MicroSoil - Investigation of alternative test methods to correctly assess the impact of plant protection products, biocides and pharmaceuticals on soil microorganisms

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Introduction

Soil microorganisms are described as drivers of the nutrient cycles of the soil, maintaining ecosystem services [1, 2]. Currently, the risk assessment of agrochemicals considers the effects on nitrogen transformation, only (OECD 216 [3]). The determination of one central function may not reflect the complex soil functions of soil microbial communities [2]. Within the project MicroSoil five alternative test methods in three differing test soils, treated with six substances each, are compared to data based on the current standard test (OECD 216) to determine systems with a higher sensitivity of respective endpoints. Here, recent partial results of effects on nutrient cycles (MicroResp™ [4]), enzymatic activities (DIN EN ISO 20130 [5]) and specialists (ammonium oxidizing bacteria, DIN EN ISO 15685 [6]) are presented.

Materials & Test structure

The study design followed the requirements of OECD 216 concerning storage, soil handling and conditions of exposure for comparability of results. Existing OECD 216 data of the test substances, provided by UBA (confidential), was used as reference data to compare the sensitivity of each method. Three nominal test concentrations with a spacing factor of five and ten were chosen:

→ Test soil: standard soil LUFA 2.1; high content of grain size sand (approx. 86%) resulting in low pH value (approx. 4.7 in 0.01M $CaCl_2$), C_{org} < 0.1%

→ Test substances and concentrations [mg a.s./kg soil dw]:
i. Ethofumesate (herbicide): 2.0, 10.0 and 20.0
ii. Propamocarb hydrochloride (fungicide): 0.003, 0.015, 0.03
iii. Pyraclostrobin (fungicide): 3.0, 15.0, 30.0
iv. Tebuconazole (fungicide): 1.0, 5.0, 10.0

Due to the low solubility in water, the substances were dissolved in acetone and applied to 1 kg soil dw via a carrier. Applications were performed using 10 g of air-dried soil.

→ Application details:
– control treatment: four control replicates
– test concentrations: triplicates each
– water adjustment: approx. 45% WHC_{max}

Short-term as well as long-term effects were investigated through measurements at test initiation, after 14 and 28 days. The tests were carried out one after another over three days, which was considered as one time point. Based on the 28 days results (e.g. effects above 25%), the test duration of the most sensitive test was extended up to 84 days.

Test methods

The chosen test methods represent miniaturised rapid test strategies to give a measure of the potential activity of the auto- and heterotrophic aerobic microorganisms.

As the methods base on 96-well microtiter plates, most standard laboratories can conduct the systems without special analytical equipment. For stimulation of the respective microbial function, subsamples were treated with diverse substrates selected on basis of literature research [7, 8] (Table 1).

Table 1: Overview of test methods and the derived endpoints.

Test method	Measurand	Type of substrate/ stimulated enzyme	Nutrient cycle	Biological endpoint
MicroResp™	Basal respiration, soil induced respiration	Deionized water, D-(+)-glucose, L-cysteine hydrochloride, L-malic acid, y-amino butyric acid, N-acetyl glucosamine, citric acid, L-alanine	Carbon, nitrogen, phosphorous, sulfur	Nutrient turnover
ISO 20130	Hydrolase activities	Arylamidase, arylsulfatase, ß-glucosidase, phosphatase, urease	Carbon, nitrogen, phosphorous, sulfur	Nutrient turnover
ISO 15685	Nitrification	Ammonium sulfate	Nitrogen	Ammonium oxidation

Results

The measurements were evaluated through guideline or manual specification under consideration of the respective validity criteria, if available. The results indicate for all four test substances:

→ ISO 20130 measurements implement higher inhibitions in general, while MicroResp™ and ISO 15685 remain relatively unaffected,
→ no concentration-response relationship between the treatments of each test substance and
→ no visible trend of inhibition over time.

Effects occurred in a non-specific pattern and did not remain stable (data exemplarily shown in Table 2). In contrast to the determined inhibitions detected with OECD 216 (UBA data), the values were not reproducible with ISO 15685. Hence, a simplified OECD 216 was initiated to validate the provided data.

Table 2: Exemplarily overview of collected data of Ethofumesate for ISO 20130. green: stimulation, yellow: inhibition < 15%, red: inhibition > 25%. n. d.: not determined.

Day of measurement	Enzyme	Inhibition [%] at test concentration		
		2.0 mg/kg	10.0 mg/kg	20.0 mg/kg
d0	Arylamidase	n. d.	n. d.	n. d.
	Arylsulfatase	-63	-61	-55
	ß-Glucosidase	14	11	13
	Phosphatase	-4	13	8
	Urease	n. d.	n. d.	n. d.
d28	Arylamidase	-5	-10	8
	Arylsulfatase	11	39	87
	ß-Glucosidase	-5	-3	-10
	Phosphatase	5	7	6
	Urease	4	12	7
d84	Arylamidase	-8	15	-6
	Arylsulfatase	4	7	40
	ß-Glucosidase	-1	5	-18
	Phosphatase	0	-1	-9
	Urease	8	5	6

Conclusion & Outlook

The methods indicate no increased sensitivity compared to the current guideline to evaluate microbial activity concerning the determined test substances and the specific test soil. Therefore, further soils, substances (an antibiotic and a quaternary ammonium compound) and methods (ARISA (Automated Approach for Ribosomal Intergenic Spacer Analysis) and ISO 10832 (Spore germination test)) will be tested for more comprehensive evaluation of alternative test system sensitivity. In addition, the provided OECD 216 data will be validated by performing tests with the respective test soils.

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