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Collembola in ecotoxicology—Any news or just boring routine?

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ABSTRACT

Despite the uncontested significance of soils for human nutrition and drinking water quality, the majority of ecotoxicological testing is confined to aquatic test systems. Among the standardised tests for soils, the reproduction test with the springtail *Folsomia candida* is among the most widely used ones. First steps towards its standardisation were undertaken in the late 1980s. Here we review major advances that have been made since then, with respect to mechanistic, pragmatic and ecological aspects. Specifically we address the ecological relevance of any modifications of the standardised tests. We introduce a miniaturised version of the reproduction test which allows reducing the amount of soil per test unit to one third and the number of synchronised individuals to 40% as compared to the standard test. In addition, we developed an assay using Collembola eggs instead of synchronised adults. First results of a three-species test indicate that the presence of other species may affect choice behaviour. We point out a potential biased view of existing ecotoxicological data with Collembola due to the fact that most results refer to metal contamination. Finally, recommendations for future research are given, with special reference to avoidance and microcosm tests involving Collembola.

1. Introduction

Along with water and oxygen, soils are a crucial resource of human life: 95% of our food is directly or indirectly based on soils. In its report on degradation of land and water resources FAO recently stated “The greatest threat is the loss of soil quality” (FAO, 2011). Global problems such as desertification, salinisation, erosion, biodiversity loss and pollution have increasingly raised public awareness and called many political initiatives into action. However, only recently considerably more emphasis was put on international action plans and initiatives (e.g. Beck et al., 2005; EC, 2012).

Despite enormous anthropogenic input and technical advancements in agriculture (e.g. soil tillage, plant breeding, mineral fertilisers, pesticides) the essential services associated with crop production are mainly provided by the extremely diverse organism communities inhabiting soils (Nielsen et al., 2011). Studying their species composition and overall performance delivers information on the ecological state and quality of a soil (Beck et al., 2005). Potential hazards of new and existing chemicals to soils are being assessed by standardised test protocols with simplified models, usually involving single species of soil invertebrates or activity measures of the microorganism community. Van Gestel (2012) just reviewed ecotoxicological testing in soils in general, with special respect to invertebrates and isopods. Invertebrate tests cover mortality, reproduction and behaviour, and the two best studied groups with standard tests are earthworms and Collembola.

In this mini-review we focus on Collembola as a group which is highly abundant in almost any environment (Hopkin, 1997) and usually dominates the individual numbers of arthropods in most arable soils worldwide (Filser et al., 2002). We sketch the development towards standardised soil assays involving Collembola since the late 1980s and throw some spotlights on pragmatic, mechanistic and biological aspects related to these. Furthermore, we introduce a miniaturised version of the Collembola reproduction test and a test with Collembola eggs. We also present a new aspect in testing choice behaviour and make suggestions for future research in ecotoxicological testing with Collembola.

2. Recent developments in Collembola ecotoxicology

2.1. Steps towards standardisation

Until 1999, no standardised test guideline involving Collembola existed. However, considerably earlier much was known about the impact of toxic substances, especially heavy metals and pesticides, on Collembola (e.g. Hüther, 1961; Fox, 1967; Tomlin, 1975; Joosse and Buker, 1979). This included the interesting fact that our present standard species, *Folsomia candida* (Fountain and Hopkin, 2005), is resistant towards the most famous pesticide of all, DDT (Manley, 1971).

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From the end of the 1980s, intensive discussions towards a standardised test system with Collembola – involving a large selection of species – took place (e.g. Wiles and Frampton, 1996; Wiles and Krogh, 1998), and finally the ISO guideline using reproduction of the parthenogenetic species *F. candida* as an endpoint came into force (ISO, 1999). Ten years later, also the OECD included this assay into its guidelines for the testing of chemicals (OECD, 2009), with some modifications such as the possibility to perform the test with another – sexually reproducing – test species, *Folsomia fimetaria*.

Due to their long duration and the multitude of animals that have to be counted in the end, reproduction tests are very time-consuming and expensive. This drawback can be overcome by behavioural observations, which may indicate negative effects in much shorter time. Following promising results of choice experiments with contaminated food or soils (Filser and Hölscher, 1997; Filser et al., 2000), Natal-da-Luz et al. (2004) suggested an avoidance test which finally resulted in a standardised guideline (ISO 17512-2, 2011).

2.2. The setting: test conditions

All test guidelines involving Collembola are performed in natural or artificial soil. Since the early days of soil ecotoxicology, a huge body of literature has been published on how soil properties such as pH, salinity, water, clay or organic matter content affect bioavailability and thus toxicity of chemicals (e.g. Sandifer and Hopkin, 1996; Smit and van Gestel, 1998; Van Gestel, 2012). For example, ageing of zinc in soil reduced its toxicity by a factor of 5–8 (Smit and van Gestel, 1998). However, toxicity of spiked soils may also considerably increase over time, which we demonstrated with sewage sludge containing silver nanoparticles (Filser et al., unpublished data).

It should be expected that the problems associated with the large diversity and heterogeneity of natural soils can be overcome by using a standardised, artificial substrate such as the OECD soil (consisting of sand, clay, peat, and $CaCO_3$). All the more striking is the outcome of a study on OECD soil prepared in 25 different laboratories: Bielská et al. (2012) reported an enormous variety of those standards, with pH ranging from 4 to 7 and organic carbon content from 1.4% to 6.1%.

The result of a test is also affected by spatial heterogeneity and food availability: both inhomogeneous distribution of the substance and the presence of separate food (yeast) patches reduced toxic effects of dimethoate (Krogh, 1995). Contrarily, nonylphenol (NP) caused stronger effects when applied together with sewage sludge than without (EC_{10} = 6 and 23 mg NP/kg, respectively; Scott-Fordsmand and Krogh, 2004). Thus, the standard assay is not always conservative, and the large variety and heterogeneity of both natural and artificial soils often complicate the interpretation of the results and even question standardisation procedures (Bielská et al., 2012).

2.3. The mechanistic perspective

Given all these soil-related problems, it is evident that a simplified procedure should additionally be available, in particular when it comes to mechanistic questions with respect to bioavailability, toxicokinetics and toxicodynamics. Especially for the latter, stress biomarkers such as heat shock proteins were already tested decades ago (e.g. Tranvik et al., 1994; Köhler et al., 1999), culminating in a seminal paper by van Straalen (2003). Since then the focus has shifted towards gene expression profiling, proteomics and microarrays, delivering a plethora of information with a single assay (e.g. Nota et al., 2013). However, with respect to ecological risk assessment these methods still suffer from "some formidable problems" (van Straalen and Feder, 2012).

With respect to simplified exposure of soil animals, there is an OECD test for earthworms using spiked filter paper (see Van Gestel, 2012), yet nothing equivalent for Collembola. It is somewhat surprising that one promising method appears to be forgotten: Houx et al. (1996) developed an acute toxicity assay in which they exposed *F. candida* to toxicants in a simple aqueous medium. We have recently tested this assay with silver nanoparticles and can only recommend it whenever the confounding effects of soil properties are to be controlled for.

2.4. Biology of the test species

Thus far we have briefly covered the regulatory, physico-chemical and biochemical aspects of Collembola ecotoxicology. Turning to the biological part of the issue, we start with the test organisms. Despite some sensitivity differences compared to other collembolan species (e.g. Wiles and Krogh, 1998; Holmstrup and Krogh, 2001), *F. candida* was considered representative for the group and selected as standard species. One main reason was its preference for warm temperatures, humus-rich soils and parthenogenetic reproduction, which makes culturing easy. The main differences of the related *F. fimetaria* are smaller size, more common occurrence in European agricultural soils and sexual reproduction (OECD, 2009). The biology of *F. candida* and *F. fimetaria* has been extensively described by Fountain and Hopkin (2005) and Krogh (2009).

The variability of the substrate does not only influence substance bioavailability (see Section 2.2) but also the performance of the test species. *F. candida* avoided OECD soil and reproduced worst in this substrate whereas they performed well in many natural soils (Domene et al., 2011). Adjustment of soil moisture, pH and organic matter content are crucial to avoid biased results of both reproduction and avoidance tests (Crouau et al., 1999; Domene et al., 2011).

Variation in other conditions may also affect the outcome of a test. Crouau and Cazes (2003) found that small temperature differences (1 °C) did not affect the variability of reproduction, yet one day difference in age did: The coefficient of variation in offspring from 11 day old *F. candida* was almost 50% higher than in offspring of 10 day old animals. In the same study, prolonging the test from 35 to 49 days reduced the variation as well, along with a 50% lower LOEC level and a slightly lower EC_{50} value (114 vs. 105 μg Cd/g soil).

Besides differences with respect to life stage and nutritional status, the main reason for variability in reproduction is genetic variability. Although this should be low in a parthenogenetic species such as *F. candida*, clones from separate laboratories may differ considerably: The EC_{50} of cadmium for four clones of *F. candida* ranged between 800 and more than 2000 μg Cd/g soil (Crommentuijn et al., 1995). A more recent study (Chenon et al., 2000) revealed no sensitivity differences of nine clones towards Cd and minor differences towards phenantrene.

Extending the selection of species, differences between their sensitivity towards chemicals become apparent (e.g. Holmstrup and Krogh, 2001), which is logical in view of their varying morphology, physiology, microhabitat or feeding preferences. Generalisations based on the animals' biology are however difficult. For instance, *Folsomia quadrioculata* is highly sensitive to copper but thrives very well in lead-contaminated soils (Filser et al., 2000).

Unfortunately, theoretical concepts seem somewhat underrepresented in Collembola ecotoxicology, although there are some powerful and promising options. In recent years, trait-based approaches have been advocated for analysing the vulnerability of wildlife to pollutants (de Lange et al., 2009) and for linking biodiversity and associated ecosystem services (de Bello et al., 2010). This concept was applied to effects of land use change on biodiversity and extended, with special attention to animal groups including