the pH by addition of $CaCO_3$ to the

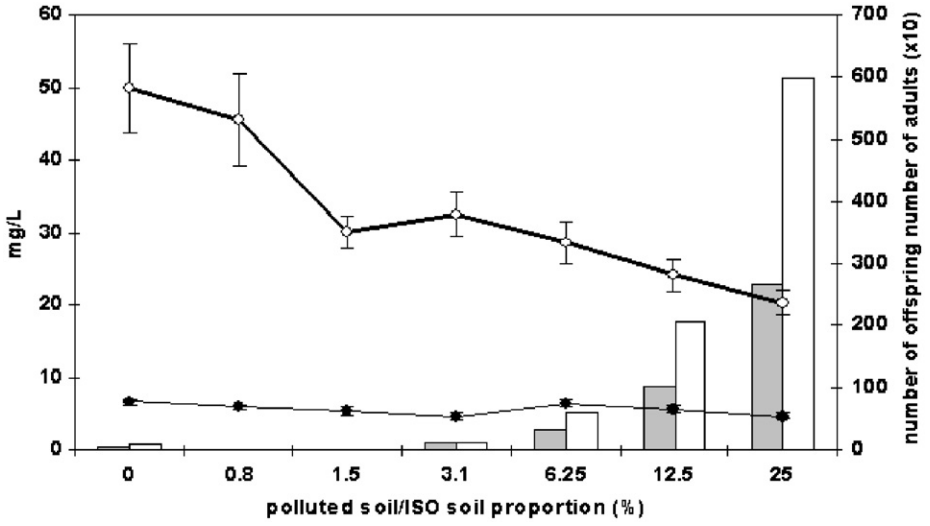

Fig. 1. Lannemezan soil assay: concentrations of aluminum (gray bars) and fluor (white bars) in the pore waters and effects of the polluted soil on juvenile production (open circle) and on mortality (full circle—number of adults at the end of the assay × 10). Means ± standard errors (S.E.M.), $n = 9$.

blank and the 12.5% series to get two different pH with about one unit difference (Fig. 5).

2.4. Chemical analysis

A/Soil analysis: After digesting the soil with HF–$HClO_4$–HNO_3 medium, the extract was evaporated to dryness, taken up in HNO_3, and the total concentrations of metals in the soil determined by ICP-MS.

B/Pore waters analysis: Pore waters were obtained by the addition of double-distilled water to soil to obtain 200% of the WHC and shaking that soil for 2 h. The suspension was left to equilibrate for 3 days and then it was centrifuged for 10 min at 8000 g (Davies and Davies, 1963). The supernatants were filtered through a 0.45 μm membrane and acidified with concentrated HNO_3. The metal concentrations were then determined by ICP-MS.

3. Results

3.1. Effects on reproduction and mortality

Concerning reproduction, the validity criteria for the controls defined by the ISO test guideline 11267 on the Collembolan tests (more than 100 juveniles per test vessel and coefficient of variation less than 30%) was always met. On the other hand, the mortality in the controls (between 20% and 35%) was higher than specified in the ISO guidelines (less than 20%).

A/Lannemezan (Fig. 1): A significant difference for reproduction relative to the control is observed from the 1.5% series (Wilcoxon test; $p \leqslant 0.05$) and the NOEC value

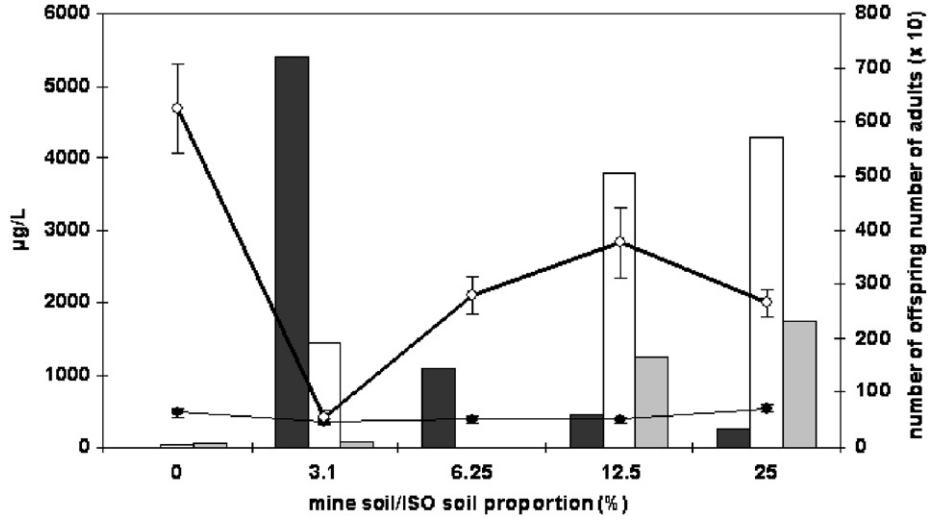

Fig. 2. Salsigne soil assay: concentrations of arsenic (black bars), manganèse (white bars) and aluminum (gray bars) in the pore waters and effects of the polluted soil on juvenile production (open circle) and on mortality (full circle—number of adults at the end of the assay × 10). Means ± S.E.M, $n = 8$.

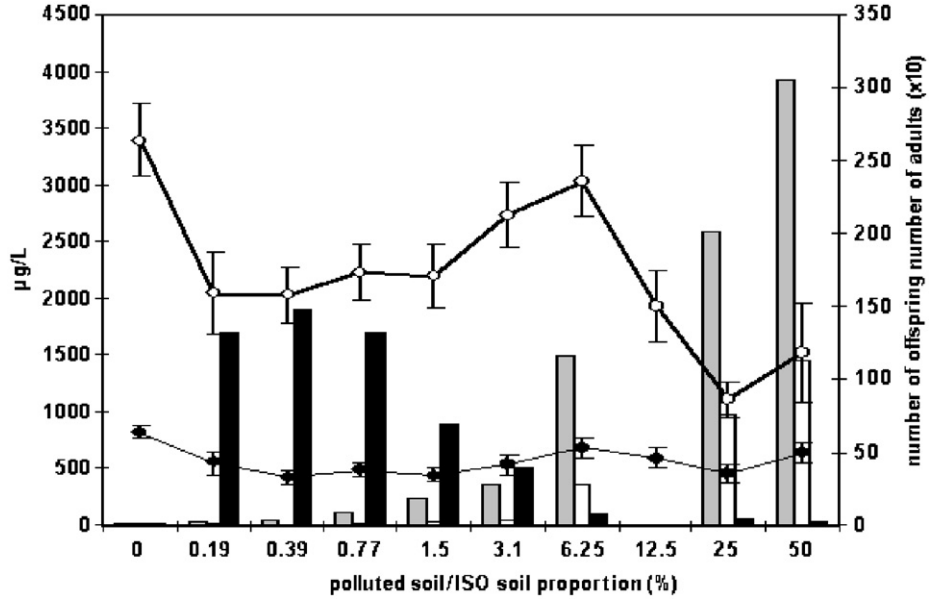

Fig. 3. Viviez soil assay: concentrations of arsenic (C × 10-black bars), zinc (C/10-gray bars), and cadmium (C/10-white bars), in pore waters and effects of the polluted soil on juvenile production (open circle) and on mortality (full circle—number of adults at the end of the assay x10). Means ± S.E.M.; $n = 9$.

is 0.8%. EC_{50} reproduction is 11% (confidence limit: 5–61%). Effects on mortality are not significant except for the 3.1% series ($p = 0.013$).

B/Salsigne (Fig. 2): the LOEC for reproduction is observed for the 3.1% series (Wilcoxon test, significance level $p \leqslant 0.01$). For series with more contaminated mixture the effect on reproduction decreases. We did not get the NOEC value in this assay. An additional assay with more diluted polluted soil gives a NOEC for the 0.19% dilution series. Probit analysis failed to yield reliable EC_{50} estimates. No significant effect on mortality is observed.

C/Viviez (Fig. 3): The LOEC for reproduction is 0.19% ($p \leqslant 0.01$; Bonferroni test). The 0.39%, 12.5%, 25% and 50% series are also significantly different relative to the control. The EC_{50} is 16.1% (Spearman-Karber) but this result is not sound on account of the curve shape. The NOEC and LOEC for mortality are 0.19% and 0.39%, respectively. The 0.77%, 1.5%, 3.1% and 25% series are also significantly different from the control.

3.2. Effects on growth (Fig. 4)

Significant effects on growth are observed for 3.1%, 12.5% and 25% of Salsigne soil and for 25% of Lannemezan and Viviez soils (Fig. 4).

3.3. Effects of pH

For the polluted soils, statistically significant differences were observed ($p \leqslant 0.01$, *t*-test homoscedastic) between two

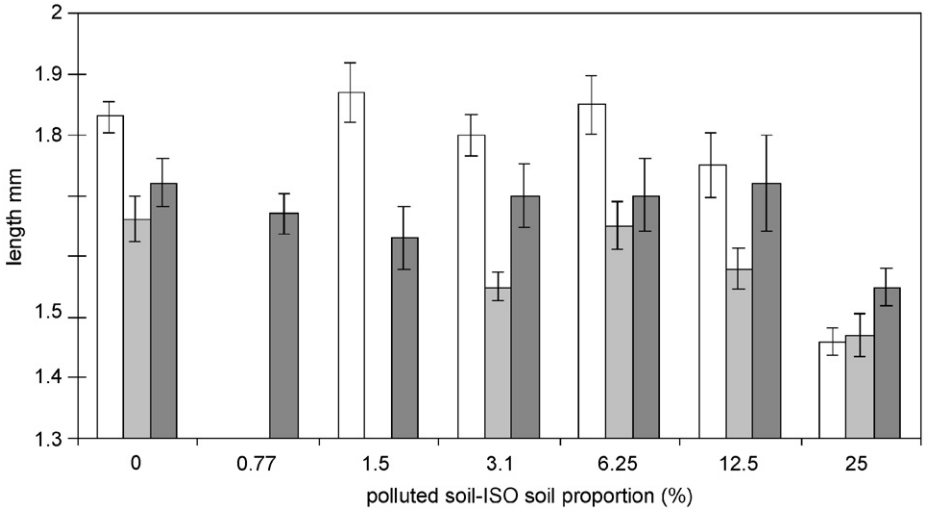

Fig. 4. Effect of the polluted soil on growth of *F. candida*. Lannemezan: white bars; Salsigne: light gray bars; Viviez: dark gray bars. Mean ± S.E.M. Significant statistical differences from the control were observed for the 25% series ($p < 0.01$) of Lannemezan and Viviez soils and for the 3.1%, 12.5% and 25% series of Salsigne soil.

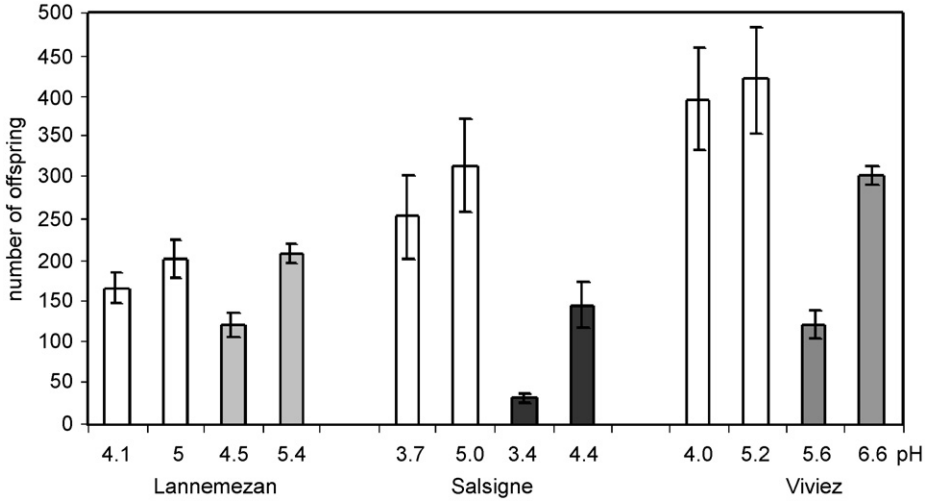

Fig. 5. Effect of soil pH on reproduction of *F. candida* in the three polluted soils (12.5%). White bars are blank series of the corresponding assay (pure ISO soils). Significant differences between two series with a one unit difference of pH are observed for the three polluted soils ($p < 0.01$). Means of the number of offspring ± S.E.M.

Table 1
Global characteristics of the three polluted soils

	CEC (meq kg^{-1})	pH (KCl)	WHC	Clay (%)	Silt (%)	Sand (%)	TOC (%)
Lannemezan	156	4.9	49	26	32	42	4.3
Salsigne	172	3.8	36	14	28	58	0.5
Viviez	89	7.1	45	23	37	40	1.9

Table 2
Concentrations of zinc, cadmium and arsenic (mg/L) in the pore waters of the Viviez assay series

Viviez/ISO soil (%)	0	0.19	0.39	0.77	1.5	3.1	6.2	25	50
Zn	0.02	0.32	0.51	1.18	2.4	3.68	14.89	25.84	28.29
Cd	<0.01	0.03	0.07	0.13	0.29	0.46	3.66	9.79	14.49
As	<0.01	0.17	0.19	0.17	0.09	0.05	0.01	<0.01	0.02

series with a one unit difference of pH (Fig. 5). These differences are not observed between the blanks showing the same pH differences.

3.3.1. Physical and chemical analyses

The cationic exchange capacity (CEC; Metson, 1961), the pH (KCl), the water holding capacity (WHC), the clay, silt and sand contents and the total organic carbon (TOC) of the three polluted soils are given in Table 1.

A/Lannemezan: Differences of pH between series at the end of the assay were less than one unit (from 4.8 for the 25% series to 4.5 for the blank). The pore water of the 0%, 3.1%, 6.25%, 12.5% and 25% contained, respectively, 0.6, 1.15, 2.8, 8.6 and 23 mg aluminum/L and <1, 1.1, 5.2, 17.8 and 51 mg fluor/L.

B/Salsigne: The results of the ICP-MS analyses for the total metal concentrations were as follows: As 3.1% (wt), Al (5.7%), Ti 2,800, Cu 1,100, Bi 445, Pb 410, Ba 400 μg/g. The arsenic concentrations in the pore waters were 10 μg/L for the blank and, respectively, 5400, 1100, 456 and 260 μg/L for the 3.1, 6.2, 12.5 and 25% mine soil proportions.

C/Viviez: The total metal concentrations in the soil were Zn 3.6%, Cu 1.5%, Pb 2.2%, Cd 0.6% and As 0.13%. The concentrations in the pore waters are given in Table 2.

4. Discussion

Large reproduction differences are observed as a function of the polluted soil sample taken. The Lannemezan's soil shows a toxicity roughly proportional to the contaminated soil percentage. The ecotoxicity curve resembles those observed testing chemicals individually with the same ecotoxicity test (Crouau et al., 1999; Sandifer and Hopkin, 1996). On the contrary, the two other soil samples taken (Salsigne and Viviez soils), give an unusual ecotoxicity curve: their effects on reproduction of *F. candida* are high for the lowest and highest contaminated series and low for the intermediate series. That unusual result is very marked in the Salsigne soil sample. Bioavailability is mainly determined by the concentration of pollutants in the pore water (van Gestel and Hensbergen, 1997). In this sample, the main contaminant of pore water is arsenic. In insects, arsenic (metalloid) is far more toxic than metals. Significant effects on reproduction of *F. candida* are observed between 100 and 200 μg cadmium/g (dry soil) (Crommentuijn et al., 1997; Crouau et al., 1999; Herbert et al., 2004), 462 μg zinc/g (Smit and van Gestel, 1996), 1200 μg copper/g, 5000 μg lead/g (Sandifer and Hopkin, 1996), 125 μg aluminum/g and 2 μg arsenic/g (Crouau and Moïa, 2006). Arsenic concentrations in the pore waters correlate well with the atypical reproduction curve. The unusual concentrations of arsenic in pore waters could be due to the differences between the series relative to the organic matter concentrations. When a small amount of contaminated soil is added to the ISO soil, the chemical characteristics of the ISO soil dominate and the arsenic moves into pore water. When more contaminated soil is mixed in, the chemistry of the soil mixtures changes, and less arsenic goes into pore water. It is known that microbial processes facilitate the release of arsenic into groundwater (Bhattacharya et al., 2004) and that microbial activity of a soil is dependent on its organic matter content. Water-soluble arsenic is significantly elevated in soil after soil amendments with compost (Rowland et al., 2007) and geomicrobial culture experiments revealed that microbial processes mediate the release of arsenic into groundwater (Dictor et al., 2001). Arsenic can be transformed to the reduced As(III) species or organic forms through biomethylation by microbes, and As(III) has higher mobility than As(V). The increased organic matter may favor microbial activity which may lower the soil redox potential (Turpeinen et al., 1999). This situation is favorable for the reduction of As(V) to As(III), and a subsequent increase in As mobility (Cao et al., 2003; Peryea, 1998). Moreover, in natural systems, dissolved organic carbon may compete with As for adsorption on mineral surfaces, hence increasing its potential bioavailability. Peat humic acids decrease As(V) and As(III) adsorption (Grafe et al., 2001).

This unusual toxicity curve is also observed in the Viviez soil but it is less prominent. This fact can partly be explained by two factors: first, arsenic concentrations are clearly lower for the Viviez pore waters than for those of Salsigne. Yet, the unusual ecotoxicological assay curve is mainly due to the behavior of arsenic which is different from that of aluminum, cadmium or zinc. Second, some

metals such as cadmium or zinc contribute to the toxicity of the Viviez soil. They are less toxic than arsenic but their concentrations in the pore waters are much higher. Contrary to that observed for arsenic, the concentrations of zinc and cadmium in the pore waters of Viviez increase with the percentages of polluted soil in the different series. It is likely that effects on reproduction are mainly due to arsenic for the series with low proportions of Viviez soils (0.19–1.5%) and to cadmium and zinc for the series with high Viviez soil contents (25% and 50%). Comparison of these results with polluted soils with those of the assays with single metals (Sandifer and Hopkin, 1996; Smit and van Gestel, 1996; Crouau et al., 1999) are difficult. EC50 for the effect of Viviez soil on reproduction is observed for higher zinc pore water concentrations than for the assays with zinc salt tested alone in ISO soil (Smit and van Gestel, 1998; Lock and Janssen, 2003). Moreover, in the Viviez soil, two additional pollutants, arsenic and cadmium, are identified. van Gestel and Hensbergen (1997) found that the effects of the mixture of cadmium and zinc on the reproduction of *F. candida* are additive. Therefore, effect of the Viviez soil on reproduction would be expected to be higher. However, ageing processes play an important role in the toxicity of zinc to *F. candida* (Smit and van Gestel, 1998) and the use of spiked soil in toxicity assays can result in an over-estimation of the effects of zinc. Zinc redistributes into more insoluble phases in historically contaminated field soils (Lock and Janssen, 2003).

The mortality in the controls is between 20% and 35%; thus it does not fulfill the validity threshold associated with the ISO 11267 test norm. However, this criterion was established for a test lasting 28 days whereas the tests presented here lasted for 35 days. It is thus normal that mortality is greater. We used 35 days duration instead of the 28 days recommended in the ISO 11267 guidelines because previous experience showed that such an increase permitted a reduction of the variability in the results and thereby an increase in the sensitivity of the tests (Crouau and Cazes, 2003). Moreover, for some persistent chemicals (e.g. metals), accumulation in the test animals during exposure and their effects are functions of the test duration which is of prime importance for the outcome of the tests (Crouau et al., 1999). Effects on mortality and growth are lower than effects on reproduction. A lower sensitivity of the growth and mortality parameters was also observed in other studies (Scott-Fordsmand et al., 1999; Crouau and Moïa, 2006). Moreover, for the Viviez soil, this observation could be partly due to the effect of the mixture of cadmium and zinc on the growth and reproduction of *F. candida*, where cadmium and zinc have a contradictory effect on growth, while their combined effect on reproduction is complementary (van Gestel and Hensbergen, 1997). Zinc competes strongly for cadmium-binding sites and consequently, the solubility of cadmium is significantly increased by the presence of zinc (Christensen, 1987).

In these assays with polluted soils, pH effect on soil ecotoxicities is obvious (Fig. 5); a decrease of one pH unit increases 2–4 times the negative effect of these soil samples on the reproduction of *F. candida*. It is probably due to the well known dependence of the solubility of the metals in relation to pH (Harter, 1983). For example, soil solution pH has a marked influence on adsorption of cadmium; increasing pore water pH leads to a rapid increase in net negative surface charge that may explain the enhanced affinity for metal ions. The ecotoxicity of soil is in effect linked to the bioavailability of the contaminants which depends on the degree of solubility of such contaminants in the pore water (van Gestel, 1997). Those results are largely in agreement with the studies on the effect of pH on ecotoxicity of metals for soil organisms (Crommentuijn, 1994; Hopkin, 1989).

5. Conclusion

Unexpected results are observed with two of the three polluted soils. This work shows that the Collembolan reproduction assay must be used cautiously, particularly when it contains arsenic. However, it can be used to test the toxicity of polluted soils. These results confirm that the growth and mortality parameters are less sensitive endpoints than reproduction and that reproduction must be preferred. Lastly, the study on the pH effects shows that, as a safety measure, the ecotoxicity assays for soil polluted by metals, must be carried out at a low pH at which the metals' solubility is higher.

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