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Comparison of the toxicity of zinc for the springtail *Folsomia candida* in artificially contaminated and polluted field soils

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Abstract

To evaluate the influence of soil characteristics and chemical application form on the ecotoxicity of zinc, the toxicity of contaminated soil samples from the vicinity of a zinc smelter was compared with the effects of $ZnCl_2$ added to two uncontaminated natural soils. Tests were performed with the springtail *Folsomia candida* (Willem).

In the artificially contaminated soils, $ZnCl_2$ had a strong effect on reproduction, whereas the effects on growth were not conclusive. In the polluted field soil however, no relationship was observed between zinc concentrations and growth or reproduction. Chemical analysis of the soils used showed that water soluble concentrations were higher in the artificially contaminated soils than in the polluted field soil. However, differences in water soluble zinc concentrations could not fully explain the differences in effects among the soils.

It is concluded that because of the influence of soil type and chemical form of zinc on bioavailability and toxicity, artificially contaminated soils should be used with caution in ecotoxicological risk assessment. It is recommended to include soil pore water analysis in future research to improve the interpretation and application of results obtained in artificially contaminated soils.

Keywords: Bioavailability; Zinc; Soil; Collembola

1. Introduction

To evaluate the potential ecotoxicological risks of contaminants, several standardised laboratory toxicity tests have been developed during the past decades. Results of these tests form the basis for the risk assessment of chemicals in ecosystems and can be used in statistical extrapolation methods to derive safe levels of chemicals in the environment (Van Straalen and Denneman, 1989; Wagner and Løkke, 1991; Aldenberg and Slob, 1993). Questions arise, however, about the predictive value of these standard toxicity tests, particularly with respect to effects at the ecosystem level. Van Straalen and Denneman (1989) summarised some factors which have to be taken into account when laboratory data are extrapolated to field situations. In general, laboratory tests are performed under constant, more or less ideal circumstances using a well defined substrate, giving reproducible and reliable results. In the field however organisms have to deal with fluctuating climate conditions that, together with characteristics of the environment, can alter both the functioning of the organism and the bioavailability of the chemical. Processes of adaptation and ecological compensation, which can occur in field situations, are not incorporated in the laboratory test designs. The difficulty of extrapolating laboratory data to field situations was illustrated

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by Van Gestel (1992) who reviewed earthworm toxicity data on four pesticides applied to soil. He concluded that differences between laboratory and field data are mainly caused by differences in the chemical behaviour of the pesticides and biology of the earthworms.

Most studies dealing with extrapolation of laboratory toxicity data have been performed with organic chemicals, although in a number of field studies the negative effects of heavy metal pollution caused by industrial works have been investigated (e.g. Strojan, 1978; Coughtrey et al., 1979; Bengtsson and Rundgren, 1988). Some authors (e.g. Beyer et al., 1984; Beyer and Anderson, 1985; Hopkin and Hames, 1994; Spurgeon et al., 1994) have linked field observations to toxicity data obtained in (standardised) laboratory tests. Results of these studies indicate that both the nature of the substrate and the application form of the metals used in toxicity tests have to be taken into account when laboratory data are compared with field observations. This is in agreement with La Point et al. (1989), who stated that one of the major causes for the lack of predictive value of laboratory tests can be found in the effect of alterations of the chemical or the environment. However, efforts to relate these phenomena to quantified differences in bioavailability have not yet led to clear results.

This paper is part of a wider study into the validation of laboratory ecotoxicity data for soils and evaluates the predictive value of a standardised laboratory test with artificially contaminated soil for effects in the field. Toxicity experiments were carried out following the ISO-guideline for assessing effects of chemicals on Collembola (ISO, 1994) using survival, growth and reproduction of the springtail *Folsomia candida* (Willem) as effect parameters. The ISO-guideline is originally drafted for tests in an artificial soil substrate. In this study, natural soils were used to compare the zinc toxicity of field-contaminated soil samples and artificially contaminated soils for *F. candida*. Different soils were used to gain insight in the influence of soil type on the performance of this springtail in the presence of zinc. In addition, effects were related to extractable zinc concentrations in both the field-contaminated and the artificially contaminated soils thus making an attempt to quantify the bioavailability of zinc for *F. candida*.

Table 1
Characteristics of Budel and LUFA soil used in Experiment (1)

	pH-KCl	OM (%)	clay (%)	CEC mmol kg^{-1} dry soil	WHC of dry soil (%)
Budel	3.46[a]	3.0	1.38	110	33.3
LUFA	6.01	3.3	3.34	61	47.5

CEC, cation exchange capacity; WHC, water holding capacity. [a]Original pH-KCl, corrected to 6.0 ± 0.5 with $CaCO_3$.

2. Materials and methods

2.1. Animals

In all experiments, the springtail *F. candida* (Willem, 1902) (Hexapoda: Collembola) was used. *F. candida* is a parthenogenetic organism, used in several laboratory studies investigating effects of soil toxicants and other factors on population growth and fecundity (Butcher and Snider, 1975; Van Amelsvoort and Usher, 1989; Crommentuijn et al., 1993). Because of its relatively short generation time and the easy way it is cultured, *F. candida* is an appropriate species to serve as a test organism in terrestrial toxicity studies.

The animals were cultured in pots with a bottom of plaster of Paris mixed with charcoal at 20 ± 1 °C at a light/dark regime of 12/12 h. The breeding culture originated from arable land at the experimental farm 'The Lovinckhoeve' at Haren, The Netherlands and was kept in the laboratory for about 8 years. The experiments were started with juveniles of the same age (10–12 days) and size (0.020 ± 0.005 mg) which were obtained by synchronising the egg laying of the culture animals. Animals were fed with dried baker's yeast (Dr. Oetker).

2.2. Soils

For the first experiment two natural soils were used. The first one was the control soil collected at 20 km from a zinc smelter at Budel, The Netherlands, as described below. The pH-KCl of the Budel soil was adjusted to 6.0 ± 0.5 with $CaCO_3$. The second soil used was obtained from the LUFA-Institute at Speyer, Germany. Main characteristics of these soils are shown in Table 1.

Both soils were contaminated with $ZnCl_2$ (>98%, Merck) leading to a nominal concentration range of 0,

Table 2
Main characteristics of the Budel gradient samples used in Experiment (2), metal concentrations indicate total amounts

Sample code	Distance km	pH-KCl original	pH-KCl corrected	OM (%)	Clay (%)	Ca	Zn	Cd $\mu g\ g^{-1}$	Cu dry soil	Pb	Fe
1	0.4	4.72	5.91	1.9	1.99	300	591	3.0	68	325	6550
2	1.1	5.25	5.91	3.6	1.43	539	1537	3.9	250	424	15686
3	1.6	3.94	6.09	2.8	1.25	304	156	0.9	29	148	6130
4	2.6	4.55	6.20	2.4	1.24	288	184	0.8	17	91	4740
5	5.0	3.09	6.21	3.6	1.26	<50	25	0.4	10	40	1960
6	6.6	2.91	5.91	6.4	1.48	74	23	0.3	5	61	2935
7	7.9	3.15	6.02	4.5	1.27	<50	17	0.4	4	20	1920
8	9.6	2.96	6.09	4.3	1.31	100	20	0.2	3	45	2820
9	12.0	2.90	6.02	5.5	1.26	<50	18	0.4	2	25	1530
10	15.0	3.26	5.93	4.7	1.55	100	10	0.3	2	10	3360
11	20.7	3.46	6.19	3.5	1.15	<50	9	0.1	2	19	2043

93.75, 187.5, 375, 750, 1500 and 3000 μg Zn g^{-1} dry soil, this range enclosing the zinc concentrations of the field-contaminated samples used in the second experiment as described below. $ZnCl_2$ was dissolved in the proper amount of deionized water needed to achieve a soil moisture content of 40% of the maximum water holding capacity (WHC).

For the second experiment, top soil samples were collected from 11 locations at different distances from the Budel zinc smelter. Heavy metal concentrations form a gradient with decreasing levels at increasing distances, with zinc being the dominant toxic metal. Soil collected at 20 km distance was considered as a control. Samples from each location were sieved, mixed, dried to a moisture content of 2% (%DW) and stored at 5°C until use. To correct for the large differences in pH between the samples, pH-KCl was adjusted to 6.0 ± 0.5 with $CaCO_3$ (Merck). Main characteristics and heavy metal concentrations are shown in Table 2.

All analyses, except pH and total zinc measurements, were done at the National Institute of Public Health and Environmental Protection at Bilthoven, The Netherlands.

2.3. Experimental

For the first experiment, the $ZnCl_2$ solutions were carefully mixed in with the Budel and LUFA soil immediately before the start of the experiment. Glass jars (100 ml) were filled with 25 g moist soil, 10 replicates for each zinc concentration. For the controls, a double quantity of jars was prepared. At the start of the experiment, ten synchronised animals were transferred into each of the jars and all jars were placed on trays in a climate room at 20 ± 1 °C, 75% RH and a 12/12 h light/dark cycle. The test containers were aerated three times a week and soil moisture content was kept constant by weighing the jars and replenishing the water loss. During the experiment the animals were fed dried baker's yeast (ad lib.).

After 4 and 6 weeks, a series of five replicate jars (ten for the control soil) was sacrificed for determination of survival, reproduction and animal weight. Each jar was emptied into a 200 ml beaker glass and 100 ml deionized water was added. The mixture was stirred carefully to let all living animals float on the surface. Surviving adults were counted by eye and in addition the water surface was photographed on diapositive material. The resulting slides were projected using a desktop slide projector and the number of juveniles was counted to determine population size. From each treatment, ten adult animals were collected randomly from the replicate jars and weighed to the nearest microgram using a Sartorius S4 super-microbalance. To obtain the dry weight (DW), the animals were lyophilised and weighed again. The adults collected after 4 weeks of exposure were used for determination of the internal zinc concentration. The soil samples sacrificed after 4 weeks were dried (50 °C, 24 h) and used for pH-KCl measurements and zinc analysis.

For the experiment with samples of the Budel gradient, soil samples were rewetted to 40% of the maximum WHC with deionized water immediately before use. The experiment was carried out as described above, with the exception that six additional jars were prepared for each location which were sacrificed in

duplicate for determination of the animal weight after 1, 3 and 5 weeks. The adults collected after 3 weeks were used for zinc analysis, whereas soil samples sacrificed after 3 weeks were used for pH measurements and zinc analysis.

2.4. pH measurement

From each soil sample, 5 ± 0.1 g was weighed and put into plastic 100 ml bottles. To each bottle 25 ml of 1M KCl was added and the bottles were placed on a shaking machine for 2 h at 200 r.p.m. After precipitation of the soil particles, pH of the supernatant was measured.

2.5. Zinc analysis

Lyophilised animals were put into pyrex 1 ml tubes and digested in a mixture of HNO_3 (Ultrex grade) and $HClO_4$ (7:1 v/v). Zinc contents were analysed by graphite furnace atomic absorption spectrophotometry (AAS) as described by Van Straalen and Van Wensem (1986). Adult animals from the breeding culture were analysed in the same way to obtain background values.

Soil samples were dried for 24 h at 50 °C. For total zinc analysis, 1 ± 0.1 g of dried soil was weighed and digested in a mixture of deionized water, HCl and HNO_3 (1:1:4 by vol.), using a microwave oven (CEM MDS 81-D). After digestion, the solution was filtered and analysed by flame AAS. A certified reference calcareous loam soil (BCR 141) was included in all analyses.

Water soluble and exchangeable zinc concentrations were determined by shaking 2 ± 0.1 g of dried soil for 2 h at 200 r.p.m. with 20 ml of deionized water and 0.01 M $CaCl_2$, respectively and analysing the filtered supernatant by flame AAS. All AAS measurements were performed with a Perkin Elmer 1100B AAS.

2.6. Statistics and calculations

$EC_{(50)}$ values were estimated using the logistic model after Haanstra et al. (1985), which was programmed in SYSTAT 5.0.

Sorption of zinc was described by a Freundlich isotherm:

$$C_s = K_f^* C_{rw}^{(1/n)}$$

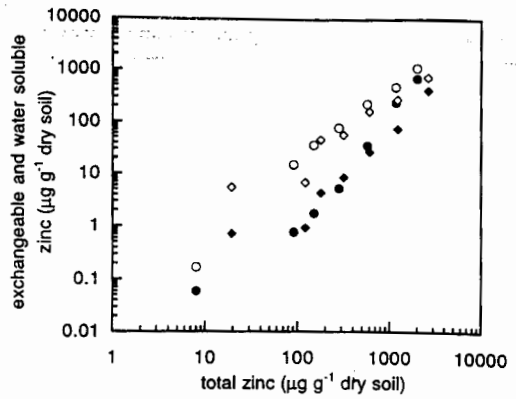

Fig. 1. Zinc concentrations in artificially contaminated Budel (○,●) and LUFA (■,◆) soil. Exchangeable concentrations are indicated by open symbols, water soluble concentrations by closed symbols.

where C_s is the concentration in soil (μg g^{-1}), C_w is the concentration in the water extract (μg ml^{-1}), K_f is the Freundlich sorption constant (ml g^{-1}) and $1/n$ is a shape parameter of the Freundlich curve. Estimates for K_f and $1/n$ were obtained by linear regression in a double logarithmic plot of C_w vs. C_s.

3. Results

3.1. Contaminated LUFA and Budel soil

Soil analysis

LUFA soil pH, measured after 4 weeks of exposure, varied between 5.26 and 5.55. Budel soil pH was somewhat higher, giving values between 5.30 and 5.76 during the experiment. No relationship between pH and $ZnCl_2$ addition was observed, although the lowest pH values were found in the three soil samples with the highest zinc concentrations.

Water soluble and exchangeable zinc are plotted against actual total zinc levels in Fig. 1. Actual zinc levels were lower than nominal concentrations, especially in the Budel soil. Recoveries of the digested reference material were higher than 96.5%. The concentrations in soil and the corresponding concentration in the aqueous extracts were used to describe the adsorption isotherm of zinc following the Freundlich equation. The estimated adsorption coefficient K_f was 220 ml g^{-1} ($r^2 = 0.986$) for LUFA soil and 238 ml g^{-1} ($r^2 = 0.983$) for Budel soil. The isotherms had 1/

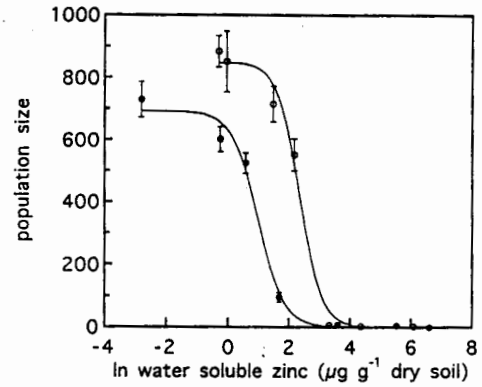

Fig. 2. Dose-response relationships for the effect of zinc on the population size of *F. candida* after 4 weeks of exposure to Budel (●) and LUFA (○) soil experimentally contaminated with $ZnCl_2$. In this figure, the natural logarithm of the water soluble zinc concentrations is given on the x-axis, error bars indicate standard error of the mean.

n values of 0.47 and 0.42 for LUFA and Budel soil, respectively.

Survival, growth and reproduction

Survival of *Folsomia candida* in the control soil was over 30% and increased with increasing zinc concentrations up to 80% survival at 750 μg Zn g^{-1} dry soil. In the contaminated LUFA soil, the highest survival of 74% was also found at 750 μg Zn g^{-1} dry soil, with a survival of 50% at the lower concentrations.

Individual growth in Budel soil was reduced with increasing zinc concentrations, $EC_{\{50\}}$ values and 95% confidence intervals (based on actual measured concentrations) were 462 (416–512), 170 (146–197) and 20.8 (15.5–27.9) μg Zn g^{-1} dry soil when expressed as total, exchangeable and water soluble zinc concentrations, respectively. In LUFA soil, fresh weight of the animals was not reduced except at 750 μg Zn g^{-1} dry soil (nominal), and $EC_{\{50\}}$ values could not be calculated.

Population size was dose-related affected by the addition of $ZnCl_2$ in both soils as shown in Fig. 2 for the 4 week exposure period. In the control treatments, average population size ($\pm$SD) of ten replicate samples at $t=4$ weeks reached values of 731 ± 179 and 885 ± 157 individuals per sample for Budel and LUFA soil, respectively. After 6 weeks average population size ($\pm$SD) of ten control samples of the Budel soil was 1500 ± 628 animals per sample, whereas in the LUFA control soil an average of 1513 ± 371 animals ($n=9$) was found. $EC_{\{50\}}$ values and 95% confidence intervals for population size are shown in Table 3, all $EC_{\{50\}}$ values are based on actual measured concentrations. $EC_{\{50\}}$ values were significantly different ($P<0.05$) between the two soils as analysed by a generalised likelihood ratio test (Sokal and Rohlf, 1969).

Internal zinc concentrations

Internal zinc concentrations of the animals kept in LUFA soil were low compared to those of the Budel animals. The average internal zinc concentration of the animals kept in uncontaminated LUFA soil was 44.5 ± 3.71 μg Zn g^{-1} DW ($n=3$). At nominal exposure concentrations of ≥ 187.5 μg Zn g^{-1} dry soil all internal concentrations were significantly higher than in the control animals ($P<0.05$; Student's t-test). The internal concentrations of the LUFA animals at the two highest exposure levels were comparable with those of the breeding culture animals, all other internal concentrations were significantly lower.

In the Budel samples, the average internal concentration of the control animals was 103 ± 34.6 μg Zn g^{-1} DW ($n=10$). Internal concentrations were significantly raised at exposure concentrations ≥ 750 μg Zn g^{-1} dry soil ($P<0.05$). At the exposure concentration of 375 μg Zn g^{-1} dry soil, the internal zinc concentration was also higher than in the control animals (128

Table 3
$EC_{\{50\}}$ values and 95% confidence intervals for the effect of zinc on the population size of *Folsomia candida* after 4 and 6 weeks of exposure in artificially contaminated Budel and LUFA soil (Experiment (1))

	$EC_{\{50\}}$ (95% confidence interval)		
Soil	Total zinc	Exchangeable zinc (μg g^{-1} dry soil)	Water soluble zinc
4 weeks			
Budel	185 (158-216)	48.6 (40.1–59.0)	2.55 (1.93–3.36)
LUFA	348 (307–393)	64.7 (54.2–77.3)	10.1 (8.19–12.48)
6 weeks			
Budel	210 (167–264)	55.6 (41.3–75.0)	3.23 (2.14–4.86)
LUFA	363 (289–455)	67.6 (47.4–96.4)	11.3 (8.29–15.50)

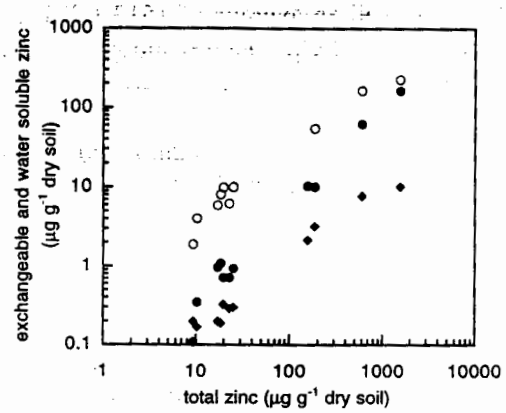

Fig. 3. Concentrations of water soluble (♦) and exchangeable (●) zinc of pH-adjusted Budel gradient samples. Exchangeable zinc concentrations of the original soil samples (not adjusted to pH 6) are indicated by open symbols (○).

μg Zn g^{-1} DW), but statistical analysis could not be performed because of the small number of animals available for analysis. Internal zinc concentrations of 56.8 ± 12.4 μg Zn g^{-1} DW (LUFA) and 115 ± 13.1 μg Zn g^{-1} DW (Budel) were measured in animals exposed to concentrations just below the actual $EC_{(50)}$ in the respective soils.

3.2. Budel gradient samples

Soil analysis

Soil pH (1M KCl) in almost all samples increased during the experiment, giving values ranging from 5.55 to 6.20 at the start of the experiment and from 5.91 to 6.19 after three weeks of exposure.

The levels of water soluble and exchangeable zinc are shown in Fig. 3; exchangeable zinc concentrations in the original soil samples (without pH correction) are shown as well. Water soluble and exchangeable fractions were positively correlated with total zinc concentrations, but addition of $CaCO_3$ to correct soil pH caused a large reduction in exchangeability of zinc. The Freundlich adsorption coefficient K_f, estimated from the concentrations in soil and the corresponding concentrations in the aqueous extracts, was 1474 ml g^{-1} ($r^2 = 0.968$). The isotherm was nearly linear, with $1/n = 1.08$.

Survival, growth and reproduction

Survival patterns of *F. candida* in the Budel field samples were comparable with those in the artificially contaminated Budel soil. Survival after 4 weeks of exposure was higher than 70% in the three samples taken closest to the smelter. Survival in the other samples decreased with increasing distances, and was below 30% in the control samples. After 6 weeks of exposure, survival in the most contaminated samples was nearly 60%, whereas in the control samples it was nearly 30%.

No correlation was found between zinc concentrations and individual growth during the 6 week exposure period. After 4 weeks the average fresh weight ($\pm$SD) of the animals from the control was 0.2042 ± 0.0298 mg ($n = 9$), whereas animals kept in the most contaminated samples had average fresh weights of 0.1946 ± 0.0208 mg and 0.2112 ± 0.0338 mg ($n = 10$), respectively.

Control population size did not differ significantly ($P < 0.05$) from population size in the highest contaminated sample and no relationship could be observed between zinc concentrations and population size (Fig. 4). Average population size of five replicate jars after 4 and 6 weeks of exposure in Sample 2 (1537 μg Zn g^{-1} dry soil) was 528 ± 162 and 1236 ± 331 individuals, respectively. In the control, average population sizes of 638 ± 146 ($t = 4$) and 1238 ± 535 ($t = 6$) individuals were determined. At $t = 6$ weeks, variation between replicates was high for most soil samples, especially for Sample 5. In this sample, population size in four out of five replicates was lower after 6 weeks of exposure compared with 4 weeks.

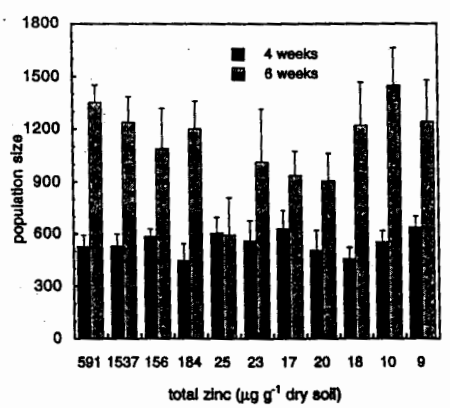

Fig. 4. Population size of *F. candida* after 4 and 6 weeks of exposure to Budel gradient samples. Error bars indicate standard error of the mean.

Internal zinc concentrations

All animals had measurable amounts of zinc and internal concentrations were slightly raised at higher soil concentrations. However, no clear relationship with total, exchangeable or water soluble zinc was observed. The average internal concentrations of animals exposed to the two samples with the lowest zinc concentrations were 54.4 ± 17.2 μg Zn g^{-1} DW and 71.1 ± 13.3 μg Zn g^{-1} DW ($n=8$). The other animals had internal concentrations ranging from 82.5 ± 29.0 μg Zn g^{-1} DW ($n=7$) to 135 ± 26.8 μg Zn g^{-1} DW ($n=8$). The average internal zinc concentration of animals from the breeding culture was 126 ± 59.5 μg Zn g^{-1} DW ($n=19$).

4. Discussion

4.1. Bioavailability of zinc

For many soil inhabiting organisms, soil pore water concentrations are supposed to be the dominant factor in the uptake of chemicals, and water soluble fractions are frequently used to estimate bioavailable concentrations. The influence of soil characteristics on the bioavailability of heavy metals has been subject of many studies. The adsorption process is dependent on various soil characteristics such as pH, clay, organic matter content and CEC (Harter, 1983; Elliott et al., 1986; Anderson and Christensen, 1988; King, 1988); adsorption coefficients of zinc can range from 0.1 to 8000 ml g^{-1} for different soil types (Baes and Sharp, 1983). In the Budel gradient samples, pH correction resulted in reduced exchangeability of zinc, and the maximum water solubility was only 2%. The adsorption coefficient of the Budel field soil (1474 ml g^{-1}) is comparable with data given by Gerritse et al. (1982) who report adsorption coefficients of 1750 to 2500 ml g^{-1} for a sandy loam soil with a somewhat higher clay content and pH. In the artificially contaminated Budel and LUFA soils, water solubility reached maximum values of 37 and 17%, which is reflected in low adsorption coefficients of 220 and 238 ml g^{-1}, respectively. The calculated $1/n$ values of 1.08 for Budel field soil and 0.42 and 0.47 for contaminated Budel and LUFA soil, respectively, indicate that different adsorption processes are taking place. The low $1/n$ values of the artificially contaminated Budel and LUFA soil can either be caused by a saturation of binding sites or by the fact that the partitioning of zinc over soil and soil pore water has not yet reached an equilibrium.

4.2. Effects on survival, growth and reproduction

In all experiments, control mortality of *F. candida* was high and exceeded by far the limit of 20% given by ISO (1994). Apparently, characteristics of both Budel and LUFA soil are not optimal for survival of *F. candida*. Since zinc is an essential element, deficiency could be a possible explanation. This is supported by the fact that internal concentrations at the higher exposure concentrations were comparable with the internal concentrations of animals from the breeding culture. At higher exposure concentrations mortality might be reduced by a reduction of parasites or other harmful organisms. Nevertheless, the numbers of offspring in the control treatments after 4 and 6 weeks of exposure were comparable with data reported for animals kept in an artificial soil substrate under the same conditions (Crommentuijn et al., 1993).

The lack of a relationship between zinc concentrations and sublethal effect parameters in the Budel gradient samples can partly be explained by the low bioavailability of zinc. In artificially contaminated Budel soil, the $EC_{(50)}$ for the effect on individual growth, expressed as the water soluble zinc concentration, was 20.8 μg Zn g^{-1}, and this concentration was not exceeded in any of the Budel gradient samples with artificially raised pH (Fig. 3). When comparing the estimated $EC_{(50)}$ values for the effect on population development (Table 3) with measured water soluble zinc concentrations of the Budel gradient samples, it follows that the $EC_{(50)}$ is exceeded only in the three most contaminated samples. Differences in bioavailability between artificially contaminated soil and polluted field soil were shown by Spurgeon et al. (1994) who found earthworms in the vicinity of a zinc smelter where total zinc concentrations exceeded the lethal concentrations determined in a laboratory test with $ZnNO_3$ mixed in with an artificial soil. Zinc emissions from smelters like in the Budel area are supposed to consist mainly of ZnO (Buchauer, 1973) which has a lower solubility than $ZnCl_2$ or $ZnNO_3$. Laboratory tests in which ZnO was used as a contaminant gave results comparable to field observations (Beyer et al., 1984; Beyer and Anderson, 1985). Given the fact that the

gradient samples vary considerably in other soil factors than zinc alone, such as bioavailability of copper, iron and other essential elements (Table 2), it can be imagined that possible zinc effects might be masked by other factors that could have stimulated population growth.

The results of Wohlgemuth et al. (1990) show a positive correlation between the NOEC values for the toxicity of cadmium for *F. candida* and calculated CEC values. From the differences in CEC, the toxicity of zinc in the LUFA soil was therefore expected to be higher than in the Budel soil. However, the $EC_{(50)}$ values for artificially contaminated Budel soil are about a factor of 1.7 lower than those for LUFA soil when expressed as total zinc concentrations. The differences between Budel and LUFA soil were not reduced by expression of the $EC_{(50)}$ values on the basis of water soluble zinc concentrations. This was unexpected since water soluble concentrations are considered to give an estimation of the bioavailable pore water concentration. Instead, the differences in $EC_{(50)}$ values were even larger when expressed as water soluble zinc concentrations. Apparently, factors other than bioavailability alone play an important role. Crommentuijn (1994) suggested that soil properties themselves may influence the sensitivity of *F. candida* for toxicants. Besides, Sheppard et al. (1992) found that extraction of ions by shaking a mineral soil with water gave different results compared to direct pore water collection. Since the differences between these two methods depend on the soil type and the ions measured, in future experiments direct measurements of pore water concentrations might be helpful for an accurate estimation of $EC_{(50)}$ values of metals in soil.

4.3. Internal effect concentrations

It is generally thought that the internal concentration of a toxicant in an organism determines the eventual effect (McCarty et al., 1992) and some authors recommend the integration of a critical body concentration in ecotoxicological risk assessment methods (Van Wensem et al., 1994). It is not clear however, how internal concentrations of zinc are related to effects on the reproductive capacity of *F. candida*. For springtails, literature data on internal zinc concentrations are scarce and show considerable variation. Carter (1983) reports a natural internal zinc concentration in Collembola of 92 μg Zn g^{-1} DW. Posthuma (1990) however, determined a value of only 33 μg Zn g^{-1} DW for the springtail *Orchesella cincta* collected in a reference forest, whereas Janssen and Hogervorst (1993) found a concentration of 102 μg Zn g^{-1} DW for the same species collected at another reference site. For *O. cincta* collected at the contaminated Budel area, internal zinc concentrations of 122 and 138 μg Zn g^{-1} DW are reported by Posthuma (1990) and Janssen and Hogervorst (1993) respectively. These values suggest that springtails are able to maintain their internal zinc concentration at a constant level. The data reported for *O. cincta* are close to the internal concentration of 126 μg Zn g^{-1} DW measured in *F. candida* individuals from the breeding culture, but comparison of data is difficult because of possible differences between species.

The average internal concentrations of animals exposed to the most contaminated Budel gradient samples are comparable with the control value of 126 μg Zn g^{-1} DW which could explain the lack of effect observed. This however does not hold for the artificially contaminated Budel and LUFA soils. The animals exposed to Budel soil at concentrations just below the $EC_{(50)}$ also had internal zinc concentrations which were close to the control level. In the LUFA soil, internal concentrations of all animals were much lower than those measured in culture animals and animals exposed to Budel soil. It is known that metal concentrations can be influenced by the individual weight of the animals (Williamson, 1980; Janssen and Bedaux, 1989) but when accumulation data are calculated as body burdens (ng per animal) LUFA animals still have accumulated only 51 to 67% compared with Budel animals. Van Gestel and Hensbergen (in preparation) found effects on population size at equally low internal concentrations of about 60 μg Zn g^{-1} DW after exposing *F. candida* to $ZnCl_2$ in an artificial soil substrate for 4 weeks. It might be possible that effects of zinc are reinforced by other factors, causing negative effects at relatively low internal zinc concentrations. Considering the above, further research is needed to determine the relationship between the internal concentration of zinc and the occurrence of various toxic effects in *F. candida*.

5. Conclusions

From the results of this study it is concluded that both soil properties and the chemical form of the con-

taminant have to be taken into account when toxicity tests are performed. When artificially contaminated soil is used for the ecotoxicological evaluation of metal polluted soils, it is necessary to base all conclusions on actual measured concentrations instead of nominal values. Efforts must be made to improve the characterisation of the bioavailable fraction and direct measurement of pore water concentrations might be an appropriate tool.

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