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Influence of Cadmium on Life-History Characteristics of *Folsomia candida* (Willem) in an Artificial Soil Substrate

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To understand the consequences of soil pollution on higher levels of biological organization, the chain of effects of cadmium on several interrelated responses was studied in a chronic toxicity experiment using the collembolan species *Folsomia candida* (Willem) in an artificial soil. The individual parameters survival, growth, and number of offspring were determined after different time intervals up to 9 weeks. The accumulation of cadmium in springtails and the population increase during the experimental period were also determined. By combining all the mentioned parameters and their development in time, a detailed picture of the action of cadmium on *F. candida* was obtained. In order of decreasing sensitivity the EC_{50} values for Von Bertalanffy growth, number of offspring, population increase, and survival were 256, >326, 475, and 850 μg Cd/g dry soil, respectively. The ultimate LC_{50} value and also the equilibrium body burden were reached after about 20 days. Reproduction started later because of retarded growth, but was not affected directly and eventually reached the control level. The results are discussed in light of the seemingly contradictory ideas of Halbach (1984, *Hydrobiologia* **109**, 79–96) and Meyer *et al.* (1987, *Environ. Toxicol. Chem.* **6**, 115–126) about the sensitivity of individual and population parameters. It appears to be very important to know how individual parameters develop in time so that the most sensitive parameter and the consequences for higher levels of biological organization can be determined. © 1993 Academic Press, Inc.

INTRODUCTION

One of the greatest challenges in ecotoxicology is to predict effects of pollutants on the population, community, and ecosystem levels from effects on the previous level of biological organization (Moriarty, 1988; Levin *et al.*, 1989). This step should be made to obtain ecologically realistic toxicity values, to close the gap between laboratory studies and the situation in the field. Such data are a valuable tool for decision makers to set reliable standards for soil pollution. At this moment standards for soil pollution are based on toxicological data at the level of single organisms which, moreover, for soil are hardly available.

Not much progress has been made in predicting the effects from a lower to a higher level of biological organization (Butler, 1984). According to Seitz and Ratte (1991) the prediction from a lower to a higher level is not done by simple extrapolation from the previous level, because at each level interactions exist which do not influence the properties of that particular level but which do influence those of the next. In the discussion of whether population parameters are more or less sensitive than individual parameters two contradicting ideas can be discerned. According to Halbach (1984) minute chronic effects, which are sublethal and hardly noticeable at the level of the individual, can be amplified through thousands of animals, leading to changes in the population dynamics. As a consequence, the population parameters are more sensitive

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than the individual parameters. This idea has been elaborated for field and laboratory experiments with *Brachionus calyciflorus* and *Brachionus rubens* exposed to pentachlorophenol. Meyer *et al.* (1987) on the contrary, state that in the case of *Daphnia pulex* exposed to cadmium and copper, population parameters are more or less as sensitive as the individual parameters because of a compensation of the unused capacity of nonfit animals by the fitter ones.

The species *Folsomia candida* seems to be a well-suited organism to study the above-mentioned question in the field of terrestrial ecotoxicology (Usher and Stoneman, 1977). This collembolan has been used in several laboratory studies to investigate the effects of different factors on fecundity and population growth (Butcher and Snider, 1975; Van Amelsvoort and Usher, 1989). *F. candida* is easy to culture and it has a relative short generation time, so it is possible to study different individual and population parameters in a single experiment. An experimental setup was designed in which *F. candida* was exposed to cadmium in artificial soil, while the number of adults, the number of offspring, and fresh weight and accumulation of cadmium in adults were determined at different time intervals.

To understand the consequences at the population level an effort has been made to unravel the chain of effects from the individual to the population level, by comparing the effects on individual parameters with each other, how they develop in time, and how (and to what extent) they interact to determine the effect on the population level. In this way it was possible to analyze the effect of different concentrations of cadmium on the measured features to obtain better insight into the way in which cadmium affects *F. candida* and to understand the consequences for the population.

MATERIAL AND METHODS

Animals

F. candida (Willem) was cultured in the laboratory at 18 ± 1°C at a light/dark cycle of 12/12 hr, in culture pots with bottoms of plaster of Paris mixed with charcoal. The culture was set up in 1986 with animals which originated from a pine stand in the Roggebotzand Forest, The Netherlands. The experiment was started with animals of the same age and weight. These synchronized animals were obtained by allowing adult animals to lay eggs, in a container with plaster of Paris bottom. After 2 days the adult animals were removed. The eggs present in the containers hatched approximately 2 weeks later. When these offspring were 1 week old the experiment was started. The food used in the culture and in the synchronization was baker's yeast (Dr. Oetker).

Preparation and Contamination of the Artificial Soil

For the experiment artificial soil was used as described in the OECD Guideline 207 "Earthworm, acute toxicity test" (OECD, 1984) and the draft for a *F. candida* test (Riepert, 1991). The substrate consists of (expressed in percentages of dry weight) 10% sphagnum peat which was air-dried, finely ground, and sieved (<1 mm); 20% kaolin clay; and 70% quartz sand (fine, 50% particles 0.05–0.2 mm).

The dry ingredients were mixed in a household mixer. After the ingredients were mixed, the pH (H_2O) was adjusted to 6 by the addition of $CaCO_3$. To contaminate the soil with cadmium, $CdCl_2.2.5\ H_2O$ (BDH, AnalaR) was added to the amount of deionized water necessary to bring the soil to the desired humidity of 40–60% of the

water holding capacity. Six cadmium treatments were applied. The nominal val were 32.7, 70.2, 150.9, 324.5, 697.7, and 1500 μg Cd/g dry soil. For each concentrati the amount of soil needed was weighed: the Cd solution was added and mixed thro it using a household mixer.

Experimental Design

For the blank, 120 containers and for each cadmium treatment, 60 containers w 15 g wet soil each were prepared. At the start of the experiment 10 synchronize animals and 0.02 mg baker's yeast were added to the containers. Besides these co tainers, extra containers were prepared for Cd measurements (6 blank and 6 per tre ment) and humidity measurements (12). All the containers were placed randomly o trays in a climate room at a temperature of 20 ± 1°C, a relative humidity of 70 ± 10%, and a light/dark cycle of 12/12 hr.

After 7, 12, 16, 19, 23, 26, 30, 35, 42, 49, 56, and 63 days, 10 blank containers a 5 containers per treatment were sacrificed for analysis of the number of adults and offspring and the weight and accumulation of cadmium of the adults. The pH (H_2O) and the pH (KCl) were determined from 3 of the 5 containers of each treatment. T containers for the soil humidity measurements were weighed at each sampling da At Day 7 and Day 63 the containers for the Cd measurements were sacrificed.

Counting and Weighing of the Animals

To each container 20 ml of demineralized water was added and stirred thorough but carefully. In this way all the animals in the soil came to float on the surface. E water surface was photographed on diapositive material. The slides were projected a desktop slide projector to count all the adults and offspring present on the w surface. If there were more than 2000 animals in a container the surface was divi in eight equal sectors and two randomly chosen parts were counted and the tot number of animals was estimated.

From each container two animals were lifted from the water surface and weig to the nearest microgram to obtain the fresh weight (FW). The animals were lyophil and weighed to the nearest 0.1 μg to obtain the dry weight (DW).

Soil Humidity and pH Measurements

The humidity was determined by weighing 12 containers with wet soil at the st and at each sampling date. At the end of the experiment the soil was dried for 1 nig in an oven at 50°C and the dry weight and the weight of the empty container w measured. The humidity (H) was calculated on the basis of the dry weight.

For pH measurements three containers of each concentration were dried overnight at 50°C. The dry soil was homogenized and two portions of 10 g were weighed an put in plastic bottles. To one sample 25 ml of deionized water and to the other 25 m of a 1 *M* KCl solution were added. The bottles were placed on a shaking machine a rotation rate of 200 rpm for 2 hr and left overnight to allow particle sedimentation pH of the supernatant was measured the next day.

Cadmium Analysis

The soil to be analyzed was dried overnight at 50°C. A portion of 1 mg was digested in a mixture of demineralized water, HCl, and HNO_3 (1/1/4 v/v) in a microwa

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(CEM MDS 81D). After digestion the solution was filtered and the filtrate was collected for analysis (total cadmium). The solutions were analyzed for cadmium by flame atomic absorption spectrophotometry at 228.8 nm. The lyophilized animals were digested in a $HClO_4$ HNO_3 (Ultrex grade) mixture (1/7 v/v) and analyzed for cadmium by oven atomic absorption spectrophotometry at 228.8 nm (Van Straalen and Van Wensem, 1986).

Statistical Analysis

The survival and growth data were analyzed with the Kruskal–Wallis multiple comparisons test ($P < 0.05$) to detect the effects of cadmium for each sampling date and between the different sampling dates. For the number of offspring the Williams test ($P < 0.05$) was used to detect significant differences from the blank. Both tests are available in the TOXSTAT-software packet (Gulley *et al.*, 1989). LC_{50} and EC_{50} values were calculated by means of the Spearman–Karber method (Hamilton *et al.*, 1977, 1978).

The increase of body weight was described using the Von Bertalanffy growth model (Kooijman, 1988)

$$W(t) = \{W_\infty^{1/3} - (W_\infty^{1/3} - W(0)^{1/3})\exp(-\gamma t)\}^3,$$

where

$W(t)$ = body weight at Day t (in mg fresh weight),
W_∞ = final weight (in mg fresh weight),
$W(0)$ = initial weight (in mg fresh weight),
γ = Von Bertalanffy growth (in Day^{-1}),
t = time in days.

In accordance with the data of Snider (1971) population increase of *F. candida* during the first 6 weeks after the first egg laying was linear and could be described by the equation,

$$P(t) = a + \alpha t,$$

where

$P(t)$ = population size at Day t in number of individuals per container,
a = intercept, population size at time zero,
α = population increase in number of individuals per container per day,
t = time in days.

The parameters of both models were estimated using a parameter estimation program (written in APL) according to the least-squares criterion.

RESULTS

Cadmium Content, pH, and Humidity of the Artificial Soil

The actual cadmium content of the artificial soil was in good agreement with the nominal values (Table 1). In the blank a background level of 0.6 μg Cd/g dry soil was measured. The pH (H_2O) was set to 6.0 at the beginning of the experiment and did not change during the experiment, except for the two highest cadmium treatments

TABLE 1

NOMINAL AND ACTUAL CADMIUM CONTENT OF THE SOILS USED IN THE EXPERIMENT, ESTIMATED VON BERTALANFFY GROWTH, AND FINAL FRESH WEIGHT OF THE *F. candida* INDIVIDUALS AND ESTIMATED POPULATION INCREASE AT THE DIFFERENT CONCENTRATIONS

Cadmium (μg Cd/g dry soil)		Von Bertalanffy growth κ (day^{-1})	(Final weight)$^{1/3}$ $W_c^{1/3}$ (mg$^{1/3}$)	Population increase α (individuals/day/container)
Nominal	Actual			
0	0.6	0.138 (0.029)	0.637 (0.009)	57.10 (3.98)
32.7	34.8	0.151 (0.025)	0.644 (0.012)	48.25 (5.05)
70.2	71.3	0.114 (0.040)	0.657 (0.019)	45.10 (6.07)
151	149	0.091 (0.029)	0.656 (0.024)	45.81 (2.20)
325	326	0.051 (0.008)	0.687 (0.019)	39.16 (5.84)
698	707	0.042 (0.013)	0.647 (0.044)	19.71 (1.64)
1500	1491	0		1.56 (1.35)

Note. Parentheses indicate SD.

for which the pH (H_2O) was 5.25 during the experiment. The pH (KCl) was 5.5 and did not change during the experiment. The humidity (H) on the basis of the dry weight of the soil decreased slightly from 48.6 to 46.8% during the experiment, which is still within the prescribed limits.

Survival

Cadmium caused a significant decrease in survival only in the two highest exposure groups (Fig. 1). After 7 days of exposure no significant mortality was observed. After 12 days significant mortality ($P < 0.05$) was found at the two highest concentrations, while there was no significant difference in mortality between these two concentrations.

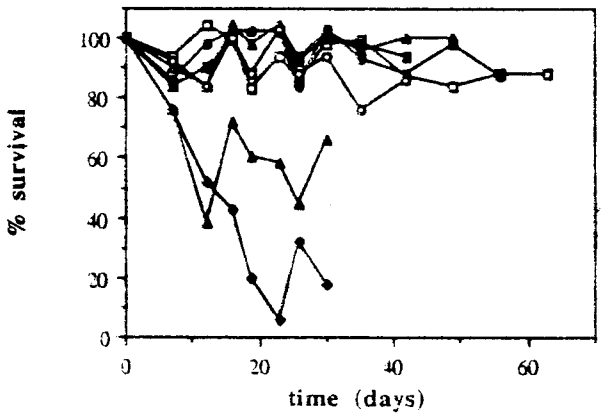

FIG. 1. Percentage survival of *F. candida* exposed to different levels of cadmium in artificial soil. Blank (□), 34.8 μg/g (●), 71.3 μg/g (△), 148 μg/g (■), 326 μg/g (○), 707 μg/g (▲), and 1491 μg/g (◇). Survival was calculated from the number of adults present in containers. These adults were sacrificed independently at various times.

TABLE 2

ESTIMATES OF MEDIAN EFFECT CONCENTRATIONS FOR SURVIVAL, NUMBER OF OFFSPRING, AND BODY WEIGHT FOR *F. candida* EXPOSED TO CADMIUM IN ARTIFICIAL SOIL

Days of exposure	LC_{50}	95% confidence range	EC_{50} offspring	95% confidence range	EC_{50} weight	95% confidence range
			μgCd/g dry soil			
19	917	708–1187			448	393–512
23	778	684–889	159	131–192	376	332–426
26	822	424–1595	204	161–258	566	501–639
30	893	736–1085	227	184–279	541	486–604
35	854	604–1208	>326		807	733–888
42			>326			

After 16 days and at all the later sampling dates there was no significant mortality at the concentrations 34.8, 71.3, 149, and 326 μg Cd/g dry soil while at the concentrations 707 and 1490 μg Cd/g dry soil significant dose-related mortality occurred.

For the blank and 34.8 μg Cd/g dry soil it was possible to distinguish adults from offspring until Day 56, for 71.3 μg Cd/g dry soil until Day 49, and for 707 and 1490 μg Cd/g dry soil until Day 30. From the survival data LC_{50} values were estimated (Table 2). The LC_{50} could not be estimated prior to 19 days because the effect was too small. Mortality stabilized at Day 19. No significant changes in mortality occurred with longer exposure and the LC_{50} values did not differ significantly from each other, so it is concluded that the ultimate LC_{50} value was reached and a mean ultimate LC_{50} was estimated to be 850 μg Cd/g dry soil.

Growth

In Fig. 2 the growth of the adult individuals during the first 50 days is demonstrated at the different Cd concentrations. The results were obtained from the surviving animals

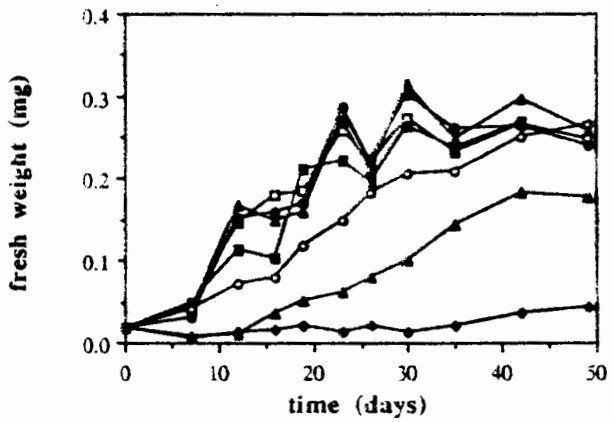

FIG. 2. Growth of *F. candida*, expressed in mg fresh weight, exposed to different levels of cadmium in artificial soil. Blank (□), 34.8 μg/g (●), 71.3 μg/g (△), 148 μg/g (■), 326 μg/g (○), 707 μg/g (▲), and 1491 μg/g (◇).

only. There is no effect on the increase in weight at the first three concentrations. At a concentration of 326 μg Cd/g dry soil the animals' weight is different from those of the blank and the first three concentrations, from Day 7 until Day 26. At Day 30 and later sampling dates there is no difference. The animals' weight at the two highest concentrations was different from those at all the other concentrations at all sampling dates, while they differed from each other from Day 19 onward ($P < 0.05$). EC_{50} values for the body weight were estimated and are reflected in Table 2. The increase in EC_{50} values with exposure time indicates that eventually exposed animals reach the same weight as the control animals.

The final fresh weight W_∞ and growth parameter γ were estimated using the Von Bertalanffy growth model. The results are reflected in Table 1. Cadmium caused a decrease of the Von Bertalanffy growth γ, while the final fresh weight W_∞ is not affected up to 707.1 μg Cd/g dry soil. At 1491 μg Cd/g dry soil no growth at all occurred and it was not possible to estimate γ and W_∞. Because there were no significant differences ($p < 0.05$) between the weights at the different sampling dates, the growth parameter γ is equal to 0.

Number of Offspring and Population Increase

The mean numbers of offspring present in the containers are given in Fig. 3 for the concentrations 34.8, 71.3, 149, and 326 μg Cd/g dry soil at different sampling dates. At these concentrations no significant mortality occurred so the offspring can be attributed to an equal number of adults. The first offspring was observed in the blank and at the first concentration on Day 19. From Day 23 onward there was also offspring present at the higher concentrations. As can be seen, there were differences between the concentrations and the blank on Days 23, 26, 30, and 35 while at Day 42 the differences ceased to exist. So, as was observed for the weight, the EC_{50} values for number of offspring also increased with time (Table 2).

To see how cadmium affects population increase, the total number of individuals present in a container at each concentration is given in Fig. 4. It seems that there was

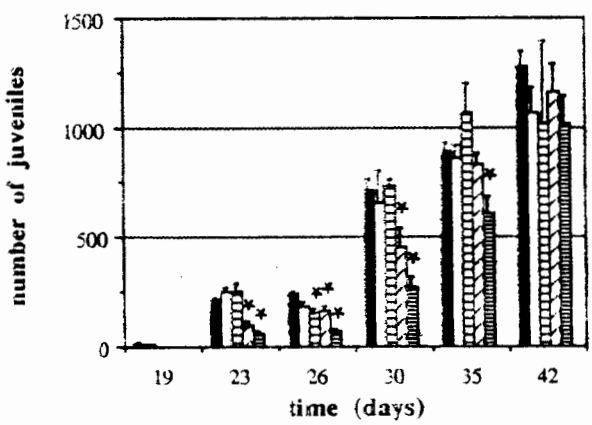

FIG. 3. Mean number of juvenile *F. candida* individuals at different sampling dates with their standard error, at different levels of cadmium in artificial soil. Blank (■), 34.8 μg/g (□), 71.3 μg/g (▤), 148 μg/g (▨), and 326 μg/g (▦). The concentrations for which the number of offspring was significantly lower from the blank are marked with an asterisk (Williams, $P < 0.05$).

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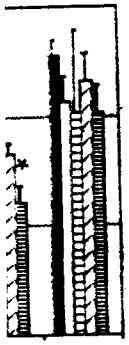

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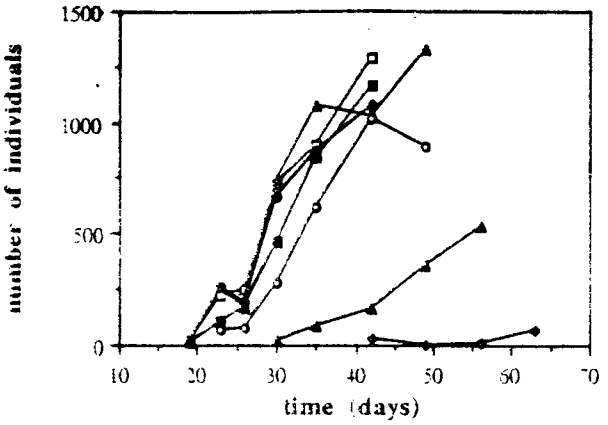

FIG. 4. Total number of *F. candida* individuals at different levels of cadmium in artificial soil. Blank (□), 34.8 μg/g (●), 71.3 μg/g (△), 148 μg/g (■), 326 μg/g (○), 707 μg/g (▲), and 1491 μg/g (◇).

no effect for the population at the first four concentrations, while population increase at the fifth concentration was retarded and at the sixth concentration the population did not develop at all. To allow comparisons between the concentrations, the population increase α was estimated using the model given above. The estimated values are given in Table 1. The table reflects a decrease in population increase with increasing cadmium concentration.

Accumulation of Cadmium

Figure 5 indicates the cadmium content of the adult animals at different times for each of the applied concentrations. Only surviving animals were analyzed. The equilibrium concentration was reached after about 20 days, which is in good agreement with the time necessary to reach an ultimate LC_{50}. The final cadmium concentration

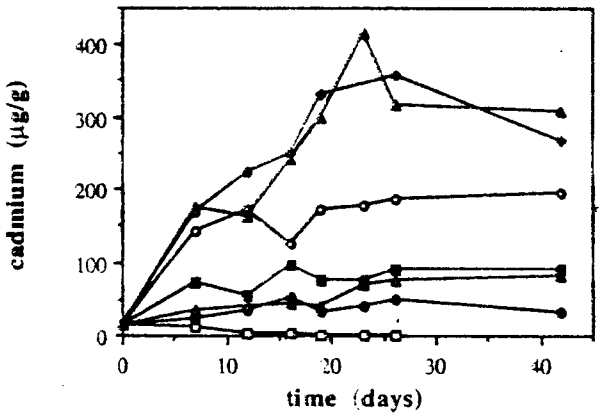

FIG. 5. Accumulation of cadmium in *F. candida* individuals expressed in μg cadmium/g dry weight at different levels of cadmium in artificial soil. Blank (□), 34.8 μg/g (●), 71.3 μg/g (△), 148 μg/g (■), 326 μg/g (○), 707 μg/g (▲), and 1491 μg/g (◇).

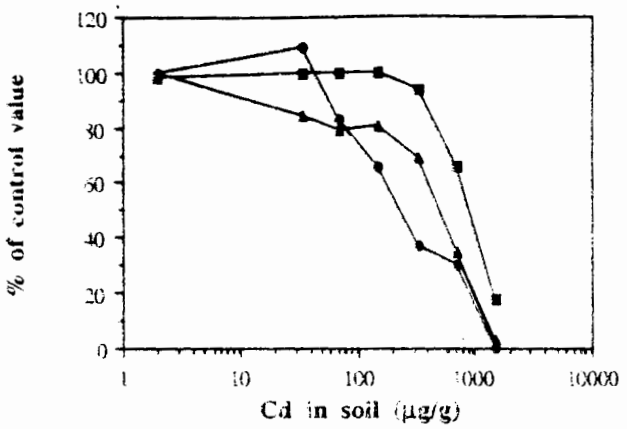

FIG. 6. Dose–effect relationships for survival at Day 35 (■), Von Bertalanffy growth γ (●), and population increase α (▲). The values at the different concentrations are expressed as the % of the control value.

for the animals exposed to 34.8, 71.3, 149, and 326 µg Cd/g dry weight is about half of the concentration present in the soil. Animals exposed to the concentrations which approach the ultimate LC_{50} value do not reach a level higher than 300 µg Cd/g dry soil. From this it can be concluded that the lethal body burden is about 300 µg Cd/g dry weight.

Comparison of Parameters

A dose–effect relationship is given in Fig. 6 for the individual parameters Von Bertalanffy growth, survival on Day 35, and the population increase. To compare the parameters with the population increase EC_{50} values were calculated and are given in Table 3, together with the EC_{50} values for body weight on Day 35 and number of offspring on Day 35. From Fig. 6 and Table 3 it is clear that when *F. candida* is exposed to cadmium the Von Bertalanffy growth is the most sensitive parameter. The body weight reached at Day 35, the number of offspring, and the population growth

TABLE 3

ESTIMATES OF MEDIAN EFFECT CONCENTRATIONS FOR SURVIVAL ON DAY 35, NUMBER OF OFFSPRING AND BODY WEIGHT ON DAY 35, VON BERTALANFFY GROWTH, AND POPULATION INCREASE FOR *F. Candida* EPOSED TO CADMIUM IN ARTIFICIAL SOIL

Parameter	EC_{50} (µg Cd/g dry soil)	95% confidence range
Survival Day 35	854	604–1208
Offspring Day 35	>326	
Body weight Day 35	807	733–888
Growth rate	256	223–293
Population increase	475	411–549

are retarded at higher concentrations. Mortality only occurs at much higher cadmium concentrations.

DISCUSSION

The ultimate mean LC_{50} value for the effect of cadmium on *F. candida* was estimated to be 850 μg Cd/g dry soil and was reached within 19 days (Fig. 1, Table 2). This LC_{50} value is in good agreement with the one found by Kratz (1984) in a loamy sand soil which is 912 μg Cd/g dry soil after 6 days of exposure. Accumulation also reaches an equilibrium within 19 days (Fig. 5). Kooijman (1981) presented a model in which he assumed a relationship between the LC_{50} value and the concentration inside the organism, which both would reach an equilibrium value at nearly the same time. This assumption is confirmed by the present study. The data also demonstrate that *F. candida*, like *Orcheseila cincta*, is a rapid cadmium-accumulating species compared to other soil invertebrates such as *Platynothrus peltifer* and *Neobisium muscorum* (Janssen *et al.*, 1991).

The growth of the animals is not influenced by the first two concentrations, is influenced only slightly at the third and fourth, and is clearly influenced at the two highest concentrations. Because the EC_{50} for body weight increases with time (Table 2) it can be concluded that eventually the same weight is reached and the effect of cadmium is a delay of growth. This is also demonstrated by the Von Bertalanffy growth (Table 1). The EC_{50} values for the number of offspring show the same pattern (Table 2). This can be explained by a retarded maturation which results in a later onset of reproduction. The explanation for this phenomenon is that cadmium acts primarily on the growth of the animals which indirectly determines their capacity to reproduce. When an animal does not gain a minimal weight, no reproduction is possible, which is the case at the highest concentration. A direct effect on the reproduction is unlikely because after 42 days the number of offspring for the first four concentrations equaled the control level. These results indicate the importance of knowing how parameters develop in time so that the action of a toxic substance can be understood.

The most sensitive parameter in this study was the Von Bertalanffy growth which has a EC_{50} value well below the LC_{50}. Although this sublethal effect occurs at relatively low concentrations, the population increase is hardly affected because reproduction is not stopped but is only retarded. This response of *F. candida* to Cd seems to be comparable with the response of the collembolan species *O. cincta* to Cd (Van Straalen *et al.*, 1989). On the other hand for the mite *P. peltifer* cadmium mainly acts on the reproduction. For the collembolan species *Onychiurus armatus* lead and copper first affected reproduction (Bengtsson *et al.*, 1985). These examples demonstrate that the effect of toxic substances is species and substance specific. The ecological consequences of delayed growth and reproduction cannot be predicted on the basis of this study, because no data of population development and age structure in the field are available.

Although the results of this study show that individual growth is more sensitive than population increase, this will not be a general rule. The difference in the relationship between individual and population parameters as found by Halbach (1984) and Meyer *et al.* (1987) can be explained by the use of different species and different toxic substances. According to Van Straalen *et al.* (1989) a greater effect on the population was observed for *P. peltifer* where reproduction is the most sensitive parameter for cadmium compared to *O. cincta* where body growth is the most sensitive parameter

for cadmium. Besides this, the distance between the concentration at which the f sublethal effects appear and the concentration which is lethal is of importance. As w demonstrated by Daniels and Allan (1981) and Allan and Daniels (1982), the ef on the population is greater when the EC_{50} values for sublethal effects and letha are far apart from each other than when they are close to each other.

In general it can be stated that knowledge of the chain of effects is very valua for understanding population effects. It is very important to know how individu parameters develop in time so that the most sensitive parameter can be determine Besides this, the distance between the concentration at which the first sublethal effe occur, and the concentration at which lethality occurs, is of importance. By looki at toxicity data in this way, a step forward is made in the field of obtaining ecologically more realistic toxicity data.

CONCLUSIONS

Unraveling the chain of effects when a species is exposed to a pollutant may con tribute to a better understanding of effects on the population level. To determin which parameter is the most sensitive it is important to look at how parameters deve in time. For *F. candida* the most sensitive parameter, when the species is exposed cadmium in artificial soil, is the Von Bertalanffy growth which indirectly affects production.

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