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Multispecies soil toxicity test

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Abstract. Most ecotoxicological tests are based on the use of a single test species. However, the various soil organisms (decomposers, producers, consumers) inhabit a common space and are thus in continuous competition. Their interactions may also influence their susceptibility to the effects of various types of toxicants or other stressors in soil. Unfortunately, these interactions cannot be detected in tests using only one species. The aim of this work was to design and optimize a multi-species laboratory test using microorganisms, plants (*Lactuca sativa*) and invertebrates (*Enchytraeus crypticus* Westheide et Graefe, 1992). Measured endpoints were the dehydrogenase activity of the microorganisms, length of the main root of the plant (*L. sativa*) and reproduction of the enchytraeids (*E. crypticus*). The preliminary results indicate that the proposed design and species selected may in the future be used as the basis for a multi-species test.

Key words. Multispecies toxicity test, enchytraeids, soil.

INTRODUCTION

Most ecotoxicological tests are done using a model species but this approach can rarely predict complex environmental interactions. A multi-species test is needed if one is predict the combined effects of competition, mutualism, predation, various chemicals and environmental conditions on fitness of organisms. Soil multispecies microtests consist of soil units that contain organisms from various trophic levels such as microorganisms (decomposers), plants (producers) and invertebrates (consumers/decomposers). They may consist of a homogenous system of sieved soil (Edwards & Boglen 1992) or intact cores taken from the field (ASTM E1197-87, 1998, Knacker & Morgan 1994, Voris et al. 1985). A homogenous system is usually simpler but intact soil cores are more ecologically relevant as they contain organisms that naturally occur in the soil. However, only one type of organism such as nematodes or enchytraeids are intensively studied in intact systems. Most commonly tested chemicals are heavy metals or the pesticide Carbendazim (CAS 10605-21-7), which is recommended as a reference chemical in some one-species tests (Burrows & Edwards 2002, Fordsmann et al. 2008, Kools et al. 2008, Moser et al. 2004a, Moser et al. 2004b, Remén et al. 2010). This substance is a widely used, broad-spectrum benzimidazole fungicide. It is a metabolite of Benomyl. It is also used as a control agent for treating golf greens and in some countries is licensed for that use only.

Briefly, there are few studies using multi-species tests but it is not possible to compare the results, because each of these studies was done using different test containers, exposure times, extraction techniques, statistical evaluations of the results and organisms.

The main goal of this study was, therefore, to combine three single-species tests in a multispecies approach, which would have several advantages: take less time, less space and less substrate. Use of a multispecies test of the suggested design can facilitate routine toxicological and ecological studies.

MATERIALS AND METHODS

Test systems consisted of glass vessels, 5×9 cm (inside diameter × height) with plastic lids each containing 30 g of artificial soil (OECD 220, 2004) the water of which was adjusted to 60% of its water holding capacity (WHC) with distilled water. There were two variants of the test vessels with three germinated plant seeds (*Lactuca sativa*) and five enchytraeids (*Enchytraeus crypticus* Westheide et Graefe, 1992) in variant A. As a source of food oat meal was added at the start of this test. In variant B there was the same amount of food but one half was added at the start of the test and the second half after 14 days. The numbers of enchytraeids and germinated seeds were the same as in the variant A. The measurements recorded at the end of the test were the reproduction of the enchytraeids, root length of lettuce seedling and dehydrogenase activity of microorganisms. The test lasted 28 days. The vessels were kept in an incubator under standard test conditions (temperature range 18–22 °C, 12/12 h light-dark cycle). At the end of the test period, all plants were extracted from the soil and the length of their main roots measured. The dehydrogenase activity (ČSN ISO, 23753-1, 2012) was measured for two of the replicates of both variants (A and B) and the remaining three replicates were used for the determining the number of enchytraeids. The soil from these test vessels was stained with Bengal red and the reddened worms were counted the next day (OECD 220, 2004).

The statistical analyses were done using statistical program Graph Pad Instat, version 3. The values for enchytraeid reproduction, growth of plant and dehydrogenase activity of microorganisms recorded for both variants were compared using the non-parametric Un-paired t-test with Welch correction.

RESULTS

Results (Table 1) for the variant A (single provision of food) were statistically different from those for variant B (food provided twice) in terms of enchytraeid reproduction (t-test with Welch correction, P=0.0183). The dehydrogenase activity, however, recorded for variant B was significantly higher (t-test with Welch correction, P=0.0030) than that recorded for the variant A, possibly because the soil was more aerated in the variant B because the soil was mixed again when the second food supplement was added to the soil. The length of the main root of lettuce (*Lactuca sativa*) was not significantly different in the two treatments.

DISCUSSION

Ten individuals are usually introduced into each container in tests using a single species of enchytraeid (OECD 220, 2004) with a subsequent recording 100 young worms (*E. crypticus*) considered to be a successful outcome. In our experiment, 182 worms were recorded on average, although only five worms were placed in each vessel at the beginning of the test. The results of this test, using enchytraeids indicates that our experiment was successful. The lettuce seedlings remained green with no visual signs of any morphological changes and their growth in height was not constrained by the height of the jar. There was no control for the DHA method because the various nonspecific natural microorganisms were present in soils used in both treatments (ČSN ISO 23753-1, 2012).

Unfortunately, it is not possible to compare our results with published data, because currently there is no data on a multi-species test using the same species as in this study.

It is possible that the mixing of the soil when second batch of food was added (variant B) adversely affected the enchytraeids. The difference in DHA recorded in the two treatments (A, B) is interesting but inexplicable. Although lettuce is known to be relatively tolerant of various stressors it is likely the difference in the two treatments was sufficient to affect plant growth.

We conclude that this test has several advantages over the currently-employed single-species method. The relative simplicity of our test makes it less expensive, easy to replicate and adjustable for measuring different endpoints, depending on the nature of the system to be studied. We suggest that the information on the toxic effects of chemicals obtained using such tests is suitable for extrapolating to field situations.

Table 1. The results of the multispecies test (variant A and variant B).The differences between results of Variant A and Variant B in the case of enchytraeid reproduction is statistically significant (Unpaired t-test with Welch correction, P=0.0183)[1] as well as in the case of DHA (unpaired t-test with Welch correction, P=0.0030)[2]

test organism	endpoint (unit)	Variant A mean number (range)	Variant B mean number (range)
Enchytraeus crypticus	reproduction (individuals)	184 (162–202)[1]	87 (51–112)
Lactuca sativa	root length (mm)	192 (60–400)	188 (50–450)
microorganisms	DHA ($\mu g.g^{-1}.h^{-1}$)	0.69 (0.57–0.80)[2]	1.50 (1.39–1.61)

In the future we plan to use particular substances (acid bromide, heavy metals, nanomaterials, pesticides) or natural soils from contaminated localities and use soil animals (e.g. springtails) and plants other than enchytraeids and *Lactuca sativa* in a multi-species system.

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