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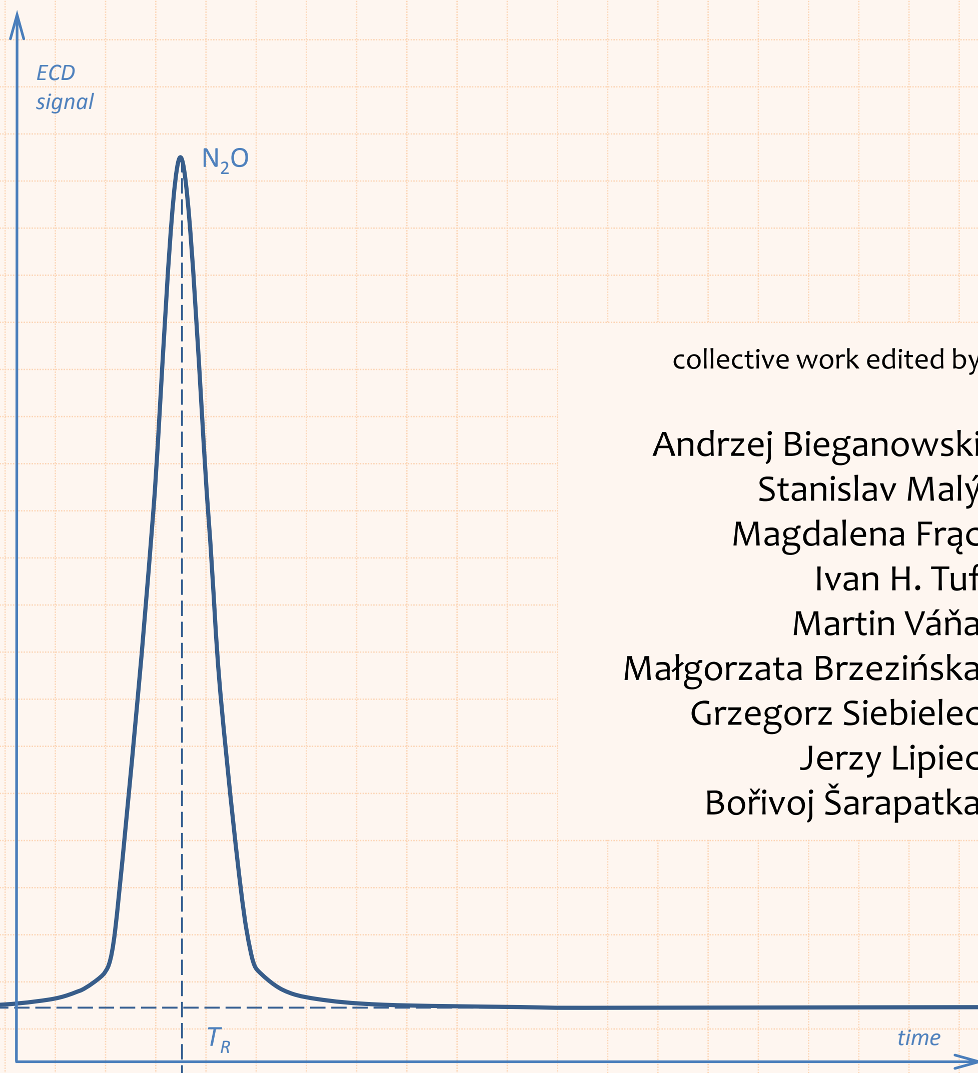
Laboratory manual



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INTRODUCTION

In the years 2013-2015 within the Operational Programme Cross-border Cooperation Czech Republic - Republic of Poland 2007-2013 the project "*Risks and benefits of introducing exogenous organic matter into the soil*" (CZ.3.22/1.2.00/12.03445) was realized. The project consortium consisted with:

- Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ), Branch Office in Brno, Czech Republic - the leader of the consortium,
- Palacký University (UPOL), Olomouc, Czech Republic,
- Institute of Soil Science and Plant Cultivation – State Research Institute (IUNG-PIB), Puławy, Poland,
- The Bohdan Dobrzański Institute of Agrophysics, Polish Academy of Sciences (IA PAS), Lublin, Poland.

The main aim of the project was to assess the effects of the use of the exogenous organic matter (EOM) as the fertilizer or soil conditioner and to prepare the guidelines for their safe and effective use in agriculture, enabling increase of the soil organic matter (SOM). These guidelines should take into account local soil and climatic conditions, the nature of agriculture and types of EOM available in the region. This aim is consistent with the priorities of the program i.e. improving the quality of the environment by improving production and environmental functions of the soil and safe disposal of wastes in the region.

The main aim was supported by the sub-goals:

- Evaluation of the state and changes of SOM content in the region using available historical data and new soil collection and analyzes
- Evaluation of EOM sources (quantitative and qualitative) available in the region. This was realized, among others, by the investigation of the effect of EOM on the soil quality and functions (in 2013 and 2014 two field experiments were carried out: one in Czech Republic - Puste Jakartice and one in Poland - Braszowice). Four types of EOM in three doses were used in the field experiments. In addition two glasshouse experiments were carried out (in Puławy, Poland) in order to test the 6 types of EOM, in 2 doses, on 3 different soils.

There the guidelines and recommendations were prepared on the basis of obtained results. We invite the Readers to view our other publications developed within the framework of this project. Nevertheless the authors realized that although tested EOMs represented typical materials available in the programme area, the experiments could cover only a narrow range of scenarios. Because of that, the guideline is focused on which analyses should be done to assess effectiveness and safety of EOM application. A laboratory manual is an indispensable tool for performing this task.

One of the strongest points of the project (beyond a reference to local conditions prevailing in the region of the Sudetenland) **was the interdisciplinarity of the investigations.** There are a lot of investigations on EOMs described in the literature. Usually they concentrate on one or two aspects - rarely on several. But the set of investigations carried out in this project seem to be quite unique because the EOM-treated soils were investigated using biological (including the presence of invertebrates), microbiological, chemical and physical methods at the same time.

Such wide spectrum of investigations required the use of many analytical methods. As one of the project deliverables was to prepare the guide for chemical, physical, microbiological, ecotoxicological and fauna analysis of soil and soil EOM the Reader receives this brochure. It is addressed to the laboratories which deal with the soil measurements. Because the methodology aspects of EOM testing are not country specific, we decided to publish this brochure in English, to

allow as wide as possible use of the book. We will be glad if it will be useful to the Readers and will be used in everyday work.

The names of the methods, their order and segregation are different than in the project proposal. This change is due to the desire to facilitate the reader to use this manual. However all methods specified in the proposal are described in this manual.

List of methods:

Biology and ecotoxicology

- Determination of the effect of agrochemicals on the reproduction of Collembolans (*Folsomia candida*)
- Determination of the effect of agrochemicals on the reproduction of Enchytraeids (*Enchytraeus crypticus*)
- Determination of the effect of agrochemicals on the reproduction of predatory mites (*Hypoaspis aculeifer*)
- Contact test using dehydrogenase activity of bacteria *Arthrobacter globiformis*
- Plant test (root growth of *Lactuca sativa*)
- Determination of the inhibitory effect of elutriates on the light emission of luminescent bacteria *Vibrio fischeri*
- Growth inhibition test with freshwater algae *Pseudokirchneriella subcapitata*
- Growth inhibition test with freshwater aquatic plants *Lemna minor*
- Sampling of soil fauna

Microbiology

- Denitrifying enzyme activity (DEA) with differentiation of denitrification products N_2O and N_2
- Assessment of basal and substrate induced respiration in soil by gas chromatography
- Determination of soil microbial biomass. Fumigation-extraction method
- Assay of β-glucosidase activity
- Assay of the dehydrogenase activity
- Assay of CM cellulase activity
- Assay of acid and alkaline phosphomonoesterase activity
- Assay of urease activity
- Evaluation of short-term nitrification activity
- Evaluation of genetic diversity of ammonia oxidizing archaea (AOA) using terminal restriction fragment length polymorphism analysis (t-RFLP)
- The estimation of soil microbial functional diversity using microbial community level physiological profiling (CLPP)

Chemistry

- Soil pH
- Electrical conductivity and salinity of soil
- Determination of total carbon and total nitrogen by dry combustion method (Dumas method) using a CN elemental analyzer
- Determination of organic carbon in soil by oxidation with a mixture of potassium dichromate and sulphuric acid
- Dissolved organic carbon as a simple measure of labile organic matter
- Sorption capacity of soil
- Determination of different forms of trace and macro elements
- Determination of PCBs, OCPs, PBDEs and PAHs by GC-MS/MS
- Determination of HBCD by LC-MS/MS
- Determination of PFAS by LC-MS/MS

Physics

- Particle size distribution measured by laser diffraction method
- Evaluation of sorptivity and water repellency of soil aggregates
- Evaluation of soil water retention
- Pore size distribution
- Strength of soil aggregates
- Infiltration in soil aggregates and saturated hydraulic conductivity of soil

BIOLOGY and ECOTOXICOLOGY

Ivan Hadrián Tuf
Martin Váňa

Present-day ecotoxicology encompasses a variety of scientific principles and methods capable of identifying and assessing the effects of substances released into the environment by mankind (Markert, 2003). It has evolved into a predictive science that aims to forecast the effects of potentially toxic agents on natural ecosystems and non-target organisms (Hoffman et al., 2003). There are two approaches how ecotoxicity can be expressed. According to the first one the concentration causing the lowest effect is calculated (LOEC). It enables to derive the highest concentration which causes no effect (NOEC) being just below LOEC. The second possibility is based on calculation of the concentration at which a specific effect is detected (e.g. percentage of mortality or percentage of inhibition of nitrification). These values are called effective concentrations (ECx). Traditionally, EC10 or EC20 are reported along with EC50. The ECx approach is recently preferred because EC values are not influenced by selected doses and the curve dose-response says more about toxicity effects than simple LOEC/NOEC.

Ecotoxicology is based on tests developed for detection of toxicity of tested substances or mixtures on enzymatic activities, bacteria, plants, water and soil organisms, mosses and lichens, but also warm-blooded vertebrates, especially rodents or birds, in general, on the living organisms and more generally on the environment. It is recommended to use bioassays with different test species, different sensitivity of test organisms, from various trophic levels. Especially effects on reproduction proved to be significant for the assessment of real environmental risk as a more sensitive parameter to predict possible adverse effects than just mortality. Currently there is a trend to develop miniaturize ecotoxicological tests, fully validated and enable to monitor adverse effects of substances on living organisms under standard, reproducible conditions (Dvořák, 2009). In parallel, there is a set of methods standardized by ISO and OECD which was taken as a base for the project.

Substrate induced respiration which characterizes a large group of microbial heterotrophs and ammonia-oxidation performed by a narrow group of chemolitotrophic microorganisms represent two ecologically different microbial activities for which standardized methods exists. The procedure for estimation of the ecotoxicity using of the rate of ammonia oxidation is described in the chapter Microbiology, it is a part of the method for determination of short-term nitrification activity. A collembollan *Folsomia candida* and a potworm *Enchytraeus crypticus* are soil invertebrates contributing to soil fertility and their activity affects the process of organic matter degradation. However, different routes of uptake are characteristic for these invertebrates. Enchytraeieds have a direct contact with soil via moist skin unlike cuticular body of *Folsomia*

candida. Main route of uptake via feeding is characteristic for springtails. Predatory mite *Hypoaspis aculeifer* can be used as a representative of the highest trophic level. In this case, toxic effects are found if pollutants are passed from EOM through the mite *Tyrophagus putrescebtiae* which serves as a prey for *Hypoaspis aculeifer*. A root length or biomass are common endpoints for ecotoxicity testing with plant. A one-week test with lettuce *Lactuca sativa* is an example of the first case, the second one is represented by an often used test with plants e.g. *Brassica rapa* or *Avena sativa*.

More challenging than testing of chemicals and their mixtures is testing potentially contaminated soils. The main problem is lack of appropriate control since control soil should have the same physico-chemical properties as tested soil because abiotic soil properties strongly influence performance of the test. Unfortunately, such soil is rarely available therefore use of soil with similar properties was recommended. However it brings practical problems because a number of combinations of individual soil characteristics is large. Another area of soil testing is searching if some pollutants can not be leached which could pose a threat for groundwater. Experimentally, model organisms are exposed to a water leachate and inhibition is evaluated using an appropriate endpoint. Measurement of inhibition of luminescence of marine bacteria *Vibrio fischeri*, growth of green alga *Pseudokirchneriella subcapitata* and water plant *Lemna minor* are examples of commonly used methods. Beside the above tests more tests were developed for testing of leachates e.g. Daphnia test, fish test, test with *Tubifex tubifex* or *Chyronomus tetans*. Above standards for individual tests there is an ISO standard (ISO 17616) which is a concise manual how to use a battery of ecotoxicity methods when contaminated soils are analyzed.

The soil subsystem is inhabited by wide spectrum of soil organisms. This so called edaphic biota can be divided into three groups according its taxonomy. In the common pasture soils, the half of biomass of soil is presented by bacteria and actinomycetes and another quarter is presented by fungi. Soil animals represent the rest of the living biomass. Basically, all soil biota is involved into decomposing food chain, with dead organic litter as main source. Beside decomposers, some herbivores (plant eating animals as e.g. wireworms, mole-crickets, or voles) and true predators (preying on other living animals) are present. Predators can regulate populations of herbivores as well as decomposers and may have a big impact to soil processes.

Decomposing food chain is based on plant litter largely, but dead bodies of animals and fungi are decomposed as well. Soil animals destroy litter mechanically (by chewing, scrambling, swallowing and formatting fecal pellets), whereas bacteria and fungi decompose organic matter (bio-)chemically using enzymes for lyses of stable substances, as lignin, cellulose, cuticle, chitin etc. Life of soil organisms is accompanied by other soil processes, as creating of stable soil organic matter (humus), creating soil structure (as particles as glued by mucus and different other substances produced by fungi, actinomycetes of animals), aerating of soil (by tunnels and holes created by burrowing animals) etc. Knowledge of soil biota is necessary for judgement if our management have any negative impact to local biodiversity of soil subsystem and related components of whole ecosystem.

Soil biota are classified into four different ecological groups according body size of its representatives. This classification is related to their abundances, which get round from tens of individuals per square meter for e.g. millipedes or earthworms to billions (i.e. 1012) for e.g. bacteria. Different abundances implicate different methods and soil sample sizes for studies of diversity and soil processes. If we omit soil microbiology (see the next chapter), soil zoological methods are divided into two main groups according soil layers inhabited by target animal taxa. Soil invertebrates can be classified as soil surface dwelling (epigeic) animals or soil dwelling (edaphic) animals. Some taxa are almost exclusively related to only one type of life-style (e.g. surface dwelling ground beetles or spiders) although there are some exceptions of it (cf. Laška et al. 2011), whereas other taxa occupy both surface as well as deeper soil layers (centipedes, millipedes, springtails etc.). The latter group is advisable to study using combination of different methods.

Methods for sampling soil surface dwelling animals:

- Pitfall traps (also called Barber traps)
- Bait traps or litter bags (e.g. Wiegert 1974, Ožanová 2001)
- Direct collection of animals under shelters (stones, bark of fallen trunks etc.)

Methods for sampling soil dwelling animals:

- Direct soil sampling
- Underground bait traps
- Subterranean pitfall traps (also called drillings)
- Octet methods for earthworms (expulsion using electricity)
- Expulsion from soil in situ (using e.g. formaldehyde liquid)

Soil samples and bait traps, i.e. substrate with living animals, can be processed in laboratory using flotation or heat extraction of animals from substrate using Kempson or Tullgren apparatuses (Tuf & Tvardík 2005). Size of those samples is related to abundance of target animal group; it varies from few square centimetres for springtails to quarter of square meter for millipedes.

Effectivity of different methods was compared and evaluated repeatedly in different conditions and biotopes (e.g. Druce et al. 2004, Prasifka et al. 2007,). Soil animals are studied to evaluate state of soil, it means we are able to evaluate, if soil conditions are sustainable and soil is self-sustaining to keep its productivity and other functions (e.g. water retention). Abundances of animals as well as composition of their assemblages are related to amount of organic matter and humus in soil and to soil structure.

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Determination of the effect of agrochemicals on the reproduction of Collembolans (*Folsomia candida*)

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What is measured in this method?

The method is used for evaluation of effect of fertilizers, soil improvers and bio-wastes on reproduction of collembolan *Folsomia candida*. Adult springtails are exposed to a range of concentrations of the test substance mixed into an artificial soil. The range of at least six concentrations is chosen in accordance with maximal recommended dose of testing fertilizers, soil improvers and bio-wastes. Maximal recommended dose is in the middle of the tested concentrations. The duration of the test is 32 days. The reproductive output of the animals exposed to the test substance is compared to that of the control.

Why this measurement is important?

Collembolans constitute one of the most numerous groups of soil organisms. They were used as representative soil organism. Collembolans can be used in laboratory tests as well as in semi-field and field studies. The duration for a reproduction test with *Folsomia candida* is the same as for *Enchytraeus crypticus*. The reproductive output of the animals is sensitive endpoint for testing of chemicals. Collembolan reproduction test is suitable bioassay for completion other plant and invertebrate tests.

Description of the measurement procedure

Preparation of artificial soil (AS)

An artificial soil is used with the following composition (based on dry weights):

- 69 % air-dried quartz sand (predominantly fine sand with more than 50 % of the particles between 0.05 mm – 0.2 mm),
- 10 % sphagnum peat (a particle size of 2 ± 1 mm); it is recommended to defaunize batch of peat by means of deep freezing (- 80 °C or 3× - 20 °C) and verify suitability for culturing the springtails before it is used in a test,
- 20 % kaolin clay,
- 0.3 to 1.0 % calcium carbonate ($CaCO_3$, pulverized, analytical grade) to obtain a pH of 6.0 ± 0.5.

AS is stored one day in laboratory condition and pH of the AS is checked. If it is necessary pH is equilibrated using calcium carbonate to reach pH of 6.0 ± 0.5. The mixed soil should be stored for three days in order to equilibrate acidity. For the determination of pH soil is mixed (1:5) with 1 M potassium chloride (KCl). One or two days before starting the test maximum holding capacity (WHC) and dry weight of AS are determined.

Synchronisation

Synchronized animals are homogeneous test animals of the same old and size. Approximately 150 adult of *Folsomia candida* are transferred on water saturated substrate plaster of Paris/charcoal (8:1; 0.5 cm layer) for egg laying. The culture is kept at 20 °C in dark (as stock

culture) one day without feeding. Adult animals are removed from the synchronization culture vessel one day later and only eggs left. After 13-14 days juveniles are hatched and in 12th day can be used for testing.

Preparation of test

Range of concentrations is chosen in accordance with maximal recommended dose of testing fertilizer, soil improvers and bio-waste, at least six concentrations. Maximal recommended dose is in the middle of the tested concentrations. For preparing a concentration series it is recommended to choose the dilution step not exceeding spaced factor two. Controls, without the test substance, are also prepared. For each concentration six replicates (five with springtails) are prepared and for control eleven (ten with springtails) replicates are prepared. It is recommended to prepare whole batch for each concentration, add tested chemical, and moist to obtain final water content 50 % of the maximum WHC. For each test concentration an amount of test soil corresponding to 30 g of dry weight is placed into the test vessel. Food for springtails (2 mg of dried yeast) is added to each vessel. The test is performed with synchronized animals. Ten juvenile springtails (12 days old) are randomly allocated to each test vessel. The springtails are carefully sucked with exhauster into each test vessel. The test vessels are covered by perforated parafilm. The test vessels are randomly positioned in the test incubator (20 °C, 400 – 800 lux) and these positions are re-randomized weekly. The test vessels are re-weighted and loss of water is replenished each week.

At the end of the test actual pH and moisture of AS is measured (vessels without animals and feeding).

Finish of the test and counting of springtails

After 32 days all springtails are counted. The method based on water-floatation techniques is recommended. Content of testing vessel is poured into counting basins, 400 ml of water is added and 0.8 ml of black ink. After gentle stirring of the suspension with spatula, springtails will drift to the water surface. Springtails in counting basins are photographed and counted (adults and juveniles).

Validity of the test

For the validity of the test the following performance criteria should be met in the controls:

- assuming that ten adults per vessel were used in setting up the test, an average of at least 100 juveniles per vessel should have been produced at the end of the test.
- the coefficient of variation around the mean of number of juveniles should not be higher than 30 % at the end of the test.

Evaluation of the test

NOEC (No Observed Effect Concentration)/LOEC (Lowest Observed Effect Concentration) – homogeneity of variances (Levene test) and normality (Shapiro-Wilk test) are calculated. Normally distributed and homogenous data are evaluated with Dunnett test. If these requirements are not fulfilled, it is recommended to use Welch test (inhomogeneity of variances) with Bonferroni-Holm correction or U-test with Bonferroni-Holm correction (non-normality).

EC_x (Effect Concentration for x% effect) – data are evaluated by probit or logit regression, or by Weibull analysis.

Instruments and equipment

- Test vessels (150 – 200) ml
- Sieve (2 mm)
- Vessel for mixing of artificial soil
- Equipment for mixing of artificial soil

- Incubator (20 °C, quantity of illumination (400 – 800) lux)
- Stereomicroscope, counter
- Photo basin (10 × 10 × 8) cm
- Tweezers, hooks or loops
- Luxmeter
- Cutting machine for cut of peat
- Parafilm
- Culture of *Folsomia candida*

Chemicals, solutions or necessary supplies

- Artificial soil (sphagnum peat - a particle size of 2 ± 1 mm, kaolin clay, quartz sand - predominantly fine sand with more than 50 % of the particles between 0.05 mm – 0.2 mm, calcium carbonate - pulverised, analytical grade to equilibrate of pH)
- Potassium chloride, KCl, c(KCl) = 1 mol/l. Preparation: 74.6 g KCl is dissolved in water and topped up with water to 1000 ml.
- Alcohol, 4 : 1 (alcohol : water)
- Plaster of Paris, charcoal (8:1; 0.5 cm layer)
- Yeast
- Black ink

Literature

ISO 11267: Soil quality – Inhibition of reproduction of *Collembola* (*Folsomia candida*) by soil pollutants. International Organization for Standardization. Geneve, Switzerland, 1999.

OECD Guidelines for the Testing of Chemicals 232, Collembolan Reproduction Test in Soil, 2009.

Determination of the effect of agrochemicals on the reproduction of Enchytraeids (*Enchytraeus crypticus*)

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What is measured in this method?

The method describes the assessment of effects of fertilizers, soil improvers and bio-wastes on reproduction of enchytraeid worm *Enchytraeus crypticus*. Adult enchytraeid worms are exposed to a range of concentrations of the test substance mixed into an artificial soil. Range of concentration is chosen in accordance with maximal recommended dose of testing fertilizers, soil improvers and bio-wastes, at least six concentrations. Maximal recommended dose is in the middle of the tested concentrations. The duration of the test is four weeks. The reproductive output of the animals exposed to the test substance is compared to that of the control.

Why this measurement is important?

Soil-dwelling annelids of the genus *Enchytraeus* are ecologically relevant species for ecotoxicological testing. Whilst enchytraeids are often found in soils containing earthworms it is also true that they are often abundant in many soils where earthworms are absent. Enchytraeids can be used in laboratory tests as well as in semi-field and field studies. The duration for a reproduction test with *Enchytraeus crypticus* is only four weeks while for earthworms it is longer (eight weeks). The reproductive output of the animals is sensitive endpoint for testing of chemicals.

Description of the measurement procedure

Preparation of artificial soil (AS)

An artificial soil is used with the following composition (based on dry weights):

- 69 % air-dried quartz sand (predominantly fine sand with more than 50% of the particles between 0.05 mm – 0.2 mm),
- 10% sphagnum peat (a particle size of 2 ± 1 mm); it is recommended to defaunize batch of peat by means of deep freezing (- 80 °C or 3× - 20 °C) and verify suitability for culturing the worms before it is used in a test,
- 20% kaolin clay,
- 0.3 to 1.0 % calcium carbonate ($CaCO_3$, pulverized, analytical grade) to obtain a pH of 6.0 ± 0.5.

AS is stored one day of rest in laboratory condition and check pH. If it is necessary equilibrate pH (6 ± 0.5) using calcium carbonate. The mixed soil should be stored for three days in order to equilibrate acidity. For the determination of pH a mixture of soil is used 1 M potassium chloride (KCl). One or two days before starting the test is determine maximum holding capacity (WHC) and dry weight of AS.

Preparation of test

Range of concentration is chosen in accordance with maximal recommended dose of testing fertilizer, soil improvers and bio-waste, at least six concentrations. Maximal recommended dose is

in the middle of the tested concentrations. For preparing a concentration series is recommended to choose the dilution step not exceeding spaced factor two. Controls, without the test substance, are also prepared. For each concentration is prepared six replicates (five with worms) and for control eleven (ten with worms) replicates. It is recommended prepare whole batch for each concentration, add tested chemical and moist with water to obtain final water content 50 % of the maximum WHC. For each test concentration, an amount of test soil corresponding to 20 g dry weight is placed into the test vessel. Food is added (25 mg of rolled oats) to each vessel for worms. Ten adult worms with clitellum are randomly allocated to each test vessel. The worms are carefully transferred into each test vessel and placed on the surface of the soil using, for example, jeweler's tweezers or hooks. The test vessels are covered by perforated parafilm. The test vessels are positioned randomly in the test incubator (20 °C, 400 – 800 lux) and these positions are re-randomized weekly. The test vessels are re-weighted and loss of water is replenished each week.

At the end of the test actual pH and moisture of AS is measured (vessels without animals and feeding).

Finish the test and counting of worms

After four weeks all worms are counted. The method based on Bengal red staining and floatation techniques is recommended. At the end of the test, the juveniles are fixed with ethanol (5 ml per replicate). The vessels are then filled with water up to a layer of 1 to 2 cm (50 ml). A few drops (400 µl) of Bengal red (1% solution in ethanol) are added and mix carefully. After 24 hours, the worms are stained a reddish colour. The soil suspension in the test vessels is transferred to a photo basin with ribbed bottom. The “ribs” restrict movement of the worms within the field of observation and should be easy to count. Then colloidal solution of SiO_2 (70 ml) is added and carefully mixed. After few minutes reddish worms will be lying on the suspension surface. Worms in photo basins are photographed and count.

Validity of the test

For the test to be valid, the following performance criteria should be met in the controls:

- assuming that 10 adults per vessel were used in setting up the test, an average of at least 50 worms per vessel should have been produced at the end of the test.
- the coefficient of variation around the mean number of worms should not be higher than 50% at the end of the test.

Evaluation of the test

NOEC (No Observed Effect Concentration)/LOEC (Lowest Observed Effect Concentration)- homogeneity of variances (Levene test) and normality (Shapiro-Wilk test)are calculated. Normally distributed and homogenous data are evaluated with Dunnett test. If these requirements are not fulfilled, it is recommended to use Welch test with Bonferroni-Holm correction (inhomogeneity of variances) or U-test with Bonferroni-Holm correction (non-normality).

ECx (Effect Concentration for x% effect)- data are evaluated by probit or logit regression, or Weibull analysis.

Instruments and equipment

- Test vessels (150 – 200) ml
- Sieve (2 mm)
- Vessel for mixing of artificial soil
- Equipment for mixing of artificial soil
- Incubator (20 °C, quantity of illumination (400 – 800) lux)
- Stereomicroscope, counter

- Photo basin (15 × 20 × 3) cm
- Tweezers, hooks or loops
- Luxmeter
- Cutting machine for cut of peat
- Parafilm
- Culture of *Enchytraeus crypticus*
- Autoclave

Chemicals, solutions or necessary supplies

- 1% solution of Bengal red. Preparation: 1 g of Bengal red dissolve in alcohol and top up with alcohol to 100 ml.
- Artificial soil (sphagnum peat - a particle size of 2 ± 1 mm, kaolin clay, quartz sand - predominantly fine sand with more than 50% of the particles between 0.05 mm – 0.2 mm, calcium carbonate - pulverised, analytical grade to equilibrate of pH)
- Potassium chloride, KCl, c(KCl) = 1 mol/l. Preparation: 74.6 g KCl dissolve in water and top up with water to 1000 ml.
- Alcohol, 4 : 1 (alcohol : water)
- SiO_2 (colloidal solution minimal 40%)
- Autoclaved rolled oats. Preparation: rolled oats sterilize 20 min 121 °C.

Literature

ISO 16387, Soil quality – Effects of pollutants on Enchytraeidae – Determination of effects on reproduction and survival, 2004.

OECD Guidelines for the Testing of Chemicals 220, Enchytraeid Reproduction Test, 2004.

Determination of the effect of agrochemicals on the reproduction of predatory mites (*Hypoaspis aculeifer*)

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What is measured in this method?

The method is used for evaluation of effect fertilizers, soil improvers and bio-wastes on reproduction of soil mite *Hypoaspis aculeifer*. Adult mites are exposed to a range of concentrations of the test substance mixed into an artificial soil. The range of at least six concentration is chosen in accordance with maximal recommended dose of testing fertilizers, soil improvers and bio-wastes. Maximal recommended dose is in the middle of the tested concentrations. The duration of the test is 14 days. The reproductive output of the animals exposed to the test substance is compared to that of the control.

Why this measurement is important?

Mite *Hypoaspis aculeifer* represent predatory mite of soil fauna. They were worldwide distributed and easily breeded in the laboratory. The reproduction cycle of this mite is faster than in the case of other invertebrates bioassays. The reproductive output of the animals is sensitive endpoint for testing of chemicals. Mite reproduction test is suitable and complementary bioassay for other plant and invertebrate tests.

Description of the measurement procedure

Preparation of artificial soil (AS)

An artificial soil is used with the following composition (based on dry weights):

- 74 % air-dried quartz sand (predominantly fine sand with more than 50 % of the particles between 0.05 mm – 0.2 mm),
- 5 % sphagnum peat (a particle size of 2 ± 1 mm); it is recommended to defaunize batch of peat by means of deep freezing (- 80 °C or 3 × - 20 °C) and verify suitability for culturing the springtails before it is used in a test,
- 20 % kaolin clay,
- 0.3 to 1.0 % calcium carbonate ($CaCO_3$, pulverized, analytical grade) to obtain a pH of 6.0 ± 0.5.

AS is stored one day in laboratory condition and pH of the AS is checked. If it is necessary pH is equilibrated using calcium carbonate to reach pH of 6.0 ± 0.5. The mixed soil should be stored for three days in order to equilibrate acidity. For the determination of pH soil is mixed (1:5) with 1 M potassium chloride (KCl). One or two days before starting the test maximum holding capacity (WHC) and dry weight of AS are determined.

Synchronisation

Synchronized animals are homogeneous test animals of the same old and size. Approximately 180 adult female of *Hypoaspis aculeifer* (are bigger) and 20 adult male of mite (are smaller and thinner) are transferred on moderate water saturated substrate plaster of Paris/charcoal (8:1; 0.5 cm layer) for egg laying. The culture is kept at 20 °C in dark (as stock culture) two days without

feeding. Adult animals are removed from the synchronization culture vessel two days later and only eggs left. After 28-35 days juveniles can be used for testing.

Preparation of test

Range of concentrations is chosen in accordance with maximal recommended dose of testing fertilizer, soil improvers and bio-waste, at least six concentrations. Maximal recommended dose is in the middle of the tested concentrations. For preparing a concentration series it is recommended to choose the dilution step not exceeding spaced factor two. Controls, without the test substance, are also prepared. For each concentration six replicates (five with mites) are prepared and for control eleven (ten with mites) replicates are prepared. It is recommended to prepare whole batch for each concentration, add tested chemical, and moist to obtain final water content 50 % of the maximum WHC. For each test concentration an amount of test soil corresponding to 20 g dry weight is placed into the test vessel. Food for mites (small amount of feeding-cheese mites) is added to each vessel. The test is performed with synchronized animals. Ten juvenile mites are randomly allocated to each test vessel by thin wet brush. The test vessels are covered by perforated parafilm. The test vessels are randomly positioned in the test incubator (20 °C, 400 – 800 lux) and these positions are randomized weekly. The test vessels are re-weighted and loss of water is replenished each week. At the end of the test actual pH and moisture of AS is measured (vessels without animals and feeding).

Finish of the test and counting of mites

After 14 days all mites are extracted and counted. The method based on heat extraction is recommended. Content of testing vessel is poured into extraction vessel (look like Tullgren funnel) and put into extractor. The duration of the extraction is crucial point and the gradient of good to moderate to bad conditions for the organisms. Infrared bulbs are in the upper section of extractor and emitted heat. In the bottom part are cooling plates. Mites are creped through hot soil to cold catching vessels according heat gradient. Heating regime: 25 °C for 24 hours, 35 °C for 20 hours, 45 °C for 4 hours (in total 48 hours). The temperature is measured in the substrate. Mites are fixed by 70% ethanol at the end of extraction and counted (adults and juveniles).

Validity of the test

For the validity of the test, the following performance criteria should be met in the controls:

- assuming that ten adults per vessel were used in setting up the test, an average of at least 50 juveniles per vessel should have been produced at the end of the test.
- the coefficient of variation around the mean number of juveniles should not be higher than 30 % at the end of the test.

Evaluation of the test

NOEC (No Observed Effect Concentration)/LOEC (Lowest Observed Effect Concentration) – homogeneity of variances (Levene test) and normality (Shapiro-Wilk test) are. Normally distributed and homogenous data are evaluated with Dunnett test. If these requirements are not fulfilled, it is recommended to use Welch test with Bonferroni-Holm correction (inhomogeneity of variances) or U-test with Bonferroni-Holm correction (non-normality).

ECx (Effect Concentration for x% effect) – data are evaluated by probit or logit regression, or by Weibull analysis.

Instruments and equipment

- Test vessels (150 – 200) ml
- Sieve (2 mm)
- Vessel for mixing of artificial soil

- Equipment for mixing of artificial soil
- Incubator (20 °C, quantity of illumination (400 – 800) lux)
- Stereomicroscope, counter
- Extraction vessel (Tullgren funnel)
- Heat extractor
- Thin brush
- Luxmeter
- Cutting machine for cut of peat
- Parafilm
- Culture of *Hypoaspis aculeifer* and cheese mite (*Typrophagus putrescentiae*)

Chemicals, solutions or necessary supplies

- Artificial soil (sphagnum peat - a particle size of 2 ± 1 mm, kaolin clay, quartz sand - predominantly fine sand with more than 50 % of the particles between 0.05 mm – 0.2 mm, calcium carbonate - pulverized, analytical grade to equilibrate of pH)
- Potassium chloride, KCl, c(KCl) = 1 mol/l. Preparation: 74.6 g KCl is dissolved in water and topped up with water to 1000 ml.
- Alcohol, 4 : 1 (alcohol : water)
- Plaster of Paris, charcoal (8:1; 0.5 cm layer)

Literature

OECD 226, Predatory Mite (*Hypoaspis* (*Geolaelaps*) *Aculeifer*) Reproduction Test in Soil, 2008.

Contact test using dehydrogenase activity of bacteria *Arthrobacter globiformis*

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What is measured in this method?

The method is used for evaluation of effect of fertilizers, soil improvers and bio-wastes on dehydrogenase activity of soil bacteria *Arthrobacter globiformis*. Bacteria are exposed to a range of concentrations of the test substance mixed with quartz sand. The range of at least five concentrations is chosen in accordance with maximal recommended dose of testing fertilizers, soil improvers and bio-wastes. Maximal recommended dose is in the middle of the tested concentrations. The duration of the test is six hours. During the test resazurin is catabolized to resorufin by *Arthrobacter globiformis* and inhibition of dehydrogenase activity is measured fluorimetically.

Why this measurement is important?

Arthrobacter globiformis is widely spread bacteria in soil. The bacteria decompose organic matter to humus. *Arthrobacter globiformis* can be used in laboratory tests as well as in semi-field and field studies. The miniaturized solid contact test with these bacteria allows the preliminary evaluate of chemicals within six hours. The dehydrogenase activity inhibition is quite sensitive endpoint to different toxic substances.

Description of the measurement procedure

Reactivation of *Arthrobacter globiformis*

Freeze dried bacteria can be used immediately after thawing, no longer than six hours. For this 0.5 ml of ice water (deionised and sterile) is added to the lyophilisate. The moist lyophilisate has to be stored in a refrigerator for 20-30 minutes. Then the lyophilisate is transferred into 20 ml of nutrient solution B, which is tempered at 30 °C.

Preparation of test

The range of at least five concentrations is chosen in accordance with maximal recommended dose of testing fertilizers, soil improvers and bio-wastes. Maximal recommended dose is in the middle of the tested concentrations. For preparing a concentration series it is recommended to choose the dilution step not exceeding spaced factor two. One day before test start quartz sand is pre-moistened. It is recommended to prepare whole batch for each concentration (20 g quartz sand) and to moist with prepared concentration of tested chemical (4 ml).

For each treatment and the control 0.6 g wet weight of sample at four replicates are weighed into the microplates (24 wells) at test day. Microplate in duplicates is prepared for each tested material (testing plate and blank plate). 0.6 ml of sterilized water is filled into each well on the test substrates. Pasteurization in the water bath is necessary (for the deactivation of the natural microbiological activity) at 85 °C for 10 min and afterwards cooling in the ice bath for 15 min. This procedure is repeated once again. The whole process of deactivation has to be performed with the closed lid of the microplate all the time. After deactivation of naturally microbial activity 0.4 ml of the inoculum is added into each well (testing plate). Blank is filled with 0.4 ml nutrient

solution B instead of inoculum. The test microplates are incubated for two hours in the dark at 30 °C on the horizontal shaker (frequency: 150 min-1). 0.8 ml resazurine is added after two hours of incubation and the relative fluorescence of the produced resofurine is measured. The measurement of fluorescence (extinction 535 nm and emission 590 nm) is repeated every 15 min during one hour. Directly after every measurement the plates are placed back into the incubator onto the horizontal shaker (at 30 °C and 150 min-1).

Validity of the test

For the test to be valid, the following performance criteria should be met in the controls:

- the fluorescence of the untreated control increased 5 × (measuring time 0 min to 60)

Evaluation of the test

Calculation of the slope of the fluorescence

$$S = 100 - \left(\frac{f_2 - f_1}{t_2 - t_1}\right)$$

Where: S - slope of the fluorescence (f) between t_1 (15 min) and t_2 (45 min)

Calculation of the inhibition in [%]

$$I = 100 - \left(\frac{S_s}{S_c} \cdot 100\right)$$

Where:

I - inhibition in [%]

S_s - slope of the fluorescence in the sample between t_1 (15 min) and t_2 (45 min)

S_c - slope of the fluorescence in the control between t_1 (15 min) and t_2 (45 min)

NOEC (No Observed Effect Concentration)/LOEC (Lowest Observed Effect Concentration) – homogeneity of variances (Levene test) and normality (Shapiro-Wilk test) are calculated. Normally distributed and homogenous data are evaluated with Dunnett test. If these requirements are not fulfilled, it is recommended to use Welch test with Bonferroni-Holm correction (inhomogeneity of variances) or U-test with Bonferroni-Holm correction (non normality).

ECx (Effect Concentration for x% effect) – data are evaluated by probit or logit regression, or by Weibull analysis.

Instruments and equipment

- Test vessels (150 – 200) ml
- Sieve (2 mm)
- Microplate fluorimeter (Ex 535 nm, Em 590nm)
- Water bath
- Incubator (30 °C)
- Autoclave
- 24-well microplates
- Freeze dried bacteria *Arthrobacter globiformis*

Chemicals, solutions or necessary supplies

- Quartz sand
- Sterilized water
- Nutrient solution A. Preparation: 10 g casein peptone, 5 g yeast extract, 5 g glucose, 5 g NaCl are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. Solution is sterilized 20 min at 121 °C.
- Nutrient solution B. Preparation: 333.3 ml of nutrient solution A is dissolved in sterile water and topped up with water to 1000 ml.
- Phosphate buffer. Preparation: 7.24 g KH_2PO_4, 12 g K_2HPO_4, 2 g CH_3COONa, 2 g glucose are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. pH value is adjusted to 7.0 ± 0.2 (with NaOH or HCl solutions). The solution is sterilized 20 min at 121 °C.
- Resazurine, $C_{12}H_6NO_4Na$. Preparation: 45 mg of resazurine is dissolved in phosphate buffer and topped up to 1000 ml. The solution is stored in refrigerator and in the dark, it is stable for one week.

Literature

Helga Neumann-Hensel, Kerstin Melbye, Optimisation of the solid-contact test with *Arthrobacter globiformis*, Journal of Soils and Sediments, 6/4, 201-207, 2006.

Plant test (root growth of *Lactuca sativa*)

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What is measured in this method?

The method is used for evaluation of fertilizers, soil improvers and bio-wastes impacts on root growth of plant *Lactuca sativa*. Pregerminated seeds of *Lactuca sativa* are exposed to a range of concentrations of the test substance mixed into an artificial soil. The range of at least six concentrations is chosen in accordance with maximal recommended dose of testing fertilizers, soil improvers and bio-wastes. Maximal recommended dose is in the middle of the tested concentrations. The duration of the test is 120 hours. The length of plant root exposed to the test substance compare to that of the control is used as output of the test.

Why this measurement is important?

Plants are the most important terrestrial primary producer of biomass. Plant fulfill ecosystem function such as food source, habitat for many animals and last but not least soil protection against erosion. Plants grow in soil and can be used as indicator of potential harmful substances contained at fertilizers, soil improvers and bio-wastes. The germinated seed (root length) could be a sensitive endpoint for testing of chemicals. This test is an indispensable part of any ecotoxicological toolbox for characterization of fertilizers, soil improvers and bio-wastes.

Description of the measurement procedure

Preparation of artificial soil (AS)

An artificial soil is used with the following composition (based on dry weights):

- 69 % air-dried quartz sand (predominantly fine sand with more than 50 % of the particles between 0.05 mm – 0.2 mm),
- 10 % sphagnum peat (a particle size of 2 ± 1 mm); it is recommended to defaunize batch of peat by means of deep freezing (- 80 °C or 3× - 20 °C) and verify suitability for culturing the springtails before it is used in a test,
- 20 % kaolin clay,
- 0.3 to 1.0 % calcium carbonate ($CaCO_3$, pulverized, analytical grade) to obtain a pH of 6.0 ± 0.5.

AS is stored one day in laboratory condition and pH of the AS is checked. If it is necessary pH is equilibrated using calcium carbonate to reach pH of 6.0 ± 0.5. The mixed soil should be stored for three days in order to equilibrate acidity. For the determination of pH soil is mixed (1:5) with 1 M potassium chloride (KCl). One or two days before starting the test maximum holding capacity (WHC) and dry weight of AS are determined.

Preparation of test

One day before test is set up seeds are germinated on wet filter paper in the incubator (24 °C). Range of concentrations is chosen in accordance with maximal recommended dose of testing fertilizers, soil improvers and bio-wastes, at least six concentrations. Maximal recommended dose is in the middle of the tested concentrations. Maximal applied dosage is in the middle of the tested

concentrations. For preparing a concentration series it is recommended to choose the dilution step not exceeding spaced factor two. Controls, without the test substance, are also prepared. For each concentration six replicates (five with plant) are prepared and six controls (five with plants) replicates are prepared. It is recommended to prepare whole batch for each concentration, add tested chemical, and moist to obtain final water content 50 % of the maximum WHC. For each test concentration an amount of test soil corresponding to 200 g of dry weight is placed into the test vessel. 15 pregerminated (24 hours) seeds are carefully put to each test vessel and gently covered with soil. The test vessels are covered by shrink wrap. The test vessels are randomly positioned in the test incubator (24 °C).

Finish of the test

After 120 hours plant roots are measured. Plants are carefully removed out of soil and roots are measured with accuracy of 1 mm. For each vessel is counted average, standard deviation and coefficient of variance for control vessels.

Validity of the test

For the validity of the test the following performance criteria should be met in the controls:

- Minimal average length of control is 15 mm at least.
- The coefficient of variation not be higher than 20 % at the end of the test.
- Average germination not be smaller than 80 %.

Evaluation of the test

NOEC (No Observed Effect Concentration)/LOEC (Lowest Observed Effect Concentration) – homogeneity of variances (Levene test) and normality (Shapiro-Wilk test) are calculated. Normally distributed and homogenous data are evaluated with Dunnett test. If these requirements are not fulfilled, it is recommended to use Welch test with Bonferroni-Holm correction (inhomogeneity of variances) or U-test with Bonferroni-Holm correction (non normality).

ECx (Effect Concentration for x% effect) – data are evaluated by probit or logit regression, or by Weibull analysis.

Instruments and equipment

- Test vessels (700 – 1000) ml
- Sieve (2 mm)
- Vessel for mixing of artificial soil
- Equipment for mixing of artificial soil
- Incubator (24 °C)
- Tweezers, hooks or loops
- Cutting machine for cut of peat
- Shrink wrap
- Seeds of *Lactuca sativa*

Chemicals, solutions or necessary supplies

- Artificial soil (sphagnum peat - a particle size of 2 ± 1 mm, kaolin clay, quartz sand - predominantly fine sand with more than 50 % of the particles between 0.05 mm – 0.2 mm, calcium carbonate - pulverised, analytical grade to equilibrate of pH)

- Potassium chloride, KCl, c(KCl) = 1 mol/l. Preparation: 74.6 g KCl is dissolved in water and topped up with water to 1000 ml.
- Alcohol, 4 : 1 (alcohol : water)

Literature

ISO11269-1, Soil quality - Determination of the effects of pollutants on soil flora – Part1: Method for the measurement of inhibition of root growth, 1993.

ISO 17126, Soil quality - Determination of the effects of pollutants on soil flora – Screening test for emergence of lettuce seedlings (*Lactuca sativa* L.), 2005.

Becker L., Scheffczyk A., Förster B., Oehlmann J., Princz J., Römbke J., Moser T. Effects of boric acid on various microbes, plants, and soil invertebrates, J Soils Sediments, 11, 238–248, 2011.

Moser H., Römbke J. (Eds.). Ecotoxicological Characterization of Waste Results and Experiences of an International Ring Test, 2009.

Determination of the inhibitory effect of elutriates on the light emission of luminescent bacteria *Vibrio fischeri*

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What is measured in this method?

The method describes the assessment of effects of elutriates on light emission of bacteria *Vibrio fischeri* during 30 min incubation under controlled conditions. The bacteria naturally emit light under optimal conditions. If this conditions are disturbed (by toxic substances), quickly fading the bioluminescence. The luminescent inhibition in the exposed wells is compared to the controls without the test samples.

Why this measurement is important?

The purpose of this test is to determine the effects of a substance containing in soil elutriates on the light emission of bacteria *Vibrio fischeri*. The tested organism naturally emitted luminescence is exposed to elutriates in 96-well micro-plates over a short time period.

Description of the measurement procedure

Reactivation of *Vibrio fischeri*

Lyofilizated culture of *Vibrio fischeri* NRRL-B-11177 is used for testing. Cool (3 °C) vibrio fischeri medium (1 ml) is added to ampule with lyofilizated culture of *Vibrio fischeri* and incubated at least 30 min in fridge.

Working suspension of *Vibrio fischeri*

Reactivated culture of *Vibrio fischeri* is diluted by vibrio fischeri medium and tempered at 15 °C. The luminescence should be in the range 300 000 to 800 000. Working suspension is stored at 15 °C no more than 15 min.

Preparation of elutriates

After sampling soil samples are homogenized, sieved (2 mm) and stored at -20 °C in a freezer. At least 7 days before measurement the samples are moved to a fridge (4 °C) to thaw. 10 g of soil (equivalent of dry weight) is weighted in 200 ml vessel. 20 ml of water is added (including water in the sample). Suspension is shaken on orbital shaker (170 RPM) overnight (24 h) at the test temperature (23 °C). Elutriates are centrifuged 10 min, 9000 RPM. In the supernatant is measured pH. pH is adjusted to 7.0 ± 0.2 (NaOH or HCl), if not in the range 6.5 – 8.0. Elutriates are centrifuged 10 min, 14000 RPM. Supernatant is diluted with 2x concentrated NaCl (LID 2- lowest ineffective dilution- the most concentrated dilution at which no inhibition, or only minor effect not exceeding the test-specific variability, were observed). Next dilutions step not exceeding spaced factor two with NaCl (LID 4, 8, 12, 16, 24, 32).

Preparation of test

Concentrations of elutriated (200 µl) are pipetted by rows randomly include well with control (NaCl) to preparative micro-plate. The covered micro-plates are tempered at 15 °C. De-ionized water (200 µl) is pipetted to the wells of outer rows of the white testing micro-plates to decrease

side effects and tempered at 15 °C. Working suspension of *Vibrio fischeri* (100 µl) is pipetted to white testing micro-plates and luminescence is measured immediately. In the next step are added randomized concentrations of elutriate (100 µl) by 8-channel pipette by row from preparative micro-plate. Luminescence is measured in 5th, 15th and 30th minute by row. It is necessary to keep whole procedure in tempered space (15 °C).

It is recommended that the sensitivity of the vibrio is checked at each set of tests with reference toxicant e.g. DCF (3,5 dichlorphenol, $Cl_2C_6H_3OH$) (inhibition 20 % - 80 % at 3.4 mg/l).

Validity of the test

For the test to be valid, the following performance criteria should be met in the controls:

- Inhibition of luminescence 20 % - 80 % caused 3.4 mg/l DCF.
- f_{kt} at 30min of incubation is 0.6 – 1.8

Evaluation of the test

Correction of initial values

$$f_{kt} = \frac{I_{kt}}{I_0} \quad (t = 5, 15, 30 \text{ min}) \qquad (1)$$

Where:

f_{kt} - correction factor

I_{kt} - luminescence of control at 5min, 15min or 30min

I_0 - luminescence of control before adding NaCl

Calculation of the concentration-dependent effect

Calculation of corrected values before adding tested samples

$$I_{ct} = I_0 \cdot f_{kt} \qquad (2)$$

Where:

I_0 - luminescence before adding tested sample

I_{ct} - corrected luminescence before adding tested sample

f_{kt} - correction factor.

Inhibition of tested sample

$$H_t = \frac{I_{ct} - I_{Tt}}{I_{ct} \cdot 100} \qquad (3)$$

Where:

H_t - inhibition of tested sample after exposition in time t (%)

I_{ct} - corrected luminescence before adding tested sample

I_{Tt} - luminescence tested sample in time t.

If inhibition is higher than 20 % in LID>8, the sample is found toxic.

Instruments and equipment

- 96-well micro-plate, white
- Sieve (2 mm)
- Vessel for shaking (200 ml)

- Incubator (15 °C)
- Luminometr
- Centrifuge

Chemicals, solutions or necessary supplies

- Natrium chloride NaCl – first solution. Preparation: 20 g NaCl dissolve in water and top up with water to 1000 ml.
- Natrium chloride NaCl – second solution. Preparation: 40 g NaCl dissolve in water and top up with water to 1000 ml
- VF medium. Preparation: 20 g NaCl, 2.035 g $MgCl_2 \cdot 6H_2O$, 0.30 g KCl are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. The solution is storied at 15 °C.

Literature

ISO 11348-3, Water quality -- Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) -- Part 3: Method using freeze-dried , 2007.

Froehner K., Meyer W., Grimme L.H.: Time-dependent toxicity in the long-term inhibition assay with *Vibrio fischeri*. Chemosphere, 46, 987 – 997, 2002.

Hirmann D., Loibner A.P., Braun R., Szolar O.H.J.: Applicability of the bioluminescence inhibition test in the 96-well microplate format for PAH-solutions and elutriates of PAH-contaminated soils. Chemosphere, 67, 1236 – 1242, 2007.

Mortimer M., Kasemets K., Heinlaan M., Kurvet I., Kahru A.: High throughput kinetic *Vibrio fischeri* bioluminescence inhibition assay for study of toxic effects of nanoparticles. Toxicology in Vitro, 22, 1412–1417, 2008.

Growth inhibition test with freshwater algae *Pseudokirchneriella subcapitata*

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What is measured in this method?

The method describes the assessment of elutriates on growth of green algae *Pseudokirchneriella subcapitata* during 72 h incubation in controlled conditions. The growth of the algae culture has to be exponential throughout the test period. The growth (cell density) is measured indirectly by in vivo fluorescent measurement which is calibrated against the cell density. The growth in the exposed wells is compared to the controls without the test samples.

Why this measurement is important?

The purpose of this test is to determine the effects of a substance containing in soil elutriates on the growth of freshwater algae. Exponentially growing test organisms are exposed to the test substance in 96 micro-plates over a period of normally 72 hours. In spite of the relatively brief test duration, effects over several generations can be assessed.

Description of the measurement procedure

Preparation of BB medium

Growth BB medium is made in from the stock solution 1-5:

10 ml stock solution 1

1 ml stock solution 2

1 ml stock solution 3

1 ml stock solution 4

1 ml stock solution 5

and replenish with de-ionized water up to 1000 ml

The pH is measured and it should be 8.3 ± 0.2. If needed pH is adjusted to this by 1 mol/l NaOH or/and HCl. The growth BB medium is autoclaved 20 min, 121 °C.

Algae pre-culture

The algae pre-culture is started 2-4 days before the toxicity test. The pre-culture should also be in exponential growth phase when used for the test. The algae are pre-cultured in BB growth media, and the cell density at the start should be around 10^4 cells/ml (± 2500 cells/ml). The algae cell density is counted with the Cyrus (Bürker) counting cuvette. The pre-culture could be kept in Erlenmeyer flask (250 ml, 100 ml BB media) at 23 °C, 60 % relative humidity and 6000 - 10000 lux light (similar to the test conditions). At the end of per-culturing the cell density should be around 10^6 cells/ml. It is recommended handling with stock culture of algae under sterile conditions.

Preparation of elutriates

After sampling soil samples are homogenized, sieved (2 mm) and stored at -20 °C in a freezer. At least 7 days before measurement of respiration the samples are moved to a fridge (4 °C) to thaw. 10 g of soil (equivalent dry weight) is weighted in 200 ml vessel. 20 ml of water is added (including water in the sample). Suspension is shaken in orbital shaker (170 RPM) overnight (24 h) to the test temperature (23 °C). Elutriates are centrifuged 10 min, 9000 RPM. Supernatant is diluted with 2x concentrated sterile BB medium (LID 2- lowest ineffective dilution). Next dilutions step not exceeding spaced factor two with sterile BB medium (LID 4, 8, 12, 16, 24, 32).

Preparation of test

Before the test the condition (shape of cells, no visible contamination) assessed, and the cell density on the pre-culture is counted. Each test contains calibration curve which is used for recalculation fluorescence vs cell density. The cell density should be 10^4 cells/ml at the start of the test. The test samples are pipetted to the micro-plates 270 µl of samples and 30 µl algae inoculum (10^5 cells/ml) is then pipetted to the wells. For the testing of background fluorescence of the samples the inoculum volume is substituted by BB medium. In the control wells the algae are grown only with the medium (270 µl BB medium and 30 µl algae inoculum). Control is pipetted to columns number 2 and 7 and to line D to prevent unequal illumination. In certain wells to get the background of the controls the inoculum is also substituted by BB medium. De-ionized water (300 µl) is pipetted to the wells of outer rows of the micro-plates in order to decrease evaporation losses.

The micro-plates are incubated covered at 23 °C, 60 % relative humidity and 6000-10000 lux light. The fluorescence of the micro-plates is measured daily (24h, 48h, 72h) after shaking without the covers. There will be condensing of water to the covers of the micro-plates, but this should not be dried.

It is recommended that the sensitivity of the algae is checked at each set of tests with reference toxicant e.g. $K_2Cr_2O_7$ (EC50 1.19±0.27 mg/l).

Validity of the test

For the test to be valid, the following performance criteria should be met in the controls:

- Number of cell increase 16x during 72 h.

Evaluation of the test

For evaluation is used calibration curve and recalculation to number of cell at 72 h. From fluorescence value in each dilution with algae is subtracted value of fluorescence without algae. Next the results are recalculated to number of cell according calibration curve. Inhibition is calculated. If inhibition more than 30 % in LID>8, the sample is find toxic.

Instruments and equipment

- 96 micro-plate
- Sieve (2 mm)
- Vessel for shaking (200 ml)
- Incubator (20 °C, quantity of illumination (6000 – 10000) lux)
- Luxmeter
- Autoclave
- Fluorimeter
- Centrifuge
- Cyrus (Bürker) counting cuvette

Chemicals, solutions or necessary supplies

- Stock solution 1. Preparation: 25 g $NaNO_3$, 3.29 g $CaCl_2$, 7.5 g MgSO4·7 H_2O, 7.5 g K_2HPO_4, 17.5 g KH_2PO_4, 2.5 g NaCl dissolve in de-ionized water and top up with de-ionized water to 1000 ml. Solution sterilize 20 min 121 °C.
- Stock solution 2. Preparation: 5 g $Na_2EDTA \cdot 2H_2O$, 3.1 g KOH dissolve in de-ionized water and top up with de-ionized water to 100 ml. Solution sterilize 20 min 121 °C.
- Stock solution 3. Preparation: 0.498 g $FeSO_4 \cdot 7H_2O$, 0.1 ml H_2SO_4, dissolve in de-ionized water and top up with de-ionized water to 100 ml. Solution sterilize 20 min 121 °C.
- Stock solution 4. Preparation: 1.142 g H_3BO_3 dissolve in de-ionized water and top up with de-ionized water to 100 ml. Solution sterilize 20 min 121 °C.
- Stock solution 5. Preparation: 0.882 g $ZnSO_4 \cdot 7H_2O$, 0.144 g MnCl2·4H2O, 0.242 g $Na_2MoO_4 \cdot 2H_2O$, 0.157 g CuSO4·5H2O, 0.049 g $Co(NO_3)$·6H2O dissolve in de-ionized water and top up with de-ionized water to 100 ml. Solution sterilize 20 min 121 °C.

All solutions are stored at +4 °C.

Literature

ISO 8692 Water quality — Fresh water algal growth inhibition test with unicellular green algae, 2004.

OECD Guideline for testing chemicals No. 201: Freshwater algae and Cyanobacteria, Growth inhibition test, 2006.

Growth inhibition test with freshwater aquatic plants *Lemna minor*

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What is measured in this method?

The method describes the assessment of elutriates on growth of freshwater aquatic plants *Lemna minor* during incubation (168 h) in controlled conditions. The growth of the duckweed culture has to be fast throughout the test period. The effect of elutriates is measured directly by number of new fronds during the tests. The growth in the exposed vessels is compared to the controls without the test samples.

Why this measurement is important?

The purpose of this test is to determine the effects of a substance containing in soil elutriates on the model organism for higher water plants. Duckweed is primary producer spread all over the world. Duckweed serves as source food for aquatic animals. This plant could reveal harmful effects of chemicals contained at tested elutriates.

Description of the measurement procedure

Preparation of Steinberg medium

Growth Steinberg medium is prepared using the stock solutions 1-8:

- 20 ml stock solution 1-3
- 1 ml stock solution 4-8

and replenished with de-ionized water up to 1000 ml.

The pH is measured and it should be 5.5 ± 0.2. If needed pH is adjusted to this by 1 M NaOH or/and 1 M HCl. The growth Steinberg medium is autoclaved 20 min at 121 °C.

Lemna pre-culture

The *Lemna* pre-culture is started 14 days before the toxicity test. The pre-culture could be kept in Erlenmeyer flask (250 ml, 2000 ml Steinberg medium) at 23 °C, 6000 - 8000 lux light (similar to the test conditions). At the end of per-culturing the number of fronds should be covered whole surface of flask. It is recommended to handle with stock culture of *Lemna* under sterile conditions.

Preparation of elutriates

After sampling soil samples are homogenized, sieved (2 mm) and stored at -20 °C in a freezer. The samples are moved to a fridge (4 °C) to thaw at least 7 days before measurement of *Lemna*. 75 g of soil (equivalent of dry weight) is weighted in 250 ml vessel. 150 ml of water is added (including water in the sample). Suspension is shaken on orbital shaker (170 RPM) overnight (24 h) at the test temperature (23 °C). Elutriates are diluted with 2x concentrated Steinberg medium (LID 2- lowest ineffective dilution). Next dilutions step not exceeding spaced factor two with Steinberg medium (LID 4, 8, 16, 32).

Preparation of test

150 ml of each dilution is contained in test vessels for one elutriate. 13 (four colony) well developed fronds of *Lemna* is placed to surface of test vessel. Optimal temperature is 23 °C and humidity is kept at 80 % optimally (use transparent lid or shrink wrap) during test. Light intensity is set on 8000 lux. The exposure time is 16 hours day and 8 hours night during whole seven days. Test vessels should have a dark bottom and sides in order to avoid exposure light.

Evaluation of the test

All vessels are evaluated the seventh day. Fronds are counted at each vessel. Inhibition is calculated. If inhibition is higher than 20 % in LID>8 compared with control, the sample is find toxic.

Instruments and equipment

- Test vessels
- Sieve (2 mm)
- Vessel for shaking (250 ml)
- Incubator (23 °C, quantity of illumination (6000 – 10000) lux)
- Luxmeter
- Autoclave
- Membrane filter 0.22 µm

Chemicals, solutions or necessary supplies

- Stock solution 1. Preparation: 17.5 g KNO_3, 4.5 g KH_2PO_4, 0.63 g K_2HPO_4 are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. The solution is sterilized 20 min at 121 °C.
- Stock solution 2. Preparation: 5 g $MgSO_4 \cdot 7H_2O$ are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. The solution is sterilized 20 min at 121 °C.
- Stock solution 3. Preparation: 14.75 g $Ca(NO_3)_2 \cdot 4H_2O$ are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. The solution is sterilized 20 min at 121 °C.
- Stock solution 4. Preparation: 120 mg H_3BO_3 are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. The solution is sterilized 20 min at 121 °C.
- Stock solution 5. Preparation: 180 mg $ZnSO_4 \cdot 7H_2O$ are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. The solution is sterilized 20 min at 121 °C.
- Stock solution 6. Preparation: 44 mg $Na_2MoO_4 \cdot 2H_2O$ are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. The solution is sterilized 20 min at 121 °C.
- Stock solution 7. Preparation: 180 mg $MnCl_2 \cdot 4H_2O$ are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. The solution is sterilized 20 min at 121 °C.
- Stock solution 8. Preparation: 1500 mg $Na_2EDTA \cdot 2H_2O$, 760 mg $FeCl_3 \cdot 6H_2O$ are dissolved in de-ionized water and topped up with de-ionized water to 1000 ml. The solution is sterilized 20 min at 121 °C.

Literature

ISO 20079, Water quality -- Determination of the toxic effect of water constituents and waste water on duckweed (Lemna minor) -- Duckweed growth inhibition test, 2005.

David w. Bowker, Anthony n. Duffield and Patrick Denny, Freshwater Biology ,10, 385-388, 1980.

Sampling of soil fauna

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What is measured in this method?

Aim of this method is to evaluate structural (qualitative) as well as quantitative parameters of soil fauna communities.

Why this measurement is important?

Soil invertebrates´ communities are strongly affected by changes in soil properties as well as changes in vegetation covers. Vegetation represent food offer for herbivores and detritivores respectively. Dead organic matter affects physical and chemical and biological parameters of soil, i.e. content of soil nutrients, amount of humus, soil structure, ability of soil to hold water, appropriate conditions for development of soil microorganisms etc. All these parameters are important for soil invertebrates; they are affected by changes in these parameters. We can evaluate:

- Number of species
- Number of individuals
- Structure (dominance) of caught community according species composition
- Structure (dominance) of caught community according functional diversity

Description of the measurement procedure

Soil invertebrates inhabiting soil is possible to divide into two groups according their life-style. The first group are *epigeic* (= surface dwelling) invertebrates, the second group are *edaphic* (=soil profile inhabiting) invertebrates. We combine two methods for obtaining data of both groups.

Epigeic invertebrates

Ground dwelling invertebrates are sampled using method called pitfall trapping. Pitfall trap consist from glass jar (kilner jar for fruits/vegetables of volume 0.7 dm^3 typically) with inserted plastic pot (0.3 dm^3 pot used for drinks in no name fastfoods). In the field, a hole is prepared with soil corer, glass jar inserted and soil adjusted around mouth of jar. Plastic pot is filled by 4% water solution of formaldehyde with added small amount of detergent (0.2 dm^3 of this fixative solution) is inserted in jar. Trap is covered by metal roof of square plate (ca 20 per 20 cm), cover is ca 2 cm above mouth of trap. Cover protect trap against rain and dirt. Three traps are installed in span of 5 m usually. Sampling period of traps is 2-3 weeks optimally.

Edaphic invertebrates

Soil profile inhabiting invertebrates are sampled using heat extraction of soil samples. Soil samples are usual quadrate method. Soil samples of size 1/20 square metre and depth of 10 cm are taken by metal circle soil corer. Samples are placed in plastic bags and transported in laboratory immediately. It is important do not sandwich too much samples during transportation because of squeezing of animals in lower layers. Samples are taken from plots once per season (at the end).

In the laboratory, samples are removed from bags in Tullgren funnels (Tuf & Tvardík 2005) and heated for 2 weeks. Heating is provided by electric bulb (classical bulb of 60 W power). Extracted invertebrates are collected in bottomed plate with fixative solution of formaldehyde (0.5%). Collected animals are taken off, sorted into taxonomical groups and placed into small ziplock-bags with 70% ethanol. Later, animals are identified into species level, counted and analysed.

Instruments and equipment

- Soil corer
- Tullgren extractors (120)
- Stereomicroscope
- Microscope

Chemicals, solutions or necessary supplies

- Formaldehyde
- Ethanol
- Detergent
- Electrical bulbs
- Plastic ziplock-bags

Literature

Tuf, I.H., Tvardík, D. (2005): Heat-extractor – indispensable tool for soil zoological studies. In: Tajovský, K., Schlaghamerský, J. & Pižl, V. (eds.): Contributions to Soil Zoology in Central Europe I. ISB AS CR, České Budějovice: 191-194. (pdf downloadable at: http://www.ekologie.upol.cz/ad/tuf/pdf/papers/Tuf_Tvardik_2005.pdf)

MICROBIOLOGY

Małgorzata Brzezińska, Magdalena Frąc, Jiří Čuhel, Bořivoj Šarapatka, Karolina Gawryjołek

The methods described in this chapter apply to determination of the biomass, diversity, metabolic processes and enzyme activity of soil microorganisms, and allow the study of the biogeochemical transformations of soil carbon (C), nitrogen (N), and phosphorous (P). These methods are recommended to study the evolution of exogenous organic matter (EOM) after its application to soils.

Numerous processes in soil, including organic matter decomposition, gas emissions, pollutants breakdown, energy flow, nutrient cycling in ecosystem, all depend on soil microbial activity. Soil physical, chemical and biological properties are involved in soil functioning and have been extensively used to measure soil quality. Physical and chemical soil properties usually change slowly. By contrast, microbial and biochemical properties respond rapidly even to small changes in soil status, and thus are considered as early indicators of soil alteration induced by agricultural management or contamination (García-Ruiz et al., 2008).

Microorganisms (bacteria, fungi, actinomycetes, algae, archaea) represent the largest fraction of organisms living in soils, both in terms of biomass and number. They play a key role in decomposition of organic matter entering the soil, including EOM. As soil organic matter (OM) is a major natural source of nutrients in terrestrial ecosystems, microbial transformation of OM is directly related to soil fertility and plant nutrient cycling. Moreover, several biogeochemical processes in agricultural soils occur with a release or consumption of gaseous carbon and nitrogen forms, thus, soil microbiota is strongly involved in emission and sink of greenhouse gases (CO_2, N_2O and CH_4).

Methods described in this chapter include:

- Basal respiration
- Substrate induced respiration
- Fumigation-extraction MBC, MBN
- Short-term nitrification activity
- Denitrifying enzyme activity
- Acid and alkaline phosphomonoesterase activity
- β-glucosidase activity
- CM-cellulase activity
- Dehydrogenase activity
- Urease activity
- Diversity of ammonia oxidizing archaea using terminal restriction fragment length polymorphism (t-RFLP)
- Functional diversity using Biolog method

Proposed selection of microbial and biochemical methods was based on their significance for the estimation of organic matter turnover, and soil C, N, and P transformations, and should be taken into account for final prediction of the impact of EOMs on soil quality. The methods include both general (e.g., dehydrogenase activity, soil respiration) and specific (e.g., urease or nitrification activity) indicators of soil microbial activity. The use of several individual methods gives a broad range of information concerning how biological status of soil (i.e., microbial biomass C and N, microbial diversity, enzyme activity, and potential to perform key biogeochemical processes) changes with time after EOMs application.

The EOM is an important and potentially growing source of material that could be applied to cultivated soils to improve their fertility (Peltre et al., 2011). Therefore, several ecological aspects of EOM application should be studied. For example, it is known that N and P availability is a key factor regulating soil productivity, and intensive organic fertilization increases the productivity. However, high input of labile C into the soil, especially the particulate organic C (50 to 2,000 µm) which is most vulnerable to decomposition (Stavi and Lal, 2013) may lead to increase emission of CO_2 and N_2O (if nitrogen is available) due to the stimulation of the respiration and denitrification processes. Information on the changes in the physical and chemical soil environments (e.g., soil pH, micronutrients availability, contamination, soil porosity, air-water conditions or hydrophobicity) allow to explain and predict some specific shifts in soil biota, for example domination of soil fungi. Therefore, understanding of the links between various physical, chemical and microbial or biological soil processes may help to anticipate which practice of the EOM amendment allows to combine the enhanced soil quality with carbon sequestration, ecosystem protection and some benefits of organic wastes disposal. There have also been developed several two- or multiparametric indices, which integrate different parameters, among which are the biological and chemical ones, such as pH, C_{org}, N_{tot}, microbial biomass C and N, respiration or enzyme activities. Integrated indices may provide a better indication of soil health than individual properties (Bastida et al., 2008). Probably most frequently used in the literature are so called ecophysiological indices specific respiration qCO_2 (respiration to microbial biomass ratio) and C_{mic}-to-C_{org} (microbial biomass C to total C_{org} ratio) (Anderson and Domsch 2010).

Literature

Anderson T.-H. and Domsch K.H. (2010) Soil microbial biomass: The eco-physiological approach. Soil Biol. Biochem. 42: 2039–2043.

Bastida F., Zsolnay A., Hernández T., García C. (2008) Past, present and future of soil quality indices: A biological perspective. Geoderma 147: 159–171.

García-Ruiz, R., Ochoa, V., Hinojosa, M. B., and Carreira, J. A. (2008) Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biol. Biochem. 40: 2137–2145.

Peltre C., Thuriès L., Barthès B., Brunet D., Morvan T., Nicolardot B., Parnaudeau V., Houot S. (2011) Near infrared reflectance spectroscopy: A tool to characterize the composition of different types of exogenous organic matter and their behaviour in soil. Soil Biol. Biochem. 43: 197–205.

Stavi I. and Lal R. (2013) Agriculture and greenhouse gases, a common tragedy. A review. Agron. Sustain. Dev. 33: 275–289.

Denitrifying enzyme activity (DEA) with differentiation of denitrification products N_2O and N_2

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What is measured in this method?

DEA values represent potential activity of denitrifying enzymes in soil at the time of sampling. The method uses inhibition of N_2O reduction to N_2 by added acetylene (C_2H_2), thus N_2O remains the only product of denitrification. Solution of nitrate and glucose is added to soil sample in a glass bottle, anaerobic conditions are settled in the bottle, acetylene is added, and after 30 and 60 min of incubation a sample of the inner atmosphere is analyzed on the N_2O content by gas chromatography. If both of the main denitrification products are assessed, production of N_2O from soil is also measured without the addition of C_2H_2. Production of N_2 is then determined as the difference between N_2O productions measured with and without added C_2H_2.

Why this measurement is important?

After carbon dioxide (CO_2) and methane (CH_4), nitrous oxide (N_2O) is the most potent greenhouse gas contributing to global warming. Moreover, N_2O contributes to the destruction of the stratospheric ozone layer and has become the most dominant ozone-depleting substance. After further reactions in the atmosphere, N_2O can also produce photochemical smog and acid rain. Its atmospheric concentration has been permanently increasing since preindustrial times. Soils are responsible for more than two-thirds of global N_2O emissions and denitrification is the main soil process evolving N_2O from soil into the atmosphere. The main denitrification products are N_2O and N_2 and their production and ratio differ between soils and depend on many environmental parameters.

Description of the measurement procedure

After sampling soil samples are homogenized, sieved (2 mm) and stored at -20 °C in a freezer. At least 7 days before measurement of DEA the samples are moved to a fridge (4 °C) to thaw. 25.0 g of field-moist soil sample is removed from the fridge, placed in 100-ml incubation bottle, and allowed to equilibrate to 25°C for 1 h in the incubator. 25 ml of the optimization solution is added to the soil samples in the bottles. Bottles are capped with rubber stoppers and enclosed with screw caps with a hole. The inner atmosphere of the bottles is exchanged for Ar by repeated evacuation (-95 kPa) and flushing with Ar (60 kPa) using special apparatus. The evacuation and flushing is conducted four times. The length of each evacuation and flushing is 1 min, i.e. 8 min for the whole procedure. The final overpressure in the bottles is released via gurgling in water. Using plastic syringe 10 ml C_2H_2 is added to the bottles through the rubber stopper and the overpressure in the bottles is released again. The bottles are placed in the incubator on the horizontal shaker and are incubated under permanent shaking at 25 °C. After 30 and 60 min, 0.5 ml headspace samples are taken with a gas-tight syringe and N_2O is immediately quantified using gas chromatography. After incubation the bottles are opened and the volume of gas phase (headspace) is gravimetrically determined with filling with water. For determining both denitrification products (N_2O and N_2) it is necessary to weigh additional replicate of soil sample and to repeat the whole operating procedure, however without the addition of C_2H_2.

Notes

- Only fresh optimization solution must be used, do not use solution older than 3 h. The solution is stored at 25 °C to have the same temperature as incubated samples.
- Before using the device for the exchange of inner atmosphere of the incubation bottles it is necessary to have it well purged with argon – at least two cycles of evacuation and filling of empty closed incubation bottles.
- Denitrification is an anaerobic process, therefore state of rubber tubes and rubber stoppers must be regularly checked to prevent contamination of inner atmosphere by oxygen.
- The whole operating procedure must be strictly standardized and the sampling of the inner atmosphere of the incubation bottles must be conducted at exact times (i.e. after 30 and 60 min of incubation). Results must be adequately recalculated, if the sampling times are slightly deviated.

Calculations

N_2O production is calculated separately for the variant with added C_2H_2 and separately for the variant without C_2H_2. However, only the variant with C_2H_2 represents the denitrifying enzyme activity (DEA) *sensu stricto*.

$$C_\Delta = C_{60} - C_{30}$$

$$G = \frac{C_\Delta \cdot V_g}{1000}$$

$$V_l = 50 - W_d$$

$$L = \frac{\propto \times G \times V_l}{V_g}$$

$$DEA = \frac{(G + L) \times 28 \times 1000}{W_d \times 22.4 \times 0.5}$$

Where:

C_{30} - N_2O concentration after 30 min of incubation (ppm)

C_{60} - N_2O concentration after 60 min of incubation (ppm)

C_Δ - difference of N_2O concentrations between 60 and 30 min of incubation (ppm)

V_g - volume of the gas phase (inner atmosphere) of incubation bottle (ml)

G - N_2O production into gas phase between 30 and 60 min of incubation (µl N_2O)

W_d - weight of dry soil, which corresponds to 25 g moist soil (g)

V_l - volume of liquid phase (optimization solution and water in 25 g moist soil) (ml)

α - Bunsen absorption coefficient for N_2O at given temperature (= 0.544 for 25 °C)

L - N_2O production into liquid phase between 30 and 60 min of incubation (µl N_2O)

DEA - denitrifying enzyme activity expressed as production of $N\text{-}N_2O$ into gas and liquid phase per 1 g dry soil per 1 h (ng $N\text{-}N_2O$ g^{-1} h^{-1})

Calculations for distinguishing of products N_2O a N_2 during denitrification activity

N_2O a N_2 production together = DEA

N_2O production = $DEA_{\text{without } C_2H_2}$

N_2 production = $DEA - DEA_{\text{without } C_2H_2}$

Relative N_2O production (%) = $\frac{DEA_{\text{without } C_2H_2}}{DEA} \times 100$

Instruments and equipment

- Incubation bottles and screw caps (GL 45) with a hole, 100 ml
- Rubber stoppers for incubation bottles
- Apparatus for evacuation of inner atmosphere from incubation bottles and their subsequent filling with inert gas (argon)
- Gas-tight chromatography syringes with PTFE plunger tips and replacement needles, 1 ml.
- Plastic syringes with replacement needles, 10 ml
- Gas chromatograph equipped with electron capture detector (ECD) and manual injection of samples, adjusted for N_2O analysis

Chemicals, solutions or necessary supplies

- Glucose, $C_6H_{12}O_6$
- Potassium nitrate, KNO_3
- Optimization solution, $c(C_6H_{12}O_6)$ = 1.0 mmol/l, $c(KNO_3)$ = 1.0 mmol/l. Preparation: 180.0 mg $C_6H_{12}O_6$ (1) and 101.1 mg KNO_3 (2) are dissolved in water, transferred into 1000-ml volumetric flask, and the flask is filled in with water to volume.
- Acetylene, C_2H_2
- Argon, Ar

Literature

M.S. Smith, J. M. Tiedje. Phases of denitrification following oxygen depletion in soil. Soil Biol. Biochem. 11, 261-267, 1979.

Assessment of basal and substrate induced respiration in soil by gas chromatography

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What is measured in this method?

The method describes the assessment of soil microbial respiration determined as CO_2 release either after addition of a substrate (substrate induced respiration – SIR) or without substrate addition (basal respiration – BR). Soil sample is incubated under aerobic conditions in enclosed bottle with or without the substrate. During incubation a sample of the inner atmosphere is analyzed for the CO_2 concentration by gas chromatography.

Why this measurement is important?

CO_2 is the most potent greenhouse gas contributing to global warming. Soil contributes to 20 % of the total emission of CO_2 to the atmosphere mainly through microbial decomposition of organic matter and microbial respiration: 60-80 % of mass of organic inputs is converted to CO_2 via microbial activity. Basal respiration represents a measure of the over-all activities of soil microorganisms and also indicates the content of mineralizable carbon. Substrate induced respiration is a measure of active biomass of soil microorganisms.

Description of the measurement procedure

Content of water in soil sample

Soil moisture should correspond to 40-60 % of maximal water holding capacity (WHC) of soils. The soil samples are moistened to 60 % WHC in the case of BR assessment and to 40 % WHC in the case of SIR assessment. The difference is caused by the fact, that mixing of substrate with soil wetted to 60 % WHC leads to sticking of soil particles, which disables effective gas exchange between soil and surrounding air. In the case of very low water content in the sample the wetting to 60 % WHC could be problematic. Then lower level of wetting should be chosen. Nevertheless, soils under one experiment should be always wetted to identical percentage of WHC.

Preincubation

After sampling soil samples are homogenized, sieved (2 mm) and stored at -20 °C in a freezer. At least 7 days before measurement of respiration the samples are moved to a fridge (4 °C) to thaw. Soils are preincubated 4 days at 22 °C. 13.5 g of soil (equivalent dry weight) is weighted in 100-ml Erlenmeyer flask. If the water content in soil is lower than 60 % or 40 % (for BR or SIR measurement, respectively) of maximal WHC, the soil sample must be moistened. The Erlenmeyer flasks are closed with parafilm and placed in incubator (22 °C).

Measurement

After preincubation, 12.5 g of soil (equivalent dry weight) is placed in 100-ml incubation bottle. In the case of SIR 0.125 g of substrate is weighted in the incubation bottle prior to the soil sample, then the soil is added and carefully homogenized with the substrate. Bottles are capped with rubber stoppers and enclosed with screw caps with a hole. The bottles are placed in the

incubator and incubation is started at 22 °C. After 2.5 and 5 h (in the case of SIR) or after 4 and 24 h (in the case of BR), 0.5 ml headspace samples are taken with a gas-tight syringe and CO_2 is immediately quantified using gas chromatography. After incubation the bottles are opened and the volume of gas phase (headspace) is gravimetrically determined with filling with water.

Calculations

$$C_\Delta = C_b - C_a$$

$$G = C_\Delta \times V_g \times 10$$

$$L = \frac{\propto \times G \times V_l}{V_g}$$

$$\text{BR or SIR} = \frac{(G + L) \times 12}{W_d \times 22.4 \times t}$$

Where:

C_a - CO_2 concentration after 2.5 h (in the case of SIR) or 4 h (in the case of BR) of incubation (v/v %)

C_b - CO_2 concentration after 5 h (in the case of SIR) or 24 h (in the case of BR) of incubation (v/v %)

C_Δ - difference of CO_2 concentrations between 5 and 2.5 h (SIR) or 24 and 4 h (BR) of incubation (v/v %)

V_g - volume of the gas phase (inner atmosphere) of incubation bottle (ml)

G - CO_2 production into gas phase between the measurements (μl CO_2)

W_d - weight of dry soil (12.5 g)

V_l - volume of liquid phase = volume of water in the soil sample (ml)

α - Bunsen absorption coefficient for CO_2 at given temperature (= 0.889 for 22 °C)

L - CO_2 production into liquid phase between the measurements (μl CO_2)

t - time between the measurements, i.e. 2.5 h for SIR and 20 h for BR

BR or SIR - basal or substrate induced respiration expressed as production of C-CO_2 into gas and liquid phase per 1 g dry soil per 1 h (μg C-CO_2 g^{-1} h^{-1})

Instruments and equipment

- Incubation bottles and screw caps (GL 45) with a hole, 100 ml
- Rubber stoppers for incubation bottles
- Gas-tight chromatography syringes with PTFE plunger tips and replacement needles, 1 ml
- Gas chromatograph equipped with thermal conductivity detector (TCD) and manual injection of samples, adjusted for CO_2 analysis

Chemicals, solutions or necessary supplies

- Glucose, $C_6H_{12}O_6$
- Ammonium sulfate, $(NH_4)_2SO_4$
- Potassium dihydrogen phosphate, KH_2PO_4
- Substrate. Preparation: Substrate is prepared by thorough mixing and grinding of 8.42 g glucose, 1.37 g $(NH_4)_2SO_4$ and 0.21 g KH_2PO_4 in a ceramic mortar.

Literature

ISO 14240-1 Soil quality – Determination of soil microbial biomass – Part 1: Substrate-induced respiration method, 1997.

ISO 16072 Soil quality – Laboratory methods for determination of microbial soil respiration, 2001.

Determination of soil microbial biomass Fumigation-extraction method

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What is measured in this method?

This method allows to determination of soil microbial biomass by measuring the total extractable organic biomass material from freshly killed microorganisms. After fumigation of soil sample, intact cells of soil microorganisms are lysed and microbial organic matter is released. Fumigation does not affect significantly for non-living soil organic matter. Soil samples are fumigated with chloroform for 24 hours. The organic carbon extracted by 0.5 M potassium sulphate is determined in fumigated and unfumigated samples and the increase in extracted organic carbon is used to determine microbial biomass carbon. This method is also applicable to the estimation of microbial nitrogen in soil

Why this measurement is important?

Soil microbial biomass is defined as an element of organic matter composed of living organisms inhabiting the soil and microfauna. Although the microbial biomass is only 1-3% of soil organic matter, it has a major part in the biochemical processes taking place in that environment. It is a major determinant of nutrient cycling and energy flow which affects the productivity of the ecosystem.

Soil microbes have the decisive influence on the metabolic processes in the soil and are also the main source of nutrients for plants because of soil microbial biomass is considered to be the main factor affecting the fertility of the soil.

Description of the measurement procedure

Line the desiccator for fumigation of the soil with moist filter paper. Weigh moist samples of soil, each containing the equivalent weight of 5 grams dry mass soils into glass beakers and place them in a desiccator with a beaker containing 25 ml ethanol-free chloroform and a few antibumping granules. Evacuate the desiccator until the chloroform has boiled for 3 minutes. Close the tap on the desiccator and incubate in the dark at about 25oC for 24 hours. After fumigation is completed, remove the chloroform and the paper from desiccator. Remove the chloroform vapour from the soil using water or electric pomp. Repeat evacuation 6 times for 2 minutes each.

To extract organic carbon add 20 ml 0.5 M K_2SO_4. Shake the samples on a horizontal shaker at 200 rev/min for 2 hours (Ghani et al., 2003) and filter the extracts through paper filter (Whatman No. 42). The procedure for control samples (unfumigated) is the same.

Concentration of total organic carbon and total nitrogen in extractant was determined by carbon analyser.

Calculation of biomass C:

Biomass was calculated as follows

$$Biomass\ C\ (B_C) = \frac{E_C}{k_{EC}}$$

where:

E_C = (organic carbon extracted from fumigated soil) - (organic carbon extracted from unfumigated soil)

k_{EC} = 0.45 (Wu et al., 1990)

All results are as µg C /g dry mass soil.

Calculation of biomass N

$$Biomass\ N = \frac{E_N}{k_N}$$

where:

E_N = (Total Nitrogen extracted from fumigated soil) - (Total Nitrogen extracted from unfumigated soil)

k_{EN} = 0.54 (Brookes et al., 1985)

All results are as µg N /g dry mass soil.

Literature

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Assay of β-glucosidase activity

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What is measured in this method?

β-glucosidase enzyme (β-D-glucoside glucohydrolase EC 3.2.1.21) is a cellulase with specificity for a variety of β-D-glycoside substrates. It catalyses the hydrolysis of β-1,4 bonds linking in disaccharide or glucose-substituted (e.g. cellobiose to produce glucose monomers).

p-nitrophenyl- β-D-glucoside (PNG) is used as a substrate to estimate β-glucosidase activity in soil. The method is based on the determination of the released *p*-nitrophenol (PNP) after the incubation of soil with *p*-nitrophenyl glucoside solution for 1 h at 37°C.

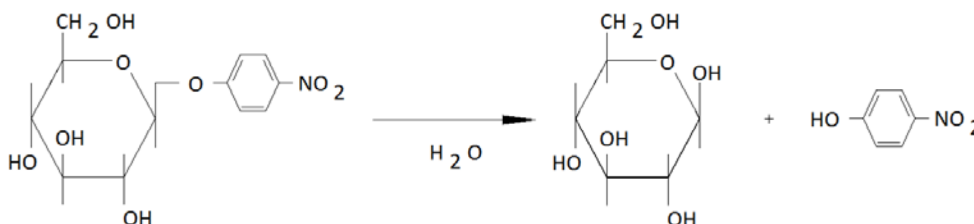

Figure 1. The hydrolysis reaction of *p*-nitrophenyl- β-D-glucoside

Why is this measurement important?

β-glucosidase enzyme plays an important role in degradation of cellulose and other carbohydrates. It means that is essential in the soil organic carbon cycle. Cellulose is the most plentiful organic compound in the biosphere, while glucose is a final product of degradation cellulose. Cellulose is a main organic carbon energy source for soil microorganisms.

β-glucosidase is useful as a soil quality indicator and is frequently used as a measure of soil organic carbon mineralization. β-glucosidase activity can provide advanced evidence of changes in organic carbon before it can be precisely measured by other routine methods. Both positive and negative effects of soil fertilization have been reported on this enzyme activity.

Description of the measurement procedure

β-glucosidase activity was determined according to the Eivazi and Tabatabai (1988) method modified by Alef and Nannipieri (1995).

- 1 g of moist soil was weighed into 15 ml test tubes.
- Soil was mixed with 0.25 ml of toluene, 4 ml of MUB solution, 1 ml PNG solution.
- Control soil was mixed only with 0.25 ml toluene and 4 ml of MUB solution.
- The tubes with soil suspension were closed and incubated for 1 h at 37°C.
- After the incubation 1 ml of $CaCl_2$ solution and 4 ml of NaOH solution were added to the samples.

- To control soil 1 ml PNG solution, 1 ml of $CaCl_2$ solution and 4 ml of NaOH solution were added.
- All suspension were mixed and centrifuged at 4000 rpm for 5 minutes.
- The colour intensity was measured at 400 nm against buffer MUB.
- The β -glucosidase activities was expressed in mg PNP kg^{-1} h^{-1}.

Instruments and equipment

- Microplate reader / spectrophotometer (wavelength of λ=400 nm)
- Water shaking bath adjustable to 37°C

Chemicals, solutions or necessary supplies

- Toluene
- Modified universal buffer (MUB) stock solution (12.1 g of Tris, 11.6 g of maleic acid, 14 g of citric acid, 6.3 g of boric acid (H_3BO_3), 500 ml of NaOH (1 M), 500 ml distilled water), pH 6.0 with HCl (0.1 M)
- $CaCl_2$ (0.5 M) solution
- NaOH (1 M) solution
- *p*-nitrophenyl- β-D-glucoside (PNG) solution (25mM)
- p-nitrophenol (PNP) for calibration curve

Literature:

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Assay of the dehydrogenase activity

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What is measured in this method?

The method allows to determine the effect of exogenous organic matter on activity of soil dehydrogenases.

The method consists in incubation of soil with artificial electron acceptor, the colourless and water-soluble 2,3,5-triphenyltetrazolium chloride (TTC), which is reduced due to the action of dehydrogenases to the red-colored triphenyl formazan (TPF), insoluble in water. Replacing oxygen and other natural acceptors, TTC intercepts electrons and protons which were detached by dehydrogenases from oxidized organic compounds. After the incubation, formazan is extracted from the soil with alcohol, and determined colorimetrically.

Why is this measurement important?

Soil dehydrogenase activity (DHA) is the result of the activity of different dehydrogenase enzymes involved in the oxidative metabolism of viable microbial cells. Under aerobic soil conditions, oxygen (O_2) is a final acceptor of electrons and protons detached by dehydrogenases, while under anaerobic conditions the function of the terminal acceptor is taken over by oxidized inorganic forms, such as nitrate, Mn(IV), Fe(III), sulfate, or by some organic compounds. Irrespective of the soil aeration, dehydrogenases are an element of the microbial respiratory metabolism related to the ATP synthesis. Since dehydrogenases occur in soil in all living microbial cells, the DHA test is used as an indicator of the overall soil microbial activity, or a measure of the general soil metabolic activity. A close relationship has been observed between DHA and soil organic matter content, microbial biomass, population size of soil microorganisms, respiration, denitrification, and other indicators of soil microbial activity. Additionally, dehydrogenase activity is often used as a measure of any disruption caused by pesticides, trace elements, etc., or management practices to the soil.

Description of the measurement procedure

Dehydrogenase activity was determined according to the Thalmann (1968) method modified by Alef and Nannipieri (1995).

- 5g of moist soil, sieved through a 2 mm mesh, was weighed into 50 ml test tubes
- Soil was mixed with 5 ml of 1% TTC solution
- The tubes with soil suspension were closed and incubated in dark at 30°C for 96 hours
- Soil samples were prepared in three replications. The controls contained only 5 ml Tris buffer (without TTC)
- After the incubation, 20 ml of methanol was added to each tube. The tubes were then shaken vigorously for 5 minutes using rotator and centrifuged for 10 min at 4000 rpm

- Next, 200 µl from the supernatant was placed into the microplate and read in microplates reader. TPF was quantified at 485 nm against methanol
- The dehydrogenase activity was expressed in mg TPF kg^{1} dry soil day^{1} (mg TPF kg^{-1} d^{-1})

Instruments and equipment

- Thermostatic incubator (30°C)
- Microplate reader / Spectrophotometer (wavelength of λ=485 nm)

Chemicals, solutions or necessary supplies

- 0.1 M Tris-HCl buffer, pH 7.4
- 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in 0.1 M Tris-HCl buffer
- Methanol
- Triphenyl formazan (TPF) for calibration curve

Literature

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Assay of CM cellulase activity

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What is measured in this method?

Cellulase activity is determined using CM-cellulose. Released reducing sugars cause a reduction in potassium hexacyanoferrate which reacted with ferric ammonium sulphate to form a complex of ferric hexacyanoferrate, which was measured colorimetrically at 690 nm.

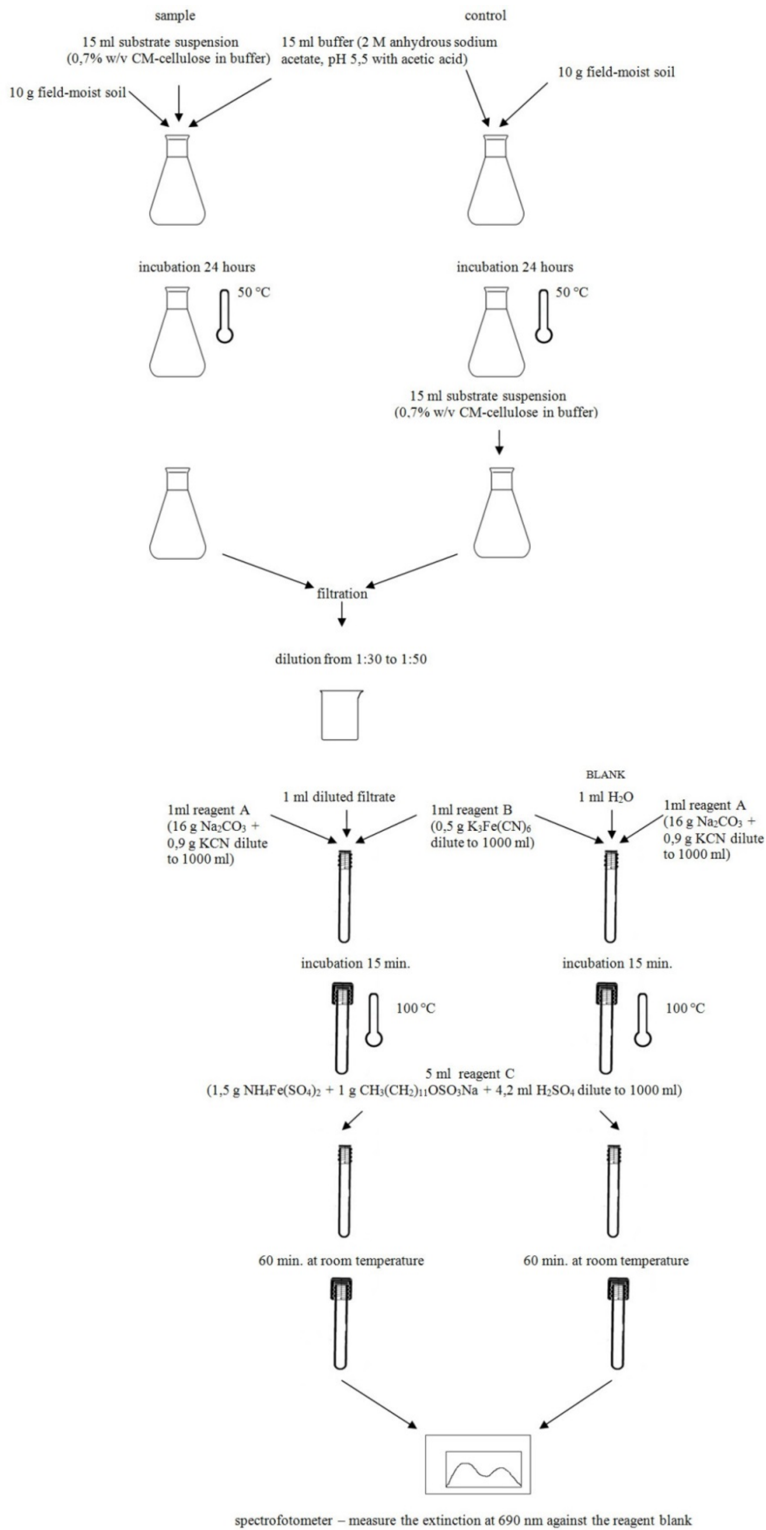
Why is this measurement important?

Cellulose is the most abundant organic compound in the biosphere synthesized by photosynthetic fixation of CO_2. Growth and survival of microorganisms which are important in agricultural soils depends on the carbon source contained in the cellulose in the soil environment. For releasing of carbon as an energy source for use by the microorganisms, cellulose in plant debris must be degraded into glucose, cellobiose and high molecular weight oligosaccharides by cellulases enzymes. This enzymes catalyze the degradation of cellulose, polysaccharides built up of β-1,4 linked glucose units. Studies have shown that activities of cellulases in agricultural soils are affected by several factors, e.g. temperature, soil pH, chemical structure of organic matter and its location in the soil profile horizon, inhibitors (trace elements, pesticides), soil management, etc.

Description of the measurement procedure

Using CM-cellulose as substrate, soil samples are incubated for 24 h at 50 °C and pH 5.5. Reducing sugars released during the incubation period cause the reduction of potassium hexacyanoferrate (III) in an alkaline solution. Reduced potassium hexacyanoferrate (II) reacts with ferric ammonium sulfate in an acid solution to form a complex of ferric hexacyanoferrate (II) (Prussian blue), which is determined colorimetrically. Results are expressed as µg Glucose equivalents$\cdot g^{-1}$ dry matter$\cdot$24 hours^{-1}. This method was developed by Schinner and von Mersi (1990).

Scheme of cellulase activity determination:



Instruments and equipment

- Spectrophotometer
- Basic laboratory equipment

Chemicals, solutions or necessary supplies

- Acetate buffer (2M, pH 5.5)
- Substrate solution (0.7 % w/v, carboxymethyl sodium salt, acetate buffer)
- Reagent A (anhydrous sodium carbonate + potassium cyanide)
- Reagent B (potassium hexacyanoferrate(III))
- Reagent C (ferric ammonium sulfate + sodium dodecyl sulfate + H_2SO_4)
- Standard stock solution (250 µg glucose·ml^{-1}, anhydrous glucose)

Literature

Schinner, F. and von Mersi, W. 1990. Xylanase, Cm-cellulase and invertase activity in soil: an improved method. Soil Biology and. Biochemistry 22*:* 511–515.

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Assay of acid and alkaline phosphomonoesterase activity

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What is measured in this method?

Phosphatase activity is determined using p-nitrophenylphosphate as substrate. The released p-nitrophenol is extracted, coloured and measured photometrically.

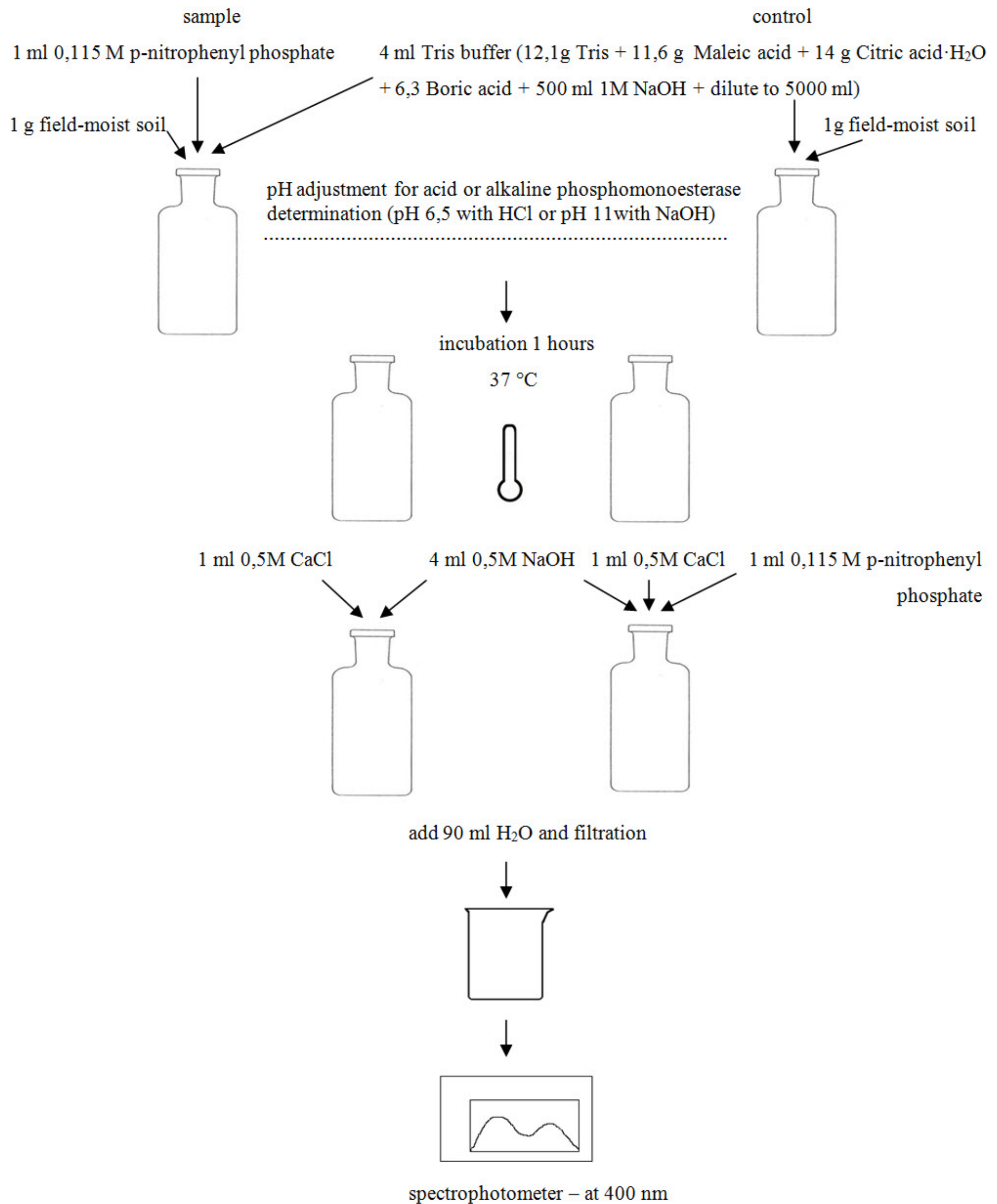
Why is this measurement important?

Phosphatases are important soil enzymes which catalyse the hydrolysis of ester-phosphate bonds, leading to the phosphate (P), which can be taken up by plants and microorganisms. Phosphomonoesterases are classified as acid (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) or alkaline (orthophosphoric monoester phosphohydrolase, EC. 3.1.3.1) according to the optimal activity at acid and alkaline pH, respectively. The activities of phosphatases depend on several factors, such a soil properties, soil tillage, soil organism interactions, plants, presence of inhibitors and activators, etc.

Description of the measurement procedure

After the addition of buffered p-nitrophenyl phosphate solution, soil samples are incubated for 1 h at 37 °C. The p-nitrophenol released by phosphomonoesterase activity is extracted and coloured with sodium hydroxide and determined photometrically at 400 nm. Results are expressed as µg Nitrophenol·g-1 dry matter·hour-1. Original methods by Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) were modified in a few points.

Scheme of acid and alkaline phosphatase activity determination:



Instruments and equipment

- Spectrophotometer
- Basic laboratory equipment

Chemicals, solutions or necessary supplies

- Substrate solution for acid phosphomonoesterase (115 mM, disodium p-nitrophenyl phosphate hexahydrate)
- Substrate solution for alkaline phosphomonoesterase (115 mM, disodium p-nitrophenyl phosphate hexahydrate)
- Modified universal buffer stock solution (tris(hydroxymethyl)aminomethane, maleic acid, citric acid monohydrate, boric acid, NaOH)
- Working buffer solution for acid phosphomonoesterase (pH 6.5, modified universal buffer stock solution + HCl)
- Working buffer solution for alkaline phosphomonoesterase (pH 11, modified universal buffer stock solution + NaOH)
- Calcium chloride solution (0.5 M)
- Sodium hydroxide solution (0.5 M)
- Standard stock solution (1 mg p-nitrophenol·cm^{-3})

Literature

Tabatabai, M. A. and Bremner, J. M. 1969. Use of p-nitrophenyl phosphate for assai of soil phosphatase activity. Soil Biology and. Biochemistry 1: 301–307.

Schinner, F., Őhlinger, R., Kandeler, E., Margesin, R. (Eds.) 1996. Methods in Soil Biology. Springer.

Eivazi, F., Tabatabai, M. A. 1977. Phosphatases in soils. Soil Biology and Biochemistry 9: 167-172.

Assay of urease activity

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What is measured in this method?

Urease activity is analysed by incubating soil samples with a urea solution. Released ammonium is extracted using a potassium chloride solution and measured colorimetrically.

Why is this measurement important?

Urease (urea amidohydrolase, EC 3.5.1.5) widely distributed in nature and detected in microorganisms, plants and animals is the enzyme that catalyzes the hydrolysis of urea to CO_2 and NH_3. This enzyme is also responsible for the hydrolysis of urea fertilizers applied to the soil. Urease activity in soils is influenced by many factors, e.g. cropping history, soil organic matter content, soil tillage, soil amendments, environmental factors, etc.

Scheme of urease activity determination:

sample

control

2,5 ml 0,72 M substrate solution (urea)

5 g field-moist soil

20 ml 0,1 M borate buffer pH 10

5 g field-moist soil

incubation 2 hours

37 °C

inkubation 2 hours

37 °C

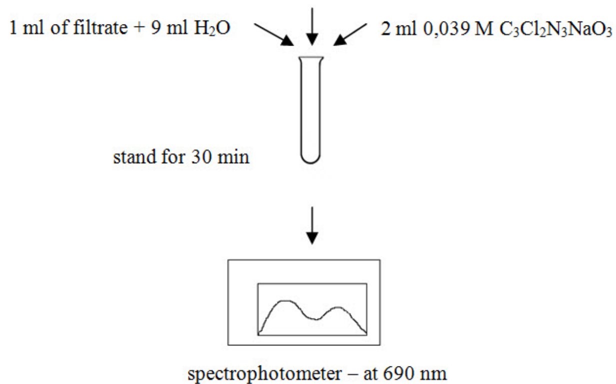
30 ml 2 M KCl

2,5 ml 0,72 M substrate solution (urea)

30 min.
on rotary shaker

filtration

5 ml reagent A (1,2 g NaOH + 17 g HOC_6H_4COONa + 0,12 g $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ diluted to 300 ml)



Description of the measurement procedure

After the addition of buffered urea solution, soil samples are incubated for 2 h at 37 °C. Released ammonium is extracted with potassium chloride solution, and determined by a modified Berthelot reaction. The determination is based on the reaction of sodium salicylate with NH_3 in the presence of sodium dichloroisocyanurate which forms a green-coloured complex under alkaline pH conditions. Sodium nitroprusside is used as a catalyst, and increases the sensitivity of the method about tenfold. Results are expressed as $\mu g\ N \cdot g^{-1}$ dry matter$\cdot 2$ hours^{-1}.

Instruments and equipment

- Basic laboratory equipment

Chemicals, solutions or necessary supplies

- Borate buffer (0.1 M, pH 10, disodium tetraborate)
- Substrate solution for the buffered method (79.9 mM, urea)
- Potassium chloride solution (2M, KCl, HCl)
- NaOH solution (0.3 M)
- Sodium salicylate solution (1.06 M)
- Reagent A (NaOH + sodium salicylate solution)
- Sodium dichloroisocyanurate solution (39.1 mM)
- Standard stock solution (1000 µg NH_4^+ - $N \cdot ml^{-1}$, NH_4Cl)

Literature

Kandeler, E., Geber, H. 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils 6: 68-72.

Schinner, F., Őhlinger, R., Kandeler, E., Margesin, R. (Eds.) 1996. Methods in Soil Biology. Springer Tabatabai, M. A. and Bremner, J. M. 1972. Assay of urease activity in soils. Soil Biology and Biochemistry 4: 479–487.

Evaluation of short-term nitrification activity

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What is measured in this method?

Effect of fertilizers, soil improvers and bio-wastes on short-term nitrification activity and estimation of nitrification potential (intensity) in soil

The method is used for assessment of inhibition effect of fertilizers, soil improvers and bio-wastes on the first step of nitrification in soils - ammonia oxidation. Tested fertilizers, soil improvers and bio-wastes is added to standard soil with known nitrification activity. The sample is incubated for six hours in a shaking suspension at substrate saturation and with addition of sodium chlorate, which inhibits the oxidation of NO_2^- to NO_3^-. This allows to determine only increment of NO_2^-. Range of at least six concentrations is chosen in accordance with maximal recommended dose of testing fertilizers, soil improvers and bio-wastes. Maximal recommended dose is in the middle of the tested concentrations. The nitrification in soil exposed to the test substance compare to that of the control soil is used as output of the test.

Alternatively, the method can be used for estimation of potential activity of indigenous ammonia oxidizing archaea and bacteria in soils.

Why this measurement is important?

Soils are an important reservoir of nitrifying bacteria. Affecting of these microorganisms can disturb nitrogen cycle in soil. This method is suitable for all soils containing a population of nitrifying microorganisms. This test can be used as a rapid screening test for evaluation of potential harmful effects of chemicals on soil microbial populations since nitrification is one of most sensitive microbially driven processes.

Description of the measurement procedure

Standard soil

Standard soil is uncontaminated soil, whose nitrification rate is (200 – 800) ng NO_2^--N/g_{dw} is used for ecotoxicity testing Any soil containing soil nitrifiers can be used for measurement of nitrification activity.

Preparation of test

15 g of pre-incubated (2 days at 25 °C) standard soil on dry weight basis is mixed with test medium containing fertilizer at different concentrations. The range of at least six concentrations is chosen in accordance with maximal recommended dose of testing fertilizers, soil improvers and bio-wastes. Maximal recommended dose is in the middle of the tested concentrations. For preparing the concentration series it is recommended to choose the dilution step not exceeding spaced factor two. Controls, without the test substance, are also prepared. Three replicates of test container were prepared for six concentrations and six replicates for controls. 60 ml of test medium is added to the mixture and all vessels are incubated on a rotary shaker (170 RPM) at 25 °C. 5 ml of samples is taken to test tubes after 2 and 6 h and 4 M KCl is added to stopped

nitrification. The suspension is immediately centrifuged and nitrites are analyzed by modified Griess–Ilosvay colorimetric determination (Forster, 1995), where nitrite forms after addition of sulfanilamide and N- (naphtyl)ethylendiamine dihydrochloride, a diazo compound. Absorbance of extracts is measured at the wavelength of 540 nm. Concentration of nitrite in the extract is determined from calibration curve. The short-term nitrification activity is expressed as the net increase of nitrite per unit of time.

The same procedure is used for measurement of nitrification activity in soils, except of that the tested medium alone is added to tested soil before incubation.

Evaluation of the test

Calculation of nitrite in soil on dry weight basis

$$NO_2 - N\ (\mu g/g_{dw}) = NO_2 - N\ (\mu g/ml) \times \frac{V + \frac{w \times m}{100}}{m}$$

Where:

NO_2-N (μg/g_{dw}) - concentration of nitrite in soil on dry weight basis,

V - volume of extracting agent (ml),

m - weight of dry soil, which corresponds to 15 g of moist soil (g)

w - moisture expressed as a percentage of the weight of water to weight of dry soil.

Calculation of short-term nitrification activity

$$SNA = \frac{C_6 - C_2}{4} \times 1000$$

Where:

SNA – short-term nitrification activity (μg NO_2^--N.g_{dw}^{-1} h^{-1}),

C_6 - NO_2^--N (ng. g_{dw}^{-1}) concentration after 6 h of incubation,

C_2 - NO_2^--N (ng. g_{dw}^{-1}) concentration after 2 h of incubation,

1000 - recalculation of µg to ng.

NOEC (No Observed Effect Concentration)/LOEC (Lowest Observed Effect Concentration) – homogeneity (Levene test) and normality (Shapiro-Wilk test) are calculated for each concentration. Normally distributed and homogenous data are evaluated with Dunnett test. If these requirements are not fulfilled, it is recommended to use Welch test with Bonferroni-Holm correction or U-test with Bonferroni-Holm correction.

EC_x (Effect Concentration for x% effect) – data are evaluated by probit or logit regression, or by Weibull analysis.

Instruments and equipment

- Test vessels (Erlenmeyer flask 250 ml)
- Centrifuge
- Monochromatic spectrophotometer
- Orbital shaking incubator
- Parafilm

Chemicals, solutions or necessary supplies

- Potassium dihydrogen phosphate, KH_2PO_4. Preparation: 13.61 g KH_2PO_4 is dissolved in water and topped up with water to 500 ml.
- Dipotassium hydrogen phosphate, K_2HPO_4. Preparation: 17.40 g K_2HPO_4 is dissolved in water and topped up with water to 500 ml
- Sodium chlorate, $NaClO_3$. Preparation: 10.6 g $NaClO_3$ is dissolved in water and topped up with water to 100 ml.
- Potassium chloride, KCl, c(KCl) = 2 mol/l. Preparation: 149 g KCl is dissolved in water and topped up with water to 1000 ml.
- Potassium chloride, KCl, c(KCl) = 4 mol/l. Preparation: 298 g KCl is dissolved in water and topped up with water to 1000 ml.
- Stock solution A: 28 ml solution KH_2PO_4, 72 ml solution K_2HPO_4, 100 ml distilled water
- Test medium: 10 ml stock solution A, 15 ml solution $NaClO_3$, 0.5 g $(NH_4)_2SO_4$

  The pH is measured and it should be 7.2, top up with distilled water to 1000 ml.

Literature

ISO 15685 Soil Quality - Ammonium oxidation, a rapid method to test potential nitrification in soil. International Organization for standardization, 2004.

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Evaluation of genetic diversity of ammonia oxidizing archaea (AOA) using terminal restriction fragment length polymorphism analysis (t-RFLP)

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What is measured in this method?

Terminal restriction fragment length polymorphism is a polymerase chain reaction (PCR) - based technique that has been used to effectively search microbial communities to determine the diversity of phylogenetic and functional markers. This method requires the isolation of community DNA from environmental samples e.g. soil and PCR amplification, performed with fluorescently labelled primers, is followed with restriction digestion. Digestion products are separated on automated genetic analyser as analysis of restriction fragments. The functional gene *amo*A is used for genetic profiling of ammonia oxidizing archaea (AOA).

Why is this measurement important?

T-RFLP analysis is a molecular technique used to study microbial communities based on variation in the gene. This analysis can be used to examine microbial community structure and community dynamics in response to changes in different environmental parameters. The most advantages of this technique is culture independent, rapid, sensitive and reproducible method of assessing diversity of communities.

T-RFLP technique was adapted to study the diversity of ammonia-oxidizing archaea (AOA). Using ammonia monooxygenase α-subunit (*amo*A) gene, the community structure and abundance of ammonia-oxidizing archaea (AOA) can be characterized. Nitrification, the oxidation of ammonia to nitrate via nitrite, is a critical process in soil N cycling and has significant agricultural and environmental consequences for the availability of nitrogen as a plant nutrient. This process includes two steps. The first and rate – limiting step, oxidation of ammonia to nitrite is performed by autotrophic ammonia- oxidizing bacteria (AOB) and ammonia – oxidizing archaea (AOA). Both AOA and AOB have ammonia monooxygenase (AMO), one of the key enzymes responsible for the conversion of ammonia to nitrite. AMO is composed of three subunits encoded by genes of *amo*A, *amo*B and *amo*C. The *amo*A gene has been much more extensively used for the study of ammonia oxidizers.

Archaeal ammonia oxidizers are ubiquitous in wide range of ecosystems and dominate in abundance over their bacterial counterparts in multiple environments, particularly in soils. These suggest that AOA may play a significant role in nitrification process. Moreover, genetic profiling of ammonia oxidizing archaea communities by t-RFLP is top-ranked as bioindicators for soil quality monitoring.

Description of the measurement procedure

Typical t-RFLP analysis comprises four major steps:

- DNA isolation
- PCR amplification, purification and restriction enzyme digestion
- Separation and detection of the digested products via automated capillary electrophoresis
- Analysis of data to generate the fragment profile of each sample

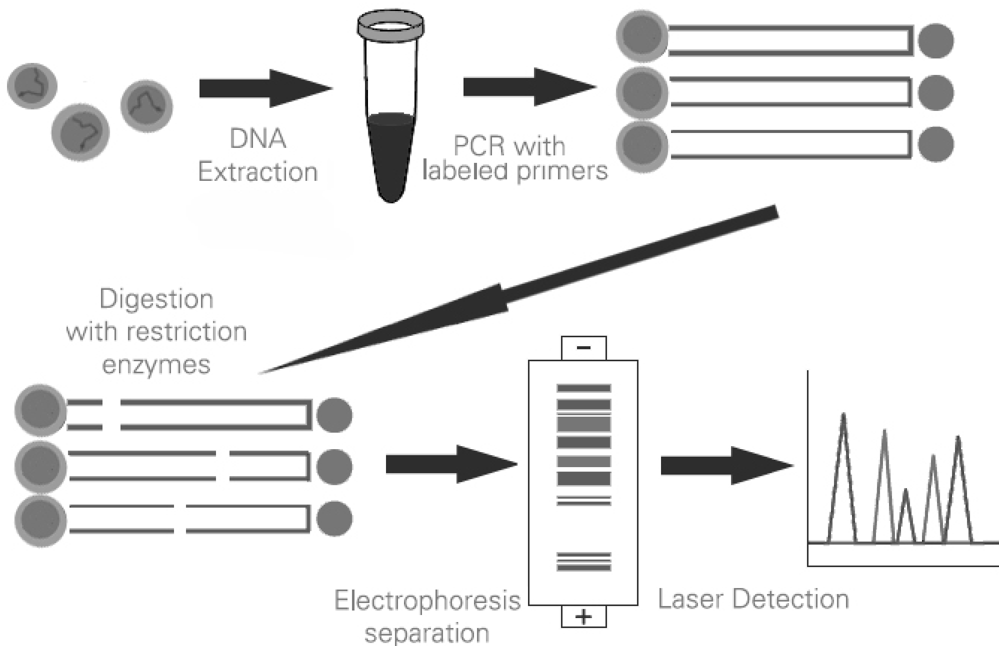

Figure 1. Scheme of steps required for t-RFLP assay

The strength of t-RFLP is in the comparative analysis of microbial communities. For such comparison to be valid from sample to sample and habitat to habitat, all experiment parameters also including data analysis, must be held constant. Most importantly this would include: the method of DNA isolation, the parameters of the PCR reaction including amounts of all components especially amount of DNA, the sequence of primers, the biochemistry of the restriction digestions, the quantity of fluorescent material loaded into the capillary and alignment of profiles and statistical analysis of the dataset.

The first step of community analysis is community DNA extraction, directly from soil. Genomic DNA was extracted from 0.5 g of soil for t-RFLP analysis using a FASTDNA SPIN kit for Feces (MP BIO®) and Fast Prep instrument (MP BIO®) following the manufacturer protocol. The amount and purity of DNA was determined by spectrophotometer NanoDrop at 260 nm. The *amo*A gene was amplified via polymerase chain reaction (PCR) using ammonia oxidizing archaea (AOA) primers. The forward primer amoA19F (5'-ATGGTCTGGCTWAGACG-3') was fluorescently labelled on the 5' by 6- FAM (6-carboxyfluorescein); the reverse primer was the following amo643R (5'-TCCCACTTWGACCARGCGGCCATCCA-3'). The PCR was performed in a total volume of 30 µl containing 4 ng of DNA template, 15 µl of RedTaq ReadyMix^{TM} PCR Reaction Mix with $MgCl_2$ (Sigma-Aldrich) with 0.06 U/µl Taq DNA polymerase. The primers used for PCR were 0.5 µM. The PCR was then performed with a thermalcycler with the following temperature cycle: 95°C initial denaturation for 5 minutes, followed by 35 cycles at 92°C denaturation for 45 s; 59°C annealing for 30 s; 72°C elongation for 60 s, and a final extension at 72°C for 7 minutes. The presence and size of the PCR products (700 bp) were checked on 1.3% agarose gel by electrophoresis (120 V, 60 minutes). The PCR products were purified using ExoSAP-IT® PCR Products Purification Kit (ABI) followed the incubation at 37°C for 15 minutes and then 15 minutes at 80°C. The purified amplification products were then digested by endonucleases (*Alu*I or *Csp6*l). The restriction-digestion mixture (10 µl), containing PCR product (50 ng DNA), 0.6 µl of buffer Tango (Fermentas®), and 0.6 µl of restriction enzyme (10 U/µl) *Alu*I or *Csp6*l (Fermentas®), respectively, was incubated at 37°C for 2 h. The reaction

was stopped by incubation at 65°C for 20 minutes. Aliquots (1 µl) of the digested samples were mixed with 9 µl of deionized formamide and 0.5 µl of DNA fragment length standard (GS-600LIZ, ABI). The mixture was denatured at 94°C for 3 minutes and snap-cooled on ice. The fluorescently labelled terminal restriction fragments (t-RFs) were run through an ABI 3130 xl capillary genetic analyser in the GeneScan mode. T-RFLP data were analysed using GeneMapper v. 4.0 software.

Instruments and equipment

- Apparatus for DNA extraction – FAST PREP®24 (MP BIO)
- Spectrophotometer for DNA concentration analysis – NanoDrop 2000
- Electrophoresis agarose system
- Gel documentation system – system for visualization and analysis of fluorescent labelled samples
- Gradient thermal cycler – Veriti 96 well Fast Thermal Cycler
- Genetic Analyser 3130 – 4 capillary analyser for electrophoresis separation of DNA fragments using capillary method for fragment analysis

Chemicals, solutions or necessary supplies

- A set of reagents for DNA extraction: FASTDNA SPIN kit for Feces (MP BIO®)
- PCR amplification reagents:
  - RedTaq ReadyMix™ PCR Reaction Mix with $MgCl_2$ with 0.06 U/µl Taq DNA polymerase (Sigma-Aldrich)
  - amoA19F (5'-ATGGTCTGGCTWAGACG-3') primer (fluorescently labelled with 6-FAM (6-carboxyfluorescein) on the 5') (10 µM)
  - amo643R (5'-TCCCACTTWGACCARGCGGCCATCCA-3') primer (10 µM)
- Product purification: ExoSAP-IT® PCR Products Purification Kit for ABI
- Restriction digestion:
  - restriction buffer TANGO 10x (Fermentas Life Sciences)
  - restriction enzyme (*Alu*I,*Csp6*I) 10 U/µl (Fermentas Life Sciences)
  - Nuclease-free water (Ambion)
- Separation of restriction fragments:
  - Size standards Gene Scan™ -600 LIZ® Size Standard (Applied Biosystems)
  - Highly deionized formamide (Applied Biosystems)
  - NanoPOP™ Polymers (Nimagen)
  - Running Buffer with EDTA (Applied Biosystems)
  - Nuclease-free water (Ambion)

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The estimation of soil microbial functional diversity using microbial community level physiological profiling (CLPP)

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What is measured in this method?

- Community Level Physiological Profiling (CLPP) is used for assessing the potential metabolic diversity of microbial communities.
- Shifts in CLPP are examined in the context of their impact on the functioning of ecosystems and evaluation of functional diversity, which is often used to estimate the changes in biodiversity of ecosystems.
- "Whole" soil communities are generated by inoculating mixed microbial assemblages in to the BIOLOG microtiter well plates and noting the response of the mixed community to the carbon sources located in.
- The plates with different carbon substrates are dedicated to evaluate the changes in community of bacteria, fungi and anaerobes.
- Those carbon substrates are in addition to a tetrazolium dye. The utilization of any carbon source by the microbial community results in the respiration-dependent reduction of the dye and purple colour formation measured as absorbance, that can be quantified and monitored over time, as biodiversity indices:
  - **Average well-color development (AWCD)** – microbial response in each microplate that express overall activity of microbial communities**.** AWCD index is determined as follows: **AWCD = Σ ODi/31,** where ODi is optical density value from each well, corrected subtracting the blank well (inoculated, but without a carbon source) values from each plate well.
  - **Richness (R)** values is calculated as the number of oxidized C substrates is calculated using an OD of 0.25 as threshold for positive response.
  - **Shannon-Weaver index (H)** – is calculated as follows: **H = −Σpi(lnpi)** where pi is the ratio of the activity on each substrate (ODi) to the sum of activities on all substrates ΣODi. This indicator is used for evaluation of microbial community evenness.

Water	B-Methyl-D-Glucoside	D-Galactonic Acid LACTONE	L-Arginine	Water	B-Methyl-D-Glucoside	D-Galactonic Acid LACTONE	L-Arginine	Water	B-Methyl-D-Glucoside	D-Galactonic Acid LACTONE	L-Arginine
Puruvic Acid Methyl Ester	D-Xylose	D-Galacuronic Acid	L-Asparagine	Puruvic Acid Methyl Ester	D-Xylose	D-Galacuronic Acid	L-Asparagine	Puruvic Acid Methyl Ester	D-Xylose	D-Galacuronic Acid	L-Asparagine
Tween 40	i-Erythritol	2-Hydroksy Benzoic Acid	L-Phenyl-alamine	Tween 40	i-Erythritol	2-Hydroksy Benzoic Acid	L-Phenylalamine	Tween 40	i-Erythritol	2-Hydroksy Benzoic Acid	L-Phenylalamine
Tween 80	D-Mannitol	4-Hydroksy Benzoic Acid	L-Serine	Tween 80	D-Mannitol	4-Hydroksy Benzoic Acid	L-Serine	Tween 80	D-Mannitol	4-Hydroksy Benzoic Acid	L-Serine
alpha Cyclodextrin	N-Acetyl-D-Glucosamine	Hydroksybutiric Acid	L-Threonine	alpha Cyclodextrin	N-Acetyl-D-Glucosamine	Hydroksybutiric Acid	L-Threonine	alpha Cyclodextrin	N-Acetyl-D-Glucosamine	Hydroksybutiric Acid	L-Threonine
Glycogen	D-Glucosaminic Acid	Itaconic Acid	Glycyl-L-Glutamic Acid	Glycogen	D-Glucosaminic Acid	Itaconic Acid	Glycyl-L-Glutamic Acid	Glycogen	D-Glucosaminic Acid	Itaconic Acid	Glycyl-L-Glutamic Acid
D-Cellobiose	D-Glucose-1-Phosphate	a-Ketobutiric Acid	Phenylethylamine	D-Cellobiose	D-Glucose-1-Phosphate	a-Ketobutiric Acid	Phenylethylamine	D-Cellobiose	D-Glucose-1-Phosphate	a-Ketobutiric Acid	Phenylethylamine
a-A-D-Lactose	DL-a-Glycerol Phosphate	D-Malic Acid	Putrescine	a-A-D-Lactose	DL-a-Glycerol Phosphate	D-Malic Acid	Putrescine	a-A-D-Lactose	DL-a-Glycerol Phosphate	D-Malic Acid	Putrescine

Carbohydrates	Polymers	Carboxylic and acetic acids	Amino acids	Amines/ amides

Fig. 1. Distribution of carbon sources in the BIOLOG ECO Plate (http://www.biolog.com/docroot_biologco/wwwRoot/download_center.html)

Water	Tween 80	N-Acetyl-D-Galactosamine	N- Acetyl -D-Glucosamine	N- Acetyl -D-Mannzoamine	Adonitol	Amygdaline	D-Arabinose	L-Arabinoza	D-Arabitol	Arbutyna	D-celobioza
α-Cyclodextrin	β-Cyclodextrin	Dextrin	i-Erythritol	D-Fructose	L-Fucose	D-Galactose	D-Galacturonic Acid	Gentiobiose	D-Gluconic Acid	D-Glucosamine	α-D-Glucose
Glucose-1-Phosphate	Glucuronamide	D-Glucuronic Acid	Glycerol	Glycogen	m-Inositol	2-Keto-D-Gluconic Acid	α-D-Lactose	Lactulose	Maltitol	Maltose	Maltotriose
D-Mannitol	D-Mannose	D-Melezitose	D-Melibiose	α-Methyl-D-Galactoside	β-Methyl-D-Galactoside	α-Methyl-D-Glucoside	β-Methyl-D-Glucoside	Palatinose	D-Psicose	D-Raffinose	L-Rhamnose
D-Ribose	Salicin	Sedoheptulosan	D-Sorbitol	L-Sorbose	Stachyose	Sucrose	D-Tagatose	D-Trehalose	Turanose	Xylitol	D-Xylose
γ-Amino-butyric Acid	Bromosuccinic Acid	Fumaric Acid	a-Hydroxy-butyric	γ-Hydroxy-butyric Acid	p-Hydroxyphenyl acetic acid	α-Keto-glutaric acid	D-Lactic Acid Methyl Ester	L-Lactic Acid	D-Malic Acid	L-Malic Acid	Quinic Acid
D-Saccharic Acid	Sebacic Acid	Succinamic Acid	Succinic Acid	Succinic Acid Mono-Methyl Ester	N-Acetly-L-glutamic Acid	Alaninamide	L-Alanine	L-Alanyl-Glycine	L-Asparagine	L-Aspartic Acid	L-Glutamic Acid
Glycyl-L-Glutamic Acid	L-Ornithine	L-Phenylalanine	L-Proline	L-Pyroglutamic Acid	L-Serine	L-Threonine	2-Amino Ethanol	Putrescine	Adenosine	Urydine	Adenosine-5'-Monophosphate

Polimers	Carbohydrates	Carboxylic and acetic acides	Miscellaneous	Amides	Amines

Fig. 2. Distribution of carbon sources in the BIOLOG FF Plate (http://www.biolog.com/docroot_biologco/wwwRoot/download_center.html)

Water	N-Acetyl-D-Galactosamine	N-Acetyl-D-Glucosamine	N-Acetyl-α-D-Mannosamine	Adonitol	Amygdalin	D-Arabitol	Arbutin	D-Cellobiose	α-Cyclodextrin	α-Cyclodextrin	Dextrin
Dulcitol	i-Erythritol	D-Fructose	L-Fucose	D-Galactose	D-Galacturonic Acid	Gentiobiose	D-Gluconic Acid	D-Glucosaminic Acid	α-D-Glucose	α-D-Glucose-1-Phosphate	D-Glucose-6-Phosphate
Glycerol	D,L-α-Glycerol Phosphate	m-Inositol	α-D-Lactose	Lactulose	Maltose	Maltotriose	D-Mannitol	D-Mannose	D-Melezitose	D-Melibiose	3-Methyl-D-Glucose
α-Methyl-D-Galactoside	α-Methyl-D-Galactoside	α-Methyl-D-Glucoside	α-Methyl-D-Glucoside	Palatinose	D-Raffinose	L-Rhamnose	Salicin	D-Sorbitol	Stachyose	Sucrose	D-Trehalose
Turanose	Acetic Acid	Formic Acid	Fumaric Acid	Glyoxylic Acid	α-Hydroxybutyric Acid	α-Hydroxybutyric Acid	Itaconic Acid	α-Ketobutyric Acid	α-Ketovaleric Acid	D,L-Lactic Acid	L-Lactic Acid
D-Lactic Acid Methyl Ester	D-Malic Acid	L-Malic Acid	Propionic Acid	Pyruvic Acid	Pyruvic Acid Methyl Ester	D-Saccharic Acid	Succinamic Acid	F9 Succinic Acid	Succinic Acid Mono-Methyl Ester	m-Tartaric Acid	Urocanic Acid
Alaninamide	L-Alanine	L-Alanyl-L-Glutamine	L-Alanyl-L-Histidine	L-Alanyl-L-Threonine	L-Asparagine	L-Glutamic Acid	L-Glutamine	Glycyl-L-Aspartic Acid	Glycyl-L-Glutamine	Glycyl-L-Methionine	Glycyl-L-Proline
L-Methionine	L-Phenylalanine	L-Serine	L-Threonine	L-Valine	L-Valine plus L-Aspartic Acid	2'-Deoxy Adenosine	Inosine	Thymidine	Uridine	Thymidine-5'-Mono-phosphate	Uridine-5'-Mono-phosphate

Carbohydrates/glucosides	Amines/ amides	Amino acids	Carboxylic acids	Miscellaneous	Polymers

Fig. 3. Distribution of carbon sources in the BIOLOG AN Plate (http://www.biolog.com/docroot_biologco/wwwRoot/download_center.html)

Why is this measurement important?

The evaluation of microbial functional diversity is important in order to understand the ecology of microorganisms in ecosystem under the influence of different kind of soil amendments and is sensitive indicator of microbial changes under the impact of anthropogenic factors.

The estimation of microbial functional diversity by using CLPP has been reported as a sensitive and rapid approach to detect changes in soil microbial communities due to soil management and treatments.

The ecological relevance of given exogenous organic matter to soil microbial communities can be easily assessed, using this method.

The number of functional groups of microorganisms (guild groups) in the assembly proves its function disorders. Decrease of functional diversity may be related to environmental pollution such as heavy metals. Increase of functional diversity usually shows improvement of the microbial soil communities, for example after the use of organic fertilization.

Description of the measurement procedure

1 g of soil was shaken in 99 mL of sterile peptone water for 20 minutes at 20°C and then was incubated at 4°C for 30 minutes.

Next 120 µl of each sample were inoculated into each well of Biolog EcoPlates, 100 µl into AN, and FF plates and then incubated at 27°C.

The AN plates were incubated in anaerobic atmosphere (85% N_2, 10% CO_2 and 5% H_2) at 27°C.

The rate of utilization was indicated by the reduction of the tetrazolium, a redox indicator dye that changed from colourless into purple if substrates were utilised. Data were recorded with a plate reader at appropriate weave length (for FF plate - 490 nm and 750 nm; for ECO and AN plates 590 nm and 750 nm) every 24 h throughout 7 days.

Instruments and equipment

MicroStation™ System:

- MicroStation Reader
- MicroStation software
- Multichannel repeating pipetor
- RetroSpect™ Trending and Tracking Software

A system for culturing anaerobes:

- the Anoxomat
- Anaerobic jar
- Packed catalyst for O_2 absorption
- Anaerobic gas mixture

Chemicals, solutions or necessary supplies

- Biolog ECO, FF and AN microplates, supplied with specific carbon sources
- Saline Peptone Water

Literature

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http://www.biolog.com/docroot_biologco/wwwRoot/download_center.html

CHEMISTRY

Grzegorz Siebielec

Chemical properties of soils constitute one of major aspects of soil quality. Such properties as pH, soil organic carbon (SOC) content, acidity, cation exchange capacity (CEC), salinity, nutrient and contaminant contents are decisive for quality of soil. Chemical status of soil builds soil potential to fulfill its agricultural and environmental functions. Production function of soil simply relates to soil pH, nutrient level or humus content that affect availability of nutrients for crops. Buffering capacity and filtering of contaminants in soil profile depend on soil pH and soil organic matter. Other soil functions like provision of biodiversity and water retention are also connected to these chemical characteristics of soil. All these functions are vital for securing food production and environmental stability of a given area.

Measurements of soil chemical properties not only provide necessary information describing ability of soil to produce high yield and high quality food. These characteristics are used to monitor changes in soil quality and degradation processes resulting from changes in agriculture and natural processes or to delineate sensitive areas to be supported through policy instruments. Chemical soil parameters analyzed in the laboratory are indicators of both soil degradation processes and threats to the environment. They are clearly measures of anthropogenic impact on the soil resources, these impacts refer to industry and transport activities (emissions of contaminants and acidifying agents) or agriculture (e.g. effects of monoculture, land abandonment, policy instruments).

SOC decline has been reported as one of major threats to soil quality across Europe (Saby et al., 2008; EC, 2012). Soil acidity is a problem especially referring to soils of sandy texture and those affected by leaching by precipitation water. Acidity hampers soil capacity to inactivate contaminants and effectiveness of mineral fertilization of soil that, in consequence, might lead to release of nutrients to ground and surface water. Acidic conditions also affect nutrient and carbon cycling in the soil ecosystem (Tan, 2010).

Analyses of extractable nutrients give a picture of nutrient availability for plants – on the other hand they simulate risk of excessive nutrient accumulation in soil (e.g. phosphorus) that can be subsequently transported to water through erosion processes.

CEC is a measure of soil resistance to anthropogenic impacts – higher CEC enables inactivation of contaminants, storage of nutrients and mitigation of acidification processes. Extractable metals might be considered as indicators of micronutrient deficiency in uncontaminated soils or potential risk of phytotoxicity or toxicity to soil organisms in metal contaminated soils (Kumpiene et al., 2014).

Total contaminant contents in soil in most of national legislations constitute basic information for labeling soil as polluted or clean. Measurement of contaminant contents in soil (e.g. extractable metals or total Policyclic Aromatic Hydrocarbons (PAH-s)) can be also utilized as tools to determine status of contaminated soils subjected to remediation techniques – as a measure of remediation effectiveness.

Such soil chemical properties as pH and metal contents are basic information required prior application of EOM as soil conditioner. A broad range of chemical parameters is used to evaluate

impacts of EOM and threats related to their application, namely electrical conductivity, metal contents, PAH-s content, nutrient extractability, etc.

Plant chemical composition provides basic information for explanation of crop yield that might be affected by deficiencies of nutrients and microelements (Mengel and Kirkby, 1982). Crop chemical composition is also a measure of crop quality in terms of sufficient level of microelements or excessive content of undesired elements (e.g. cadmium, arsenic, lead) or compounds (e.g. pesticide residues, nitrates). Analyses of contaminants in crops in scientific studies or screening activities tell about risk for transfer of contaminants to the food chain. Similarly, level of certain elements (potentially toxic metals, sulphur, chloride) in plant biomass describes its suitability for composting or combustion purposes.

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Soil pH

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What is measured in this method?

The concentration of H^+ ions in soil water or KCl slurry.

Why is this measurement important?

Soil pH is probably the most informative single measurement that can be made to determine soil characteristics since soil pH is closely correlated to many other soil properties. At a single glance, pH tells much more about a soil than merely indicating whether it is acidic or basic. For example, availability of essential nutrients and mobility of potentially toxic trace elements is highly dependent on pH.

Most of soil properties (chemical, physical, biological) are greatly affected by pH. Growth of microorganisms, that play key role in the processes of nutrient cycling, depends greatly on soil pH. Soil pH itself influences the growth and yield of plants. Cultivated plants grow in certain pH range, usually from 5.6 to 7.8 but the optimal pH for most crops is in the neutral range. . The factors that regulate the soil pH are presence soil carbonates and soil treatment with limestone. Measuring pH in EOM-treated soils is important due to potential impact of pH shift on rate of contaminant and nutrient release from the EOMs.

Description of the measurement procedure

Prior the measurement soil sample is air dried and sieved through 2mm mesh to separate the skeletal parts and the remains of plants, animals etc.

- Prepare 1M KCl (dissolve 75 g of KCl in 1000 ml of H_2O – pH of this solution should be 5.2 – 5.6. In case the pH does not meet the range, it should be adjusted by adding either a few drops of HCl or NaOH).
- Weight 2g of soil into two separate plastic containers of the volume of at least 40 ml.
- Pour 25 ml of 1M KCl into one of the containers and stir.
- Pour 25 ml of deionized H_2O into second container and stir.
- Leave the samples for 18 h.
- Check the calibration of the pH meter with the pH 4.0, pH 7.0 and 10.0 buffers.
- Measure the pH in soil suspension prepared in water and in 1M KCl.

Instruments and equipment

- pH – meter
- Glass electrode
- Analytical balance – accuracy 0.01g

Chemicals, solutions or necessary supplies

- KCl
- HCl
- NaOH
- Deionized water
- pH standard buffers: pH 4.0, pH 7.0, pH 10.0

Literature

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Electrical conductivity and salinity of soil

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What is measured in this method?

Electrical conductivity of the filtrate of soil-water slurry.

Why is this measurement important?

The term salinity, refers to the presence of the major dissolved inorganic salts, essentially represented by ions Na^{+}, Mg^{2+}, Ca^{2+}, K^{+}, Cl^{-}, SO_4^{2+}, HCO_3^{-}, CO_3^{2-}, in aqueous samples. As applied to soils, it refers to the soluble plus readily dissolvable salts in the soil, or more usually in an aqueous extract of a soil sample. Salinity refers to total concentration of such soluble salts. The diagnosis, assessment, management and need for reclamation of saline soils are evaluated using the information on soil salinity.

Description of the measurement procedure

The sample is air dried and sieved through 2mm mesh to separate the skeletal parts and the remains of plants, animals etc.

- Weight 10g of soil into plastic 100 ml containers.
- Pour 50 ml of H_2O into container.
- Shake on the laboratory rotary shaker for 1 hour at 140 rpm.
- After shaking, filtrate the solution through hard filters into the plastic container.
- Prepare the standard solution –1000µS/cm at 25°C (dissolve 0.2617g of KCl in 500 ml of H_2O).
- Check the calibration of the conductivity meter with the prepared solution.
- Measure the electrical conductivity in filtrate.
- Electrical conductivity can be converted to salinity by multiplying the result of electrical conductivity by factor 0.264.
- For contamination control run this procedure for blank samples.
- For quality control purpose run this procedure for Certified Reference Material.

Instruments and equipment

- Electrical conductivity meter
- Conductivity sensor
- Laboratory rotation shaker
- Analytical balance – accuracy 0.01g

Chemicals, solutions or necessary supplies

- KCl
- Distilled water

Literature

Methods of soil analysis. Part 3 – Chemical methods, Editor: Sparks D.L. , Soil Science Society of America, Inc., American Society of Agronomy, Inc, Madison, Wisconsin, USA, Chapter 14: Rhoades J.D. Salinity: Electrical Conductivity and Total Dissolved Solids.

Determination of total carbon and total nitrogen by dry combustion method (Dumas method) using a CN elemental analyzer

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What is measured in this method?

Total content of carbon (TC) and nitrogen (TN) in solid soil samples

Why is this measurement important?

Soil carbon and nitrogen have a major impact on the quality and quantity of soil organic matter which in turn improves soil structure, water retention, increases biodiversity, reduces soil susceptibility to erosion and compaction.

C/N ratio indicates degree and rate of organic matter decomposition and can be used, as well as the quality and self-sufficiency of the compost, sewage sludge, digestates and other high organic materials as organic fertilizers or soil conditioners.

Measurements of carbon and nitrogen are important tools for monitoring of carbon sequestration in soil and greenhouse gas (CO_2, CH_4 and N_2O) emissions from soil.

Description of the measurement procedure

Sample preparation

The soil samples are sieved (2mm) and air dried. Then they are weighed (100 – 300 mg), wrapped and sealed in tin foil using the Sample Pressing Tool.

Analytical process and measurement

A range of commercial analyzers are available for the determination of carbon and nitrogen in soils by dry combustion. The basic principle of operation of these analyzers is high temperature (900 - 1600°C) digestion of the sample in a stream of pure oxygen and in the presence of catalysts. The gaseous products of combustion are transported by carrier gas (usually helium) through the various traps and scrubbers to remove sulfur oxides, halogens and water vapor. After reduction of nitrogen oxides the final gas mixture is separated into its components (N2 and CO2) which are sequentially sent to a detector (e.g. TCD). Percent contents of the elements are calculated from the detector signal in relation to the sample weight and the calibration curve.

Measurement procedure

- Weigh 0.05 – 2 g of soil into a metal foil, porcelain boat or ceramic crucible (depending on type of analyzer).
- Prepare the standard samples with known content of the N and C (e.g. phenylalanine, sulfanilic acid) to quality control of measurement.
- Place the soil samples and the standard samples in the autosampler of analyser.
- Set the parameters of the process with software (combustion temperature, oxygen dosing, carrier gas pressure etc.) and run the analysis.

Total contents of carbon (TC) and nitrogen (TN) are recalculated based on dry matter content DM [%] (total solids, 105°C) of soil:

$$T(C,N)_{105°C} = \frac{T(C,N)}{DM} \cdot 100\%$$

Instruments and equipment

- CN Elemental Analyzer
- An analytical balance, readability min. 0.1mg
- Sample Pressing Tool (in some of analyzers)

Chemicals, solutions or necessary supplies

- Metal foils (e.g. tin, aluminium, silver, platinum) or porcelain boats or crucibles (steal, ceramic)
- Catalyst (e.g. CuO, Al_2O_3, V_2O_5, WO_3)
- Reduction agent and traps (e.g. copper wire, tungsten granulate, silver wool)
- Drying agent (e.g. P_2O_5, $Mg(ClO_4)_2$)
- Standard samples (e.g. phenylalanine, sulfanilic acid)
- Helium pure
- Oxygen, pure

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Determination of organic carbon in soil by oxidation with a mixture of potassium dichromate and sulphuric acid

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What is measured in this method?

The method enables measurement of oxidizable carbon in soil samples. The inorganic carbon contained in the carbonate minerals in soil such as calcite or dolomite is not oxidized and not measured.

Why is this measurement important?

Organic carbon is a measure of soil organic matter (SOM), which impacts on soil fertility and nutrient cycling, soil structure, buffering and sorption capacity, biological diversity and water retention. Additionally organic matter affects mobility of pollutants.

Measurements of soil organic carbon (SOC) are important for monitoring of soil quality, impact of agriculture on SOC levels and trends in carbon sequestration in or release from soil.

Description of the measurement procedure

The method and its various modifications are better known as the Tiurin method in Central and East European countries and as the Walkley-Black method in Western Europe. For the purposes of this methodological guide the modification of the Tiurin method according to (Ostrowska et all, 1991) and the modification of the Walkley-Black method similar to (ISO14235 : 1998) were adopted.

A measured volume of potassium dichromate mixture solution is added to a soil sample. The sample is heated on a hot plate at boiling point for 5 min. (Tiurin method) or into a digestion system at 125°C for 45 min. (Walkley-Black method). The organic carbon in the soil is oxidized to CO_2 by the dichromate, which itself is reduced. The dichromate which has not been consumed to oxidise soil carbon is determined by titrating with ammonium iron sulphate. Soil organic carbon (SOC) is calculated from the volume of dichromate remaining in the solution.

Sample preparation

The soil samples are air dried and sieved (2 mm).

Tiurin method

- Step 1: Weigh 0.1 – 0.4 g of soil (depending on expected carbon content) into a 100 ml Erlenmeyer flask. Prepare three flasks with a pure quartz sand as a blank sample.
- Step 2: Add exactly 10 ml of 0.067 M potassium dichromate solution to the flask from a burette and swirl the flask gently to mix the soil and dichromate solution. Cover the Erlenmeyer flask with glass funnel, which act as a reflux condenser, to minimize loss of chromic acid.
- Step 3: Place the flask on a hot plate, wait until the solution starts to boil and heat at boiling point for 5 min.

- Step 4: Remove the sample from the hot plate and allow it to cool.
- Step 5: Rinse the funnel with about 20-30 ml of distilled water.
- Step 6: Add 4 drops of phenanthroline complex and titrate ammonium iron sulphate solution (Mohr salt). Mix the sample during titration. The end point is a red.
- Step 7: Calculate the percentage of soil organic carbon according to this formula:

$$SOC\ [\%] = \frac{(V_0 - V_s) \cdot c_s \cdot 0.3}{w}$$

where:

V_0 – volume of Mohr salt consumed on blank sample titration [ml]

V_s – volume of Mohr salt consumed on soil sample titration [ml]

w – weight of soil sample [g]

c_s - molarity [mol/dm^3] of Mohr salt solution calculated from the following:

$$c_s = \frac{c_p \cdot V_p \cdot 5}{V_s}$$

where:

$c_p = 0.04$ M – molarity of $KMnO_4$ solution

V_p – volume of $KMnO_4$ solution required for titration V_s of Mohr salt

V_s – volume of Mohr salt titrated by $KMnO_4$

- Step 8: The SOC is recalculated based on dry matter content DM [%] (total solids, 105°C) of soil:

The SOC is recalculated based on dry matter content DM [%] (total solids, 105°C) of soil:

$$SOC_{105°C} = \frac{SOC}{DM} \cdot 100\%$$

Walkley-Black method

- Weigh 0.1 – 0.4 g of soil (depending on expected carbon content) into a 250 ml quartz tube.
- Add exactly 10 ml of 0.067 M potassium dichromate mixture to the quartz digestion tube from a burette and swirl the tube gently to mix the soil and dichromate solution. Cover the tube with glass condensed ball to minimize loss of chromic acid.
- Insert the digestion tube into a special rack and place it into a digestion system with a controller Program the digestion system for heating the samples at 125°C for 45 min.
- After cooling, transfer the sample into a 300 ml Erlenmeyer flask, thoroughly rinse the tube and the condensed ball with distilled water into the Erlenmeyer flask.
- Next, proceed according to the Tiurin method (steps 6-8).

Instruments and equipment (Tiurin method)

- A hot plate with adjustable temperature
- An analytical balance capable of accurately weighing 0.1 mg
- 50 ml Erlenmeyer flasks
- 10 ml burette
- 50 ml burette with scale 0.1 ml

Instruments and equipment (Walkley-Black method)

- A digestion system with controller and suitable rack
- 250 ml quartz digestion tubes with condensed balls
- An analytical balance capable of accurately weighing 0.1 mg
- 300 ml Erlenmeyer flasks
- 10 ml burette
- 50 ml burette with scale 0.1 ml

Chemicals, solutions or necessary supplies (Tiurin method and Walkley-Black method)

- 0.067 M Potassium dichromate mixture:

  Dissolve 40 g potassium dichromate ($K_2Cr_2O_7$) in 900 ml of distilled water. Transfer the solution to a 2000 ml volumetric flask. Place the flask into a bucket with cold water. Extremely carefully, with a small portions, add 1000 ml concentrated sulphuric acid (d = 1.84 g/cm^3), mix gently. Make to volume with distilled water.
- 0.2 M Ammonium iron(II) sulphate (Mohr salt):

  80 g of Mohr salt [$(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$] dissolve in 600 ml of distilled water, transfer to a 1000 ml volumetric flask, add 20 ml sulphuric acid (d = 1.84 g/cm^3), make to volume with distilled water.
- 0.04 M Potassium permanganate:

  Analytical weighed amount of $KMnO_4$ (0.02 mol/l; 0.1 N) dissolve in 400 ml of distilled water, transfer to a 500 ml volumetric flask and make to volume with distilled water.
- Phenanthroline complex (indicator):

  Dissolve 1.485 g of 1.10 – phenanthroline in approximately 80ml of distilled water, add 0.695 g Iron(II) sulphate ($FeSO_4 \cdot 7H_2O$), mix well and make to volume with distilled water.

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Dissolved organic carbon as a simple measure of labile organic matter

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What is measured in this method?

Dissolved organic carbon (DOC) concentrations in soil solutions extracted from soil sample.

Why this measurement is important?

Many monitoring programs advocate that soil organic matter contents should be measured to assess effects of agricultural practices on soil quality and SOM balance. Rates of change in the total organic pool are relatively slow and difficult to detect in the short term perspective or within lifetime of any research project. Research over recent years has focused on the labile fraction (LF), as it is considered a quickly reacting and sensitive indicator to monitor trends in soil organic matter quality and processes affecting SOM accumulation. It is more sensitive to tillage, manuring, fertilisation, crop rotation and other interventions than total organic matter.

The DOC is a component of the labile SOM connected to other labile fractions of SOM such as microbial biomass - C, water soluble C, extractable carbohydrates, mineralisable - N and easily decomposable non - humic organic substances such as carbohydrates, polysaccharides, proteins, organic acids, amino acids, waxes, fatty acids, and other non-specific compounds. DOC constitutes the fraction of organic particles smaller than 0.45 μm that are suspended in aqueous solution which account for only a small proportion of the total organic matter in the soil. However this potentially dissolved pool of C is an important contributor to transport of nutrients in the soil system.

Description of the measurement procedure

Specific dissolved organic carbon species are isolated based on defined operational conditions. The DOC is extracted from fresh soil samples with hot water o by a method of Ghani (2003). The extraction of DOC is conducted in simple steps as shown in fig. 1. The proposed temperature of water used for the extraction of labile C is 80ºC.

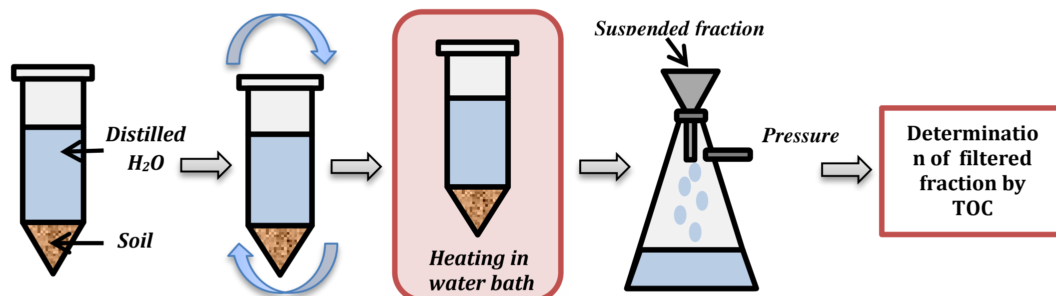

Fig. 1. Scheme of the extraction procedure for isolation dissolved organic carbon from soil samples.

Soil samples (equivalent 9 g oven dry weight) are weighted into 100 ml polypropylene centrifuge tubes. Soil DOC is then extracted with 90 ml of deionised water (Liquid /Soil 10:1). The tubes are shaken for 10 s to suspend the soil in the water. Then the tubes are capped and left for 16 h in a hot-water bath at 80 °C. At the end of the extraction period, each tube is shaken for 10 s by hand to resuspend the soil to ensure that hot water extractable carbon released from the SOM is fully suspended in the extraction medium. These tubes arethen centrifuged for 10 min at 3500 rpm. The supernatants are filtered through 0.45 μm cellulose nitrate membrane filters on Buchner funnel. Total carbon (inorganic and organic C) in both the first and second extracts is determined on a liquid CN analyser. Volume of 40 μl of the extracts is injected in the detection chamber for the analysis of total C. Three injections of the same volume is analysed for each sample. The analysis time for each sample is about 10 min. The DOC is the dissolved organic fraction of the total extractable C that is determined by subtracting the inorganic C values from the total hot-water extractable C.

Instruments and equipment

- Total Carbon Liquid Analyser C-N
- Buchner Funnel + 0.45 μm cellulose nitrate membrane

Chemicals, solutions or necessary supplies

- Deionised water or water of equivalent purity ($5 < pH < 7.5$) with a conductivity < 0.5mS/m according to grade 3 specified in ISO 3696

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Sorption capacity of soil

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What is measured in this method?

This method is a simplified procedure to assess cation exchange capacity of soil based on accounting for exchangeable basic cations and hydrogen ions, retained by soil. It consists of two methods:

- Exchangeable cations capacity – measures the content of exchangeable basic cations
- Exchangeable acidity – measures the content of exchangeable hydrogen ions.

Sorption capacity of soil (T) equals the sum of content of alkaline cations (S) and hydrogen ions, known as hydrolytic acidity (Hh) and is expressed in cmol(+) kg^{-1} of soil

$$T = S + Hh$$

The status of soil sorption complex can be also described as the degree of its saturation with major basic cations (V).

$$V = \frac{S}{T} \cdot 100\%$$

Why is this measurement important?

This parameter depends on soil texture, mineral composition, content of organic matter and its quality, as well as on intensity of physical, chemical and microbiological processes. Fine-textured soils, reach in organic matter are characterized by greater sorption capacity than sandy soils with low content of organic matter.

Sorption capacity for cations, in most cases, increases with the pH increase. When the pH is acidic, the hydrogen exchangeable cations are bound to the persistent charges of clay minerals and humus. When the pH is shifted to neutral or alkaline, hydrogen ions transfer to are neutralized in soil solution while basic cations are exchangeably sorbed by the soil solid fraction. Sorption capacity of soils is mostly shaped by the content of clay and humus and by soil pH. It is generally greater in soils with higher humus and clay contents.

Hydrolytic acidity (Hh)

Description of the measurement procedure

- Use dry soil samples sieved through <2mm mesh.
- Weight 30g of soil to 100ml plastic containers and add 75 ml of 1M calcium acetate and shake it on the laboratory shaker for 60 minutes at 140 rpm.
- Filter the suspension to plastic container and transfer 50 ml of filtrate to Erlenmeyer flask and add 2 drops of 1% solution of phenolphthalein.

- Titrate the above solution with 0.1M solution of sodium hydroxide until it reaches weakly pink colour that is not disappearing in one minute.

Calculation of the results

Hydrolytic acidity is expressed in ml of 0.1M NaOH used for the titration or in cmol (+)kg^{-1} of soil

$$Hh\ in\ ml\ 0.1\ mol\ NaOH/100\ g\ soil\ = a \cdot 5 \cdot 1.5$$

$$Hh\ in\ cmol(+)/\ kg\ soil\ = \frac{a \cdot 5 \cdot 1.5}{10}$$

Where:

a – amount in ml of 0.1M NaOH used for titration

5 – factor for recalculation for 100g of soil as a is an equivalent of 20g of soil

1.5 – empirical factor for the calculation of total hydrolytic acidity

Hydrolytic acidity can also be expressed as a rate of CaO q/ha

$$CaO\ q/ha = \frac{Hh \cdot 0.0028 \cdot 10 \cdot 3000000}{1000 \cdot 100}$$

Where:

Hh – hydrolytic acidity expressed in ml of 0.1M NaOH/100g of soil

0.0028 – amount of CaO in g, equivqalent of 1ml of 0.1M NaOH

10 – recalculation from 100g for 1 kg

3000000 – amount of kg of soil in arable layer of soil

1000 – recalculation from g to kg

100 – recalculation from kg to quintal

Or can be directly calculated from Hh

$$CaO\ t/ha = \mathrm{Hh} \cdot 0.84$$

Instruments and equipment

- Automatic burette
- Laboratory horizontal shaker

Chemicals, solutions or necessary supplies

- Calcium acetate $(CH_3COO)_2Ca \cdot H_2O$
- Sodium hydroxide NaOH
- Phenolphthalein
- Ethanol
- Distilled water
- 1M calcium acetate – weight 88.09g of calcium acetate, dissolve in distilled water, transfer to 1000 ml volumetric flask and fill it to the 1000 ml marker
- 0.1M sodium hydroxide – weight 4g of sodium hydroxide and dissolve in distilled water, transfer to 1000 ml volumetric flask and fill it to the 1000 ml marker
- 1% phenolphthalein – dissolve 1g of phenolphthalein in 96% ethanol

Exchangeable cations

Description of the measurement procedure

- Use dry soil samples sieved through <2mm mesh.
- Weight 2g of soil to 120ml plastic containers.
- Add 100 ml of 1M ammonium chloride (carbonate soils) or ammonium acetate (acidic soils), depending on the type of soil.
- Shake the samples for 2 hours at 145 rpm.
- Filtrate the suspension to 100 ml plastic containers.
- Analyse the content of elements with AAS technique.
- Concentrations (mg/100cm^3 of Ca, K, Na, Mg are determined on the basis of the read out from calibration curves. To recalculate the concentrations of the metals in soil sorption complex for cmol(+)/kg of soil, it is required to divide the concentrations in mg/100g by the masses of meq of individual cations, expressed in mg/mmol(+). They are: Ca: 40.08/2=20.04 mg/mmol(+), Mg: 24.49/2= 12.25 mg/mmol (+), K: 39.1 mg/mmol (+), Na: 23.0 mg/mmol (+).

  Ca^{2+} = 50.0/20.04·a [mmol(+)/100g of soil],

  K^{+} = 50.0/39.1·a [mmol(+)/100g of soil],

  Na^{+} = 50.0/23.0·a [mmol(+)/100g of soil],

  Mg^{+} = 50.0/12.25·a [mmol(+)/100g of soil],

[mmol(+)/100g of soil = cmol(+)/kg of soil]

Where:

a – concentration read out from calibration curve, mg/100cm^3

Total amount of alkaline cations in sorption complex of soil is calculated by addition of concentrations (cmol(+)/kg of soil) of all major basic cations

$$S=Ca^{2+} + K^{+} + Na^{+} + Mg^{2+} \ [cmol(+)/kg\ of\ soil]$$

Instruments and equipment

- Laboratory horizontal shaker
- pH – meter
- Atomic absorption spectrometer

Chemicals, solutions or necessary supplies

- Acetic acid -CH_3COOH - 80%
- Ammonia - $NH_3 \cdot H_2O$) - 20%
- Ammonium chloride - NH_4Cl
- Magnesium foil
- Calcium chloride - $(CH_3COO)_2$ Ca
- Potassium chloride – KCl
- Calcium chloride – $CaCl_2$

- 1M ammonium acetate – add 70 ml of acetic acid and 75 ml of ammonia to 1000 ml volumetric flask and fill with distilled water to the marker; obtained solution should be pH 7.0 – check with pH – meter; if not, add few drops of ammonia or acetic acid.
- 1M ammonium chloride – weight 53.5 g NH_4Cl and dissolve in 1000 ml of distilled water; pH should be 8.2; if not add few drops of ammonia or acetic acid.
- Magnesium basic standard solution – dissolve 0.5 g of magnesium foil in 30 ml of hydrochloric acid (HCl) in 500 ml volumetric flask and then fill up to the marker with distilled water. This solution contains 1 mg of magnesium in 1 ml.
- Magnesium working standard solution – add 50 ml of Magnesium basic standard solution to 500 ml volumetric flask and fill up to the marker with distilled water. This solution contains 0.1 mg of magnesium in 1 ml.
- Potassium and sodium basic standard solution – weight 1.907 g of potassium chloride and 2.5418g of sodium chloride, dried previously in 130 ^{o}C and transfer both to 1000ml volumetric flask. Fill up with distilled water to the marker. This solution contains 1 mg of K and 1 mg of Na in 1 ml.
- Potassium and sodium working standard solution – add 100 ml of potassium and sodium basic standard solution to the 1000 ml volumetric flask and fill up with distilled water to the marker. This solution contains 0.1mg of K and 0.1mg of Na in 1 ml.
- Calcium basic standard solution – weight 4.3957 g of calcium acetate, transfer to 1000 ml volumetric flask and will up with distilled water to the marker. This solution contains 1 mg of Ca in 1ml.
- Prepare working calibration standards from basic standard solutions.

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Determination of different forms of trace and macro elements

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What is measured in this method?

The content of a given form of trace and macroelements in soil.

Why is this measurement important?

The knowledge on occurrence, properties and behaviour of elements is essential to understand geochemical and biochemical processes in the soil-plant system. The distinction of trace elements (TE) took place because of their low concentrations <0.1% (1000mg/kg) and their specific functions in different processes especially in biochemical processes. This division is not unambiguous, because some elements may be in trace amounts in one matrice and in large quantities in a different media. The example is iron, which is one of the major elements in geological formations and soils but a trace element in plants, animal and human tissues.

Occurrence of trace elements in the environment and their importance for plants, animals and humans is the subject of many studies. There is a significant increase in knowlegde in this field observed, both because of awareness of role of these elements, as well as due to fast development of chemical analytical methods and measuring equipment.

Understanding behaviour of elements in the chain: soil / water / air / plant / animal / human is the basis for a proper assessment of their deficiency or excess. The maintenance of the chemical equilibrium of the natural environment, particularly with regard to the prevalence of trace elements, is very important for human health.

This issue has recently become particularly important as global population grows, while the area of cultivated fields reduces, that might result in intensification of agriculture, e.g. more intensive fertilization. Additional factors contributing to changes in the level of trace elements in different parts of the natural environment are industry and urban development.

The soil is the main source of trace and macro elements in food and fodder. Their intake by plants is influenced by soil factors, forms of elements and plant species or cultivar specific properties. The contamination of soils with trace elements is considered as one of major threats for agro-ecosystems worldwide, which might result in contamination of food as a consequence.

Various forms of elements are analyzed in soil depending on what fraction of given elements or what type of element availability we want to predict. Total contents of trace elements and their relation to certain threshold values are most often a basis for classification of contaminated and non-contaminated soils or wastes.

Soil macroelements are most often tested for their availability to plants or risk for nutrient migration to groundwater. For this purpose extraction methods, such as e.g. Mehlich 3 test are utilized.

Extractable trace elements are commonly measured to simulate risk of their phytotoxicity or ecotoxicity in contaminated soils or deficiency in typical un-contaminated soils. Such trace elements as zinc, copper, nickel are most commonly considered as potentially phytotoxic whereas cadmium, lead, mercury, arsenic pose a risk of toxicity for soil organisms, animals and human.

Such trace elements as copper, zinc, manganese, iron, selenium, boron can be deficient in crops and in consequence in human diet.

Total content of elements

Description of the extraction procedure

Method principle

This method involves extraction of trace and major elements with aqua regia with the use of microwave mid – pressure (30 bar) digestion system. Such extraction represents so called pseudototal content of element, most commonly used for classification of contaminated soils or high organic wastes.

Sample preparation

The sample is air dried and sieved through 2mm mesh to separate the skeletal parts and the remains of plants, animals etc. Then the sieved soil is milled.

Digestion procedure

- Weigh 0.5 g to 1 g of milled soil to the 75 ml digestion vessels.
- Add 10 ml of previously prepared aqua regia to each digestion vessel.
- Leave the samples for 10 – 15 minutes open for initial digestion.
- Seal the vessels.
- Place the sealed vessels in the rotor and then place the rotor in the microwave oven.
- Set the following digestion parameters:
  a. Max. power: 1600W
  b. Temperature: 170°C
  c. Ramping time: 15 minutes
  d. Holding time: 20 minutes
  e. Cool down time: 20 minutes.
- Transfer the obtained solution to 50 ml falcon vials and top up to 50 ml with 0.055 µS/cm ultrapure water.
- For contamination control run this procedure for blank samples.
- For quality control purpose run this procedure for Certified Reference Material.
- Leave the Falcon vials for 24 h to let the undissolved remains of soil to sediment or centrifuge the vial for 5 minutes at 3000 rpm.
- Analyse the concentration of elements with ICP-MS technique.

Instruments and equipment

- Laboratory balance – accuracy 0.001g
- Microwave closed vessel digestion system with vessels up to 30 bars and available power of at least 1400 W
- Inductively Coupled Plasma – Mass Spectrometer (ICP-MS)

Chemicals, solutions or necessary supplies

- Concentrated nitric acid (HNO_3) – 69% - 70% (Merc Suprapure grade)
- Concentrated hydrochloric acid (HCl) – 36.5% - 38% (Merc Suprapure grade)
- Aqua regia – mixture of concentrated hydrochloric and nitric acid v/v 3:1

Determination of trace elements soluble in 1M HCl

Description of the extraction procedure

Method principle

The method describes the extraction of trace elements with 1M hydrochloric acid to simulate trace element plant availability in agriculturally used soils. It is most commonly used to predict micronutrient deficiencies, however there is an increasing interest in adopting this method to contaminated soils as a substitute of more expensive extractions with concentrated acids.

Sample preparation

The sample is air dried and sieved through 2mm mesh to separate the skeletal parts and the remains of plants, animals etc.

Extraction procedure

- Weigh 2 g of soil into the plastic containers of volume of at least 50 ml.
- Add 20 ml of 1M HCl.
- Seal the containers and shake them on the laboratory shaker for 1 h at the speed of 40 rpm.
- Filter the extract into the plastic containers of volume of at least 30 ml.
- Analyse the content of elements with ICP-MS technique.

Instruments and equipment

- Laboratory balance – accuracy 0.001g
- Laboratory rotation shaker
- Inductively Coupled Plasma – Mass Spectrometer (ICP-MS)

Chemicals, solutions or necessary supplies

- Concentrated hydrochloric acid (HCl) – 36.5% - 38% (Merc Suprapure grade)

Mehlich 3 Extraction for Macro- and Micronutrients

Description of the extraction procedure

Method principle

Mehlich 3 (M3) estimates plant availability of most macro- and micro-nutrients in soils of acidic to neutral pH using a dilute acid-fluoride-EDTA solution adjusted to pH 2.5. The method has shown to be well correlated to crop phosphorus (P) and applicable for the determination of plant available potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and micronutrients, such as manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn). Due to the corrosive nature of chloride in the Mehlich 2 extractant (Mehlich, 1978) and its inability to extract micronutrients on a wide range of soils, particularly copper in organic soils, Mehlich 3 was first implemented in North Carolina soil testing program in 1981, after Mehlich 2 had been used for two years. The overall goal of research conducted by Mehlich was to develop a universal extractant that would be applicable across a wide spectrum of soils in regard to their reaction (pH) and mineralogy (Mehlich, 1984). The method has been widely adopted by many laboratories across the world.

Sample preparation

The sample is air dried and sieved through 2mm mesh to separate the skeletal parts and the remains of plants, animals etc.

Extraction procedure

- Weigh 5.0± 0.05 g of air dried soil pulverized to pass 2 mm mesh sieve (< 2.0 mm) in a 50-mL glass or plastic flask.
- Add 50.0 mL of Mehlich 3 extracting solution. Include a method blank and check samples as determined by the lab.
- Place extraction flask(s) on end – over-end mechanical shaker (35 ±5 rpm) for 5 minutes.
- Filter or centrifuge suspension immediately and collect the extract in 50 mL plastic vials.
- Analyse the content of elements with ICP-MS technique.

Instruments and equipment

- Laboratory balance – accuracy 0.001g
- Laboratory rotation shaker
- Inductively Coupled Plasma – Mass Spectrometer (ICP-MS)

Chemicals, solutions or necessary supplies

Solutions are made with ASC reagent grade chemicals and distilled water unless otherwise noted.

- Ammonium nitrate (NH_3NO_3)
- Ammonium fluoride (NH_4F)
- Nitric acid (HNO_3), 68-70%
- Ethylenediamine tetraacetic acid (EDTA), $(HOOCCH_2)_2NCH_2CH_2N(CH_2COOH)_2$
- Acetic acid, glacial [CH_3COOH]
- Ammonium fluoride-EDTA stock solution (3.75M NH_4F—0.25M EDTA): Dissolve138.9g of NH_4F in 600 mL of deionized water and add 73.06 g EDTA (or 93.06 g of Na_2-EDTA·$2H_2O$), dissolve and dilute to 1000 mL.

- Mehlich-3 extracting solution (0.2 N CH_3COOH—0.25N NH_4NO_3-0.015N NH_4F-0.013N HNO_3-0.001 M EDTA@pH-2.50 ± 0.05). Dissolve 80.05 g NH_4NO_3 in about 3,000 mL of deionized water. Add 16.0 mL of 3.75M NH_4F -0.25M EDTA stock solution and mix well. Add 46 mL of concentrated glacial CH_3COOH and 3.3 mL of concentrated HNO_3 and bring to 4,000 mL final volume. The final pH should be 2.50 ± 0.05.

Description of the measurement procedure

Method principle

This section describes the determination of element concentration in aqua regia or other extraction solutions of soil with ICP-MS technique.

Samples

Use the samples obtained in one of the above procedures.

Measurement procedure

- Prepare the multi-element working standard solution with the elemental composition suitable for measurement.
- Prepare the calibration standards from working standard solution according to the expected ranges of elements content.
- Simultaneously prepare the calibration blank solution.
- Prepare the internal standard solution to added online.
- Dilute the samples additionally according to the ranges of calibration standards. An extra care must be taken in order to have the same concentration of acids in calibration standards and in the samples.
- Optimize the performance of the ICP-MS instrument. Tuning must be performed to obtain desired sensitivity $^{7}Li^{+}$ >2000cps, $^{89}Y^{+}$> 3000cps, $^{205}Tl^{+}$ >2000cps, along with the stable and robust plasma conditions Ce^{+}/CeO^{+} <2%, Ce^{+}/Ce^{2+} <3%.
- Set up the sequence and run analysis.
- Calculation of element concentration is done automatically by the software of the instrument and is based on the calibration performed before every set of samples.

Instruments and equipment

- Inductively Coupled Plasma – Mass Spectrometer (ICP-MS)

Chemicals, solutions or necessary supplies

- Single element standard stock solutions of the elements to be measured (Merc Suprapure grade)
- Concentrated nitric acid (HNO_3) – 69% - 70% (Merc Suprapure grade)
- Concentrated hydrochloric acid (HCl) – 36.5% - 38% (Merc Suprapure grade)

Literature

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Agilent 7500ce ICP-MS Operating manual.

Determination of PCBs, OCPs, PBDEs and PAHs by GC-MS/MS

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What is measured in this method?

Selected representatives of persistent organic pollutants (POPs) listed in the Stockholm Convention, namely indicator polychlorinated biphenyls (PCBs – PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180), organochlorine pesticides (OCPs – hexachlorobenzene, hexachlorocyclohexanes, DDTs and metabolites), polybrominated diphenyl ethers (PBDEs – BDE28, BDE47, BDE99, BDE100, BDE153, BDE154, BDE183) and polycyclic aromatic hydrocarbons (PAHs – 16 EPA PAHs) listed on the UNECE Protocol to the Convention on LRTAP on POPs.

Why this measurement is important?

POPs under the Conventions stated above are persistent, bio accumulative and toxic compounds, therefore their production and use is banned or significantly restricted because they might cause adverse effects on humans and the environment. There is a need to monitor their presence in materials intended to application into the environment, e.g. compost, sludge, fertilizers, etc., and their behavior in soil.

Description of the measurement procedure

2.5 g of air-dried, ground and sieved soil sample is extracted for 1 hour by dichloromethane using an automated Soxhlet device. Extract is spiked by internal standards, transferred to evaporation vial and concentrated under a gentle nitrogen steam down to 1-2 ml. Extract is cleaned up using silica (2 g, activated at 150 °C/16 hours) column. Analytes are eluted with 10 ml of hexane followed by 15 ml of dichloromethane:hexane (50:50; v/v). The combined eluate is concentrated up to 0.5 ml using an automated evaporation device, transferred to autosampler vial, concentrated up to 0.25 ml under a gentle nitrogen steam and spiked with injection standards. The final extracts are analysed by gas chromatography (GC) coupled to tandem mass spectrometry (MS/MS). The GC system is equipped with programmable-temperature vaporizer (PTV) used for sample injection in splitless mode at 270°C. Chromatographic separation is carried out on 20 m × 0.18 mm × 0.18 µm capillary column with stationary phase of 5% phenyl/95% dimethylpolysiloxane. The mass spectrometer is operated in multi reaction monitoring (MRM) mode. Argon is used as collision gas.

Instruments and equipment

- Extraction system for accelerated Soxhlet extraction
- Concentration device
- Gas chromatograph coupled to triple quadrupole mass spectrometer

Chemicals, solutions or necessary supplies

- Dichloromethane (SupraSolv grade)
- *n*-hexane (Unisolv grade)
- silica gel 60 (0.063-0.200 mm)
- *Iso*-octane (Pestanal grade)
- Individual and mixture reference standards of native and isotopically labelled compounds

Literature

EN ISO 22032, Water quality – Determination of selected polybrominated diphenyl ethers in sediment and sewage sludge – Method using extraction and gas chromatography/mass spectrometry.

ISO 18287, Soil quality – Determination of polycyclic aromatic hydrocarbons (PAH) – Gas chromatographic method with mass spectrometric detection (GC-MS).

EN 16167, Sludge, treated biowaste and soil – Determination of polychlorinated biphenyls (PCB) by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD).

Determination of HBCD by LC-MS/MS

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What is measured in this method?

Hexabromocyclododecane (HBCD – α-, β-, γ-isomer), used as additive flame retardants, e.g. in polystyrene foams, listed in Annex A to the Stockholm Convention on POPs.

Why this measurement is important?

The HBCD amendment of Annex A to the Stockholm Convention entered into force on 26 November 2014. HBCD use and production is restricted with specific exemptions such as e.g. use in polystyrene insulation foams in buildings. Similarly to other POPs, HBCD might cause adverse effects on humans and the environment. So there is a need to monitor their presence in materials intended to application into the environment, e.g. compost, sludge, fertilizers, etc., and thus to reveal their potential source of contamination.

Description of the measurement procedure

1 g of air-dried, ground, sieved sample is weighted into 15ml centrifuge tube and spiked by internal standard. The spiked sample is extracted with 5 ml dichloromethane/acetonitrile (1:1, v/v) for 15 min by sonication. The extract is centrifuged at 3900 rpm for 5 min, supernatant is transferred to 15ml tube and extraction was repeated once. The combined extract is concentrated under gentle nitrogen steam down to volume of 2 ml, treated with 100 mg PSA (1 min shaking) and centrifuged at 3900 rpm for 5 min. Supernatant is transferred to 15ml tube, concentrated under gentle steam of nitrogen near to dryness, and reconstituted in methanol to 0.5 ml. Extract is passed through 0.2 µm PFTE filter to vial and spiked with injection standard. Final extract is analysed by ultra performance liquid chromatography coupled to tandem mass spectrometry. Chromatographic separation is carried out on 50 mm × 2.1 mm × 1.7 µm C18 column. Mobile phase consisted of 0.1% acetic acid in water and 0.1% acetic acid in methanol. The mass spectrometer is operated in MRM mode. Argon was used as collision gas.

Instruments and equipment

- Sonication bath
- Centrifuge
- Device for sample concentration under nitrogen stream
- Ultra performance liquid chromatograph coupled to triple quadrupole mass spectrometer

Chemicals, solutions or necessary supplies

- Dichloromethane (SupraSolv grade)
- Acetonitrile (Lichrosolv grade)
- Bulk PSA (primary secondary amine) bonded silica sorbent

- Native and isotopically labelled standards
- Solvents and chemicals used for mobile phase preparation

Literature

García-Valcárcel, Ana Isabel a José Luis Tadeo. Determination of hexabromocyclododecane isomers in sewage sludge by LC-MS/MS. Journal of Separation Science. 2009, 32(22), 3890-3897. DOI: 10.1002/jssc.200900424.

Determination of PFAS by LC-MS/MS

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What is measured in this method?

Perfluoroalkyl substances (PFAS), namely perfluorooctane sulfonate (PFOS) listed in Annex B to the Stockholm Convention on POPs along with representatives of perfluorocarboxylic acids (PFCA): perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA). PFOS is currently used in electric and electronic parts, fire fighting foam, photo imaging, hydraulic fluids and textiles while PFOA is dominantly used as an emulsifier for the emulsion polymerization of fluoropolymers such as polytetrafluoroethylene (PTFE, or Teflon).

Why this measurement is important?

PFOS is extremely persistent and has bioaccumulating and toxic properties. Due to these properties PFOS use and production is worldwide restricted. PFOA is not as bioaccumulative as PFOS and longer PFCA, however PFOA is detected widely in surface waters, and is present in numerous mammals, fish, and bird species. So monitoring of PFAS in materials intended to application into the environment, e.g. compost, sludge, fertilizers, etc., is prerequisite to reveal a potential source of PFAS contamination.

Description of the measurement procedure

1 g of air-dried, ground, sieved sample was weighted into 15ml centrifuge tube and spiked by internal standard (e.g. 2 ng – 10 ng of isotopic labelled PFAS). The spiked sample was treated with 0.5 ml of 0.2M NaOH for 30 min, then neutralized with 50 µl of 2M HCl and extracted with 5 ml methanol for 15 min by sonication. The extract was centrifuged at 3900 rpm for 5 min, supernatant was transferred to 15ml tube and extraction was repeated once. The combined extract was concentrated under gentle nitrogen steam down to volume of 2 ml, treated with 100 mg GCB and centrifuged at 3900 rpm for 5 min. 1 ml of treated extract was transferred to Eppendorf tube, concentrated under gentle steam of nitrogen down to 0.5 ml, spiked with injection standard (e.g. 2 ng – 10 ng of isotopic labelled PFAS) and filled in water to 1 ml. The final mixed extract was centrifuged at 12000 rpm for 5 min, then filtered through 0.2 µm nylon filter to vial and analysed by ultra performance liquid chromatography coupled to tandem mass spectrometry.

Chromatographic separation was carried out on 50 mm × 2.1 mm × 1.7 µm C18 column. Mobile phase consisted of 2 mM ammonium acetate in water and 2 mM ammonium acetate in methanol. The mass spectrometer was operated MRM mode. Argon was used as collision gas.

Instruments and equipment

- Sonication bath
- Centrifuge
- Device for sample concentration under nitrogen stream
- Ultra performance liquid chromatograph coupled to triple quadrupole mass spectrometer

Chemicals, solutions or necessary supplies

- Individual and mixture reference standards of native and isotopically labelled compounds
- Solvents and chemicals used for extraction and mobile phase preparation
- Bulk GCB (graphitized carbon black) sorbent

Literature

POWLEY, Charles R., Stephen W. GEORGE, Timothy W. RYAN a Robert C. BUCK. Matrix Effect-Free Analytical Methods for Determination of Perfluorinated Carboxylic Acids in Environmental Matrixes. *Analytical Chemistry*. 2005, vol. 77, issue 19, s. 6353-6358. DOI: 10.1021/ac0508090.

PHYSICS

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Soil investigations wouldn't be comprehensive without physics. The physical tests allow to describe the gas/liquid/solid system which is the matrix for microbial and biological activity. Unless the alteration of air-water relations can occur relatively quickly (what has the consequence particularly in changes of microbial activity in the soil) that the solid phase is subjected to a slow soil-forming processes. The presence of organic matter may, in the longer term, these processes modify.

Organic matter is the main binding agent responsible for the formation of clay humus complex that impacts formation and stabilization of soil aggregation. Studies of the effects of external organic matter application on soil quality require knowledge of physical properties and processes of the soil. Addition of organic matter mediates volume and distribution of pores, specific surface area, water repellency, water retention that affect many physical processes and functions and thus soil physical quality. Soil physical processes influenced by soil organic matter content include crusting, densification, infiltration, erosion, gaseous diffusion and warming. These processes strongly determine soil functions and ecosystem services such as the soil capacity to supply water to plants through changes in available water capacity, chemical composition of the atmosphere through rendering soil a source or sink of greenhouse gases, protection of soil organic matter against microbial mineralization by enhancing the stability of soil aggregates, filtration and purification of natural waters, adaptation to climate changes and formation of habitat and energy source for soil microorganisms and other biota. As a consequence they influence crop yield and productivity. Moreover, the soil physical properties and processes are important components of mathematical models for studying or predicting water flow, solute transport and use efficiency of water and nutrients in agriculture.

Amendment of soil with an external extra source of organic matter may show positive or adverse effect on soil physical quality. For example, increased water repellency and slower wetting rates of soil will reduce infiltration and water retention, leading to enhanced run-off across and a smaller amount of plant available water. On the other hand repellent soil aggregates may increase structural stability of soil. Moreover, some organic amendments may decline the soil stability through the increase in proportion of monovalent exchangeable cations such as Na^+ and $^{K}+$ or NH_4^+ at the expense of bivalent cations Ca^{2+} and Mg^{2+} that favorably affect soil structure. These effects depend on source and application rate of external organic matter.

Literature

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Particle size distribution measured by laser diffraction method

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What is measured in this method?

Particle size distribution can be measured in two ways. The sedimentation methods can be used as one possibility. These methods are based on the Stokes Law. There are two main methods which are used in most laboratories:

- the pipette method in which the strictly measured volume of a suspension, sampled after timely and from adequate depth is dried. The weight of the dry mass is the basis for the calculation of the content of a given fractions
- hydrometer method in which reading from the hydrometer after a specified period is recalculated on the contents of a given fractions.

In both cases the sensitivity of the method is relatively small.

The second possibility of measuring of particle size distribution is laser diffraction method. This method was developed at the end of previous century. It is based on the completely different (from the sedimentation methods) physical phenomenon – the scattering of the light on the particles which quickly passed through the laser beam. According to Fraunhofer or Mie theory the pattern of scattered light can be recalculated into particle size distribution.

Why is this measurement important?

Particle size distribution is one of the basic physical characteristic of the soil. The knowledge of this quantity is necessary for interpretation of other physical soil properties such as water transport, thermal properties or gas exchange. Moreover, the particle size distribution indirectly affects the transport of chemicals in the soil.

Description of the measurement procedure

- Sieve the air-dry soil through a 2 mm mesh.
- Wet the small amount of the sample with a few drops of distilled water on the plate. The moistened soil should have the consistency of a paste.
- Add the moistened soil to the measuring system. The amount of added soil should establish the obscuration in the range of 10 – 20%. The information about the obscuration level is available in the software of diffractometer.
- Set the stirrer and pump speed. These parameters should be validated for each device, and soil type. The Mastersizer 2000 (Malvern, UK) with Hydro G dispersion unit was used in this Project. Therefore, according to the previous validation, the stirrer and pump speeds were: 700 rpm and 1750 rpm respectively.
- Use the ultrasounds to disperse soil aggregates. The ultrasound probe is usually built in in the laser diffractometer. The power and time of ultrasounds duration should be validated for each device, and soil type. According to the previous validation, the maximum power (35W) for 4 minutes was used.

- Set the light lengths. The Mastersizer 2000 has two sources of light beams: laser (633nm - red) and diode (466nm - blue). Both were used during the measurement.
- Set the duration of the individual measurement. One minute was chosen in the Project measurements (30 s for red and 30 s for blue lights).
- Set the measurement frequency. It was used the frequency of 60000 per minute.
- Choose the theory for recalculation of light intensity registered on the detectors. If the Mie theory will be chosen, define the optical parameters. Because in the measurements Mie theory was used the following parameters were adopted: refractive index of continuous phase (water) – 1.33; refractive index of the soil – 1.52; absorption coefficient of the soil – 0.1.
- Set the No. of replications of the measurement after the given adding of the sample to the measuring system. 3 replications were set.
- Make the measurement.
- Replicate the whole procedure at least 3 times.

Instruments and equipment

- 2mm sieve
- Laser diffractometer

Literature

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Evaluation of sorptivity and water repellency of soil aggregates

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What is measured in this method?

Soil water repellency is a reduction in the rate of wetting and retention of water in soil caused by the presence of hydrophobic coatings on soil particles. To obtain an index of water repellency (hydrophobicity) the sorptivity of water (influenced by repellency) is compared to the sorptivity of ethanol (not influenced by repellency).

Why is this measurement important?

Soil water repellency (hydrophobicity) resists wetting and it has substantial hydrological and geomorphological consequences. These comprise the reduced infiltration capacity of soils, enhanced overland flow and accelerated soil erosion, development of preferential flow and the accelerated leaching of agrichemicals. Potentially hydrophobic organic materials are produced by; plant root exudates, certain fungal species, surface waxes from plant leaves, and decomposing organic matter. The level of repellency depends on the proportion of soil particles with a hydrophobic surface coating. In an extremely water repellent soil sorptivity of water e will be 0. Water repellency is an important process in the physical stabilisation of soil. (Doerr et al. 2006; Hallett, 2008). Potentially water repellent organic materials are produced by; plant root exudates, certain fungal species, surface waxes from plant leaves, and decomposing soil organic matter Water repellency is a key process in the physical stabilisation of soil. The level of repellency depends on the proportion of soil particles with a hydrophobic surface coating.

Description of the measurement procedure

The water and ethanol sorptivities were determined using an infiltration device described by Leeds-Harrison et al. (1994). The infiltration of the liquids into the aggregate occurred via a small circular area (1.5 mm diameter) with a sponge inserted at the tip. When connection between the aggregate and the tip appeared, the flow of liquid from the horizontal capillary tube connected with the device occurred. It enabled determining the rate of uptake, Q, as the rate of liquid movement in the horizontal capillary tube. The steady rate of the Q through a circular surface area was determined by Wooding in 1968 (Leeds-Harrison et al., 1994) (Eq. 1).

$$Q = \frac{4brS^2}{f} + 4rKh \tag{1}$$

where:

b - parameter affected by soil-water diffusivity function (assumed as 0.55),

r - radius of infiltration tip (0.75 mm),

S - sorptivity of liquid,

K - hydraulic conductivity,

h - pressure head,

f - fillable porosity, obtained with standard wax method.

After few modifications (quoted by Leeds-Harrison et al., 1994), the sorptivity could be calculated as:

$$S = \sqrt{\frac{Qf}{4br}} \tag{2}$$

The sorptivity of water and ethanol was determined for 14-16 mm soil aggregate fractions. The water repellency index (R) of soil aggregates was quantified by comparison of sorptivity of water S_W, and ethanol S_E (Tillman, et al., 1989), from the equation:

$$R = 1.95\left(\frac{S_E}{S_W}\right) \tag{3}$$

The ratio of S_E to S_W informs about how much of sorptivity is reduced by water repellency (Hallett et al., 2004). Ethanol was used because of the solid- liquid contact properties. Its infiltration into the soil is not affected by hydrophobic compounds but only by the soil pore structure (Hallett et al., 2001). The R values of 1, >1.95 and >50 indicate respectively no repellency, subcritical repellency and high repellency (Tillman et al., 1989; Urbanek et al., 2007).

Instruments and equipment

- Device for determining the infiltration of soil aggregates

Chemicals, solutions or necessary supplies

- Ethanol

Literature

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Evaluation of soil water retention

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What is measured in this method?

The soil water retention curve, y(h), describes the relationship between the water content, y, and the energy status of water (soil-water potential) at a given location in the soil (other names used in the literature: soil moisture characteristic curve, soil-water retention curve, pF curve). In a saturated soil at equilibrium with free water, the actual pressure is atmospheric, and the hydrostatic pressure and suction are zero. At this pressure, the soil-water content is approximately equal to the soil porosity and the soil is saturated. As energy is applied and suction is increased, water is drawn out of the soil and more of the relatively large pores, which cannot retain water against the suction applied, will empty out. A gradual increase in suction will result in the emptying of progressively smaller pores, until, at high suction values, only the very narrow pores retain water. The amount of water remaining in the soil at equilibrium is a function of the sizes and volumes of the water-filled pores.

It specifies how much water is retained by the soil at a given soil-water potential. This relationship is characteristic for each soil and must be measured experimentally.

Why is this measurement important?

Knowledge of y(h) is essential for the hydraulic characterization of a soil, since it relates an energy density (potential) to a capacity (water content). The shape of the curve reflects the particle or pore-size distribution of the soil.

Description of the measurement procedure

The soil water retention curve (WRC) will be determined in cylinders of 100 cm^3 volume, 5 cm diameter. The soil water retention curve was determined by gradually desaturating an initially saturated soil by applying increasingly higher suctions, thus producing a main drying curve. The suctions applied successively in a standard pressure chambers (low and high pressure chambers produced by the Soil Moisture Equipment Corp. USA) were as follows : 1 – 1 hPa, 2 – 10 hPa, 3 – 31 hPa, 4 – 100 hPa, 5 – 160 hPa, 6 – 500 hPa, 7 – 1 000 hPa, and 8 – 15 000 hPa, corresponding to the soil water potentials: -0.1, -1, -3.1, -7, -16, -50, -100 and -1 500 kJ m^{-3}, respectively. All values of the water content were expressed volumetric percents. The shape of the soil-water retention curve depends on soil management and texture. Figure 1 shows typical soil water retention curves for relatively coarse-textured (e.g., sand and loamy sand), medium-textured (e.g., loam and sandy loam), and finetextured (e.g., clay loam, silty loam, and clay) soils. The curves in Figure 1 may be interpreted as showing the equilibrium water content distribution above a relatively deep water table where the pressure head is zero and the soil fully saturated. The plots in Figure 1 show that coarse-textured soils lose their water relatively quickly (at small negative pressure heads) and abruptly above the water table, while fine-textured soils lose their water much more gradually. This reflects the particle or pore-size distribution of the medium involved. While the majority of pores in coarse-textured soils have larger diameters and thus drain at relatively small negative pressures (suctions) and once these large pores have been emptied only a small amount of water remains. The majority of pores, however, in fine-textured soils do not drain until very large suctions (negative pressures) are applied. Hence, more of the water is adsorbed, and

increasing suction causes a more gradual decrease in water content. In general, the higher the clay content, the higher water retaining properties, both at saturation and any other level of suction. This results in a much flatter shape of the soil-water retention curve for the sandy soil. In a clayey soil, the pore-size distribution is more uniform.

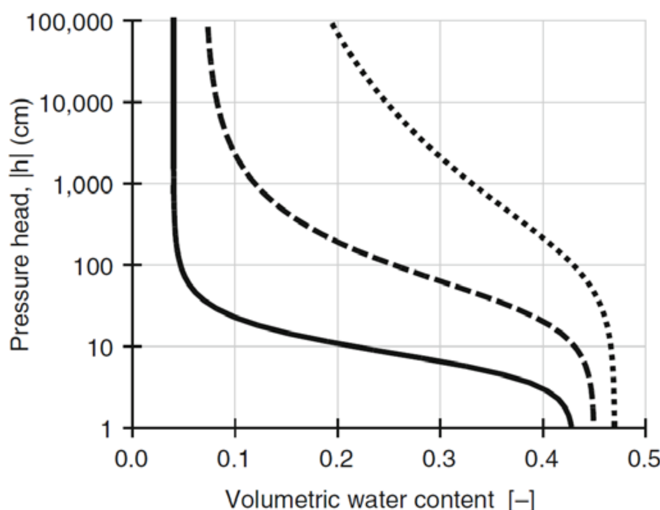

Figure 1. Typical soil water retention curves for relatively coarse- (solid line), medium- (dashed line), and fine-textured (dotted line) soils (after Van Genuchten and Pachepsky, 2011).

Instruments and equipment

- Standard pressure chambers

Chemicals, solutions or necessary supplies

- Cellulose membrane

Literature

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Pore size distribution

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What is measured in this method?

Evaluation of soil water retention curve (as described in "Evaluation of soil water retention" is often used to calculate soil pore-size distributions (PSD) of equivalent radius or diameter (Anderson et al., 1990).

Why is this measurement important?

The calculation of PSD based on water retention allows characterizing pore structure response to both soil management practices and texture. The amount of water retained at suction levels below 1000 cm H2O (or approximately pF = 3) depend primarily on capillary effects and pore-size distribution, and is therefore strongly affected by soil structure as a result of soil management. However, at higher suction levels, is due increasingly to adsorption and is influenced less by the structure and more by the texture and specific surface of the soil particles.

Description of the measurement procedure

Based on the capillary rise equation, the relationship between suction and equivalent pore radius can be approximated by the below equation (Sobczuk 1998).

$$Req\ (mm) = \frac{1.459854}{\Delta p}$$

where

Req is the equivalent pore radius emptied at a given level of suction, Δp (in cm H_2O).

The retention curve historically was often given in terms of pF, which is defined as the negative logarithm (base 10) of the absolute value of the pressure head measured in centimeters. The volume of water retained at suction levels below 1000 cm H_2O (or approximately pF = 3) depend mainly on capillary effects and pore-size distribution, and is therefore strongly affected by soil structure. Water retention at higher suction levels, however, is due increasingly to adsorption and is influenced less by the structure and more by the texture and specific surface of the soil particles.

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Strength of soil aggregates

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What is measured in this method?

The crushing strength (CS) refers to the force required to break an aggregate and is a very sensitive indicator of the structural stability of the whole soil. Soils with higher aggregate CS offer higher resistance to mechanical disturbance . The CS is a dynamic measure of the inter- and intra-aggregate bonds and depends on soil organic concentration .

Why is this measurement important?

A high mechanical stability of soil aggregates is fundamental for maintenance of proper tilth and provides stable traction for farm implements but limits root growth inside aggregates. The combined effect of the internal aggregate strength and wettability can result in increased soil stability and water infiltration. The mechanical properties of individual aggregates affects he macroscale behavior of the soil.

Description of the measurement procedure

The tensile strength of soil aggregates q (MPa) will be determined using strength testing device (Zwick/Roell) by putting an air-dry aggregate into its most stable position for crushing and calculated as suggested by Dexter and Kroesbergen, (1985), from the equation:

$$q = 0.576 \cdot F \cdot d^{-2}$$

where:

F- is the vertical breaking force (N);

d- is the mean aggregate diameter (taken along the longest, intermediate and the smallest axis)

0.576 - is the coefficient.

The porosity (%) of soil aggregates was determined using standard wax method (after weighing, for determination of the volume, necessary for calculating bulk density, the aggregates were covered with the paraffin and immersed in water).

Instruments and equipment

- Strength testing device

Literature

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Infiltration in soil aggregates and saturated hydraulic conductivity of soil

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What is measured in this method?

Infiltration denotes the entry of water into the soil aggregate through its surface. The saturated hydraulic conductivity (Ks) represents average hydraulic conductivity, which depends mainly on the size, shape and distribution of the pores.

Why is this measurement important?

Infiltration of soil aggregates is an important factor affecting water storage and movement because large inter-aggregate pores are dewatered first and the transport of water and solutes is influenced by the properties of the individual aggregates. It assesses the rate water flow in very conductive pores inside aggregates. Low aggregate and soil infiltrability favors water runoff and surface erosion. On the other hand, slight water repellency may stabilize soil against slaking and aggregate breakdown. Water infiltration into soil is a complex process that in field conditions varies for every precipitation event due mainly to its dependence of antecedent soil moisture. Saturated hydraulic conductivity can be used as a descriptor of the infiltration process.

Description of the measurement procedure

Infiltration of water into soil aggregates, Q, as the rate of liquid movement in the horizontal capillary tube. The steady rate of the Q through a circular surface area will be determined using equation 1 (Leeds-Harrison et al., 1994) described in section „Evaluation of sorptivity and water repellency of soil aggregates".

Measurements of the saturated hydraulic conductivity (m d^{-1}) are conducted on undisturbed soil samples contained in metal cylinders. (e.g. the Kopecky rings of 100 cm^3) (Oosterbaan and Nijland, 1994). Before measurements of the flow of water the samples are saturated with water and subjected to a hydraulic overpressure.

Instruments and equipment

- Device for determining the infiltration of soil aggregates
- The set for measurement of saturated hydraulic conductivity
- Cylinders of 100 cm^3 volume

Literature

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Laboratorní manuál

Editoři: Andrzej Bieganowski, Stanislav Malý, Magdalena Frąc, Ivan H. Tuf, Martin Váňa, Małgorzata Brzezińska, Grzegorz Siebielec, Jerzy Lipiec, Bořivoj Šarapatka

V letech 2013 až 2015 byl v rámci Operačního programu přeshraniční spolupráce Česká republika - Polská republika 2007-2013 realizován projekt „*Rizika a přínosy aplikace exogenní organické hmoty na půdu*“ (CZ.3.22/1.2.00/12.03445). Projektové konsorcium se skládalo z těchto institucí:

- Ústřední kontrolní a zkušební ústav zemědělský, Brno, Česká republika – vedoucí partner konsorcia,
- Univerzita Palackého v Olomouci, Olomouc, Česká republika,
- Ústav pěstování rostlin a pedologie – Národní výzkumný ústav (IUNG-PIB), Puławy, Polsko,
- Institut agrofyziky Bohdana Dobrzanskiho, Polská akademie věd, Lublin, Polsko.

Hlavním cílem projektu bylo zhodnotit vliv aplikace exogenní organické hmoty (EOM) jako hnojiva či půdního přípravku na různé složky půdních ekosystémů a připravit metodický postup pro jejich použití v zemědělství, aby bylo možné aplikací EOM bezpečně a účinně zvýšit obsah půdní organické hmoty (SOM). Připravený metodický postup by měl zohlednit lokální půdní a klimatické podmínky, charakter zemědělské praxe a druhy EOM dostupné v oblasti česko-polského pohraničí. Tento cíl je v souladu s prioritami Operačního programu, t.j. zlepšení kvality životního prostředí ochranou produkčních a environmentálních funkcí půdy a bezpečná likvidace odpadu v regionu.

Hlavní cíl řešeného projektu byl podepřen následujícími dílčími úkoly:

- Hodnocení současného stavu a změn v obsahu SOM v regionu pomocí dostupných historických dat a analýz v nových půdních vzorcích.
- Hodnocení zdrojů, množství a kvality EOM dostupné v regionu. Toto hodnocení bylo mimo jiné realizováno zkoumáním vlivu EOM na kvalitu půdy a její funkce. V letech 2013 a 2014 byly provedeny dva polní experimenty – jeden v Pustých Jakarticích (Česká republika) a jeden v Braszowicích (Polsko). V rámci těchto polních pokusů byly šetřeny čtyři druhy EOM ve třech rostoucích dávkách. Dále byly založeny dva skleníkové pokusy (v Puławách, Polsko), kde bylo testováno šest druhů EOM ve dvou dávkách, a to po aplikaci na tři různé půdy.

Na základě získaných výsledků byl připraven zmíněný metodický postup a doporučení. Tímto bychom rádi vyzvali čtenáře, aby vyhledali i naše jiné publikace, které jsme v rámci tohoto projektu zpracovali. Plně si uvědomujeme, že ačkoli testované EOM představují typické materiály dostupné v programovém území, tak provedené experimenty byly schopny pokrýt pouze omezený rozsah možných scénářů jejich aplikace na půdu. Proto připravená metodika je zaměřena spíše na laboratorní analýzy, které by měly být prováděny pro zhodnocení účinku a bezpečnosti aplikace EOM, než na poskytnutí konkrétních doporučení pro zemědělskou praxi. Předkládaný laboratorní manuál je nezbytným nástrojem pro provedení těchto analýz.

Jedním z nejvýznamnějších přínosů projektu (mimo spojitost se specifickými místními podmínkami v oblasti Sudet) **je především mezioborová povaha provedeného testování EOM**. V literatuře lze nalézt řadu údajů o testování EOM, avšak tyto práce jsou zaměřeny spíše na jeden či dva aspekty studia, zřídkakdy na několik. Mezioborový přístup, který byl podstatou řešení tohoto projektu, je proto velice unikátní a přínosný. Půdy ošetřené EOM tak byly současně testovány na celou řadu různých parametrů – biologických (včetně výskytu bezobratlých organismů), mikrobiologických, chemických i fyzikálních.

Pro testování tak široké řady různých parametrů je nutné využít mnoha analytických metod. A protože jedním z výstupů tohoto projektu bylo připravit metodiku pro provedení chemických, fyzikálních, mikrobiologických a ekotoxikologických analýz půdy a EOM, předkládáme čtenářům tento laboratorní manuál, který je určen především pro laboratoře zabývajícími se půdními analýzami. Jelikož metodologický aspekt testování EOM není specifický pro konkrétní zemi či region, rozhodli jsme se sepsat tento manuál v angličtině (pro jeho co nejširší možné využití). Budeme rádi, pokud čtenáři shledají manuál užitečným a budou jej využívat při jejich každodenní práci.

Názvy metod, jejich pořadí a rozdělení se od projektového návrhu liší, a to kvůli lepší přehlednosti a jednoduššímu používání manuálu. Nicméně všechny metody uvedené v projektovém návrhu jsou v tomto laboratorním manuálu uvedeny a popsány.

Přehled metod:

Biologie a ekotoxikologie

- Hodnocení vlivu agrochemikálií na reprodukci chvostoskoků (*Folsomia candida*)
- Hodnocení vlivu agrochemikálií na reprodukci roupic (*Enchytraeus crypticus*)
- Hodnocení vlivu agrochemikálií na reprodukci dravých roztočů (*Hypoaspis aculeifer*)
- Kontaktní test s využitím dehydrogenasové aktivity bakterie *Arthrobacter globiformis*
- Rostlinný test (délka kořene *Lactuca sativa*)
- Hodnocení inhibičního vlivu výluhů na luminiscenci bakterie *Vibrio fischeri*
- Inhibice růstu sladkovodní řasy *Pseudokirchneriella subcapitata*
- Inhibice růstu vodních rostlin *Lemna minor*
- Vzorkování půdní fauny

Mikrobiologie

- Aktivita denitrifikačních enzymů (DEA) s rozlišením denitrifikačních produktů N_2O and N_2
- Stanovení bazální a substrátem indukované respirace v půdě pomocí plynové chromatografie
- Stanovení půdní mikrobiální biomasy fumigačně-extrakční metodou
- Stanovení β-glukosidasové aktivity
- Stanovení dehydrogenasové aktivity
- Stanovení CM celulasové aktivity
- Stanovení aktivity kyselé a alkalické fosfomonoesterasy
- Stanovení ureasové aktivity
- Hodnocení krátkodobé nitrifikační aktivity
- Hodnocení genetické diversity amoniak oxidujících archaeí (AOA) s využitím analýzy polymorfismu délky terminálních restrikčních fragmentů (t-RFLP)
- Funkční diversita půdních mikroorganismů stanovená metodou hodnocení metabolických profilů (CLPP)

Chemie

- Půdní pH
- Elektrická konduktivita a salinita půdy

- Stanovení celkového uhlíku a dusíku metodou suchého spalování (dle Dumase) s využitím CN elementárního analyzátoru
- Stanovení organického uhlíku oxidací ve směsi dichromanu draselného a kyseliny sírové
- Rozpuštěný organický uhlík jako míra labilní organické hmoty
- Sorpční kapacita půdy
- Stanovení různých forem stopových a makroprvků
- Stanovení PCB, OCP, PBDE a PAH pomocí GC-MS/MS
- Stanovení HBCD pomocí LC-MS/MS
- Stanovení PFAS pomocí LC-MS/MS

Fyzika

- Stanovení rozdělení velikosti půdních částic metodou laserové difrakce
- Hodnocení sorptivity a hydrofobicity půdních agregátů
- Vyhodnocení retenční křivky půdy
- Rozdělení velikosti půdních pórů
- Pevnost půdních agregátů
- Infiltrace vody a saturovaná hydraulická konduktivita půdy

Podręcznik metodyczny

Redakcja: Andrzej Bieganowski, Stanislav Malý, Magdalena Frąc, Ivan H. Tuf, Martin Váňa, Małgorzata Brzezińska, Grzegorz Siebielec, Jerzy Lipiec, Bořivoj Šarapatka

W latach 2013-2015, w ramach Programu Operacyjnego Współpracy Transgranicznej Republika Czeska - Rzeczpospolita Polska 2007 – 2013, realizowany był projekt „Zagrożenia oraz korzyści wynikające z wprowadzania do gleb egzogennej materii organicznej" (CZ.3.22/1.2.00/12.03445). Partnerami konsorcjum byli:

- Ústřední kontrolní a zkušební ústav zemědělský (Centralny Instytut Nadzoru i Badań w Rolnictwie), Oddział w Brnie, Republika Czeska – partner wiodący,
- Univerzita Palackého (Uniwersytet Palackiego) w Ołomuńcu, Republika Czeska,
- Instytut Uprawy Nawożenia i Gleboznawstwa – Państwowy Instytut Badawczy (IUNG-PIB) Puławy, Polska,
- Instytut Agrofizyki im. Bohdana Dobrzańskiego PAN, Lublin, Polska.

Głównym celem projektu było określenie wpływu stosowania egzogennej materii organicznej (EOM) jako nawozu lub kondycjonera glebowego, oraz przygotowanie wytycznych dla bezpiecznego i efektywnego wykorzystania EOM w rolnictwie, zapewniającego przyrost glebowej materii organicznej (SOM). Wytyczne te miały uwzględniać lokalne warunki glebowe i klimatyczne, charakter rolnictwa oraz różne, dostępne w regionie, rodzaje EOM. Cel ten jest zgodny z priorytetami Programu w zakresie poprawy jakości środowiska przez podwyższenie produkcyjności, poprawę zdolności gleby do pełnienia funkcji środowiskowych oraz umożliwienie bezpiecznej utylizacji odpadów w regionie.

Cel główny realizowany był przez cele szczegółowe:

- Ocena stanu i trendów zmian zawartości glebowej materii organicznej (SOM) w regionie na podstawie dostępnych danych historycznych oraz danych uzyskanych na podstawie analiz przeprowadzonych na próbkach pobranych z pola.
- Ocena źródeł EOM (ilościowa i jakościowa) dostępnych w regionie. Realizacja tego celu uwzględniała m.in. badanie wpływu EOM na jakość i funkcje gleby (w 2013 i 2014 r. przeprowadzono dwa doświadczenia polowe: jedno w Czechach - Puste Jakartice, oraz jedno w Polsce - Braszowice). W doświadczeniu polowym zastosowano cztery typy EOM w trzech dawkach. Wykonano również dwa doświadczenia w warunkach szklarniowych (Puławy, Polska) dla przetestowania 6 typów EOM w 2 dawkach, na 3 różnych glebach.

Na podstawie uzyskanych wyników przygotowano wytyczne i wskazówki do stosowania EOM. Zapraszamy Czytelników także do innych naszych publikacji powstałych w ramach niniejszego projektu. Autorzy opracowania zdają sobie sprawę z tego, że chociaż testowane dodatki EOM reprezentują typowe materiały dostępne w regionie objętym projektem, to eksperymenty mogły objąć jedynie niewielki zakres możliwych scenariuszy. Dlatego w niniejszym opracowaniu uwaga koncentruje się raczej na przedstawieniu kompletu analiz koniecznych dla oceny skuteczności i bezpieczeństwa stosowania EOM. Przedstawiony przewodnik metodyczny jest narzędziem niezbędnym do realizacji tego zadania.

Jednym z najmocniejszych punktów projektu (poza odniesieniem do lokalnych warunków panujących na obszarze Sudetów) **jest interdyscyplinarny charakter badań**. W literaturze naukowej dostępnych jest wiele badań dotyczących EOM. Zazwyczaj jednak koncentrują się one na jednym lub dwóch aspektach - rzadko na kilku. Natomiast badania przeprowadzone w ramach niniejszego projektu wydają się być wyjątkowe ze względu na to, że gleby nawożone EOM

zostały poddane badaniom kompleksowym z jednoczesnym wykorzystaniem metod biologicznych (w tym uwzględniających obecność bezkręgowców), mikrobiologicznych, chemicznych i fizycznych.

Tak szerokie spektrum badań wymagało zastosowania wielu metod analitycznych. Jednym z rezultatów projektu było przygotowanie przewodnika do analiz chemicznych, fizycznych, mikrobiologicznych, ekotoksykologicznych i dotyczących fauny, specjalnie opracowanych do badania gleby oraz dodatków EOM. Broszura, którą przedstawiamy Czytelnikowi, adresowana jest do laboratoriów zajmujących się pomiarami w glebie. Ponieważ aspekty metodyczne testowania EOM nie mają specyfiki narodowej, podjęliśmy decyzję publikacji tego opracowania w języku angielskim, aby umożliwić jego jak najszersze wykorzystanie. Będzie nam miło, jeśli książka okaże się przydatna dla Czytelników i będzie wykorzystywana w codziennej pracy.

Nazwy metod, ich kolejność i przyporządkowanie są nieco inne, niż w propozycji projektu. Zmiana ta została wprowadzona dla łatwiejszego korzystania z podręcznika, lecz wszystkie metody wskazane w projekcie zostały opisane w niniejszym przewodniku.

Lista metod:

Biologia i ekotoksykologia

- Określenie wpływu substancji chemicznych stosowanych w rolnictwie na rozmnażanie skoczogonków (*Folsomia candida*)
- Określenie wpływu substancji chemicznych stosowanych w rolnictwie na rozmnażanie wazonkowców (*Enchytraeus crypticus*)
- Określenie wpływu substancji chemicznych stosowanych w rolnictwie na rozmnażanie drapieżnych roztoczy (*Hypoaspis aculeifer*)
- Test kontaktowy z wykorzystaniem aktywności dehydrogenaz bakterii *Arthrobacter globiformis*
- Test z roślinami (wzrost korzeni *Lactuca sativa*)
- Oznaczanie inhibicyjnego działania odcieków na emisję światła przez bakterie luminescencyjne *Vibrio fischeri*
- Test hamowania wzrostu glonów słodkowodnych *Pseudokirchneriella subcapitata*
- Test hamowania wzrostu rośliny słodkowodnej *Lemna minor*
- Pobieranie próbek fauny zasiedlającej glebę

Mikrobiologia

- Aktywność enzymów denitryfikacyjnych (DEA) z rozróżnieniem produktów denitryfikacji N_2O i N_2
- Oznaczenie respiracji podstawowej oraz respiracji indukowanej substratem w glebie metodą chromatografii gazowej
- Oznaczenie biomasy mikroorganizmów glebowych metodą fumigacji – ekstrakcji
- Oznaczenie aktywności β-glukozydazy
- Oznaczenie aktywności dehydrogenaz
- Oznaczenie aktywności CM celulazy
- Oznaczenie aktywności kwaśnej i alkalicznej fosfomonoesterazy
- Oznaczenie aktywności ureazy
- Oznaczenie aktywności nitryfikacyjnej (pierwszy etap procesu nitryfikacji)

- Ocena różnorodności genetycznej archaea utleniających amoniak (AOA) z wykorzystaniem analizy polimorfizmu długości terminalnych fragmentów restrykcyjnych (t-RFLP)
- Ocena różnorodności funkcjonalnej mikroorganizmów glebowych metodą profilowania fizjologicznego poziomu populacji (CLPP)

Chemia

- pH gleby
- Przewodnictwo elektryczne i zasolenie gleby
- Oznaczenie węgla całkowitego i azotu całkowitego poprzez spalanie na sucho (metoda Dumasa) w wykorzystaniem analizatora elementarnego
- Oznaczanie węgla organicznego w glebie poprzez utlenianie w mieszaninie dichromianu potasu i kwasu siarkowego
- Rozpuszczalny węgiel organiczny jako prosta miara labilnej materii organicznej
- Pojemność sorpcyjna gleby
- Oznaczanie różnych form mikro- i makroelementów
- Oznaczanie PCB, OCP, PBDE i PAH z wykorzystaniem GC-MS/MS
- Oznaczanie HBCD z wykorzystaniem LC-MS/MS
- Oznaczanie PFAS z wykorzystaniem LC-MS/MS

Fizyka

- Wyznaczanie rozkładu granulometrycznego gleby metodą dyfrakcji laserowej
- Wyznaczenie pojemności sorpcyjnej i wodoodporności agregatów glebowych
- Ocena retencji wody w glebie
- Wyznaczenie rozkładu porów glebowych
- Wyznaczenie wytrzymałości agregatów glebowych
- Określenie infiltracji wody w agregatach glebowych oraz wyznaczenie nasyconego przewodnictwa wodnego gleby

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Laboratory manual

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