3.2. Impact on the soil ecosystem through natural and genetically engineered organisms. Effects, methods and definition of damage as contribution to risk assessment

S. Scheid[1], A. Fliessbach[2], P. Mäder[2], J. Mayer[1], K. Nowack[2], B. Oehen[2], F. Widmer[1], H.-R. Oberholzer[1*]

[1] Agroscope Reckenholz-Tänikon Research Station (ART), Reckenholzstrasse 191, CH-8046 Zürich, www.art.admin.ch

[2] Research Institute of Organic Agriculture (FiBL), Ackerstrasse, Postfach, CH-5070 Frick, www.fibl.org

[*] **Contact: hansrudolf.oberholzer@art.admin.ch**

In the ordinance relating to impacts on the soil (OIS 1998), genetically engineered organisms are considered, along with pathogenic and exotic organisms as “biological impacts on soil”. The handling of these organisms is regulated by the ordinance on deliberate release and the law on gene technology. In principle, all organisms that are released can proliferate, disseminate and undergo evolutionary changes, resulting in additional requirements for the release of organisms, independent of their genetically engineered status. The project of the Agroscope Reckenholz-Tänikon Research Station (ART) and the Research Institute of Organic Agriculture (FiBL) was intended to establish a risk assessment to the soil ecosystem of exposure to natural and genetically engineered organisms. The project was sub-divided into four modules. **Module 1** encompassed the theoretical basis of the research project. **Module 2** and **module 3** tested the implementation of these theories in a model experiment with the organism *Pseudomonas fluorescens* strain CHA0 and a field experiment using the compound “Effective Microorganisms” (EM). **Module 4** presents the combination of the results from modules 1 to 3.

Module 1 – Theoretical basis (ART and FiBL)

The foundation for the work in the first part of module 1 was the analysis of international concepts for determining soil quality and for surveying the effects of soil exposure to organisms with respect to physical, chemical, as well as biological indicators. In addition, literature was surveyed for references to genetically engineered plants, both genetically and non-genetically engineered organisms (which were added to the soil in a controlled way), and for reference to invasive exotic organisms introduced to soil. The culmination of these surveys resulted in the compilation of a set of indicators recommended throughout the world to determine soil quality and the currently acknowledged effects of soil exposures to micro-organisms. Furthermore, existing concepts for the definition of damage to environment, respectively soil, were studied and collated.

In the second part, possible effects of biological exposures to the soil ecosystem were defined. 17 Effects were identified, which fall into the categories of soil physics, soil chemistry, soil biology, and soil function (see Fig. 1 Poster). An effect was defined to be change of a soil feature, respectively –function. A readily updatable list of methods for the detection of such effects was compiled and includes 90 methods. Each method was qualified for the adequacy to detect the postulated effects. Each method was judged by 16 criteria. Such criteria included the standardisation and reproducibility of sensitivity of a method. In addition, methods were analysed for their cost-effectiveness. A protocol for biosafety aspects was determined with regard to whether effects, measured by a particular method, could be rated and whether such results could help determine the levels of risk to the soil ecosystem. Furthermore, a risk definition was developed in order to be able to assess and evaluate the impact of changes in soil attributes and soil functions.

For the application in risk assessment, a procedure for an effect-based selection of methods was developed. First, expert opinions on the expected effect on soil of biological exposures were identified and categorized among 17 effects. Following this, the appropriate methods for covering the effects can be selected and the advantages and disadvantages of each method can be judged based on the proposed criteria.

Module 2 – Model experiment with the organism *Pseudomonas fluorescens* strain CHA0 (FiBL)

Pseudomonas fluorescens is applied in agriculture in order to promote plant growth and to protect plants from soil-borne diseases. Besides such intended effects, potential effects on non-target-organisms may exist. They must be examined prior to the mass release of such organisms. The intended and unwanted effects of a released organism depend on where it is applied. Theoretical ecological concepts are based on the assumption that societies with a high diversity are less prone to changes by invading species. Based on this theory, a model experiment was performed in the greenhouse using soils from loess sites (*Lössstandorten),* whose microbial biomass and activity had developed differently through differing soil cultivation. At the time of sowing, spring wheat was inoculated with a *P. fluorescens* strain CHA0 that is naturally resistant to rifampicin (rif^{+}) and its establishment in the experimental fields was monitored. The *P. fluorescens* strain CHA0 used in this study is not commercially available, but shows similar features to *P. fluorescens* in the approved compound „Biofitac PF1". Its capacity to suppress diseases is based mainly on the production of the antimicrobial substance 2,4-Diacetylphloroglucinol.
The microbial biomass (C_{mic}, N_{mic}), basal respiration, metabolic ratio qCO_2, dehydrogenase activity (DHA), bacterial count, mycorrhizal-root colonisation and the bacterial pattern of usage of substrate were assayed on the 18^{th} and 60^{th} days post sowing and application of *P. fluorescens* strain CHA0. At the start of the experiment, the soil's biological parameters showed large differences, which were derived from the selected soils. As the experiment progressed, the changes in the biological parameters that resulted from plant growth were measured. The effect of the inoculum was, however, small and for most of the parameters only transient. For the soil with the smallest microbial biomass at the start there was, however, a sustained measurable change, which lasted over 60 days. The bacterial patterns of use of substrate, which indicate changes in the microbial societies, changed primarily as a result of plant growth, while the differences in soil as well as the sampling time could be ignored. The sensitivity of the methods used depended on the selected soils in a decreasing order from N_{mic}, DHA, C_{mic} to qCO_2. Besides the selective determination of the germ count of *P. fluorescens* strain CHA0 (found only in treated soils), the methods DHA, C_{mic} and the C_{mic}/N_{mic}-ratio were well suited to determine the inoculum effect. The time-based effect or the effect based on the growing plant was determined in the most sensitive way through N_{mic}, DHA, C_{mic} and qCO_2. The results support the hypothesis that an established abundant microflora can buffer against effects of a deliberately released invading species. In other words, the bacterial inoculum was more effective in relatively poor soils compared to variegated soils.

Module 3 – Field research with the compound "Effective Microorganisms" (ART)

In a biologically farmed field experiment, which lasted four years from 2003-2006, the effect of the compound "Effective Microorganisms" was studied. The experiment was performed at the Agroscope Reckenholz-Tänikon Research Station (ART), location Reckenholz. The experimental design comprises (i) direct spraying of the compound EMA as well as (ii) the introduction of EMA in combination with the EM fertilizer compound Bokashi (fermented wheat bran) and (iii) EMA in combination with Bokashi and a specific dung (*Rottemist*).
According to the supplier, EM consists of about 80 different microorganisms (e.g. lactose producers, photosynthetically active bacteria). The EM-compound is approved in Switzerland by the BWL as an additive for fertilizer, as soil conditioners, as compound for composting, and for improving biological activities in the soil.
In order to differentiate the effects of the microorganisms in the EM compound from the organic carrier, a treatment without EM-application as well as three procedures with autoclaved EM compound was included in the experiment as controls.
The yearly yield, as well as the soil biological parameters of microbial biomass (SIR, CFE), basal respiration, dehydrogenase activity, pattern of use of substrate, and DNA-profile were assayed in spring and autumn 2005 and in spring 2006. By incubation experiments in the lab, the degradation of cellulose, the N-mineralisation potential, as well as the N-mineralisation output were determined. If the EMA spray compound was used, no significant differences between the EM-procedure and the untreated control were measurable for all biological soil parameters examined. Effects were found on yield and the biological soil parameters after the application of Bokashi and *Rottemist*. These effects, however, were neither found for all parameters examined nor at all sampling times. The differences measured could be simply attributed to the organic material added (Bokashi) and to the concomitant input of nutrients into the soil. The sampling time had an effect on the amount of microbial biomass, the basal respiration and the pattern of use of the substrate.
It can be concluded from the results of the experiment that the addition of "Effective Microorganisms" did not increase the plant yield and, in the medium-term (4 years), did not show an influence on soil quality in agriculture under the climatic conditions of central Europe.

Module 4 – Synthesis of the Modules 1-3 (ART und FiBL)

The stepwise process for the effect-based selection of methods, as developed in this project, enables a systematic and fact-based choice of methods to survey effects of biological impacts on the soil ecosystem. This methodology is well suited for practical application. While studying the possible effects of *P. fluorescens* strain CHA0 and the "Effective Microorganisms", it has become obvious, that if a more detailed knowledge of the target organism, with respect to its association with other organisms is available, it allows to define possible effects of introducing such organisms on the soil quality more precisely.
Based on the results of the experiment, the sensitivity of the applied methods to survey changes in soil quality was discussed. It became evident that the methods in the model experiment were sensitive to cover changes due to the inoculation of soils with *P. fluorescens* strain CHA0. This did not apply for the field experiment in respect to changes based on the addition of "Effective Microorganisms". The methods used were, however, sensitive enough to detect changes based on the application of organic material through the EM-application. Changes based on EM-addition that have the same impact as the addition of organic material, can thus be detected through these methods. It was found that the sensitivity of the methods to

detect effects on soil, used in both the model- as well as the field-experiment, varied. This means that the form of the experiment must be taken into account when selecting a method.

Based on damage definition criteria, the changes measured were evaluated for the presence of damage. A comparison between the procedure of adding the *P. fluorescens* strain CHA0 plus EM vs. the corresponding controls showed only minor or no influences on the tested biological soil parameters.

The need to develop physical, chemical and biological soil analysis methods to survey the exposure of organisms to soil can be divided into the two following areas:

- *Standardized methods,* which are generally used in the evaluation of soil quality: For these, the necessary methodology exist, so that a practical application is possible. For these methods, the fundamentals of interpretation are to be worked out.
- *Non-standardized methods*: These are mainly the molecular methodologies, for which a standardized process would have to be developed so that they can be integrated into routine tests. Again the interpretation of results would have to be worked out.

Fundamental to the risk assessment of soil is to qualify parameters, which identify what level of alteration in soil quality constitutes soil damage. Besides continued development of methodologies and soil quality surveys, which help to identify the “normal fluctuations” of soil biological parameters, it will be necessary to close existing gaps of knowledge in the area of data interpretation.

References

Fließbach, A., Lutz, M., Oberholzer, H.-R., Mäder, P. (2008). Soil amendment with *Pseudomonas fluorescens* CHA0 is more effective in soils low in microbial biomass and activity. submitted

Mayer, J., Scheid, S., Widmer, F., Fließbach, A., Oberholzer, H.-R. (2007). Wirkungen von ‚Effektiven Mikroorganismen EM’ auf pflanzliche und bodenmikrobiologische Parameter im Feldversuch - Effects of ‚Effective Microorganisms EM’ on plant and microbiological parameters in a field experiment. Vortrag 9. Wissenschaftstagung Ökologischer Landbau: Zwischen Tradition und Globalisierung. Universität Hohenheim, DE-Stuttgart, Mar. 20-23, 2007.
Beitrag archiviert unter http://orgprints.org/9691/01/9691_Mayer_Vortrag.pdf

Mayer, J., Scheid, S., Oberholzer, H.-R. (2008). How effective are ‘Effective Microorganisms’? Results from an organic farming field experiment. 16th IFOAM Organic World Congress, Modena, Italy, June 16-20, 2008.

Mayer, J., Scheid, S., Widmer, F., Fliessbach, A., Oberholzer, H.-R. (2008). Effects of ‚Effective Microorganisms (EM)’ on crop yields and soil microbial parameters in a field experiment in temperate climate. In preparation.