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Rapid communication

The relative sensitivity of growth and reproduction in the springtail, *Folsomia candida,* exposed to xenobiotics in the laboratory: An indicator of soil toxicity

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Abstract

The *Folsomia candida* reproduction test [ISO, 1998. Soil quality—Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. International Standard Organization Report 11267, 1998, Geneva] is used to evaluate the ecotoxicological risks of contaminants in soils. The aim of this study was to compare the sensitivity of growth and reproduction of *F. candida* to four xenobiotics: two metals (Cd, Al), one metalloid (As), and one organic compound (pentachlorophenol). We showed that reproduction is a slightly more sensitive parameter than growth: EC_{20} for reproduction was 1.25 μg/g dry soil for arsenic, 56 μg/g for cadmium, 97.5 μg/g for aluminum, and 41.7 μg/g for pentachlorophenol. The corresponding EC_{20} values for growth were 2.8, 65, 630, and 94.6 μg/g. Keeping in mind that a growth test needs fewer juveniles and less time than a reproduction test, we conclude that the two parameters are complementary and could be used for a better ecotoxicological evaluation of contaminants. However, the relative growth and reproduction sensitivities should be tested with more chemicals before growth could be considered as a good alternative for a faster sublethal test.

Keywords: Toxicity testing; Soil pollution; Collembolan; Growth; Reproduction

1. Introduction

Chemical analyses are essential for the evaluation of soil pollution but do have some drawbacks: (i) they cannot be exhaustive, requiring prior knowledge of the pollutants with at least a rough estimate; (ii) they do not always give information on the degradation products of the pollutants, whose toxicity can be as high as that of the initial pollutant; and (iii) they give little information on the overall toxicity of the polluted soil, which is particularly related to interactions between pollutants and between the pollutants and the soil matrix they are mixed in with. Ecotoxicity tests are necessary to complete chemical analyses. Five tests using soil animal models have been standardized for use in Europe: the mortality and reproduction tests with the earthworm *Eisenia fetida* (International Standard Organization (ISO), 1994, 1997), the reproduction test with the Collembola *Folsomia candida* (ISO, 1998), the Enchytraeids reproduction test (ISO, 16387), and the assay for field testing with earthworm (ISO, 1999). The reproduction test with *F. candida* is increasingly used because it is very often sensitive and the breeding of this species is easy (Riepert, 1995). The effects of several different parameters such as temperature (Grégoire-Wibo and Snider, 1983), pH and soil moisture (Holmstrup, 1997), feeding (Smit et al., 1998), and the variability between strains have been studied (Crommentuijn et al., 1995; Chenon et al., 2000). However, it has two drawbacks: it needs numerous 10–12-day-old animals (400–800 juveniles depending of the assay), and it is rather time consuming. So, it would be useful to design an ecotoxicological assay with *F. candida* which would be

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sensitive and more rapid and would need fewer animals. Reproduction depends largely on the body size of the adult (Hopkin, 1997). Growth is easy to measure and does not require expensive apparatus. Today, digital cameras are cheap and some image analysis softwares are free of charge (Image Tool, Scion, Image J); they decrease greatly the amount of work necessary to measure dimensions for a lot of animals.

Growth of an animal is dependent upon the efficiency of its metabolism, which is lowered by xenobiotics. So, it could be a sensitive parameter for the toxicity of chemicals (Crommentuijn et al., 1993; Tranvik et al., 1993; Folker-Hansen et al., 1996). Some researchers found growth to be a less sensitive parameter than reproduction (Scott-Fordsmand et al., 1997, 1999; van Straalen et al., 1989; Fountain and Hopkin, 2001; Smit et al., 2004); others found opposite results (Folker-Hansen et al., 1996).

First, we determined the growth curve of *F. candida* and the relationship between growth and reproduction. Such a relationship would be ecologically pertinent to growth. We then compared the relative sensitivity of growth and reproduction rates with respect to aluminum, cadmium, arsenic, and pentachlorophenol. Our aim was to verify if there is a relation between the two parameters and if growth can be a sensitive and pertinent parameter for ecotoxicological tests.

2. Materials and methods

2.1. Growth rate of F. candida

Forty one-day-old individuals were isolated, each in one cylindrical plastic pot (30 cm^3), on a moist substrate of 9:1 (w/w) plaster of Paris:charcoal mixture. One granulated dry yeast was added weekly on the soil surface as food. Digital photographs were taken (Nikon Coolpix 990; image quality fine; macro-mode) weekly and the length of the animals, from the end of the posterior abdominal segment to the anterior margin of the head, was measured by on-screen viewing by means of the Image Tool software. A small plastic ruler with millimetric graduation was placed near the animal as a calibration scale. The growth of 40 Collembola was measured from hatching to day 64. Trend was defined by least-squared fitting of a quadratic regression of the length of the animals against their age (Systat).

2.2. Relation between growth and reproduction

This experiment was conducted in the same way as the first assay except for the following details. The animals were 7 days of age at the beginning of the experiment. Photographs were taken at day 29 for length measurements and the number of juveniles was counted at day 36 using Image Tool software. Regression of growth on reproduction was calculated with Systat software.

2.3. Comparison of the sensitivity with respect to xenobiotics of growth and reproduction

2.3.1. Effect of cadmium

We simultaneously carried out two cadmium sensitivity tests; one assay was devoted to reproduction, the other to growth. For each treatment concentration of the two assays, ten 10–12-day-old juveniles were introduced into 100-mL glass jars (screw top) containing 32 g of wet OECD soil. The OECD soil consisted of 70% quartz sand, 20% kaolinite, and 10% peat ground, dried, and sieved to 0.5 mm with the pH adjusted to 5 ± 0.5 by the addition of $CaCO_3$. It was moistened to 50% of the water holding capacity with distilled water. The nominal cadmium concentrations were 0, 38.5, 76.5, 153, and 306 μg/g dry soil. Cadmium was dissolved in the distilled water used for moistening of the soil. The pots were placed within an incubator giving a controlled environment of 20 ± 1 °C and continuous darkness. They were opened twice a week for aeration and every 2 weeks for feeding with baker's yeast (about 2 mg). The first assay (growth assay) was terminated after 15 days and the second assay (reproduction assay) after 35 days; we used a shorter exposure time for the growth assay because of the results with the pentachlorophenol; for the reproduction assay, we used 35 days duration instead of the 28 days recommended in the ISO guideline because we found in previous work that an increase in the assay time decreases the variability of the results (Crouau and Cazes, 2003). The pots were flooded with tap water and gently stirred; then the animals were transferred to a box with a plaster of Paris–harcoal mixture for photography with a digital camera. Length measurements were taken using the same method as for the first assay. Counting was done by on-screen viewing by means of the Image Tool software.

2.3.2. Effect of aluminum

The experimental conditions were similar to those used for the cadmium assay. Nominal concentrations of aluminum in soil were 0, 62, 125, 250, 500, and 1000 μg/g dry soil.

2.3.3. Effect of arsenic

The test was carried out on artificial OECD soils (artificial soil prepared according to the ISO, 1994, guideline) containing 0, 0.003, 0.009, 0.026, 0.08, 0.24, 0.74, 2.22, 6.67, 19.9, and 60 μg/g dry soil (sodium arsenate $Na_2HAsO_4 \cdot 7H_2O$; Sigma; purity >98%). Arsenic was dissolved in the water used to wet the soil. This test was performed on isolated animals (15 replicates/treatment; one animal/replicate) in plastic