MIBIREM – Toolbox for Microbiome based Remediation

Deliverable 1.6

Initial handbook for standardised sampling

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1 Publishable summary

MIBIREM aims to identify, analyse, cultivate and up-scale microbiomes that can degrade contaminants in soil and groundwater. To reach this goal taking samples at selected contaminated sites is the first step. Due to the involvement of different partners that have different backgrounds, it is necessary to harmonise the way how samples are taken, stored and treated. This facilitates a better comparability of samples and data taken by several partners in different countries in the project.

This initial handbook describes the strategies and procedures, how soil and groundwater samples are taken in the project, how they are transported, treated and stored. A proper sample code that is used by all partners is prepared to facilitate an easy and unambiguous identification of each sample. Here it must be noted that not all sites are in partner-countries, but two potential sites are in Poland and Spain, which are not directly involved in MIBIREM. During the whole process from taking the sample to analysis it is essential to ensure that there are minimal changes in the sample so that the measured results most probably reflect the conditions at the site.

For groundwater samples, on-site parameters are measured before sampling. Samples are then sent to an external chemical laboratory that analyses the contaminants that are relevant for the respective use-case and additional chemical parameters that are necessary to characterise the sampling site. In addition, samples are taken for DNA extraction and for enrichment cultures and degradation experiments. For the latter it is important to use sterile bottles and keep samples cooled until further treatment.

Soil samples are sent to an external chemical laboratory that analyses the contaminants that are relevant for the respective use-case. For determination of basic parameters in the lab, DNA extraction and enrichment cultures samples are sieved to 2 mm. Additional chemical parameters are only analysed, if the sample is further used in the project for community analysis, enrichment cultures, or degradation tests.

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2 Introduction

2.1 Aim

The aim of this deliverable is to provide a guideline that ensures that samples are taken, transported, stored and treated in a comprehensible way according to quality criteria that guarantee generation of reliable results.

This Handbook is a "living document" in a sense that this is a first draft based on the current knowledge, experience and assumptions of the project consortium. The progress in the project will show the feasibility of the procedures described in this handbook. The handbook will be adapted based on the experience made during the project and will lead into an improved version that will be part of the MIBIREM-toolbox.

2.2 Principles

The sample shall be taken in a way that ensures that the parameters to be examined during and after taking the sample is influenced as little as possible to enable the best representation of the "real value" at the site.

Samples must be packaged and preserved in such a way that ensures minimal change of the parameter(s) to be analysed in the sample:

- Adhesion of substances to sampling devices and packaging materials
- Volatilisation of substances from samples
- (Pho to)chemical conversion of substances in samples (under the influence of light)
- Biological conversion of substances in samples (under the influence of microorganisms)
- Contamination by other substances or microorganisms

It is important that all deviations from this guideline are recorded in the on-site protocol!

3 Coding of samples

The following coding of samples is used in the project MIBIREM:

[Country code]_[Site code]_[Sample number]_[Subcode of sample (e.g. different depth)]_[Water/Soil]

e.g.: FR_PLO_01_A_S

Sample from France (FR), Ploufragan (PLO), Sample No. (01_A), taken from soil (S)

Country codes, codes for individual sites and sample codes are given in Table 1 below:

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Table 1: Abbreviations for sample codes (additional site codes will be included, in case other sites might be selected; sites not included in MIBIREM will be excluded from a later version of this handbook)

Country codes		**Site codes**		**Sample codes**	
AT	Austria	SIM	Gaswerk Simmering	S	soil
		ERD	Gaswerk Erdberg	GW	Groundwater
BE	Belgium	GEN	Lumco in Gent		
DE	Germany	RIN	Betriebshof Stadtwerke Rinteln		
		KIE	Former Gaswork site Kiel		
		STO	Stockach		
		BIT	Bitterfeld		
ES	Spain	LEM	Jata, Lemoiz, Bizkaia		
FR	France	PLO	Renaud-Lucienne soap Factory, Ploufragan		
		TAV	Tavaux site (Jura)		
		GRE	Le Pont de Claux (Grenoble)		
		WIN	PCUK Wintzenheim (Colmar)		
IT	Italy	COL	Colleferro		
NL	The Netherlands	AME	Vetgasfabriek Amersfoort		
		UTR	Griftpark Utrecht		
		KRA	Gasfabriek Kralingen, Rotterdam		
PO	Poland	JAW	Jaworzno, Poland		

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4 Groundwater

This chapter describes the procedure on how to take groundwater samples to facilitate comparability between samples taken from different partners on different sites. Groundwater samples are taken according to the following tiered approach to avoid analysis of samples that are not further used in the project.

Tier 1: Take samples and measure on-site parameters (by partner taking the samples); Table 2

Tier 2: Measure contaminant concentration; Table 3

Tier 3: Measure extended parameters in samples with high contaminant concentrations, that are used for enrichment cultures, or further analysis, but also in some samples that are not contaminated, or show only a low level of contamination to get background-values for parameters (external lab); Table 4

4.1 Preparation and Pre-pumping

- Determine the depth of the aquiclude and groundwater table in metres below ground level (m bgl).
- Put a pump with hose into the water well by ensuring that the hose does not get in contact with the ground.
- When using a motor pump (if permitted by the site owner), it is placed a few metres downwind of the water well, to prevent contamination with exhaust gases.
- Use a flow cell, or (if not available) pump the groundwater into a bucket and put the sensors into the bucket to measure on-site parameters (Table 2) continuously.
- Pump three times the water volume of the water well (provided that the inflow is sufficient, depending on the hydraulic conditions at the site and the volume of the water well). After that time Electric Conductivity (EC) should be stable. If not, record that EC is still unstable and wait for another 5 minutes. If EC is getting stable in an earlier stage, pumping only between 1 and 3 times the volume of the water well might be enough.

4.1.1 Recording of on-site parameters

When pre-pumping is finished (i.e. stable EC, or waiting for 5 more minutes) record on site parameters according to Table 1:

Table 2: On-site parameters for groundwater samples

On-site parameters - groundwater	Unit	Comments
Temperature	°C	
pH	-	
Oxygen	mg/L	
Electrical conductivity	µS/cm	
ORP	mV	NB: reliability often low
Visual deviations	-	Smell (passively); colour; free product

4.2 Taking groundwater samples

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After on-site parameters were recorded immediately start with taking samples for chemical parameters, DNA extraction and for bacterial cultivation.

4.2.1 Contaminants in groundwater samples

For each use case, samples are taken to analyse the relevant contaminants (Table 3). If additional contaminants are also expected at a site (or are known to be there), additional samples are taken to analyse these contaminants.

Table 3: Contaminants in groundwater measured according to use case

Contaminants - groundwater	**Unit**	**Comments**
PHC (C_{10}-C_{40} + C_{10}-C_{22})	µg/L	
Σ16 EPA PAH	µg/L	
BTEXN (Ortho-, Meta-, Para)	µg/L	
Trimethylbenzene (1,3,5- / 1,2,3- / 1,2,4-)	µg/L	
Indane	µg/L	
Ethyltoluene (Ortho & Meta)	µg/L	
Indene	µg/L	
Naphthalene	µg/L	
Propylbenzene	µg/L	
HCH (all isomers: α, β, γ, δ, ε)	µg/L	
Cyanides (total and free)	µg/L	
Other potentially relevant contaminants:		
Chlorobenzenes	µg/L	Only at HCH-sites
chlorophenols	µg/L	Only at HCH-sites
DDT	µg/L	
aldrin	µg/L	
Chlordane	µg/L	

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4.2.2 Extended chemical parameters in groundwater samples

Samples are taken for analysis of extended chemical parameters to facilitate a characterisation and comparison of different sampling sites. The list of basic chemical parameters is given in Table 4:

Table 4: Extended chemical parameters for characterisation of sampling sites

Chemical parameters - groundwater	Unit	Comments
Nitrate	µg/L	
Nitrite	µg/L	
Ammonium	µg/L	
Dissolved Manganese (Mn(II))	µg/L	
Dissolved iron (Fe(II))	µg/L	
Total iron	µg/L	
Sulfate	µg/L	
Sulfite	µg/L	
Phosphate	µg/L	
Dissolved Organic Carbon (DOC)	µg/L	
Methane	µg/L	

4.2.3 DNA extraction from groundwater

The following procedure describes how to take samples for DNA extraction from groundwater and how they are treated until DNA is extracted:

- Take 2x1L of groundwater samples (use autoclaved glass bottles and rinse them with the pumped water before filling them up)
- Cool the samples during transport to the lab (ideally samples should be cooled at 4°C, and transport time should be kept as short as possible)
- Keep samples at 4°C until filtering
- Filter bacteria using a 0.45µm nitrate cellulose filter (same day, or next day, preferably not later; record time between sampling and filtration).
  If the filter is partly clogged, extend the time for filtering until the filter is clogged completely and then record the volume of filtered groundwater. In case of complete clogging before a sufficient amount of water is filtered, scratch the surface with autoclaved wooden picks (soft rounded ones) to unclog the filter. As final option change to a new filter. If two filters per sample are used, the extraction is first done separately and in a later step of the extraction protocol pooled again.
- Immediately after filtration, freeze and store the filter at -20°C by rolling them into a plastic vial (e.g. 50 ml Falcon tube) using sterile tweezers. The filter has to be rolled in a way that the "sample-side" (= upper side) of the filter does not touch the inner wall of the tube. (*Remark: If DNA extraction is first established in a project, it is necessary to do blank filtering as a negative control*).
- Extract DNA with DNeasy Power Water Kit (DNeasy PowerWater Kit (qiagen.com)) following manufacturer's instructions.

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- Store DNA at -20°C

4.2.4 Groundwater samples for enrichment cultures/microcosm experiments

- Take 2-3L of groundwater samples (use autoclaved glass bottles and rinse them with the pumped water before filling them up)
- Cool the samples during transport to the lab (ideally samples should be cooled at 4°C, and transport time should be kept as short as possible)
- Since in the MIBIREM project the focus is on aerobic microbial degradation and the aim is to enrich bacteria that are able to degrade contaminants under these conditions, there is no need to maintain anoxic/micro-oxic conditions of the samples.

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5 Soil

This chapter describes the procedure on how to take soil samples to facilitate comparability between samples taken from different partners on different sites.

5.1 Taking soil samples

Soil samples are taken according to the following tiered approach to avoid analysis of samples that are not further used in the project.

Tier 1: Take samples and measure standard parameters; Table 5

Tier 2: Measure contaminant concentration; Table 6

Tier 3: Measure extended parameters only in samples with high contaminant concentrations, that are used for enrichment cultures, or further analysis (external lab); Table 7

In addition to contaminated samples it is also essential to take samples that are not contaminated, or show only a low level of contamination to get background-values for parameters and thus facilitate better understanding of potential limiting factors for degradation and how the contaminants influence these parameters!

5.1.1 Standard parameters

Table 5: Basic physico-chemical parameters for soil samples

Standard parameters - soil	Unit	Comments
pH	-	
Dry matter content	% (m/m)	
Electrical conductivity	µS/cm	
Visual deviations	-	Smell (passively); colour; free product

5.1.2 Contaminants in soil

Table 6: Contaminants in soil measured according to use-case

Contaminants - soil	Unit	Comments
PHC (C_{10}-C_{40} + C_{10}-C_{22})	µg/kg	
Σ16 EPA PAH	µg/kg	
BTEXN	µg/kg	
HCH (all isomers: α, β, γ, δ, ε)	µg/kg	
Cyanides (bound and free)	µg/kg	
Other potentially relevant contaminants:		
PCB	µg/kg	Possible in Hydrocarbon contaminated sites (present up to 1900µg/kg in some points at Ploufragan French site)
chlorobenzenes	µg/L	Only at HCH-sites

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chlorophenols	µg/L	Only at HCH-sites
DDT	µg/L	
aldrin	µg/L	
Chlordane	µg/L	

5.1.3 Extended physico-chemical parameters in soil

The extended parameters (Table 7) are only measured in samples that are selected for further use in the project due to their elevated concentration of contaminants.

Table 7: Extended physico-chemical parameters for soil samples

Extended parameters - soil	Unit	Comments
Inorganic carbon (carbonate) / buffer capacity		
Total Nitrogen	%	
Available phosphorous	mg/kg	
Available Potassium	mg/kg	
Available Magnesium	mg/kg	
C/N-ratio	-	
Metals (redox):		
Iron (II) and total	mg/kg	
Manganese (II) and total	mg/kg	
Heavy metals:		
Copper	mg/kg	
Zink	mg/kg	
Lead	mg/kg	
Cation Exchange Capacity (CEC)	$mmol_c/kg$	
Exchangeable Calcium	$mmol_c/kg$	
Exchangeable Magnesium	$mmol_c/kg$	
Exchangeable Potassium	$mmol_c/kg$	
Exchangeable Sodium	$mmol_c/kg$	
Exchangeable Aluminium	$mmol_c/kg$	
Exchangeable Iron	$mmol_c/kg$	
Exchangeable Manganese	$mmol_c/kg$	
Total Organic Carbon (TOC)	%	
Particle-size distribution (sieve fraction)	-	
Organic matter content	%	

5.1.4 DNA extraction from soil

The following procedure describes how to take samples for DNA extraction from soil and how they are treated until DNA is extracted:

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- Produce a composite sample for each sampling spot by mixing well by hand with a shovel in a suitable container (e.g. bucket) to get enough material for all physico-chemical analysis, DNA extraction and experiments in the lab (e.g. degradation tests, enrichment cultures).
- For DNA extraction put about 50 g of the composite sample into a Falcon tube (or comparable containers e.g. zip lock bags). For physical-chemical analysis use the respective tubes provided by Agrolab.
- Make sure to clean soil sampling material between sampling spots (e.g. dWater, deconex, EtOH)
- Transfer soil samples to the lab cooled at 4°C
- Sieve the soil to 2 mm (if not possible use a bigger mesh and record)
- Store aliquot of soil (e.g. 5 g) at -20°C; aliquots should not go through repeated thaw/freeze cycles - once it is take out and defrosted sample should not be put back to -20°C
- Use part of the composite sample of the soil to determine water content (measure fresh soil weigh and dry soil weight after dry at 105°C) for calculation of the amount of DNA per dry weight of soil
- Use FastPrep (FastPrep-24™ Classic Instrument | MP Biomedicals) to grind soil sample: (parameters (duration and intensity) can be adapted, in case of different soil types instead use manufacturer recommendation; tubes should be filled up to two third of the volume (more soil volume might reduce DNA yield, record the amount of soil (weight) used for the gDNA extraction)
- Use MP Kit (FastDNA™ Spin Kit for Soil DNA Extraction | MP Biomedicals) for extraction of DNA
- Store DNA at -20°C

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5.1.5 Soil samples for enrichment cultures/microcosm experiments

- Take soil from the composite sample produced as described under 5.1.4 and ideally sieve it to 2 cm at the site; if this is not possible, record why and take the sample to the lab and sieve it there (see below).
- Transfer samples in a sterile box/bag to the lab cooled at 4°C
- If soil was not immediately sieved in the field, sieve it in the lab to 2 mm (soil should not be dried before sieving to avoid loss of volatile contaminants); if soil was dried before sieving record drying conditions (at least temperature, time and thickness of layers)
- Store at 4°C
- Record time, treatment and storage conditions between sampling and further working steps.

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