Arch. Environ. Contam. Toxicol. 49, 343–352 (2005)
DOI: 10.1007/s00244-004-0220-z

ARCHIVES OF
Environmental
Contamination
and Toxicology

Effects of Different Soil Types on the Collembolans *Folsomia candida* and *Hypogastrura assimilis* Using the Herbicide Phenmedipham

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Received: 26 October 2004 /Accepted: 5 February 2005

Abstract. Soil ecotoxicology studies are usually performed in standard soils such as Organization for Economic Cooperation and Development artificial soil or LUFA ST. 2.2, a natural soil. When assessing the toxic effects in the environment, soil properties are often different from those in standard soils, which might lead to a different exposure situation for the test species and therefore to misleading conclusions. Selected to cover a broad range of properties and based on the Euro-Soils concept, 17 different soils were studied regarding their suitability to two test species: *Folsomia candida* and *Hypogastrura assimilis* (Collembola). In reproduction tests, the test species reacted differently to the soils. *F. candida* was less affected by soil properties: 500 to 1200 juveniles/vessel were found in untreated soils (*i.e.*, controls). These differences can be attributed to normal interindividual variability. *H. assimilis* showed a significant correlation with maximum water-holding capacity and also a tendency to lower the reproductive output in soils with a low pH (<4). Therefore, some soils were revealed to be inappropriate for tests with *H. assimilis*. In the main tests, the effect of the reference test substance Phenmedipham (formulation Betosyp) was studied in those soils where sufficient reproduction was determined beforehand. Clearly, the chronic end point was more sensitive than survival when testing Phenmedipham. In *H. assimilis*, because of high variability and low effects in the tested dosages, no conclusions could be drawn. In *F. candida*, different soils caused different toxic effects: Juveniles preferred soils with high C-to-N ratios. Higher microbial activity might support a quicker metabolization of the test substance. In general, the toxic response is caused by a synergistic action of several soil properties with each of them exerting an effect too small to be clarified with the available set of data.

In toxicity testing, the standardization of methods is required for reasons of comparability and quality assurance. Most ecotoxicologic soil tests are performed using two different standard soils: either an artificial soil (Organization for Economic Cooperation and Development [OECD] 1984) or the natural LUFA 2.2 soil (Schinkel 1985). However, the toxicity of chemicals may be affected by properties of the soil in which the chemical is tested. This problem can be addressed in two ways: either as many soils as practically feasible can be tested (Jepson *et al*. 1994) or a unifying concept can be developed (Van Gestel 1997) Despite that many complex processes occurring in soil are at least partially known (Gawlik *et al*. 2003), the sheer number of different soils with their many combinations of main soil properties complicates the definition of such a unifying concept, so the problem is often ignored. For example, approaches to establish threshold values for organic xenobiotics or heavy metals, *e.g.*, in the context of the sludge amendment, do not consider sufficiently the influence of general soil parameters on the mobility, availability, and ecotoxicity of the compounds being regulated (Langenkamp and Marmo 2001).

For these reasons, a set of reference soils—the so-called Euro-Soils—was introduced in 1990 to create a common basis for better comparison and quality control of soil sorption data (Kuhnt *et al*. 1994). First five and finally six regionally representative soils were identified, collected, prepared, and characterized as reference soils for chemical testing in the European Union (Gawlik *et al*. 1996). The demand for Euro-Soils grew dramatically after their introduction, and it quickly became evident that the original Euro-Soils were not a suitable source for ecotoxicologic standard tests because the available amount simply was not sufficient.

Recently, Römbke and Amorim (2004) suggested that each soil similar in terms of their main properties (*i.e.*, texture, pH, C-to-N ratio, and organic matter content) to one of the six Euro-Soils could be used for ecotoxicologic tests. To validate this hypothesis, such tests were performed in the original Euro-Soils, similar soils, and the standard OECD and LUFA 2.2 soils for comparison purposes. Although several species were chosen as test individuals, here we present the results using two collembolans: *Folsomia candida* and *Hypogastrura assimilis*. They were selected as test species because of their ease of culture and high reproductive rates. An international test guideline exists for the first species (International Organization for Standardization [ISO]

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1998), and a standard procedure has been proposed for the latter (Folker-Hansen *et al.* 1996). *H. assimilis* differs from *F. candida* mainly in its manner of reproduction: The former reproduces sexually, whereas the latter is a parthenogenetic species. In addition, *H. assimilis* shows some social behavior, which has not been observed in *Folsomia* (or onychiurid spp.) (P.H. Krogh, personal communication, 2001). Reproduction tests were performed with both species in several soils. Median effective concentration (EC_{50}) as well as no observed–effect concentration (NOEC) values were calculated, and the influence of the respective soil properties on the test results was statistically evaluated. Thus, in this work we aimed (1) to assess whether the two collembolans were suitable as test species; (2) to determine in which soils the collembolans could be tested; and (3) to investigate the influence of soil properties on test results.

Materials and Methods

Test Species

F. candida Willem 1902 (Collembola: Isotomidae) is a blind, unpigmented, euedaphic collembolan reproducing parthenogenetically (Hopkin 1997). *Hypogastrura assimilis* Krausbauer (Collembola: Poduridae) is an epi- to hemiedaphic species, pigmented, and has eyespots. It is a sexually reproducing species. The female individuals are approximately twice the size of male individuals and are lighter in color (Folker-Hansen *et al.* 1996). Both species were easily cultured in the laboratory in a moistened substrate of plaster of Paris and charcoal (mixture 8:1) prepared according to Usher and Stoneman (1977). Individuals were maintained in laboratory at 20°C, in the dark, and fed dried baker's yeast (*Saccharomyces cerevisiae*).

Test Substance

The test substance Phenmedipham, a herbicide, was applied as the formulation Betosyp [formerly known as Betanal; STÄHLER AGROCHEMIE, 157g/L active ingredient (a.i.)] to the soils in the following concentrations: 0.1, 0.32, 1, 3.2, and 10 mg a.i./kg soil dw. In the case of the OECD soil, the concentrations were 1, 3.2, 10, 32, and 100 mg a.i./kg soil dw. These concentrations were based on the results of a range-finding test during which no effects occurred with concentrations up to 10 mg a.i./kg. In the case of the LUFA 2.2 soil, the concentrations were 5, 8.75, 12.5, 16.25, and 20 mg/kg soil dw based on the results of a ring test (Hund-Rink *et al.* 2002a). The contamination of all test substrates was done by mixing an aqueous solution of the test chemical into the premoistened soils separately for each test concentration. After homogeneous mixing, subsamples of soil were introduced into the individual test vessels.

Test Procedures

Synchronized cultures were established for the experiments by removing egg clusters from stock cultures into new culture vessels. Two days after the start of hatching, juveniles were transferred into a new vessel where they were fed and watered. After approximately 10 days for *F. candida* and 16 days for *H. assimilis*, the individuals were in the correct life stage to start the test.

Test procedures were as described in ISO guideline 11267 for *F. candida*. Ten individuals, 10 to 12 days old, were placed in each test vessel already containing the premoistened test soil and the food supply. The vessels were covered with a parafilm layer in which a few holes for airing were made. Food and water were replenished weekly. After 4 weeks, the test ended, and each test vessel was filled with distilled water that was gently mixed with a spatula. Afterwards, juveniles and adults were floating on the surface. The addition of a few drops of dark ink provided a higher contrast between the white individuals and the black background. A digital photograph of the water surface plus collembolans was taken. The collembolans on the image could later be easily counted using computer software SigmaScan Pro 5(SPSS 1999). Some replicates were randomly selected and the pictures were checked by hand to validate the accuracy of the program. Adults and juveniles were easily distinguished by their size.

The test procedure for *H. assimilis* was similar except that the individuals were 16 to 19 days old at the start of the test to distinguish between male and female individuals. Ten male and 10 female individuals were introduced per test vessel. The test duration was 3 weeks. *H. assimilis* forms clusters when floating on the water surface, so the counting of individuals with software is not possible. To individualize the organisms, test vessels were covered with a narrow net and connected to an empty vessel by a double lid. Afterwards, the vessels were inverted, covered with black paper, and placed under a heating source for 24 hours. Because the individuals tended to escape from the heat in the upper vessel, they fell into the empty vessel at the bottom. Finally, the individuals were immobilized with alcohol; digital photos were taken; and the same software package was used for counting. Adults and juveniles were not distinguishable from each other.

Test Soils

The main properties of the test soils (pH, organic matter, C-to-N rstio, cation exchange capacity [CEC], maximum water-holding capacity (WHC), clay, silt, and sand content) are listed in Table 1. Their selection is described in detail by Römbke and Amorim (2004). Artificial soil (OECD 1984) is composed of 69% sand, 20% kaolin clay, 10% sphagnum peat, and 0.3 to 1% of $CaCO_3$ for pH adjustment (6 ± 0.5). LUFA 2.2 is a natural standard soil from Speyer (Germany). The codes given for the natural soils are translated as follows: ES = Euro-Soil; ESo = the soil is a sample from the same site as the original ES; numbers = the soil is similar to a certain ES number (X = the soil could not classified to a certain ES class); and the other codes represent the first three letters of the soil original place name, *i.e.*, Nat1 = Natzungen; Hoh2 = Hohenlimburg; Coi2 = Coimbra; Sch3 = Schmallenberg; Mon4 = Mönninghausen; Tau4 = Taubenheide; KarX = Karlsruhe (Schluttenbach); and Ren7 = Gladbeck-Rentfort. At least one natural soil from each class was tested. Not surprisingly, it was most easy to find soils belonging to ES class because this class represents ``normal" agricultural soils in Central Europe. In some cases, in particular the original EURO-Soils, the amount of available soil was so small that not all tests could be performed. With the exception of soil ES7, bought from the University of Vienna (Austria), the other samples were from the European Chemical Bureau (Ispra, Italy), where only a small amount remained available.

Experimental Setup

The study was conducted in two parts. In the first set of experiments, the survival and reproduction of the two species was tested in all soils in a control situation, *i.e.*, without any contamination. The goal was to verify the suitability of the individual soils for a certain species. In the second set of experiments, only the soils where reproduction was

Table 1. Main characteristics of the tested soils and the relative Euro-Soils properties: pH, OM, C:N ratio, grain size distribution, CEC, and WHC

Soil	pH ($CaCl_2$)	O.M. (%)	C:N Ratio	Clay (%)	Silt (%)	Sand (%)	CEC (mval/100 g)	WHC (%)
ES1	5.1	2.7	7.7	75	22	3	29.9	62.6
Nat1	6.2	1.7	8.7	33	66	5	40.7	58.4
ES2	7.4	6.4	18.5	23	64	13	28.3	68.5
Hoh2	6.2	12.9	25.0	6	61	33	78.3	73.9
ES3	5.2	6.5	13.3	17	37	46	18.3	42.6
Eso3	5.2	6.0	11.8	18	38	44	74.5	–
Sch3	5.4	4.1	10.4	23	45	32	68.5	67.4
Coi3	6.7	6.5	17.0	26	60	14	75.8	68.1
LUFA 2.2	5.8	4.4	14.0	6	17	77	11.2	55
ES4	6.5	2.9	9.7	20	76	4	17.5	42.9
Mon4	6.5	2.5	9.7	11	77	12	20.7	53.2
Tau4	6.9	2.9	9.7	17	79	4	61.3	63.1
ES5	3.2	15.9	30.8	6	13	81	32.7	38.7
Eso5	3.2	9.2	29.7	10	12	79	87.0	100.1
ES7	4.4	11.5	14.2	19	35	46	5.0	80.6
Ren7	3.8	8.7	11.0	18	40	42	132	121.8
EsoX	6.3	8.9	23.5	31	33	36	–	64.0
KarX	3.6	10.6	45.9	13	58	29	173	71.9
OECD artificial	6.0	8.0	Ca. 40	10	10	80	45.8	Ca. 90

CEC = Cation-exchange capacity.
Col = Columbia.
ES = Euro-Soil.
Hoh = Hohenlimburg.
Kar = Karlsruhe.
Mon = Mönninghausen.
Nat = Natzungen.
OECD = Organization for Economic Cooperation and Development.
OM = Organic matter.
Ren = Gladbeck-Rentfort.
Sch = Schmullenberg.
Tau = Taubenheide.
WHC = Water-holding capacity.

within the validity range (mortality <20% and number of juveniles >100/test vessel) as defined in the ISO guideline (1998) were tested with the test substance Phenmedipham.

Statistical Procedures

Two main hypotheses were tested: (1) that the measured soil properties would influence the survival and reproduction of the test individuals (tests without chemicals) and (2) that the measured soil properties would influence the toxicity of Phenmedipham either by directly altering the exposure (*e.g.*, because of different adsorption and bioavailability) or by adding an extra stress factor for the individuals (*i.e.*, in addition to the chemical).

Redundancy analysis (RDA) was applied to the results of survival and reproduction of enchytraeids and collembolans maintained in different soil types in the absence of toxicants. The analysis was performed with Canoco for Windows 4.5 (Ter Braak and Smilauer 2002) using survival and reproduction of each species to play the role of species and the physical and chemical parameters of each soil to play the role of environmental data. All data, except for pH values, were log-transformed before the analysis. Additionally, because of the interdependence of the individual parameters, only the two extreme categories (sand and clay excluding silt) of the three texture classes were used. Species data were centered and normalized within Canoco for Windows. A similar procedure was used to analyze toxicity data from exposure of collembolans in different soil types. However, in this case toxicity parameters (EC_{50} and NOEC) were used to play the role of species. Conditional effects of environmental data on species data were assessed using Monte Carlo permutation tests with automatic variable selection from within Canoco for Windows.

Stepwise multiple regression models were developed using the statistical software package SPSS 12.0 (SPSS 2003) to quantify the relationship of the biological data with soil data. All but the pH data were also normalized using logarithms (X+1) and silt was excluded. Analysis of variance and Bivariate Spearman Correlations were calculated using SPSS 12.0 (SPSS 2003). EC_{50} and NOEC values were calculated using the ToxRatPro program (ToxRat 2003).

Results

First Set: Control Experiments

In total, the mortality and reproduction of *F. candida* were tested in 18 soils (Fig. 1). Some animals died in all soils; however, in nearly all cases the mortality was $\leq 10\%$. Only in three of the original Euro-Soils (ES1, ES3, and ES4), up to 20% mortality occurred. The number of juveniles varied by a factor of approximately 2 (minimum $\sim$ 450 in ES4; maximum $\sim$ 1000 in LUFA St. 2.2) but in any case was at least four times higher than the validity criterion as defined by the ISO guideline. No statistical differences could be determined be-

tween OECD soil (reference) and the other soils in terms of the number of juveniles (one-way analysis of variance [ANOVA], Dunnetts' two sided; $p > 0.05$).

The second collembolan species, *H. assimilis*, could be tested in only 13 soils (the amount of the original Euro-Soils was too small). The results differed completely from the tests with *F. candida*. The number of individuals in the various soils was highly variable (Fig. 2) and fluctuated between 0 (ESo5) and 350 (ESoX) individuals/test vessel. In the soil Ren7, adults survived, but no reproduction was possible. The results in these soils were also statistically significantly different from the results in the OECD soil (one-way ANOVA, Dunnetts' two sided; $p < 0.05$).

The variability of the results for *H. assimilis* was clearly higher and the species more sensitively reactive to soil properties than *F. candida*. In addition, minimum and maximum average numbers of juveniles obtained were considerably different for each species: a minimum of 493 to a maximum of 953 for *F. candida* and a minimum of zero to a maximum of 359 for *H. assimilis*.

The RDA with collembolans data showed a high correlation between species parameters and soil properties for the first two axes (0.657 and 0.544, respectively). Species parameters alone explained 33.1% and 3.7% of the total variance associated with the first and second axes, respectively, whereas the interaction between species parameters and soil properties accounted for an additional 54.4% and 6.1% of the total variability, respectively.

Species were grouped along the first axis in association with higher values of water-holding capacity (WHC) and C-to-N ratio (Fig. 3). Juveniles and adults of *F. candida* were separated along the second axis in association with a gradient of WHC and cation-exchange capacity (CEC) (higher values are associated with juveniles) and pH (higher values are associated with adults). No significant conditional effects were found.

Stepwise multiple regression analysis showed significant a relationship between the number of individuals of *H. assimilis* at the end of the test and the Log(WHC) (negative effect) (see stepwise model in Table 2). A negative correlation between individual numbers and pH was found, although pH was not included in the final regression model for *H. assimilis*.

Second Set: Experiments With Phenmedipham

In the case of *F. candida*, all soils tested as control were tested with Phenmedipham (as Betosyp), and results can be seen in Figure 4. In the case of *H. assimilis*, ESo5 and Ren7 showed none or nearly no reproduction in the control test run; hence all but these two soils were used as test substrates. The test results with *H. assimilis* were very variable (among replicates and between treatments) and without any dose-response relationship. Because of this, effect levels (EC_{50} or NOEC values) could not be calculated. In the controls, a higher range concerning the number of individuals was found than in the first tests without contamination. This was caused by the differences found in the soil ESoX where 990 (± 252.2) individuals were found—nearly three times the maximum observed before. The effect values of the tests with *F. candida* are summarized in Table 3.

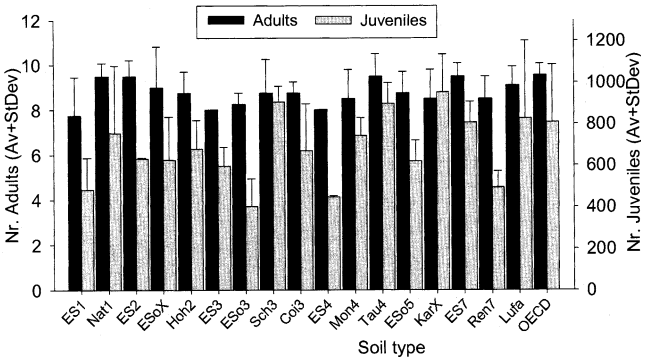

Fig. 1. Results obtained after exposing *F. candida* to different soil types (OECD soil, LUFA 2.2, Euro-Soils, and similar soils). Graph shows the average number + SD.

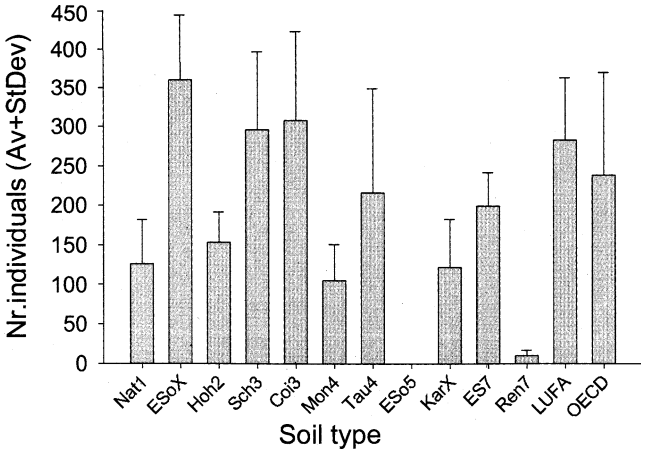

Fig. 2. Results obtained after exposing *H. assimilis* to different soil types (OECD soil, LUFA 2.2, Euro-Soils, and similar soils). Graph shows the average number + SD.

Because the concentrations were chosen in a way that the main end point of reproduction (= number of juveniles) could be determined, it was hypothesized that the effect values for adults (EC_{50} and NOEC) would usually be higher than the highest test concentration (10 mg a.i./kg). The only exceptions were the two acidic soils, ESo5 and KarX, where the NOEC values were determined as 0.1 and 10 mg a.i./kg, respectively. It should be noted that the concentration range for the two standard soils was chosen so the effects on mortality could be also determined. Although in the artificial soil both effect values were relatively high (51.9 and 32 mg a.i./kg, respectively), they were clearly lower in the LUFA St. 2.2 soil (10.6 and <5 mg a.i./kg, respectively).

The effect values for the juveniles varied between 4.4 and 39.2 mg a.i./kg (EC_{50}) and < 0.1 and ≥ 32 mg a.i./kg (NOEC), so the EC_{50} values varied by a factor of $\sim$ 10, whereas the NOEC values covered an even broader range with a factor of > 320. Clearly, different results were obtained in the different soils. In addition, there was also a distinction between the different Euro-Soils groups in terms of toxicity. At first, the results in the standard soil LUFA St. 2.2 and in particular the acid KarX soils are difficult to understand. In both soils the NOEC values for adult mortality are lower than the NOEC

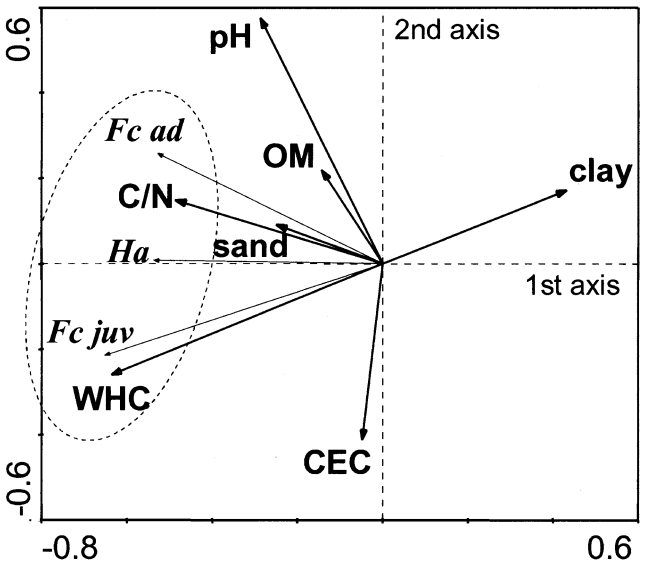

Fig. 3. RDA biplot of species and soil parameters. Fc = *F. candida*; Ha = *H. assimilis*; ad = adults; juv = juveniles). The dashed circle represents the association between species and soil parameters. RDA = redundancy analysis.

Table 2. Stepwise regression models for the test species *F. candida* and *H. assimilis* with and without the chemical substance Betanal and its relation with the soil parameters[a]

Model	r^2 adjusted	*F*	*n*	$p < .05$
Log (**Ha**) = 7.186−2.749 * **log(WHC) (53.2>WHC**	0.429	8.510	10	0.017
Log **(Fc_Bet_EC50juv)** = 0.036 + 0.773 * **log(C/N) (7.7>C/N<45.9)**	0.629	16.267	11	0.004

[a]Bioassay end points and environmental parameters, except pH, were log-transformed (X + 1).
ad = Adult organisms.
Bet = Betanal.
Fc = *F. candida.*
Ha = *H. assimilis*.
Juv = Juvenile organisms.
WHC = Water-holding capacity.

values for reproduction. In the case of the LUFA St. 2.2 tests, the effect, given as EC_{50}, on both end points is quite similar.

In nearly all cases control mortality was ≤ 10%. The number of juveniles in controls of the chemical testing varied by a factor of approximately 5 (minimum 226.7 in KarX soil and maximum 1189.7 in Nat1) but in any case was at least two times higher than the validity criterion as defined by the ISO guideline.

An RDA with *F. candida* data could not be conducted because the available data set had too many undetermined values (*e.g.*, EC_{50} survival >10; Table 3). Nonetheless, a significant correlation was found between the Log(EC_{50}) for juveniles and the Log(C-to-N ratio) (positive effect) (see stepwise regression model in Table 2).

Discussion

First Set: Control Experiments

In the tests with *F. candida*, no major differences were observed in terms of survival and reproduction in the different soils. The validity criteria were always fulfilled. Nevertheless, the juvenile numbers varied in the different soils, meaning that the soil properties somehow influenced reproduction. As expected, the end point of reproduction is more sensitive to soil differences than survival, *i.e.*, the chronic end point is more appropriate for these kind of studies.

However, any relationship between changes in the number of juveniles and soil properties must be discussed with caution because these changes can also be partially attributed to normal interindividual differences. For example, Crouau and Cazes (2002) found that the variability in juvenile numbers in the standard reproduction test (*i.e.*, performed with artificial soil) with *F. candida* had several causes; among them were the mortality of adults and the variability induced by the use of animals that hatched on 3 successive days.

An influence of pH occurred in some but not all tested soils because there was a tendency to see a lower number of *H. assimilis* individuals at lower pH values. Sandifer and Hopkin (1996) tested *F. candida* in artificial soil at pH values of 6.0, 5.0, and 4.5 in a standard laboratory test. There was no clear relationship between adult survival or juvenile production and soil pH, but an overall decrease in reproduction was observed in the control samples with pH values of 5.0 and 4.5 compared with those at pH 6.0. In a similar experiment, Greenslade and Vaughan (2003) studied an even wider range of pH values and found an optimum of juvenile numbers at pH values of 5.38 to 6.62. Interestingly, at lower pH values (<3.47) the number decreased to approximately 50% of the optimum number, whereas at higher pH values (7.65 and 8.03) there was a strong decrease down to zero. The individuals seem to be sensitive to very basic soils. The soils tested in our experiment covered a pH range between 3.2 and 7.4. Basically, our results confirm the work of former investigators: There is an influence of the pH on reproduction (especially for *H. assimilis*), but within a range of 3.2 to 7.4 this influence is not large enough to significantly impede the number of juveniles in *F. candida*.

Stepwise multiple regression analysis showed a significant relationship only between the number of individuals of *H. assimilis* at the end of the test and the Log(WHC) (negative effect). Similar results were observed by Van Gestel and Diepen (1996), who studied the effect of different soil moisture contents on *F. candida* in OECD artificial soil: 25%, 35%, 45%, and 55% corresponding to 28%, 40%, 51%, and 63% of WHC. These investigators found that the collembolans produced more eggs at lower moisture contents, but these eggs hatched somewhat later than those produced at higher moisture levels. It is not known at which moisture level eggs are no longer produced, but because of the well-known susceptibility of insects to dryness, it must be somewhere close to the lowest level tested (Edney 1977; Bursell 1970). Perhaps there is a similar effect in *H. assimilis*.

Apparently, other soil properties are exerting an effect, but no statistically significant relation to any other indi-

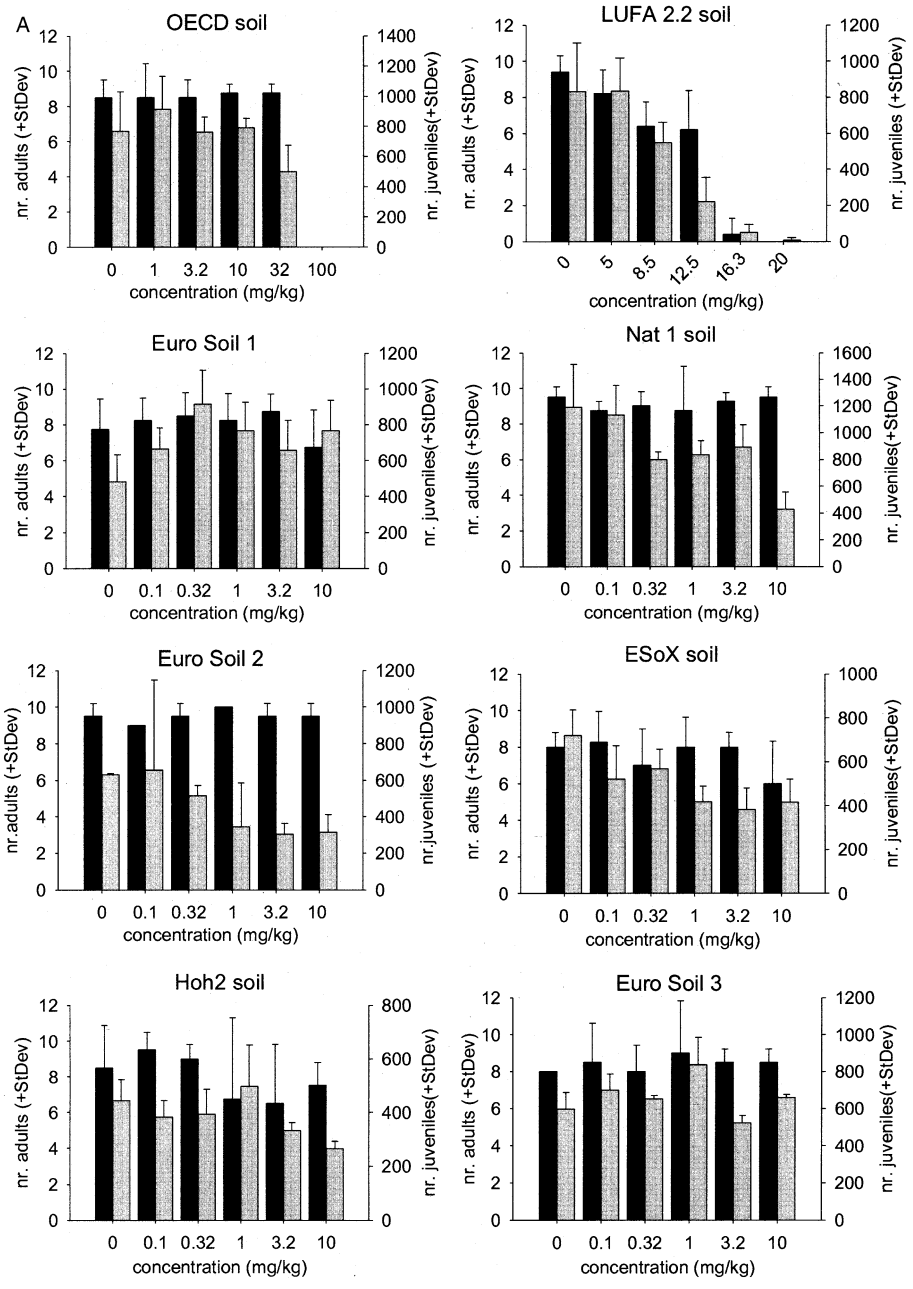

Fig. 4. Results obtained by exposing *F. candida* in different soils to Phenmedipham (average numbers).

vidual parameter could be observed. From the RDA, although no significant conditional effects were found, one can see that juveniles and adults of *F. candida* are separated along the second axis in association with a gradient of WHC and CEC (associated with juveniles) and pH (associated with adults). In any case, the variability of the results strongly impedes the identification of these probably additive effects.

Second Set: Experiments With Phenmedipham

Because *H. assimilis* showed no consistent effects at the tested concentrations of Phenmedipham and a high variability within treatments, no conclusions could be drawn concerning the interactions between the chemical and the soil properties. This may be related to the sexual mode of reproduction of the species, a factor of increased variability compared with the parthenogenetically reproducing *F. candida*. Additionally, the extraction and counting method for *H. assimilis* was less accurate then the one for *F. candida*, which probably also contributed to the variability in results. Therefore, it is strongly advised to use the improved methodology adopted by Krogh *et al.* (1998) to decrease this variability.

Phenmedipham is used as a reference substance according to ISO guideline 11267 (1998), so a broad data set from tests in standard LUFA St. 2.2 is available (Achazi *et al.* 2000). This substance has also been used as an external control in a ring test sponsored by the German Federal Environmental Foundation (Hund-Rinke *et al.* 2002b). According to these sources,

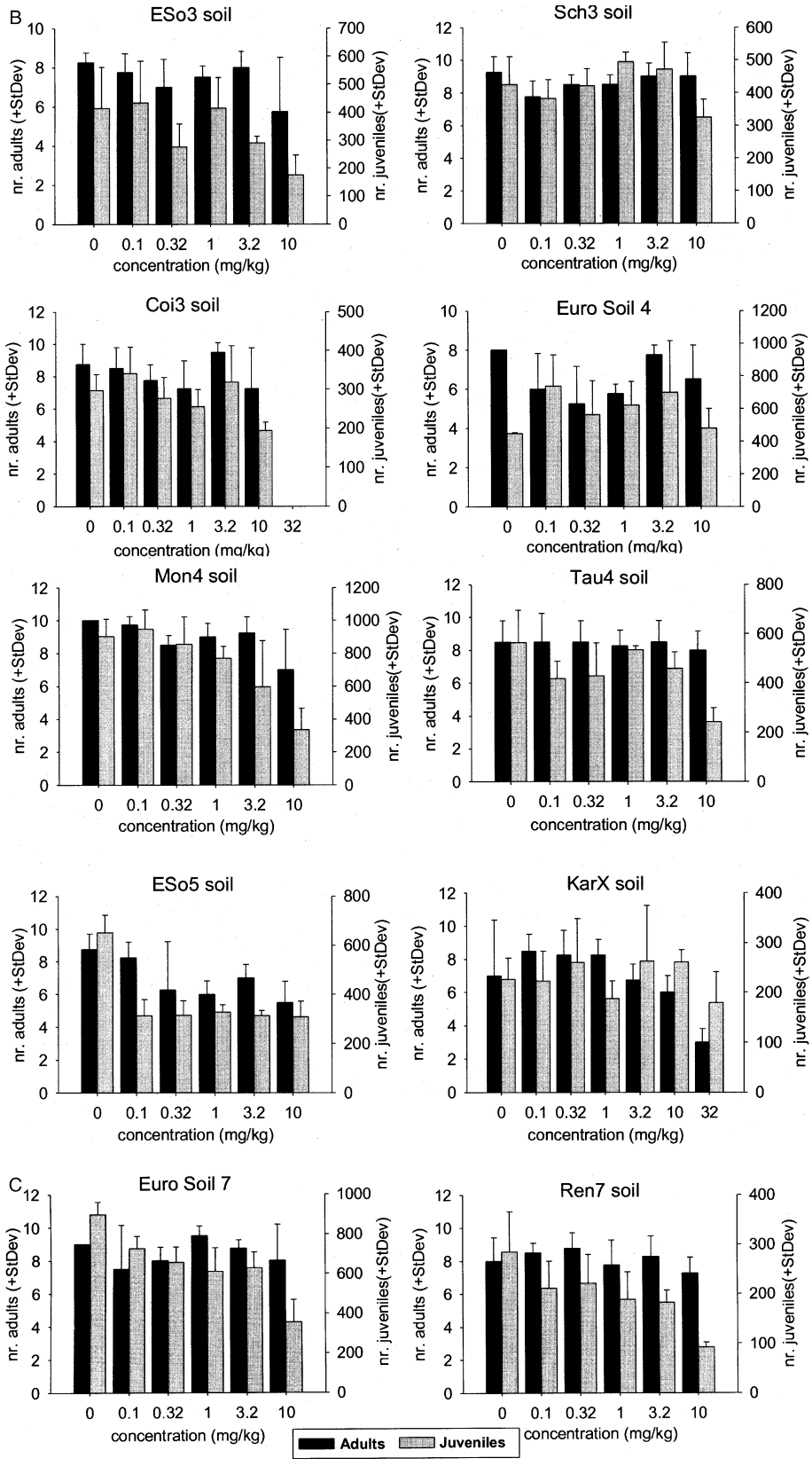

Fig. 4. Continued.

the LC_{50} values for *F. candida* differ between 14.8 and 15.4 mg a.i./kg, which is very close to the value of 10.6 mg a.i./kg as was determined in this study. Actually, effects on reproduction occurred at nearly the same concentration with EC_{50} values between 9.1 and 10.8 mg a.i./kg, which is in agreement with our results (10.1 mg a.i./kg). For the sake of thorough-

Table 3. EC_{50} and NOEC values from the exposure of *F. candida* to Phenmedipham (a.i.)

	F. candida			
	EC_{50}		NOEC	
Soils	Survival	Reproduction	Survival	Reproduction
ES1	>10	>10	≥10	≥10
Nat1	>10	6.8	≥10	0.1
ES2	>10	4.4	≥10	0.32
Hoh2	>10	22.8	≥10	3.2
ES3	>10	>10	≥10	≥10
ESo3	>10	9.4	≥10	3.2
Sch3	>10	>10	≥10	3.2
Coi3	>10	12.2	≥10	3.2
LUFA 2.2	10.6	10.1	<5	5.0
ES4	>10	>10	≥10	≥10
Mon4	>10	6.0	≥10	1.0
Tau4	>10	8.3	≥10	3.2
ESo5	n.d.	n.d.	0.1	<0.1
ES7	>10	7.9	≥10	<0.1
Ren7	>10	4.5	≥10	0.32
ESoX	>10	n.d.	≥10	<0.1
KarX	>10	>10	10	≥32
OECD	51.9	39.2	32	10

Col = Columbia.
ES = Euro-Soil.
Hoh = Hohenlimburg.
Kar = Karlsruhe.
Mon = Mönninghausen.
Nat = Natzungen.
OECD = Organization for Economic Cooperation and Development.
Ren = Gladbeck-Rentfort.
Sch = Schmullenberg.
Tau = Taubenheide.

ness, it should be mentioned that fulfillment of the validity criteria (*i.e.*, mortality of adults and number of juveniles in the control) was also very similar in the cited studies and in the tests reported here, *i.e.*, the reproducibility of this collembolan test is very good. To assess the general sensitivity of *F. candida*, it is interesting to note that in an avoidance test this species was affected by Phenmedipham at concentrations >3.5 mg a.i./kg, whereas other collembolan species were slightly more sensitive, reacting at approximately 2 mg a.i./kg (Heupel 2002).

The sensitivity of *F. candida* toward Phenmedipham was different in the various soils. A significant correlation was found between the Log(EC_{50}) for juveniles and the Log(C-to-N ratio) (positive effect). Juvenile toxicity was related with higher values of C-to-N ratio (range, 7.7–40): higher microbial activity might support a quicker metabolization of the test substance. It is known that the degradation of Phenmedipham in soil begins with microbially induced hydrolization (Domsch 1992).

Few investigators have studied the effect of soil properties on the toxicity of chemicals to *F. candida*. Martikainen (1996) studied the toxicity of dimethoate to *F. candida* in three different soil types (artificial soil, clayey soil, and humus sandy soil). Because of its use in the SECOFASE project (Sublethal Effects of Chemicals on Fauna in the Soil Ecosystem), this insecticide has often been used as a reference substance (Løkke and Van Gestel 1998). The organic matter content of the soil was negatively correlated with the toxic effects of dimethoate. Phillips *et al.* (2002) investigated the ecotoxicity of the chemical-warfare agent (CWA) HD (Mustard) using *F. candida*. Toxicity tests were conducted using standard artificial soil (10% OM; 6 pH), O'Neill-Hall sandy loam (natural soil with 4.3% OM; 5.1 pH), and Sassafras sandy loam (SSL; natural soil with 2% OM; 4.9 pH). HD toxicity to both adults and juveniles was greater in SSL than in the two other soils. Smit and Van Gestel (1998) evaluated the influence of soil characteristics and the means of contamination on the bioaccumulation and toxicity of zinc for *F. candida*. In contaminated soils without further treatment, zinc toxicity was related to organic matter and clay content of the soil (for percolated and aged soils the variation in effect concentrations between test soils was decreased). Van Gestel and Mol (2003) studied the effects of cadmium on survival, growth, and reproduction of *F. candida* in four soils differing in organic matter (3.0% to 10.9%) and clay content (1.4% to 5.2%) but not in pH (∼6.0). EC_{50} values for effects on reproduction ranged between 53.7 and 193 mμg Cd/kg dry soil (after 4 weeks). The absence of a consistent relationship between cadmium toxicity and soil properties suggested that differences of less than a factor of 3 to 4 in organic matter and clay content, in soils with the same pH, do not lead to significant differences in cadmium toxicity to collembola. Because the OECD artificial soil had the highest content of organic matter and clay, as well as the highest CEC, a very low toxicity would be expected for this soil. This was indeed the case after 4 weeks, as comparable with our own experiments, but at other time intervals toxicity was lower in other soils.

Conclusion

The most important result of this study is that important soil properties in a wide range do not limit the use of *F. candida* in ecotoxicologic standard tests. Soil had an important influence on *F. candida* when tested with Phenmedipham, *i.e.*, the EC_{50} values of juveniles changed by a factor of approximately 10. Clearly, juveniles prefer soils with a high C-to-N ratio, whereas their preferences relative to other soil properties are less clear. More data using more soils and species are required to understand the effect of soil properties in soil ecotoxicology.

H. assimilis was more sensitive to different soils than *F. candida*, although the inherent high variability of results in *H. assimilis* and the less feasible and accurate extraction and counting procedures acted as negative factors. Therefore, this species cannot be recommended for ecotoxicologic standard tests unless technical improvements are made. However, the variability caused by the sexual mode of reproduction may be a problem.

The further role of OECD artificial soil in ecotoxicology should be discussed. Test results with this soil were often those showing the lowest toxicity. Despite that this observation was based on tests with just one chemical, it is clear that the relatively high organic matter content is the main cause of an often decreased bioavailability of a test substance and thus its toxicity. For example, a decrease in peat content (*e.g.*, to 5% peat) would probably be more realistic while still being acceptable for soil invertebrates. In addition, the use of natural

peat in a standard substrate should be reconsidered because different kinds of peat are known to induce changes in the fate of chemicals and the behavior of individuals. The possibility of obtaining comparable results by using this artificial substrate is worthwhile, but it is simply not representative of the diversity of natural soils (and might underestimate toxicity). However, it is unlikely that OECD artificial soil will be completely discharged in the future. Instead, it might serve as an external control (probably with a decreased amount of organic matter) to secure the quality of the individual test system. The results presented here show that soil toxicity testing should not rely solely on tests with artificial soils but rather should include assays with reproductive end points using natural soils with varying physical and chemical parameters to adequately assess the toxicity of chemicals.

Acknowledgments. This study was sponsored by Fundação para a Ciência e Tecnologia, Portugal, through a doctoral grant to Mónica Amorim (SFRH BD 1348 2000). The authors thank Hans-Joachim Schallnass for his help with statistics. Special thanks go to Paul Van den Brink for advice on multivariate statistics and to H. Muntau and B. Gawlik (formerly at European Chemical Bureau, Ispra, Italy), for providing samples of the original Euro-Soils.

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