The use of *Folsomia candida* (Collembola, Isotomidae) for the bioassay of xenobiotic substances and soil pollutants

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**Abstract**

The impacts of cadmium, pentachlorophenol, phenanthrene and of two artificially polluted soils, on reproduction by the collembolan *Folsomia candida* were studied using a test that is in the process of becoming a European standard. The first artificially polluted soil was contaminated with metals (cadmium, chromium, lead and zinc), the second with organic chemicals (pentachlorophenol, trichlorophenol and phenanthrene). The EC50 values for reproduction were 129, 87 and 175 μg/g, respectively, for cadmium, pentachlorophenol and phenanthrene. Additional experiments dealing with the effects of soil pH and humidity, and of reproduction timing were carried out. A number of problems concerning the experimental conditions and the soil (soil moisture and pH) are discussed. It is concluded than the *F. candida* reproduction test could be a suitable ecotoxicological test for soil with some technical improvements in relation to organic matter content and test duration, and with more precise specification of the pre-normative ISO guidelines on soil humidity and structure. Further adaptation of the test for soil toxicity evaluation is needed.

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**1. Introduction**

Progress in soil ecotoxicology has fallen far behind aquatic ecotoxicology, particularly in relation to testing protocols. Evaluating the toxicity of a substance or mixture of substances using these tests usually consists of exposing a biological model and quantifying the toxic effects on one or several parameters related to, for instance, the biology, biochemistry or physiology of the model. In the words of Giesy and Graney (1989), ‘the ultimate goal of toxicity testing is to monitor or predict the effects of single compounds, elements or mixtures on the long-term health of individual organisms, populations, communities or ecosystems.’ Numerous ecotoxicological tests have been developed for aquatic environments using bacteria (Microtox test, Büllich and Isenberg, 1981), protozoa (rotifer test, Couillard et al., 1987), algae (*Selenastrum capricornutum* test, Steeman-Nielsen, 1952), and animals (nematode test, Samoiloff et al., 1980; daphnia test, Baird et al., 1989) as biological models. Some of these tests are now used as standards and have been adopted for routine use in the assessment of water pollution (algal growth test: ISO, 1989; daphnia reproduction test: OECD, 1981). However, very few ecotoxicological tests using soil animal models have been developed. A single test has been standardised for use in Europe: the mortality test on the earthworm *Eisenia fetida* (OECD, 1984). This test
is not sufficient to evaluate ecotoxicity towards the soil fauna for at least two reasons: firstly, it is a test of acute toxicity and is thus generally less sensitive than a chronic toxicity test, and secondly, earthworms only represent part of the soil fauna. Moreover, since *E. fetida* is not a true soil dweller, results obtained with this species may not be applicable to earthworms generally. It would be particularly useful to have one or more tests based on soil arthropods. Since arthropods, arachnids, insects, myriapods and terrestrial crustaceans make up a large proportion of the soil fauna it would be useful to know what the impacts of pollutants on these animals are.

Various ecotoxicological tests based on the use of soil arthropods have been proposed (Fisher et al., 1994; Løkke et al., 1994; Secofase, 1994; van Gestel and Doornekamp, 1994). One of the most advanced uses the collembolan *Folsomia candida* (Isotomidae, Collembola) and the parameter used to assess the effects is reproduction (Riepert, 1993; Riepert and Kula, 1996). This springtail is one of the most abundant soil arthropods; its diet is mainly composed of litter, fungi and bacteria, and it reproduces by parthenogenesis. Krogh and Petersen (1995) have shown that reproduction is a more sensitive parameter and supplies more information than mortality. Ring tests have been performed between various European laboratories with this ecotoxicological test (Riepert and Kula, 1996).

We used the *F. candida* reproduction test to evaluate the toxicity of three different types of xenobiotics: a heavy metal, cadmium; two organic compounds, pentachlorophenol and phenanthrene; and two polluted soils. In the light of the results from these tests and from some additional experiments on hatching kinetics, fertility variations and effects of pH and moisture, we propose some modifications to the experimental protocol and suggest precautions that should be taken to improve its reliability and sensitivity and reduce or eliminate certain problems.

2. Materials and methods

2.1. Test organisms

Adult *F. candida* to start a culture were provided from the Biologisches Bundesanstalt für Land- und Forstwirtschaft Institut im Pflanzenschutz, Berlin. The animals used in the test were mass-bred in plastic boxes containing a regularly dampened charcoal/plaster mixture, kept in darkness at about 20°C, and fed on baker’s yeast. The test required juveniles 10–12 days old; 10 animals were used per experimental glass container (100 ml capacity) and six or seven containers per contaminant concentration. As these small animals cannot be handled directly, they were transferred from the hatching boxes to the experimental pots by means of a suction device.

2.2. Experimental soils

The experimental soil used for the toxicity tests corresponds to the ISO standard 11268-1, that is, it was composed of 70% quartz sand with more than 50% of the particles having a size of between 0.05 and 0.2 mm, 20% kaolinite clay, and 10% peat ground, dried and sieved to 0.05 mm and CaCO₃ added to adjust the pH (KCl) to 6 ± 0.5%. It was moistened to 53% of its water-holding capacity (WHC measured using protocol ISO 11274). CdCl₂ was dissolved in an appropriate amount of distilled water to reach a soil moisture content of 53% of the WHC. For pentachlorophenol and phenanthrene (Sigma), the compounds were dissolved in an organic solvent (acetone, Prolabo, quality grade) and mixed with the soil before moistening. Organic solvent alone was added to the control soil. The solvent was then left to evaporate, and the full amount of moistening water added. The soil with its various components was then homogenised to obtain a crumbly structure. Five concentrations of each chemical compound were tested (Cd: 15, 30, 60, 120, 240 μg/g dry soil; pentachlorophenol: 38.5, 76.5, 153, 229.5, 306 μg/g dry soil; phenanthrene: 80, 100, 140, 220, 380 μg/g dry soil).

Two artificially polluted natural soils (luvisol, FAO classification) were also tested. The first (S1) was contaminated with metals (50 μg/g dry soil cadmium; 800 μg/g chromium; 800 μg/g lead; 2000 μg/g zinc), the second (S2) with organic chemicals (800 μg/g phenanthrene; 80 μg/g 2,4,6 trichlorophenol; 80 μg/g pentachlorophenol). The polluted soils were mixed with the ISO 11268-1 soil in various proportions (in percentage: 2.1; 4.2; 6.2; 8.3; 16.6 for S1 and 6.2; 12.5; 16; 25; 33; 50 for S2).
2.3. Experimental design of the *F. candida* reproduction test

The *F. candida* reproduction test consists of exposing juveniles to contaminated soil and comparing the rate of reproduction with that of animals placed in non-contaminated control soil. Once the animals are placed in the experimental pots, they are left for 33–34 days in a chamber maintained at 20 ± 2°C under lighting of about 400 lux with a 12/12 h photoperiod. The pots were opened twice a week for aeration and every 2 weeks for feeding with yeast.

At the end of the period of exposure to the toxic compound under test, water was added and, following thorough stirring, the animals floated to the top of the suspension where they were counted under the binocular microscope.

The Wilcoxon’s two-sample test was used to detect significant differences from the blank (LOEC: Lowest Observed Efficient Concentration); the EC50 values were calculated by means of the maximum likelihood–probit procedure (ToxCalc 5.0 software).

2.4. Effects of pH and of soil moisture on reproduction

Reproduction rates were compared for various pH values of the ISO soil, without added xenobiotic compounds. The test conditions and composition of the soil were the same as for reproduction trial blanks except for the CaCO3 content which varied between 0.1 and 0.6% to give soils with four different pH values: 4.2, 5.6, 6.5 and 6.9. This test was run in the same way as the chemical toxicity tests.

Reproduction rates were also compared for three levels of moisture in the soil tested. The test conditions and the composition of the soil were the same as for the reproduction trial blanks except for the quantity of water added to the soil which was 20, 24 or 28 ml for 100 g dry soil, that is, 37, 45 or 53%, respectively, of the WHC of the experimental soil. No xenobiotic compound was added in the ISO soil.

2.5. Hatching kinetics

An experiment was carried out to determine the mean period between hatching of an individual and that of the next generation in order to determine if the duration of the reproduction test was compatible with the period of the reproductive cycle. We placed juveniles (10–12-day-old) in four glass pots (20 juveniles per pot) with a layer of charcoal/plaster at the bottom and we counted the number of hatchlings every day for 6 weeks.

3. Results

3.1. Effects of chemicals on *F. candida*

The LOEC and EC50 values for reproduction were 120 μg/g (significance level \( p \leq 0.05 \)) and 87 μg/g (64–94, 95% confidence interval), respectively, for pentachlorophenol (Fig. 1) and 153 μg/g (\( p \leq 0.05 \)) and 129 μg/g (119–138) for cadmium (Fig. 2). For the effect of phenanthrene on reproduction (Fig. 3), the LOEC was 220 μg/g (\( p \leq 0.05 \)) and the EC50 175 μg/g (148–192, 95% confidence interval). A significant effect on mortality was observed at a concentration of 380 μg/g of phenanthrene. The response of mortality to pentachlorophenol was similar to the corresponding reproduction curve. This was not the case for cadmium and phenanthrene.

3.2. Effect of polluted soils

The LOEC and EC50 values were 16.6 (\( p \leq 0.05 \)) and 18.3%, respectively, (95% confidence limits: 14.6–29.1) for the S1 soil (Fig. 4) and 12.5 (\( p \leq 0.05 \)) and 8.6% (95% confidence limits: 7.9–9.2) for the S2 soil (Fig. 5). No significant effect on mortality was found for S1; the effect of S2 on mortality was similar to its effect on reproduction.

3.3. Effect of soil pH and soil moisture

Mortality did not seem to be influenced by the pH of the soil, while the rate of reproduction decreased steadily with the rise in pH (Fig. 6). At moisture contents of 37, 45 and 53% of the WHC means population densities of 170 (± 34), 187 (± 41) and 390 (± 52) individuals, respectively, were recorded.

3.4. Hatching kinetics

The hatching kinetics of *F. candida* followed the patterns shown in Fig. 7. There was a rather regular
Fig. 1. Dose-response relationships for the effect of pentachlorophenol on juvenile production (bars; left Y-axis; means and standard errors) and adult survival in *F. candida* (curve; right Y-axis).

Fig. 2. Dose-response relationships for the effect of cadmium on juvenile production (bars; left Y-axis; means and SEM) and adult survival in *F. candida* (curve; right Y-axis).

Fig. 3. Dose-response relationships for the effect of phenanthrene on juvenile production (bars; left Y-axis; means and SEM) and adult survival (curve; right Y-axis) in *F. candida*. 

106

Fig. 4. Reproduction (bars; left Y-axis) and survival of adults (curve; right Y-axis) under the influence of increasing concentrations of S1 soil (means and SEM).

Fig. 5. Reproduction (bars; left Y-axis) and survival of adults (curve; right Y-axis) under the influence of increasing concentrations of S2 soil (means and SEM).

Fig. 6. Juvenile numbers (bars; left, Y-axis; means and SEM) and adult survival (curve; right, Y-axis) pH in experimental soil with different pH volume (4.2–6.9).
alternation of small and large peaks of reproduction. Hatching started on the 25th day after the introduction of the juveniles into the experimental pots. The first notable peak in hatchlings occurred around the 27th day. The four experimental boxes were synchronous for the first five hatching peaks, then the synchrony tailed off.

4. Discussion

4.1. Effects on reproduction and survival

The EC$_{50}$ for the reproduction parameter which we found for cadmium (129 \( \mu \text{g/g dry soil} \)) was between the values found by Crommentuijn et al. (1993), 227 \( \mu \text{g/g} \), and by van Gestel and van Diepen (1997), 60 \( \mu \text{g/g} \). Sandifer and Hopkin (1996), however, reported a much higher EC$_{50}$: 480–780 \( \mu \text{g/g} \) depending on the pH. These differences can at least partly be explained by differences between the clones used in the various laboratories carrying out the test. A similar problem has been reported for mortality in the cladoceran Daphnia magna, with LC$_{50}$ values ranging from 0.06 to 100 \( \mu \text{g/g} \) for cadmium (Baird et al., 1990). Crommentuijn et al. (1995) also found differences, albeit smaller, among clones of F. candida. One solution to this problem is that all laboratories carrying out the F. candida reproduction test use the same strain which would be reared in a single European laboratory and distributed upon request. As pointed out by Forbes and Forbes (1994) ‘for the purpose of assessing laboratories, it may be perfectly appropriate to restrict testing to a single genetic clone’; however, Forbes and Depledge (1992) warned against suppressing natural variability. It would be better to perform the test on three or four different clones and to introduce a safety factor choosing the lowest observed EC$_{50}$. Another solution would be to perform the reproduction test with juvenile offspring of recently collected adults. Our results for pentachlorophenol are in agreement with those of the laboratories participating in the European ring test (LOEC reproduction between 32 and 316 mg/kg dry weight, Riepert and Kula, 1996). Pentachlorophenol and phenanthrene toxicities are in approximately the same range; to our knowledge, there is no other work dealing with the effects of phenanthrene on Collembola.

The results from the S1 soil assay are in rather good accordance with those of Smit and van Gestel (1996) who found an EC$_{50}$ for the effect of zinc on the reproduction of F. candida of around 350 \( \mu \text{g/g dry soil} \) (LUFA soil). The S1 cadmium concentration was rather low so it probably only had a weak effect on reproduction in this experiment. Similarly, with regard to the LOEC of S1 (16.6%), the chromium concentration was about 130 \( \mu \text{g/g} \), that is, much less than the LOEC value for chromium (1200 \( \mu \text{g/g} \)) found by Riepert and Kula (1996). These not very concentrated metals could bring down the LOEC of S1 by additive effects or by synergy with zinc. For the S2 soil, the LOEC was observed for a 1/8 dilution which corre-

Fig. 7. Numbers hatching over time in 4 experimental pots. 20 juveniles were introduced per pot on Day 0.
sponds to a 100 µg/g phenanthrene concentration, that
is, half the LOEC of phenanthrene tested separately.
The LOEC of the S2 soil corresponds to a pentachlo-
rophenol concentration of about 10 µg/g; this value is
obviously lower than the LOEC for pentachlorophenol
alone. This discrepancy can be explained by different
effects: on the one hand, synergism could occur between the xenobiotic compounds in S2; on the
other, the organic matter content which partly condi-
tions the bioavailability of xenobiotics (Harris, 1972;
Crommentuijn et al., 1997) was lower in the S2 soil
than in the ISO soil; consequently, xenobiotic bioa-
vailability was higher in the S2/ISO mixture than in
pure ISO soil. In the same way, xenobiotic bioavail-
ability could be higher in the assays with S2 soil than
in the assays of pure xenobiotic compounds in pure
ISO soil.

4.2. Preparation of the experimental soil

Various recommendations in regard to the method
for the determination of effects on reproduction (Rie-
pert, 1993; Document ISO/TC 190/Sc 4/WG 2)
require modification or greater clarity in an attempt
to decrease the variability of results between labora-
tories. These concern pH, water content and structure
of the experimental soil, the duration of the trials and
the age of the juveniles at the beginning of the assay.

The instructions relating to the preparation of the
experimental soil containing the contaminant and in
which the springtails are placed are too vague. The
quantity of water to be added to the peat/sand/kaolinite
mixture is fixed at between 40 and 60% of its WHC. It
would be preferable to fix this level more precisely
since, as shown by our tests, the reproduction rate
depends to a large extent on this factor. van Gestel and
van Diepen (1997) did not find effects of soil moisture
on cadmium EC50; however, further studies are neces-
sary to generalize this result (particularly to organic
chemical compounds with a low water solubility). A
more restrictive condition of 55–60% of WHC would
be a suitable level for the collembolan F. candida
which needs a very damp substrate and whose rate of
reproduction is very sensitive to even very slight
drying out of the substrate. It is impossible to obtain
an appropriate structure of the experimental soil
(“crumbly structure”) with a soil moisture higher
than 60% of the WHC.

Our trials show that soil pH also affects the repro-
ductive rate in F. candida. As the pH also influences
the availability of the contaminants (Pedersen et al.,
1997), it is important to respect the restrictions of the
pre-norm document concerning this parameter
(pH = 6 ± 0.5). The soil pH was shown to have an
impact on reproduction in the earthworm E. fetida
(Spurgeon and Hopkin, 1996). For the testing of
polluted soil, if the pH of the ISO soil is very different
from the pH of the polluted sample, the final pH of the
mixed soils could be dependent on the proportion of
the two soils; these differences in pH could induce
differences in the rates of reproduction which could be
erroneously attributed to polluted soil toxicity. (This is
not the case for S1 and S2, which had rather similar pH
values to the ISO soil pH; pH (KCl) = 6.3 ± 0.3).

Moreover, the final structure of the soil could be
much better defined than the simple description
“crumbly” that is used. For example, peat particle
size partly conditions xenobiotic sorption by soil and
so their bioavailability. The toxicity of any chemical
partly depends on the particle sizes of the substrate it is
mixed with, if only because the WHC of the substrate –
and thus the quantity of water that must be added – is
also dependent on the particle size. Likewise, 10% of
peat constitutes a “high organic matter content”
(Riepert and Kula, 1996). As numerous xenobiotics
are adsorbed by organic matter and therefore become
less bioavailable, organic matter content conditions
the apparent toxicity of some chemical compounds.
For example, the LOEC of potassium bichromate is
3300 µg/g for a soil with 10% peat content and
562 µg/g for 5% peat content (Riepert and Kula,
1996); thus, as a precaution, it would be better to
use a soil of lower organic matter content. Thomson
and Gore (1972) used a soil with a low organic content
(0.7%) to study the toxicities of 29 insecticides to F.
candida. Since the organic matter content of S1 and S2
was markedly less than the ISO soil (S1, S2 = 1.6%;
ISO = 10%), the higher the proportion of ISO soil in
the mixture the higher the organic matter content and
the lower the bioavailability of toxic compounds with
a high affinity for organic matter. So, for testing
polluted soil it would be better, if possible, to use
as a blank, and for dilution of the polluted soil, the
same soil but without contamination; this choice will
remove the problems associated with differences in
pH and organic matter content.
4.3. Hatching kinetics

Fig. 7 reports the hatching kinetics over a period of 6 weeks. It can be seen that the first hatching peak only occurred about 4 weeks after the introduction of the parent animals, that is, near the end of the recommended 28-day duration of the reproduction test. Part of this delay can be explained by the very artificial conditions in which the animals were kept (animals placed on a flat, bare surface small in area) in this experiment. We do feel, however, that a total duration for the test of 28 days is too short. In order for the results to be more meaningful, we would prefer to see the duration of the trials extended to 33–34 days that is, as can be seen from Fig. 7, after the second hatchings peak. Moreover, for some persistent chemicals (e.g. metals) accumulation in the test animals during exposure and their effects are functions of the test duration which is of prime importance for the outcome of the tests.

4.4. Miscellaneous remarks

A certain number of other modifications deserve consideration. One concerns the age of the individuals at the time they are transferred to the experimental pots: the pre-norm document specifies animals aged 10–12 days. In the *F. candida* reproduction test, the animals are not exposed during the first 10 days of their life – the period when they are likely to be most sensitive. If such a difference in sensitivity with age does exist, it would be preferable to use younger animals, for example, aged 4–5 days (for practical reasons, it is difficult to use younger animals).

5. Conclusion

In spite of these and other problems such as temperature homogeneity, ambient light intensity, and solvent evaporation, the *F. candida* reproduction test appears to be a very useful assay and should be included for soil ecotoxicological testing of xenobiotic chemical compounds. One of its main advantages is that it is more sensitive than the earthworm mortality test, probably owing to the fact that it is a test of chronic toxicity using the reproduction parameter and not a test of acute toxicity. Moreover, the parthenogenetic mode of reproduction of *F. candida* makes it particularly easy to rear in large numbers and suited to testing based on the reproduction parameter. However, the question is open as to whether parthenogenesis is a source of variability and if the sensitivity of *F. candida* actually is of the same order as that of sexually reproducing springtails (Jepson et al., 1995).

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