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Applying a GLM-based approach to model the influence of soil properties on the toxicity of phenmedipham to *Folsomia candida*

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Abstract
Purpose Soil properties are the main explanation to the different toxicities obtained in different soils due to their influence on chemical bioavailability and the test species performance itself. However, most prediction studies are centred on a few soil properties influencing bioavailability, while their direct effects on test species performance are usually neglected. In our study, we develop prediction models for the toxicity values obtained in a set of soils taking into account both the chemical concentration and their soil properties.
Materials and methods The effects on the avoidance behaviour and on reproduction of the herbicide phenmedipham to the collembolan *Folsomia candida* is assessed in 12 natural soils and the Organisation for Economic Co-operation and Development (OECD) artificial soil. The toxicity outcomes in different soils are compared and explanatory models are constructed by generalised linear models (GLMs) using phenmedipham concentrations and soil properties.
Results and discussion At identical phenmedipham concentrations, the effects on reproduction and the avoidance response observed in OECD soil were similar to those observed in natural soils, while effects on survival were clearly lower in this soil. The organic matter and silt content explained differences in the avoidance behaviour in different soils; for reproduction, there was a more complex pattern involving several soil properties.
Conclusions Our results highlight the need for approaches taking into account all the soil properties as a whole, as a necessary step to improve the prediction of the toxicity of particular chemicals to any particular soil.

Keywords Avoidance · *Folsomia candida* · Phenmedipham · Reproduction · Soil properties

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1 Introduction

In ecotoxicological studies, even when the same species and pollutant are assessed, the toxicity observed can differ between soils. In addition to the variability due to test species individual variability (Crouau and Cazes 2003) or genetic differences (Diogo et al. 2007), soil properties are the key factors explaining these inconsistencies through their direct influence on a pollutant's bioavailability, as well as species performance itself.

Soil properties explain differences in chemical bioavailability in different soils (Lock and Janssen 2001b; Amorim et al. 2005a, b), as they determine the sorption equilibrium

of a pollutant between soil solid-phase and porewater, thus the pollutant's concentration in porewater, which is widely accepted to be the main exposure route in soil-dwelling species (Smit and Van Gestel 1998; Van Gestel and Koolhaas 2004; EFSA 2009). In addition, soil properties directly influence test species performance according to their ecological niches, as suggested in several studies (Amorim et al. 2005b, c, d; Römbke et al. 2006; Domene et al. 2011).

This inconsistency in the toxicity observed in different soils has partly been treated by the development of models which allow the extrapolation of toxicities from standard substrates to natural soils taking into account only some of the soil properties (organic carbon, pH or cation exchange capacity). However, the total influence of soil properties, including their effects on both chemical bioavailability and test species performance, is rarely used in the interpretation of results. The main reason for this omission is the existence of complex interactions between different soil properties, usually intercorrelated, which impedes the derivation of general guidelines to allow the toxicity extrapolation between soils.

In our study, we assess the influence of soil properties on the toxicity of the herbicide phenmedipham to the soil collembolan *Folsomia candida*. Phenmedipham has been proposed as reference substance to be tested on *F. candida* at least once a year for quality assurance of the results obtained in different laboratories (Fountain and Hopkin 2005). Our study has two main aims: (1) to compare the outcomes in the Organisation for Economic Co-operation and Development (OECD) artificial soil with those observed in natural soils in order to show their representativeness, and (2) to show how to cope with the variability of toxicity results in different soils through models derived from empirical data. This work can be taken as an exercise on how to improve the toxicity risk prediction for soils with known properties.

2 Materials and methods

2.1 Test organism

The test organism was the soil collembolan *F. candida* (Isotomidae), a species commonly used in soil ecotoxicity tests. Cultures were maintained in polyethylene containers 17.5×12.5×7.5 cm, filled with a 1 cm layer of a wet plaster of Paris and charcoal mixture (9:1, *v*/*v*). Cultures were kept in darkness in a climatic chamber at a constant temperature of 21 °C. The assay was performed in different soils according to ISO 11267 (ISO 1999), using 10 individuals aged 10–12 days old per replicate. The effects on survival and reproduction at increasing concentrations of the test chemical were assessed after 28 days of exposure.

2.2 Chemical

The tested chemical was phenmedipham, a phenylcarbamate herbicide also known as the commercial product Betosyp (Stähler Agrochemie, 157 g l^{-1} a.i.). Phenmedipham is a broadleaf herbicide that inhibits photosystem II (Abbaspoor and Streibig 2007), but toxic effects of phenmedipham have been reported for fishes, birds, mammals and aquatic invertebrates (EPA 2005), as well as soil invertebrates (Amorim et al. 2005a, b, c, d; Idinger et al. 2006; Kuperman et al. 2006).

Based on a preliminary assay, five different test concentrations were selected for each soil tested, which corresponded to phenmedipham concentrations of 0, 1.5, 3, 6, 12, 24, and 48 mg kg^{-1} in the avoidance tests, and 0, 0.75, 1.5, 3, 6, 12, 24, and 48 mg kg^{-1} in the reproduction tests.

2.3 Soils collection and preparation

Twelve natural soils were selected for this study, all from European Mediterranean regions: Alentejo (Portugal), Catalonia (Spain) and Liguria (Italy). Soils were mainly agricultural, without agrochemical treatments the last 5 years, and the remainder were collected from fallows and pastures. The main criterion for their selection was the obtention of a set of soils with widely-ranging physico-chemical and organic content properties. Topsoil samples (0–20 cm depth) were collected, 5-mm sieved, and air-dried. Then, soils were defaunated by two freezing–thawing cycles of −20 °C for 4 days followed by 4 days at 20 °C. OECD artificial soil was prepared according to OECD (1984) and used in order to compare their outcomes with those of natural soils.

Soil properties together with heavy metal contents shown in Table 1 were analysed by the methods reported in Domene et al. (2011). Soilwater content for the tests was adjusted in order to provide to each soil a moist and crumbly substrate, which corresponded to a range between 35 and 60 % of the maximum water-holding capacity (WHC). The reason for choosing a moisture content below 40 % of the WHC in some of the soils was to avoid a doughy structure in the more fine-textured soils. The moisture content in Table 1 corresponded to soil moisture at the beginning of the test. Water losses were negligible, since tests were carried out in sealed containers periodically aerated.

2.4 Experimental procedure

2.4.1 Avoidance tests

Tests were performed according to ISO (2010). Each experimental unit consisted of a translucent cylindrical container (7×6 cm) filled with two adjacent 30 g wet soil portions (control and test soil), each occupying half the container.

Table 1 Properties of the soils used to determine the effect of soil properties on the toxicity of phenmedipham to *F. candida*

Soil	Use	pH	Coarse sand	Fine sand	Silt	Clay	C	N	CEC	MaxWHC	%WHCav	%WHCrep	Moisture av	Moisture rep	Cd	Cr	Cu	Ni	Pb	Zn
		Soil/ water, 1:5	%	%	%	%	%	%	meq/ 100 g	%	%	%	%	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
OECD	Artificial soil	7.0	9.7	76.9	2.7	10.7	3.36	0.03	7.0	63.1	59.4	55.5	37.5	35.0	0.1	8.0	20.0	3.0	10.0	15.0
BR	Fallow	7.6	10.0	17.9	23.6	48.5	1.45	0.11	26.8	61.1	39.9	40.0	24.4	24.4	<5.6	67.0	46.0	66.0	18.0	55.0
GAN	Vineyard	8.3	1.5	74.5	12.0	12.0	0.35	0.04	6.0	37.6	37.0	36.2	13.9	13.6	<0.1	16.0	20.0	32.0	11.0	32.0
GRA	Olive field	8.2	2.1	25.7	48.5	23.7	0.99	0.11	14.2	49.8	35.9	36.2	17.9	18.1	0.2	19.0	26.0	28.0	10.0	42.0
IT2	Agricultural	7.7	23.6	15.7	44.4	16.3	2.78	0.25	18.6	39.8	52.3	50.0	20.8	19.9	0.6	61.0	172.0	48.0	59.0	170.0
IT3	Fallow	7.7	23.1	28.1	36.4	12.4	1.62	0.16	18.4	43.3	50.1	50.0	21.7	21.7	<0.1	71.0	48.0	54.0	18.0	76.0
IT4	Fallow	7.9	20.2	29.1	34.7	16.0	1.62	0.13	18.8	47.4	50.0	50.0	23.7	23.7	<0.1	67.0	34.0	54.0	21.0	76.0
LIT	Fallow	5.2	41.9	24.8	21.6	11.7	2.44	0.16	8.6	42.4	50.0	50.0	21.2	21.2	<5.6	21.0	40.0	48.0	16.0	67.0
LUV	Pasture	5.5	29.8	38.2	20.3	11.3	1.16	0.08	9.9	32.1	49.9	50.0	16.0	16.0	<5.6	24.0	23.0	28.0	19.0	54.0
POR	Vineyard	6.9	46.2	21.4	20.5	11.9	2.49	0.22	18.6	38.8	36.9	36.9	14.3	14.3	0.2	67.0	92.0	46.0	147.0	420.0
PRA	Grainfield	5.1	42.4	35.0	12.1	10.6	1.28	0.12	11.2	39.4	38.1	32.2	15.0	12.7	<0.1	13.0	38.0	5.5	40.0	86.0
PZ	Pasture	5.3	69.8	21.3	5.8	3.2	1.28	0.07	4.0	30.7	50.5	50.0	15.5	15.4	<5.6	<16	<15	<28	7.0	6.0
RIU	Grainfield	7.3	23.7	34.9	13.8	27.6	1.10	0.13	14.9	45.0	34.7	33.8	15.6	15.2	<0.1	22.0	26.0	18.0	19.0	64.0

The soils were collected in three European Mediterranean regions: Alentejo (BR, LIT, LUV and PZ), Catalonia (GAN, GRA, POR, PRA, RIU), and Liguria (IT2, IT3 and IT4). All values referred to dry matter

Coarse sand 2–0.2 mm, *fine sand* 0.2–0.02 mm, *silt* 0.02–0.002 mm, *clay* <2 μm, *C* organic carbon, *N* total nitrogen, *CEC* cationic exchange capacity, *MaxWHC* maximum water-holding capacity, *%WHC av* soil moisture in avoidance tests expressed as percent of the maximum WHC, *%WHCav* soil moisture in avoidance tests expressed as percent of the maximum WHC, *%WHCrep* soil moisture in avoidance tests expressed as percent of the maximum WHC, *Moisture av* soil moisture in avoidance tests, *Moisture rep* soil moisture in reproduction tests

Then, 20 *F. candida* individuals (10–12 days old) were transferred to the centre of the container, and left under controlled climatic conditions for 48 h (20±2 °C and 16:8 h light/dark photoperiod). After this period, each soil portion was taken separately, poured into a 200-mL Erlenmeyer flask, and flooded with water. Soil was gently stirred in order to force the individuals to float on the water surface and enable counting.

For each of the soils, all of the combinations of the unpolluted soil (control soil, left side) and each of the different test concentrations (test soil, right side) were prepared. The main interest of these tests was determining the influence of phenmedipham together with soil properties on the avoidance behaviour of this species. In order to determine if the individuals' distribution was affected by factors other than soil properties, we carried out control-dual tests, where both portions were composed of the same soil (Hund-Rinke and Wiechering 2001). Both for the control-dual tests and the avoidance tests, five replicates were prepared for each pairwise comparison of soils.

2.4.2 Reproduction tests

28-Day reproduction was determined in the 12 natural soils and in OECD artificial soil according to the ISO Guideline 11267 (ISO 1999). Five replicates were prepared for each soil, consisting of a wet soil (30 g dry weight) in a sealed 150-mL glass flask. The test ran for 28 days under constant climatic conditions (20±2 °C and 16:8 h light/dark photoperiod). At the start of the test and the 14th day, 3 mg of granulated yeast were added to each replicate as a food source. At the end of the test period, soil was poured into a 200-mL Erlenmeyer flask and flooded with water and stirred in order to float the individuals on the water surface. After that, a picture was taken in order to count the adults and juvenile collembolans by the image the analysis software ImageTool 3.0 (University of Texas, Health Science Center, San Antonio, TX, USA).

2.5 Statistical treatment

2.5.1 Avoidance tests

The avoidance rate was assessed in each experimental unit, calculated by the equation $A = [(C - T)/N] \times 100$, where A = percent avoidance, C = number of individuals in the control soil, T = number of individuals in the test soil, and N = total number of individuals, as described in the draft of ISO 17512 (ISO 2007). A positive value indicates avoidance of the test soil, a value of zero indicates equal distribution in both sides, and a negative value indicates that individuals are attracted by the test soil.

Using the avoidance rate for each replicate, we calculated, for each soil, the avoidance median effective concentration values (EC50) and their 95 % confidence limits by probit regression (Minitab version 13.2, State College, PA, USA). A normal or a logistic distribution was assumed based on the Kolmogorov–Smirnov normality test. The positive avoidance values were used for regression, while the negative avoidance values (lack of avoidance) were also included in the regression but transformed to zero.

In order to relate avoidance behaviour to the phenmedipham concentrations together with soil properties, we constructed a regression model through generalised linear models (GLM; Brodgar version 2.5.2, Highland Statistics Ltd, Newburgh, UK). In this model, the response variable was the number of individuals in test soil, while the explanatory variables were phenmedipham concentration and known soil properties. The inclusion of the chemical concentration as explanatory variable in the model allowed the prediction of response at a given concentration in any particular soil whose soil properties were in the range of the set of soils used to derive the model. To our knowledge, this approach has never been used, since typically IC50 values are used as response without including phenmedipham toxicity as explanatory variable (e.g. Son et al. 2009), an approach which allows the comparison of the potential toxicity of the chemical in different soils but not the prediction of the actual effects.

The explanatory variables showing high intercorrelation were not used for the model construction (those showing correlation coefficient >0.8 or VIF >10). The explanatory variables retained were used for the model construction assuming a Poisson distribution and using logarithm as link function. After different trials, the model containing the variables with the best adjustment to our data was obtained by an automatic backward selection procedure. We assumed that models with a low value of Akaike information criteria (AIC) were most suitable. The suitability of the model was evaluated by the assessment of the homogeneity of the residuals (visual checking of the residuals versus fitted values), and their normality (by means of a normal Q–Q plot).

2.5.2 Reproduction tests

Median lethal concentration (LC50) and reproduction median effective concentration (EC50) were calculated for phenmedipham in each soil type using Statistica 6.0 (Stat Soft, Inc., Tulsa, OK, USA). These values and their 95 % confidence intervals were calculated from suitable regression models (Gompertz, hormesis or linear), based on best fit.

The assessment of the phenmedipham concentrations and the main soil properties influencing the reproduction of

F. candida was done by the construction of a regression model through GLM with Brodgar 2.5.2 (Highland Statistics Ltd, Newburgh, UK). In this model, we used as response variable the number of juveniles in the replicates of each test concentration. In addition, we also constructed another model using the percent of reproduction (percent of juveniles compared to that in controls) as response variable. In both cases, the explanatory variables were phenmedipham concentration and known soil properties. The reason for using both the absolute number of juveniles and the percent of reproduction as response variables was to compare the resulting models. This comparison allows the identification of properties which influenced both the bioavailability of phenmedipham and thus the direct impact on the reproduction outcome (model based on the number of juveniles) and soil properties influencing bioavailability (model based on percent of reproduction). The percent of reproduction was taken as a standardised measure of reproduction (based on the reproduction in controls), allowing the construction of a model that minimises the direct influences of soil properties on reproduction and maximises the influence on phenmedipham's bioavailability.

As before, the explanatory variables displaying high intercorrelation were not used for the models' construction, while the explanatory variables retained were then used for the models construction assuming a Poisson distribution and using logarithm as link function. Using an automatic backward selection procedure, we selected the model containing the variables with the best adjustment to our data, which corresponded to that with lower value of AIC. The model suitability was assessed by the homogeneity and normality of the residuals.

3 Results

3.1 Avoidance tests

The number of dead or missing individuals during the assay was generally below 10 % in all treatments, fulfilling the validity criteria of the ISO Guideline 17512-2 (ISO 2010). The only the exception was PRA soil, with 24–38 % of the initial individuals absent in the different test concentrations, and agreeing with the results in reproduction tests, where all the individuals died after a month. This outcome suggested unreported pesticide or fertiliser application in this agricultural soil, something that made us exclude this soil from further statistical analysis. The EC50 values for effects for avoidance are shown in Table 2.

As a general rule, avoidance was observed in all the soils tested. In most soils, an attraction to the polluted soil was observed in the lowest phenmedipham concentrations as indicated by their negative avoidance rate. However, at higher concentrations, collembolans showed higher avoidance rates with increasing phenmedipham concentrations (Fig. 1).

Table 2 Phenmedipham toxicity values in *F. candida* in different soils, expressed as milligram per kilogram and with the 95% confidence intervals shown between brackets

Soil	EC50 avoidance	EC50 reproduction	LC50
OECD	9.23 (8.60, 9.87)	8.04 (6.14. 10.4)	>24
BR	8.00 (7.67, 8.36)	6.12 (5.42. 6.89)	6.46 (4.39. 9.35)
GAN	5.28 (5.13, 5.45)	2.50 (2.15. 2.90)	5.14 (4.33. 6.94)
GRA	10.8 (10.4, 11.2)	6.25 (4.84. 7.99)	8.45 (7.18. 9.92)
IT2	11.8 (11.3, 12.4)	8.02 (6.57. 9.75)	>12
IT3	9.18 (8.50, 9.86)	5.10 (3.89. 6.61)	>12
IT4	10.0 (9.65, 10.5)	6.19 (5.37. 7.12)	8.59 (0.52. 59.5)
LIT	15.2 (14.5, 15.9)	10.4 (8.45. 12.7)	>12
LUV	7.25 (6.83, 7.70)	8.69 (7.57. 9.96)	>12
POR	15.1 (14.5, 15.7)	7.74 (6.14. 9.70)	>12
PRA	12.7 (11.6, 13.9)	–	–
PZ	7.27 (6.38, 8.13)	7.21 (5.35. 9.61)	>12
RIU	8.36 (7.88, 8.86)	6.45 (5.19. 7.97)	>12

See Table 1 for soil abbreviations

According to the GLM model, the number of individuals in the test soil was significantly influenced by the phenmedipham concentration, soil moisture expressed as percent of the WHC (%WHC), and silt and carbon content (Table 3). The model was described by the equation $\text{Number of individuals}(T) = e\text{^}[(2.518 - (0.102 \times \text{phenmedipham}) - (0.011 \times \%\text{WHC}) + (0.004 \times \text{silt}) + (0.213 \times C)]$, where T is the number of individuals in test soil. The model was able to explain 60 % of the variance in the avoidance response ([(null deviance−residual deviance)/(null deviance)]×100).

3.2 Reproduction tests

Concerning the validity of reproduction tests, mortality was below 20 %, the number of juveniles in controls was above 100 and the variation coefficient was always below 30 % in the controls of all soils, fulfilling the validity criteria. The only exception was PRA soil, where all the original individuals died. As already mentioned, we excluded this soil from further statistical analysis.

Reproduction was inhibited with increasing phenmedipham concentrations, with hormesis in the lowest concentrations in some of the soils (Fig. 2). The LC50 for phenmedipham was above 12 mg kg^{-1} in most soils, and above 24 mg kg^{-1} in OECD soil. LC50 values below 10 mg kg^{-1} were observed in BR, GAN, GRA, and IT4 soils. Regarding phenmedipham's EC50 values for reproduction, most soils presented values ranging from 5 to 10 mg kg^{-1}, while GAN presented an EC50 of 2.5 mg kg^{-1} (see Table 2).

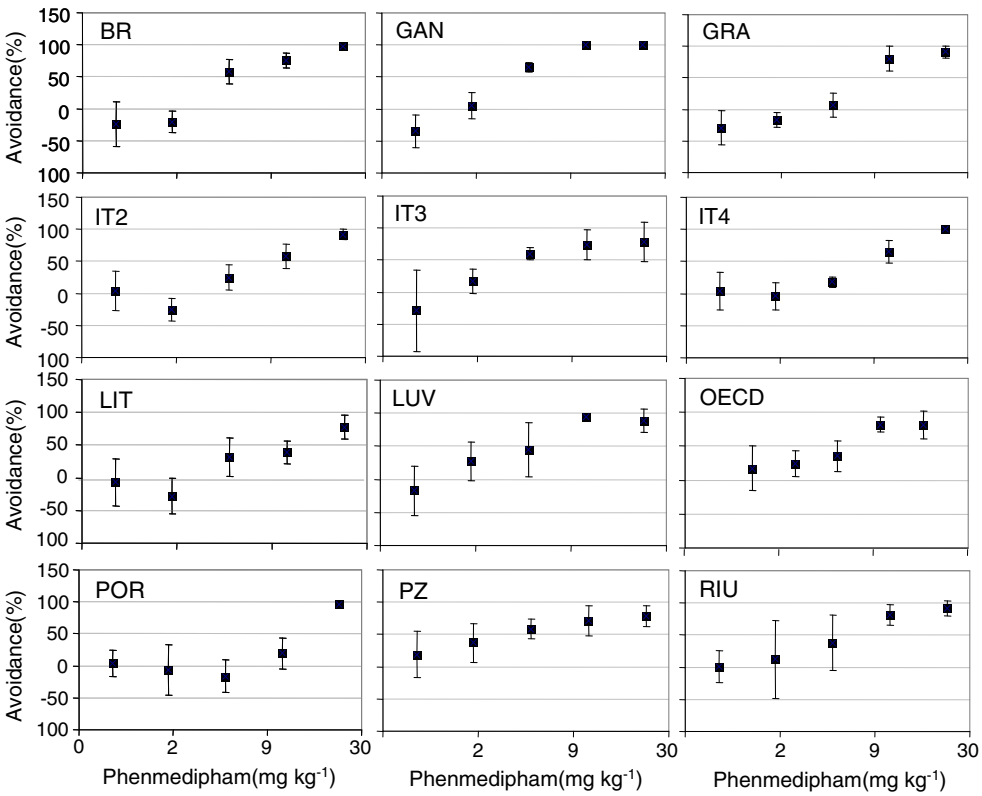

Fig. 1 Avoidance (%) of *F. candida* to increasing phenpedipham concentrations in different soils. Avoidance= $[(C-T)/N]\times 100$, where C = number of individuals in the control soil, T = number of individuals in the test soil, and N = total number of individuals. *Bars* standard deviation

According to the GLM model, the number of juveniles was significantly influenced by the phenmedipham concentration, moisture, pH, coarse sand, silt, clay, and total nitrogen contents, C/N ratio and cation exchange capacity (CEC; Table 4). The model was described by the equation: Number of juveniles $= e^{\wedge}[(7.518) - (0.142 \times \text{phenmedipham}) + (0.062 \times \text{moisture}) - (0.283 \times \text{pH}) - (0.017 \times \text{coarsesand}) + (0.004 \times \text{silt}) - (0.026 \times \text{clay}) - (1.211 \times N) + (0.005 \times C/N) + (0.049 \times \text{CEC})]$. The model explained 72 % of the variance of the reproduction response expressed as number of juveniles.

Concerning the model derived for reproduction (the number of juveniles in controls), this parameter was influenced by the phenmedipham concentration, pH, coarse sand, silt, clay and total nitrogen content, and C/N ratio (Table 5). The model was described by the equation: Reproduction(%) $= e^{\wedge}[(4.713) - (0.142 \times \text{phenmedipham}) \quad -(0.056 \times \text{pH}) + (0.003 \times \text{coarsesand}) + \quad (0.004 \times \text{silt}) + (0.006 \times \text{clay}) + (0.331 \times N) + (0.003 \times C/N)]$. The model for the reproduction response, expressed as the percent of reproduction, explained 70 % of the variance.

Table 3 Model fit of the generalised linear model (using a Poisson distribution) and numerical output for the avoidance behaviour of *F. candida* to phenmedipham, expressed as the number of individuals in the polluted soil side

	Estimate	Standard error	*t* Value	*p* Value
Intercept	2.518	0.161	15.60	<0.001
Phenmedipham	−0.102	0.005	−21.41	<0.001
%WHC	−0.011	0.004	−2.814	0.005
Silt	0.004	0.002	0.015	<0.001
Carbon	0.213	0.033	6.497	<0.001

Null deviance: 1,318.04 on 324 *df*

Residual deviance: 560 on 320 *df*. AIC: 1558

4 Discussion

4.1 Fate of phenmedipham in the environment

Phenmedipham, applied as herbicide, is released into the environment in sprays, concentrates, or other routes of application, directly or indirectly by dry or wet deposition. Phenmedipham remains in the top layers of soil after herbicidal applications and has a low leaching potential (Laitinen et al. 2006; NLM 2012).

Phenmedipham is a xenobiotic compound but that be easily degraded by some soil bacteria and fungi (Bellinck and Mayaudon 1979; Knowles and Benezet 1981; Bellinck and Mayaudon 1983a, b; Pohlenz et al. 1992), but also by chemical hydrolysis and photolysis (NLM 2012). At recommended application rates, the half-life of phenmedipham is about 23–39 days (Tena et al. 1982; WSSA 1989; Wauchope et al. 1992). Higher persistence has been shown in a slightly acidic soil with low humus content (50 % remaining

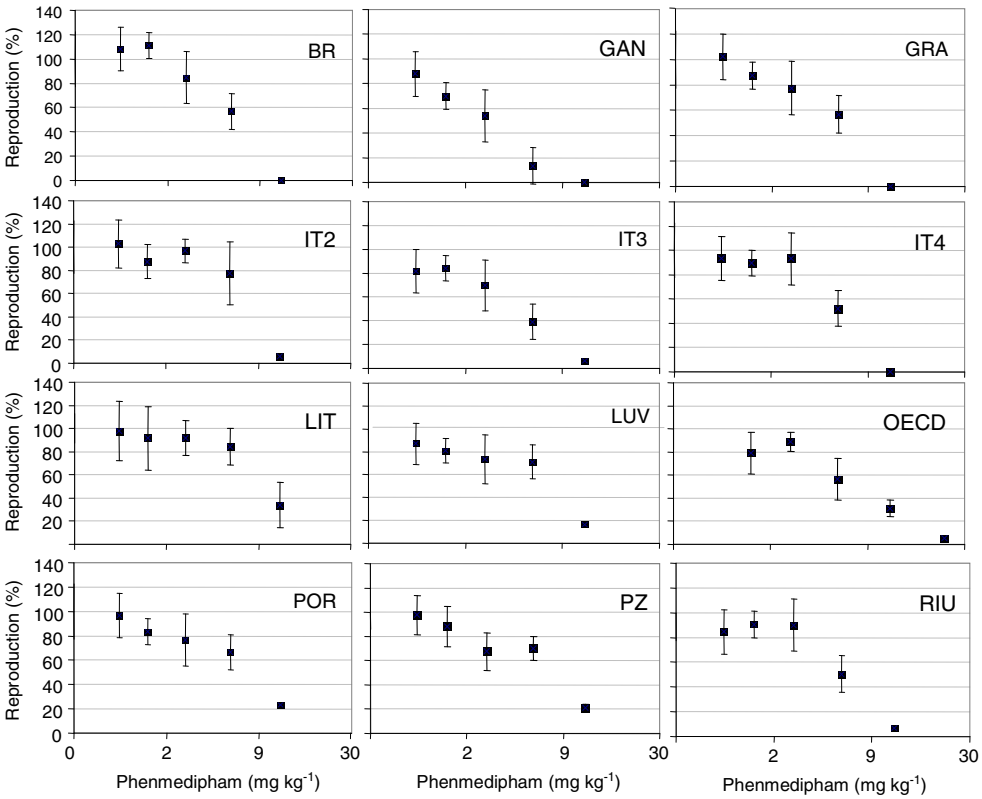

Fig. 2 Reproduction of *F. candida* with increasing phenmedipham concentrations in different soils, expressed as percent compared to the reproduction in controls. *Bars* standard deviation

after 28–55 days; Kossmann 1970), while lower persistence has been described in alkaline soils (11 % after 32 days; Sonawane and Knowles 1971). The US Department of Agriculture's Pesticide Properties Database lists for this pesticide a soil half-life of 30 days, but the rate can be slower in acidic soil or faster in alkaline soil (NLM 2012). Degradation is also enhanced with the addition of an organic matter source to soil (Bellinck and Mayaudon 1983c). According to the information available, the exposure to phenmedipham was ensured during the whole experimental time both in the avoidance and reproduction tests.

4.2 Representativeness of results from OECD artificial soil

Ecotoxicological soil studies are usually performed in standard soils such as the artificial soil OECD soil or the natural German soil LUFA 2.2, with soil properties generally different than those in natural soils. Hence, toxicity results obtained in

Table 4 Model fit of the generalised linear model (using a Poisson distribution) and numerical output for the reproduction of *F. candida* expressed as the number of juveniles in soil polluted with phenmedipham according to different soil properties

	Estimate	Standard error	*t* Value	*p* Value
Intercept	7.518	0.0427	175.9	<0.001
Phenmedipham	−0.142	0.0008	−169.4	<0.001
Moisture	0.062	0.0014	45.84	<0.001
pH	−0.283	0.0048	−59.03	<0.001
Coarse sand	−0.017	0.0004	−43.27	<0.001
Silt	0.004	0.0004	9.302	<0.001
Clay	−0.026	0.0005	−52.48	<0.001
Nitrogen	−12.11	0.0551	−22.00	<0.001
C/N	0.005	0.0001	41.39	<0.001
CEC	0.047	0.0010	48.97	<0.001

Null deviance: 73,979 on 359 *df*

Residual deviance: 20,443 on 350 *df*. AIC: 23113

Table 5 Model fit of the generalised linear model (using a Poisson distribution) and numerical output for the reproduction of *F. candida* expressed as the rate of juveniles produced (compared to control soil) in soil polluted with phenmedipham according to different soil properties

	Estimate	Standard error	*t* Value	*p* Value
Intercept	4.713	0.0893	52.801	<0.001
Phenmedipham	−0.142	0.0022	−65.210	<0.001
pH	−0.056	0.0101	−5.560	<0.001
Coarse sand	0.003	0.0007	3.427	<0.001
Silt	0.004	0.0009	4.434	<0.001
Clay	0.006	0.0007	8.625	<0.001
Nitrogen	0.331	0.1342	2.470	0.013
C/N	0.003	0.0003	8.817	<0.001

Null deviance: 8,944.4 on 359 *df*

Residual deviance: 2,719.1 on 352 *df*. AIC: 4761

the laboratory with standard soils might strongly differ from those obtained from natural soils, and even more with respect to studies carried out at field conditions. This is why regulatory agencies have began to emphasise the importance of assessing chemical toxicity in natural soil types instead of artificial soil (Kuperman et al. 2006).

Several studies have pointed to the underestimation of toxicity of metals when the OECD artificial soil is used compared to natural soils (Spurgeon and Hopkin 1995; Lock and Janssen 2001a, b), but also for some organic pollutants (Amorim et al. 2005a, c, d). Other authors have indicated similar toxicities to those obtained in natural soils (Martikainen 1996). The main reason is the high organic content, fine texture, and high CEC of OECD and other similar artificial soils, which results in a lower pollutant bioavailability (Boyd and Williams 2003; Crouau and Tan Tchiam 2006). For this reason, some authors have suggested reducing the peat content of the OECD from 10 to 5 % in order to increase its field relevance (Amorim et al. 2005c). As an example, the mite reproduction test (OECD Guideline 226) uses only 5 % peat (OECD 2008). Furthermore, besides its contrasted soil properties with respect to natural soils, it has been indicated that OECD soil is not completely representative of real situations because it contains an uncommon clay mineral in natural soils (kaolin) that lacks of aluminium, iron, and manganese oxides, which are important in the bioavailability of metals (Römbke et al. 2006). On the contrary, other studies have suggested an overestimation of heavy metals toxicity in OECD compared to naturally polluted soils due to ageing processes acting in these soils (Spurgeon and Hopkin 1996; Fountain and Hopkin 2004).

In our study, the mortality caused by phenmedipham in OECD soil was lower compared to natural soils. The same trend was generally observed for reproduction, where OECD showed an EC50 of 8 mg kg^{-1}, below the values reported in similar studies, ranging from 13.3 to 50 (Idinger et al. 2006; Diogo et al. 2007), but still within the range of the set of natural soils tested in our study. In avoidance tests, the avoidance behaviour to phenmedipham, however, appeared at lower concentrations in OECD soil (see Table 2). These contradictory results can only be attributed to the different mechanisms influencing each biological endpoint.

It should be remarked that these apparent biases are small for avoidance and reproduction, since the values obtained in OECD soil are always within the range of toxicity values observed in the natural soils studied. The only exception was where the phenmedipham effect on survival in OECD soil was clearly lower compared to the other soils (over 24 mg kg^{-1}).

In addition, there is a positive correlation between EC50 values for avoidance and reproduction (Pearson, r=0.630, p=0.028), agreeing with the proposed use of the avoidance test with this species as early screening tools (Natal-da-Luz et al. 2004).

4.3 Influence of soil properties in test results

The toxicity observed in soil ecotoxicological tests is strongly influenced by soil properties through their direct effects on the bioavailability of chemicals but also by the ecological preferences of the test species. Soils far from the ecological requirements of the test species could overestimate the toxicity of the chemical since the toxic stress adds to the stress derived from an unsuitable environment (Højer et al. 2001). Several studies have shown how test species performance change in different soils according to their ecological preferences (Van Gestel et al. 1992; Sandifer and Hopkin 1996; van Gestel and van Diepen 1997; Crouau et al. 1999; Greenslade and Vaughan 2003; Jänsch et al. 2005; Amorim et al. 2005b,c, d; Römbke et al. 2006; Domene et al. 2011).

It has been suggested that in collembolans, the uptake process is mainly associated with solid soil phases, in contrast to soft-bodied oligochaete species and plants, which are more strongly influenced by porewater characteristics (Vijver et al. 2001). Other studies have supported the validity of the soil porewater hypothesis for *F. candida* (Martikainen and Krogh 1999; Lock and Janssen 2003). In the context of the derivation of general rules for the extrapolation of toxicity data between soils, the establishment of cause-and-effect relationships between bioavailability and soil properties is impaired by the fact that most soil properties are intercorrelated. As an example, CEC is generally related to pH, as cation-exchange sites are pH dependent, but it is also related to clay and organic carbon content, because they contain cation-exchange sites (Dayton et al. 2006). It is widely accepted that pH, CEC, and clay and organic matter content are the most important soil parameters affecting the toxicity of pollutants (Van Gestel et al. 1995; Lock et al. 2000; Boyd and Williams 2003; Simini et al. 2004). However, for each pollutant, and combinations of soil properties, the main influencing properties may differ (Van Gestel 1997; Peijnenburg et al. 1999). Thus, approaches assessing the influence of all soil properties have not been used to date.

In our study, we assessed the influence of soil properties on the toxicity of phenmedipham to *F. candida* through an integrated approach which took into account all the soil properties as a whole by means of generalised linear models. Different results were obtained in 48 h avoidance tests and 28-day reproduction tests.

In the avoidance tests, we found that avoidance was higher at higher phenmedipham concentrations and soil moisture (expressed as %WHC), while it was lower at high silt and carbon contents in the tested soils. The response to increasing concentrations of phenmedipham is obvious since this species is able to avoid polluted soil over a certain threshold. It is also meaningful that the observed lower avoidance to phenmedipham occurred in soils with an expected lower bioavailability, since fine-textured soils

(Simini et al. 2004) and also those with high organic content (Martikainen 1996) show a lower toxicity of organic pollutants. On the contrary, the higher avoidance in soils with higher moisture is difficult to explain, and contradicts the known significant role of soil moisture in avoidance behaviour of this species (Domene et al. 2011), but also given the low solubility in water of this chemical (4.7 mg l^{-1} at 25 °C). In any case, these are the most influential properties, since they explained most of the variation in the avoidance behaviour of the set of soils studied.

A lower number of juveniles were produced at higher phenmedipham concentrations, pH, coarse sand, clay, and total nitrogen content. A higher number of juveniles were produced at increasing soil moisture, silt content, C/N ratio, and CEC. The positive effect on reproduction of moisture and the lower offspring in soils with very coarse/fine texture in this species is in accordance with a previous study carried out by the authors using a wider set of soils (Domene et al. 2011). The model derived for reproduction is integrating both the indirect influence of soil properties on reproduction through the bioavailability and the direct influence on reproduction. However, if we look at the model developed for the percent of reproduction, the direct influence should have been removed, since it is a standardised measure of reproduction, expressed with respect to the reproduction in controls, and hence should only reflect the influence on bioavailability. When the percent of reproduction is used as response variable, the model did not change importantly, since lower percent of reproduction were observed at higher phenmedipham concentrations and pH values. On the other hand, lower percent of reproduction were observed at lower coarse sand, silt, clay, and total nitrogen content and C/N ratio. This suggests that the changes in the toxicity observed are mainly related to changes in the bioavailability of phenmedipham rather than the direct effects of soil properties on the test species. This also agrees with the consideration of this species as relatively insensitive to soil properties (Jänsch et al. 2005).

The fact that organic matter content did influence avoidance to phenmedipham but not reproduction is surprising given its known influence on decreasing the bioavailability and toxicity of other organic chemicals (Martikainen 1996; Martikainen and Krogh 1999; Phillips et al. 2002; Simini et al. 2004). The reason might be a phenmedipham degradation which was faster than expected according to the available literature, which have reported half-degradation times around 28–55 days (Kossmann 1970), less in alkaline soils according to NLM (2012). Hence, the phenmedipham loss during the 28-day reproduction test could have hidden the influence of organic matter in avoidance tests. In addition, it has been shown for this species that higher C/N values were associated with a lower toxicity of phenmedipham (Amorim et al. 2005b), which is in agreement with the results from our study.

Regarding soil pH, it has been shown that decreasing pH enhances the toxicity of some organic chemicals (Phillips et al. 2002), but we found the opposite trend for phenmedipham, with higher toxicity with increasing alkalinity. This relationship is strong, but goes against the known easier degradation of this chemical when soil pH is alkaline (NLM 2012). Phenmedipham's half-life in aqueous solution ranged from 7.5 days to 1.8 h at pHs of 6 and 8, respectively (WSSA 1989). This apparent contradiction could be explained by the limited range of pH covered by the soils used on this study, mostly alkaline (only three soils were acidic, with pH around 5.3).

The total nitrogen content is also very influential: it decreased the number of juveniles but increased percent of reproduction. The direct influence of nitrogen on individual performance has been suggested in literature for soil fauna after amendments with organic or nitrogenated fertilisers (Neher 1999; Seniczak et al. 1994; Domene et al. 2007), but the influence on bioavailability in this study remains unclear.

Regarding the influence of texture, it is widely accepted that fine-textured soils generally show lower toxicity to heavy metals (Lock and Janssen 2001a) and organic pollutants (Simini et al. 2004) due to a lower bioavailability. However, in our study, the toxicity for reproduction, measured as the number of juveniles, was higher in soils with extreme textures (high percentage of coarse sand or clay content). This result agrees with the suggested negative influence of extreme textures in the number of juveniles produced in an unpolluted soil (Natal-da-Luz et al. 2008). However, when toxicity was measured as percent of reproduction, this trend disappeared, and coarse sand, silt, and clay contents appeared associated with a lower toxicity, but their low estimate values in the model suggests a low influence of texture in the final outcome.

There is a positive influence of moisture on the number of juveniles produced, but any influence disappears when the percent of reproduction is assessed. This agrees with the known influence of this parameter in the reproduction of collembolans (Pedersen et al. 1997; Crouau et al. 1999), and also with its low influence on bioavailability if maintained in the range of tolerance of this species (Van Gestel and Van Diepen 1997). Moisture could only have a negative influence if it would be below the tolerance limit, i.e. when a synergistic interaction between the toxic chemical and drought stress appears (Bauer and Römbke 1997; Van Gestel and van Diepen 1997; Højer et al. 2001).

CEC did not influence the percent of reproduction, suggesting a low influence on the bioavailability of phenmedipham of this parameter, as expected due to the neutral charge of this chemical, in contrast to heavy metals. Unexpectedly, a positive influence of CEC on the number of juveniles produced was observed, something that can only be an artefact.

In order to ensure the validity of the models derived in this study, the significant correlation between the predicted and the observed toxicity values should be assessed in a different set of soils, something that we did not address given the scope of our study.

5 Conclusions

In toxicity tests using *F. candida* as test species and phenmedipham as test chemical, the validity of OECD soil as a surrogate of natural soils was evaluated as acceptable for avoidance and reproduction tests. However, concerning effects on survival, toxicity was clearly lower in OECD soil. This suggests that results obtained using this substrate for the extrapolation of effects to natural soils should be interpreted with care and that its use should be restricted to standardisation purposes.

Avoidance test results correlated with those in reproduction tests, thus demonstrating the usefulness of avoidance tests with this species as an early screening tool. In avoidance tests, phenmedipham presented lower effects in soils with higher carbon and silt contents, probably due its lower bioavailability. This pattern, found in the 48-h avoidance tests, disappeared in the 28-day reproduction tests, in which many other soil properties were influencing the results. More precisely, reproduction was mainly affected by the pH and total nitrogen content of the tested soils.

The results from our study showed that it is difficult to predict the toxicity of chemicals if only based on few soil properties. At the same time, the simultaneous consideration of all soil properties added variables to the explanatory models that are sometimes difficult to interpret. Approaches taking into account soil properties as a whole are necessary to increase our understanding of these complex interactions, which is the basis of an improved prediction of the toxicity of a chemical in soil.

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