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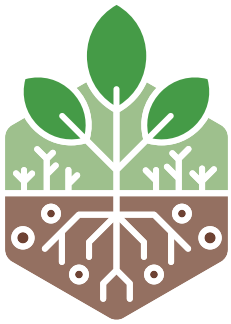
D1.1 REQUIREMENTS FOR SITE MONITORING AND CHARACTERISATION



pHYBi

Phytomanagement for a bio-based textile industry

PHYtomanagement as a sustainable feedstock source of lignocellulosic-based high-value BIo-based products for textile applications

Grant Agreement Number 101156439

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ABBREVIATIONS

Abbreviation	Definition
AAS	Atomic Absorption Spectroscopy
ABA	Abscisic Acid
API	Application Programming Interfaces
ASTM	American Society for Testing and Materials
BCF	Bioconcentration Factor
CHNS	Carbon, Hydrogen, Nitrogen, and Sulfur
CAT	Catalase
CEC	Cation Exchange Capacity
CETIM	Centro Tecnológico de Investigación Multisectorial
CK	Cytokinin
CS	Case Study
dbh	Diameter at breast height
DEM	Digital Elevation Models
DMF	Dimethylformamide
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
EC	Electrical Conductivity
Eh	Redox Potential
EPA	U.S.A Environmental Protection Agency
FAAS	Flame Atomic Absorption spectrophotometry
FAO	Food and Agriculture Organization of the United Nations.
FC	Field Capacity
GC-MS	Gas Chromatography-Mass Spectrometry
GIS	Geographic Information System
GPS	Global Positioning Systems
HAC	Acid Hydrolysis
HMF	Hydroxymethylfurfural
HPLC	High-Performance Liquid Chromatography
HYPRES	HYdraulic PRoperties of European Soils.
INT	Iodonitrotetrazolium Chloride
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
Igeo	Geoaccumulation Index
IRGA	Infrared Gas Analysis
ISO	International Organization for Standardization
LAI	Leaf Area Index
LUCAS	Land Use/Cover Area frame Survey
MAOM	Mineral -Associated Organic Matter
MBC	Microbial Biomass Carbon
MDA	Malondialdehyde
NDVI	Normalized Difference Vegetation Index
NREL	National Renewable Energy Laboratory.
NTT	Next Technology Tecnotessile
OM	Organic Matter
PAH	Polycyclic Aromatic Hydrocarbons
pH	Potential of Hydrogen

PLFA	Phospholipid Fatty Acid Analysis
POM	Particulate Organic Matter
POD	Peroxidase
PRI	Photochemical Reflectance Index
PWP	Permanent Wilting Point
ROS	Reactive Oxygen Species
SA	Salicylic Acid
SIC	Soil Inorganic Carbon
SOC	Soil Organic Carbon
SOD	Superoxide Dismutase
SOM	Soil Organic Matter
SPAD	Soil Plant Analysis Development
TC	Total Carbon
TE	Trace Elements
TF	Translocation Factor
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
TPH	Total Petroleum Hydrocarbons
UAV	Unmanned Aerial Vehicle
UBU	University of Burgos
UMLP	Université Marie & Louis Pasteur
UNI	Ente Nazionale Italiano di Unificazione
UniOvi	University of Oviedo
USLE	Universal Soil Loss Equation
VOC	Volatile Organic Compounds
WHC	Water Holding Capacity
WP	Work Package

EXECUTIVE SUMMARY

Deliverable D1.1 presents the requirements for the monitoring and characterisation of key environmental, soil, and plant indicators for the pHYBi project. This deliverable is designed to provide a structured data collection and analysis approach across the project's multiple study sites, enabling the assessment of phytomanagement impacts on soil health, plant growth, and biomass quality. The project adopts a holistic approach that includes physicochemical and microbiological parameters by focusing on site attributes, soil properties, and plant/biomass characteristics.

The data collected will enable the assessment of pollutant fate, particularly the movement of trace elements and organic pollutants (i.e.: TPH, PAH) through the soil-plant system, and their effects on the plant, via biochemical stress indicators, such as ROS, MDA, and antioxidants. Key to the project's success is the integration of remote sensing technologies (e.g., UAV drones), offering significant advantages such as large-scale, non-destructive, and continuous real-time monitoring of plant health, biomass yield and environmental conditions.

In line with European regulations, pHYBi will employ a collaborative approach to standardise data collection protocols, ensuring that monitoring techniques are tailored to the technological capabilities and resource limitations of the project's partners. The overall aim is to generate a comprehensive dataset that supports sustainable soil restoration and feedstock production. The monitoring efforts will contribute to biomass valorisation in sectors like the textile industry while considering the impact of pollutants on feedstock quality. Additionally, the project will assess soil health indicators related to chemical, ecotoxicological, and physical stress factors, improving our understanding of contaminant transfers and soil health improvement.

1. INTRODUCTION

1.2 DESCRIPTION OF THE DOCUMENT AND PURSUE

Monitoring and understanding the health of soil and plant systems are fundamental to the success of pHYBi. The project's approach is designed to assess and optimise phytomanagement strategies for soil restoration and biomass production, with a focus on mitigating environmental stressors such as high contamination and salinity. To achieve this, we propose a comprehensive set of indicators to measure key factors at different stages of the project. This approach allows us to establish baselines, evaluate the effectiveness of phytomanagement interventions, and track long-term changes, capturing both spatial and temporal variability.

Soil health is a central concern, as it determines the capacity of land to support sustainable plant growth and provide ecosystem services. The project draws on a wide range of indicators, focusing on the soil's physical, chemical, and biological properties to assess its health under various stress conditions (i.e., salinity, and the presence of metals and/or organic pollutants, which may occur individually or together). These indicators will help identify baseline conditions and track changes in soil functions over time, enabling the identification of effective interventions for improving soil quality. Equally important is monitoring plant growth and biomass yield, which will be influenced by the soil's health and ability to remediate pollutants. Understanding how plants respond to the abovementioned environmental stressors and land use changes provides essential insights into the success of the proposed phytomanagement strategies. Remote sensing technologies, such as Unmanned Aerial Vehicles (UAV drones), will be utilised in some of the sites, to monitor plant growth, health, and biomass yield, offering advantages in large-scale, continuous, and non-destructive monitoring. Finally, we will investigate the dynamics of microbial populations, which play a key role in soil health and pollutant degradation. The project will monitor microbial diversity and functionality over time to better understand their contribution to soil remediation. Microbial indicators, alongside plant and soil health measures, will allow for a holistic assessment of the phytomanagement process, ensuring that all environmental compartments are considered.

The wide range of indicators herein proposed is grouped into key categories presented in different sections of this document: site attributes, soil parameters, and plant and biomass characterisation. UBU, UMLP, and UNIOVI will lead the general assessment of the sites and conduct the analyses regarding both soil and plant/biomass indicators as case site managers. CETIM will be responsible for performing the analysis to define biomass quality indicators, in collaboration with NTT (Supplemental Information 1). These indicators were selected based on scientific expertise and stakeholder input, ensuring protocols are harmonised yet adaptable and tailored to the specific project needs and partner capabilities. They are designed to assess various aspects of the phytomanagement process, from soil structure and contamination to plant resilience and the quality of biomass for industrial applications, such as lignocellulosic fractionation. By comparing the resulting data before and after phytomanagement intervention, we can evaluate its effectiveness and ensure that the environmental risks associated with pollution and salinity are minimised.

It should be noted that the monitoring strategies may be influenced by three key constraints: time, cost, and site-specific factors. The nature of the analyses themselves will limit the frequency and number of samples that can be analysed, particularly for time-consuming and high-cost indicators. Frequency and sample size will also depend on whether certain parameters change rapidly enough to warrant frequent sampling. These constraints will be further

influenced by the history of the sites—some were selected for their long-term phytoremediation history, while others are newly established, and effects will manifest at different rates. Indicators will be selected or tailored based on the capacities of partners, with basic coverage and a minimum consortium effort implemented across all sites. This will include **ten core soil indicators** that will be analysed at each site (see Supplemental Information 1). Additional indicators will be considered based on available resources, ensuring that the scope remains within feasible limits. These decisions will be made in coordination with WP2 and the plant introduction phase of phytomanagement strategy validation.

Sampling sheets for soil and plant (Supplementary Information S2 and S3) will be used to systematically record immediate information about the samples, including their identification, collection conditions, and methodologies. This approach ensures accurate data capture, facilitates the replication of sampling procedures, and helps interpret observations that may explain the results, ultimately enhancing the reliability of the research findings.

By integrating these monitoring approaches, pHYBi aims to optimise phytomanagement strategies, enhance soil restoration, soil-based ecosystem services and improve feedstock quality, all while adhering to European environmental regulations. The indicators will inform how to align the production systems with both bioremediation and industrial applications, specifically monitoring the development of sustainable lignocellulosic feedstock using bio-formulations. This will ultimately support pHYBi's proposed value chain for creating dyes and coatings for application on textile-based and fibrous substrates.

1.3 WPS AND TASKS RELATED WITH THE DELIVERABLE

This deliverable refers to Task 1.1 (Definition of monitoring requirements and quality indicators [M1-M6]) included in WP1: Site investigation and feedstock characterisation.

2. SITE ATTRIBUTES

Understanding the environmental and physical attributes of the sites where phytomanagement strategies are applied is essential for creating effective restoration plans. Site attributes encompass a broad range of factors, such as climate conditions, topography and land use history, all of which significantly influence the success of phytoremediation. Moreover, the interactions among these factors, as well as between vegetation and landforms, can further impact restoration outcomes. Therefore, successful strategies in environmental management must account for spatial and temporal variability in these attributes.

Topographic features such as slope, aspect, and landforms influence solar radiation, water retention, and nutrient availability, all of which control tree growth and productivity (Korkalainen & Laurén, 2006). For instance, in forests, productivity tends to increase downhill due to better water and nutrient access, although microtopographic depressions may experience frequent frosts that reduce productivity (Marquis et al., 2021). Variability in soil properties, such as organic carbon content, is also shaped by these topographic features (Jendoubi et al., 2019), impacting land use and land management decisions. Furthermore, the temporal and spatial distribution of soil characteristics, such as soil thickness and moisture, is influenced by both natural factors (e.g., climate) and human activities (e.g., land use changes) (Y. Zhang et al., 2024). These factors can drive significant ecological consequences, influencing species composition, function and dynamics, recovery rates, and ecosystem resilience (Jakovac et al., 2021; Muscarella et al., 2020).

The pHYBi project is designed to collect and analyse these site-specific characteristics to determine the most appropriate phytomanagement strategies. Establishing a baseline will allow us to assess the initial conditions of the sites before any interventions are made. By aligning these data with European guidelines and stakeholder input, the project ensures that the interventions are scientifically sound and adaptable to varying site conditions. This section introduces the key site attributes that will be monitored across all participating sites, ensuring that interventions are tailored to each unique location.

To collect real-time data on site characteristics, pHYBi aims to integrate innovative monitoring techniques, including remote sensing, UAV and the Veris® sensor platform for mapping apparent electrical conductivity (EC_{app}) in high salinity fields. For some indicators, the data will be gathered at multiple points during the project, with initial surveys performed to create a baseline and periodic updates throughout the duration of the project to assess any changes. The data collected will guide the optimisation of phytomanagement strategies, ultimately contributing to long-term environmental restoration. The evaluation of site attributes is crucial to the success of this project, as these factors provide a fundamental understanding of the environmental context in which interventions will place.

2.1 ENVIRONMENTAL CHARACTERISTICS

In the context of the pHYBi project, assessing site characteristics is essential for establishing baseline conditions and informing the development of the proposed phytomanagement strategies. These site attributes are vital for determining the suitability of different locations for phytoremediation and understanding how environmental variables interact with soil and plant health.

Geospatial and Temporal Data are essential for accurately locating and tracking the experimental sites over time. **Longitude, latitude**, and **elevation** provide fundamental geographical information that ensures precise site characterisation. These variables are measured using Global Positioning Systems (GPS) or geospatial tools, with elevation data collected from either GPS or Digital Elevation Models (DEM). Sampling for these parameters is done once at the beginning of the project. Additionally, when monitoring at different intervals, both the **date** and **season** will be recorded to track landscape changes over time and provide temporal context (i.e., seasonality), while capturing any environmental changes that may influence sampling and the project's outcomes.

Climatic variables such as **temperature, humidity, precipitation, wind speed**, and solar radiation significantly impact plant growth, soil conditions, and pollutant dynamics (Certini & Scalenghe, 2023; Gomes et al., 2021; Hamidov et al., 2018; Kumar et al., 2020; Večeřová et al., 2022). While these parameters won't be directly measured during the monitoring campaigns, we will rely on weather station data, weather tools (e.g., Atlas, meteorological Application Programme Interfaces APIs), or climatic databases to collect this information (e.g.: the NASA Global Climate Data Platform https://power.larc.nasa.gov/data-access-viewer/, the meteorological data platform https://www.historique-meteo.net/). If possible, we will use field tools such as thermometers, hygrometers, or pyranometers. Seasonal averages from the monitoring periods will help track fluctuations that could affect our results and the phytoremediation process. Variations in climatic conditions across seasons influence plant metabolism, growth, and soil microbial activity, ultimately impacting the plants' ability to absorb or immobilise contaminants (Pandey et al., 2022). This data is essential for interpreting results and refining our understanding of how environmental factors interact with soil health and biomass production.

Topographic Variables, including **slope steepness, slope direction**, and **drainage**, are critical in understanding water flow, erosion risk, and the overall suitability of the site for phytoremediation (Çellek, 2022; Jiang et al., 2014). These attributes measured using DEM and specific Geographic Information Systems (GIS) tools, GPS, and field observations, will be recorded once at the beginning of the project and provide context for the long-term monitoring of soil and plant systems. This information is particularly important for understanding potential water stress, nutrient movement, and contamination dispersion on the site.

Vegetation and Ecosystem Structure provide essential information about the existing plant communities, their health, and their potential for supporting phytoremediation. **Vegetation coverage, species diversity**, and **canopy structure** will be assessed using UAV remote sensing mounting multispectral cameras, offering a non-destructive and efficient way to monitor changes in plant growth and ecosystem composition over time. Sampling for these indicators will occur three to four times throughout the project, helping to track how vegetation establishes and adapts to the landscape, as well as its role in soil restoration. Initially, around M7 to M9, an initial assessment of the field conditions will be carried out before any new phytomanagement strategy is implemented. The second sampling will coincide with WP2 progress, specifically around the time of the field pilot studies, between M21-M23. Finally, a third sampling will be conducted towards the end of the project, around M35-M37, for a final assessment. In all cases, the sampling will take place during late spring to early fall to optimize the monitoring of plant and soil activities, including microbial processes. Additional sampling opportunities may also be defined depending on site-specific conditions, project progress, and historical data. Key metrics like the **Leaf Area Index (LAI)** and **chlorophyll estimation** by Soil Plant Analysis Development (SPAD) measurement or Normalized Difference Vegetation Index (NDVI) will be used to gauge plant health and resilience, which are critical for understanding

how plants respond to environmental stressors and pollutants. This data will also inform biomass yield and the suitability of the site for biomass-related industrial applications.

Table 1. Overview of Geospatial, Climatic, Topographic, and Vegetation Indicators.

Category	Indicator	Units	Methodology/ Source	Frequency	Notes/ Remarks
Geospatial and Temporal Data	Longitude	Degrees (°)	GPS or Geospatial Tool	Single time	
	Latitude	Degrees (°)	GPS or Geospatial Tool	Single time	
	Coordinate reference system	CRS / EPSG code	GPS or Geospatial Tool	Single time	
	Stand ID/ block ID	N/A	Field design	Single time	
	Stand / plantation area	Hectares (ha) or squared meters (m^2)	GIS / management record	Single time	Include area covered during sampling or harvest
	Block area	Hectares (ha) or squared meters (m^2)	Field design	Single time	
	Date	N/A	Date of sampling	Three times	
	Season	N/A	Seasonal classification based on date	Three times	
Climatic Variables	Temperature	°C	Thermometer, Weather Station	Three times	Seasonal average
	Humidity	% Relative Humidity	Hygrometer, Atlas, Weather Tool	Three times	Seasonal average
	Precipitation	mm	Weather Station, Atlas, Rain gauge	Three times	Seasonal average
	Wind speed	m/s or km/h	Atlas, Weather Station	Three times	Seasonal average
	Solar radiation	kWh/m²	Pyranometer; Meteo API, Databases	Three times	Seasonal Average
	Elevation	meters (m)	GPS or DEM, Atlas	Single time	
Topographic Variables	Slope steepness	Degrees (°)	DEM, GPS, or Slope Analysis Tool	Single time	
	Slope direction	Degrees (°)	GPS, DEM, or Field Measurement	Single time	
	Orientation of slope	N/A	Field Observation, DEM	Single time	

	Drainage	N/A	Field Observation, Hydrological Analysis	Single time	
Vegetation and Ecosystem Structure	Species diversity and richness	Species Count - Biodiversity Index	Field Survey	Three times	Attention to invasive species
	Site Layout	Image 2D	UAV imagery, Satellite Imagery	Three time	
	Vegetation coverage (%)	%	UAV Remote Sensing	Three times	
	Leaf Area Index	m^2/m^2	UAV Remote Sensing	Three times	
	Tree density	Trees/ha	Survey, UAV Remote Sensing	Three times	
	Crop/ Trees average age	Years	Stand history, Field Survey	Three times	
	Canopy	N/A	Field Observation, UAV Remote Sensing	Three times	
	Chlorophyll estimation	Unitless or SPAD	SPAD Meter, UAV	Three times	Photochemical Reflectance Index (PRI), Normalized Difference Vegetation Index (NDVI)

2.2 SITE MANAGEMENT AND LAND ECOLOGY

The pHYBi project will focus on developing phytomanagement strategies across a diverse range of sites, each presenting unique challenges and opportunities for ecological restoration. These sites include: (1) former agricultural cropland irrigated with raw domestic wastewater; (2) a post-industrial brownfield from an abandoned fertilizer plant; (3) land polluted with hydrocarbons due to an oil spill; (4) a site formerly used for petroleum; and (5) a salt-affected mining area. In this context, site management and land ecology indicators are crucial for understanding the historical and current influences on land use, which ultimately affect both soil health and vegetation performance.

We propose indicators that will help assess how previous land use activities have shaped the site's ecological conditions and its capacity to support phytomanagement strategies. By examining the **history of the sites in terms of the type, intensity, frequency and duration of land amendments, irrigation practices, and any natural or artificial barriers**, we can better understand the challenges posed by the landscape's current state. Additionally, **the spatial extent** of these factors will be considered to assess their broader impact on soil restoration efforts. This comprehensive approach will provide valuable insights into how these past and ongoing practices influence the success of phytomanagement interventions.

The approach to monitoring these indicators includes both historical data sources and on-site observations, complemented by advanced geospatial technologies such as GIS, remote sensing, and field surveys. For instance, land use history, irrigation practices, and the presence of natural and artificial barriers will be recorded using GIS-based tools, remote sensing imagery, and field surveys to provide a detailed, multi-dimensional understanding of the site's characteristics. Furthermore, the landscape ecology indicators—such as **habitat connectivity, fragmentation, and surrounding land use**—will be assessed through GIS analysis and field mapping. This will offer insight into how the surrounding environment might influence soil and vegetation restoration efforts. By monitoring these factors, the pHYBi project will ensure that interventions are designed to optimize site-specific conditions, promote habitat connectivity, and support sustainable ecological management over time.

Monitoring and data collection is planned to occur at strategic intervals throughout the project, such as at the beginning and end, to capture both temporal and spatial changes on the sites and in their surroundings, influenced by both human intervention and natural factors.

Table 2. Land Management, Disturbance Factors, and Landscape Ecology Indicators.

Category	Indicator	Methodology/ Source	Frequency	Notes/ Remarks
Land Management and Disturbance Factors	Land use history	Historical records, field surveys, land use datasets (e.g., national/regional)	Single time	e.g.: mining, agriculture, forestry, waste disposal, city park
	Amendments' history	Field surveys, farm records, environmental management records	Single time	e.g.: mulching, compost, biochar, bioaugmentation
	Remediation practices implemented	Environmental management records, site reports, project reports	Single time	e.g.: phytoremediation, bioremediation, soil washing, excavation, chemical stabilization
	Irrigation practices	Field surveys, agricultural practice records	Single time	e.g.: record water quality
	Natural barriers	GIS-based, remote sensing, field observations	Single time	e.g.: rock outcrop, soil hardpan, watercourses
	Artificial barriers	GIS, road infrastructure data, remote sensing, field mapping	Single time	e.g.: subsurface pipes, concrete foundations, manholes, ditches, roads, fences.
	Perturbations	Field surveys, remote sensing (e.g., deforestation, construction activities)	Single time	e.g.: tillage, ploughing, subsoiling, cover crop
Landscape Ecology	Connectivity	GIS-based analysis of habitat corridors, remote sensing data, field mapping	Two times	Presence and quality of corridors (riparian, wildlife, greenbelt, urban parks, etc.).

	Fragmentation	Patch size and shape metrics, edge-to-area ratio, patch density, fragmentation index, GIS-based analysis	Two times	Degree of forest and habitat loss due to urban areas, roads, fences, agriculture. Structure and distribution of patches.
	Land use	GIS data (land use/land cover maps), remote sensing, regional/national datasets	Two times	Surrounding habitat availability. Dominant land cover type: deforestation, restoration, industry, agriculture, urban development, settlements, mining, wastewater treatment, landfill, etc.

3. SOIL PARAMETERS

Soil health is a key indicator of ecosystem functioning, directly influencing plant growth, pollutant degradation, and overall productivity. In phytomanagement, soil parameters—such as physical structure, chemical composition, and biological activity—offer insights into the soil's capacity to support plant growth and degrade contaminants (Raupp et al., 2024; Xing et al., 2025). The indicators proposed here encompass a range of physical, chemical, and biological factors, considering not only soil quality but also the impact of stressors like pollution, salinity, and land degradation. These indicators are essential for understanding pollutant transfer within the soil-microbe-plant system (N. Zhang et al., 2021), designing effective interventions, and enhancing soil carbon sink properties.

This section outlines the selection of soil parameters and methodologies for assessing soil health under various environmental stressors. Modern soil monitoring approaches integrate chemical, ecotoxicological, and biological indicators to evaluate soil resilience and functionality. Soil health indicators will be measured at key stages of the project, starting with baseline assessments to establish pre-intervention conditions. Regular monitoring will then track changes in soil quality, providing essential data to adjust phytomanagement strategies and assess the success of interventions over time.

3.1 SOIL SAMPLING PROTOCOL

Soil sampling is a fundamental step for accurately characterising the soil properties of each case study area. The goal is to collect samples that reflect the true conditions of the soil, providing reliable data for environmental monitoring, crop management, and further scientific research. This can only be achieved through a standardised procedure that minimises bias and ensures reproducibility.

1. Sampling Locations and Depths

For precise soil characterization, composite samples will be collected using the LUCAS methodology (Fernández-Ugalde et al., 2017) from multiple locations within each plot. The

initial step involves identifying and defining sampling plots, if applicable. Within these plots, central LUCAS sampling points will be randomly selected by generating random coordinates within the plot boundaries or using random number generators. As an alternative, systematic sampling techniques, such as a zigzag pattern or a W-shaped traverse, may be employed to capture spatial variability across the plot. Once the central point is established, four additional core sampling points will be placed 2 meters to the north, east, south, and west of the central point. If it is impossible to collect the subsample in the appropriate location, a suitable location within 2m from the central point should be found (Figure 1). The number of sampling points should be determined based on plot size and site variability. Smaller or more homogeneous plots may require fewer points, while larger or more heterogeneous areas may necessitate additional points to capture the site's variability.

After removing surface debris, soil sampling should be conducted at two primary depths, whenever possible. Additional depths may be considered if specific indicators suggest the need to assess vertical distribution according to soil horizons:

- Topsoil (typically 0-20 cm)
- Subsoil (typically 20-40 cm)

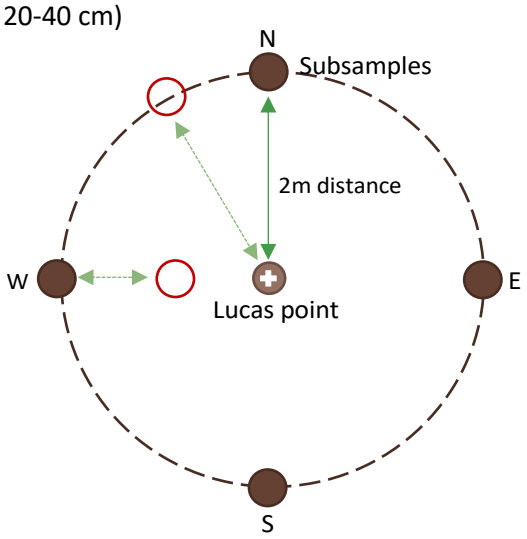


Figure 1. *Soil Sampling Scheme according to the LUCAS Design; based on Fernández-Ugalde et al. (2017).*

2. Amount of samples

At each identified LUCAS point, soil cores will be collected from both the topsoil and subsoil depths, whenever possible, considering site-specific challenges. In such cases, surveyors should adapt the sampling approach accordingly, which may involve adjusting sampling depth or technique to ensure the collection of representative soil samples while minimizing potential biases. Given the moderate heterogeneity of the sites, the goal is to collect approximately 0.5 kg of soil from each of at least 4 to 6 LUCAS sampling points. The ten individual cores (5 locations × 2 depths) from each point will be combined to create a single composite sample per point, representing the variability of the site. However, if an indicator requires analysis by depth, a separate composite sample can be created from the 5 points at each depth. Analyses will be performed on these composite samples, not on the individual cores. This range ensures both biological and technical replication, providing a good balance between accuracy, reliable representation of the site, and cost-effectiveness.

However, to monitor or compare conditions (e.g., pre- vs post-treatment), the objective is to collect enough samples to detect a true effect, such as a meaningful change in soil properties. This requires sufficient statistical power (typically set at 0.8 or 80%). The number of samples needed depends on the precision required in the data. A power analysis should be conducted to determine the minimum sample size. A preliminary characterization with a small number of samples or, if available, previous data can help inform the analysis. If such data is unavailable, initial samples should be collected, and the power analysis will determine if the sample size is adequate. If additional samples cannot be collected, particularly in long-term comparisons, insufficient sample size may compromise the ability to detect significant differences, leading to unreliable conclusions. In such cases, the analysis may need to be adjusted.

3. Sampling Methods and Tools

For soil sampling, tools such as augers, spades, trowels, and split spoon samplers are commonly used. The tool selection depends on the depth of sampling and the need for undisturbed samples:

- Near-surface soils (0-30 cm) can be sampled with spades, trowels, or shovels.
- Deeper subsoils (30-50 cm) require augers or split spoon samplers for effective soil core collection.

4. Composite Sampling

After collecting subsamples from several points within a defined area, these subsamples are mixed to form a composite sample. This ensures that the final sample is representative of the entire sampling area, accounting for heterogeneity in the soil. If the site has trees, the subsamples should be collected between the trees, ensuring consistency.

5. Handling and Sieving

Once collected, the soil samples should be carefully handled to avoid contamination. In most cases, the samples should be sieved to 2 mm to remove large debris and ensure uniformity. If sieving cannot be done immediately, it's best to air-dry the samples before sieving. All samples should be placed in labelled containers and stored appropriately until further analysis. If soil compaction or agglomeration occurs during the drying process, the sample must be disaggregated using a rubber hammer or wooden roller.

6. Sites with Trees vs. Unplanted Sites

- For unplanted sites or plots: It is recommended to follow the LUCAS protocol, as outlined earlier, by sampling at least four different points, with five - ten soil cores collected per point. If available, plot segmentation based on EC_{app} values will be used to help accurately select or define the specific sampling points within the terrain.

- For sites with trees: To ensure standardized soil sampling within tree rows, a systematic approach combining elements of transect sampling and the LUCAS methodology should be applied. Sampling will be conducted between the rows of trees, following a linear pattern with the LUCAS point as the central reference (Figure 2). Subsamples will be collected equidistantly from the trees and mixed to form a composite sample. If the LUCAS point falls within a tree, the nearest point outside the tree row will be selected instead.

If sampling between rows is not feasible due to obstacles, the soil sample should be collected near the edge inside the field, as close as possible to the original LUCAS point and as far as possible from artificial surfaces. If this is not possible, the sample can be taken at the hedge of the field. Sampling near the trees will capture high levels of tree influence on the soil, while samples taken at increasing distances will reflect medium, low, or negligible influence. The distance to the closest tree should be reported. This method provides insight into the spatial variability of soil properties relative to tree proximity. At each sampling point, soil cores should be collected from both the topsoil and subsoil layers, whenever possible, to provide a comprehensive assessment of soil conditions. Any special arrangements or deviations made for soil collection should be clearly reported.

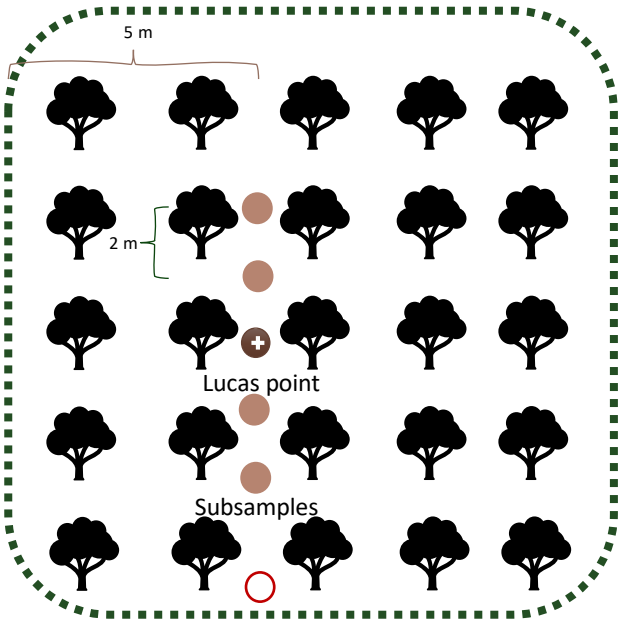


Figure 2. *Soil Sampling Scheme for Planted Sites Within Tree Rows; following Fernández-Ugalde et al. (2017).*

3.2 SOIL STRUCTURE AND PHYSICOCHEMICAL PROPERTIES

3.2.1 Total Carbon and Soil Organic Carbon (SOC) Stock

The measurement of total carbon and soil organic carbon is essential for understanding how soil health improves and its potential for carbon sequestration (Nayak et al., 2019). SOC is a critical parameter for assessing the effectiveness of phytoremediation techniques in enhancing soil fertility and stability. As plants take up CO_2 during growth, SOC accumulation reflects the success of the project in improving soil conditions and mitigating the impacts of climate change by storing carbon.

To measure SOC, soil samples are collected from multiple depths using soil augers to ensure uniformity across the site. The samples are air-dried, sieved (2mm Ø mesh), and ground to a fine powder for analysis. Total carbon content is determined following ISO 10694:1995 ISO by dry combustion using a CHNS elemental analyser or a high-temperature furnace (typically 550-900°C), which burns the organic material releasing gases which are measured to calculate TC.

To distinguish between organic and inorganic carbon, a replicate sample is treated with diluted HCl to remove inorganic carbon (carbonates), which releases CO_2. The second measurement,

after HCl treatment, provides soil organic carbon (SOC), and the difference between the two measurements gives soil inorganic carbon (SIC).

If available, a TOC analyser can be used as an alternative to traditional dry combustion methods. This instrument can simultaneously measure total carbon (TC), total inorganic carbon (TIC), and total organic carbon (TOC), which is equivalent to soil organic carbon (SOC) when TIC is subtracted.

The SOC stock is then calculated for each sample with the following formula:

$$C_t\ (t\ ha^{-1}) = SOC \times 10000 \times d \times D_a \times \left(\frac{100 - V}{100}\right)$$

In it: SOC, content of soil organic carbon in %; d, depth of soil in m; Da, apparent density of soil in kg m-3; V, volume of gravel (Ø>2 mm) in %.

For **DOC (Dissolved Organic Carbon),** which is crucial for microbial activity and plays a key role in soil health, nutrient cycling, and contaminants degradation, the soil is extracted using distilled water or a weak buffer solution. The extract is then filtered and analysed using a TOC analyser, where the organic carbon is oxidized to CO_2, which is measured by an infrared detector to determine the DOC concentration.

This process will be repeated three times during the project to track changes in SOC over time.

3.2.2 Erosion (Rate)

Erosion is a critical factor in soil degradation, particularly concerning phytomanagement as it increases the risk of non-point source pollution, thereby further threatening human safety and socioeconomic development (Tian et al., 2023). The pHYBi project seeks to prevent further soil loss and ensure the stability of the restored ecosystems. Monitoring erosion provides valuable data on the impact of the restoration process, especially in relation to vegetation coverage, which plays a key role in minimising soil erosion by stabilising the soil surface. For such efforts, we will document, if encountered within a 500-meter radius of the LUCAS points, the types of erosion, such as sheet, rill, gully, mass movement, wind erosion, and areas where soil has been redeposited. Understanding these aspects enables targeted interventions to mitigate soil loss and enhance land productivity.

While erosion is a natural process affecting all land, it can lead to the loss of topsoil, severely impacting crop yields, degrading water quality, damaging drainage systems, and releasing greenhouse gases. Water and wind erosion account for approximately 84% of all degraded land globally (Gholami et al., 2024). In cases where erosion is evident, photographs of the affected area will be taken, along with information regarding the distance and direction (north, east, south, or west) relative to the LUCAS point.

The erosion rate will be estimated using the Universal Soil Loss Equation (USLE), a well-established model that predicts soil erosion based on factors such as rainfall, soil type, slope, vegetation cover, and management practices. Data for these factors will be gathered using Soil Erosion Calculator Tools and relevant databases. If necessary, slope angles and steepness can be measured using UAV technology and GIS tools, or alternatively with a clinometer or topographic total station, as needed. Soil samples will be collected to assess texture, organic matter content, and bulk density—all key parameters for the USLE. The collected data will be

integrated into the USLE formula to estimate the expected annual soil loss (tons per hectare per year). This assessment will be conducted once the project has started to establish a baseline before vegetation restoration.

3.2.3 Herbaceous Layer Presence and Composition

The herbaceous layer is vital for soil stability and biodiversity. It helps control erosion, enriches the soil through organic matter inputs, and supports microbial communities (Kervroëdan et al., 2021; Kong et al., 2024; Stefanowicz et al., 2022). Monitoring its presence, establishment, and composition helps assess the success of phytomanagement interventions in terms of vegetation establishment and ecosystem recovery.

Within the framework of this project, it will be surveyed through a combination of field observation and remote sensing. Field surveys will measure plant cover in representative plots, using a Nested Quadrat Design, where smaller quadrats (e.g., 1m²) are placed within larger plots (e.g., 5 or 10 m²), with sizes adjusted depending on the site. This method captures variability at different spatial scales, providing detailed data from smaller quadrats and broader patterns from larger plots. By using both small-scale and large-scale sampling, we can better understand plant community structure across various spatial levels.

Within these quadrats, all herbaceous plants are recorded and taxonomically identified in the field using a plant database or field guide. Species that cannot be reliably identified on-site will have voucher specimens collected for further analysis. Herbaceous cover will be visually estimated as a percentage of the total area. To assess diversity at the quadrat level, species richness and diversity indices (e.g., the Shannon-Wiener index) will be calculated. In addition, UAV imagery (drone-based) will be utilised to capture high-resolution images of the site to estimate vegetation cover over larger areas. These methods will be repeated three times throughout the project to monitor temporal changes in plant establishment, covering different growth phases and environmental conditions.

3.2.4 Soil Map (3D GIS-based)

A 3D soil map is essential for understanding the spatial distribution of soil properties across the site. By mapping variations in texture, bulk density, organic content, and other key soil characteristics, the project can better tailor phytomanagement strategies to local conditions, enhancing the restoration process. This mapping technique provides a detailed and dynamic representation of soil variability and is crucial for monitoring soil recovery.

To create a 3D soil map, the project will integrate data from field sampling, remote sensing, and GIS (Geographical Information System) analysis. Soil samples will be taken at regular intervals across the site to measure key properties like bulk density, texture, and moisture content. These data will be supplemented with UAV imagery to assess surface properties and create high-resolution elevation models. A digital elevation model (DEM) will be constructed from the UAV data and combined with the soil property measurements using GIS software. The 3D map will be generated to visualise the spatial distribution of soil properties, allowing for the identification of areas needing specific phytoremediation interventions. This mapping process will occur once at the beginning of the project to establish a baseline and may be updated if new site information is collected.

3.2.5 Bulk Density

Bulk density is a critical indicator of soil health, with higher bulk density values typically resulting from compaction. This compaction reduces pore space and increases mechanical resistance to root growth, impeding root penetration and limiting the soil's capacity to support plant development. Such compaction also disrupts water infiltration and drainage, as compacted soils with reduced large pore spaces hinder water movement, leading to poor water retention and increased runoff. Furthermore, elevated bulk density can restrict microbial biomass and diversity, as the reduction in pore space limits oxygen availability and alters moisture conditions (Frene et al., 2024). Monitoring this indicator is essential for evaluating the soil's physical recovery and its suitability for plant growth, particularly in areas that have been disturbed or degraded.

The determination will be based on the ISO 11272:2017 methodology, which proposes three sampling methods. The Core Method is typically used for undisturbed soil samples, where a soil auger or cylindrical core sampler (with a known volume) is used. The Excavation Method is often used for soils with significant coarse fragments or gravel, where core sampling may not be feasible. The Clod Method is suitable for shallow, fragile soils or areas where core sampling is impractical. The samples are weighed immediately after collection and then dried in an oven at 105°C until a constant weight is achieved. Bulk density (g/cm³) is calculated by dividing the dry weight of the sample by the volume of the core. Samples will be taken from different soil depths (e.g., 0-20 cm, 20-40 cm, 40-60 cm) to capture vertical variations in soil compaction. This will help assess the feasibility for root development, water infiltration, microbial habitat, and soil aeration. This procedure will be conducted at least once during the project to establish a baseline for soil suitability for plant growth, and again towards the end of the project to assess any changes influenced by tree planting.

3.2.6 Particle Density

Particle density is essential for understanding the composition and texture of soil. It helps assess the degree of soil mineralization and is a key factor in calculating soil porosity and understanding soil structure. This indicator is important as it influences soil compaction, porosity, and water retention. Higher particle densities often indicate soils rich in heavy minerals like magnetite or hematite. In contrast, elevated organic matter content generally leads to lower particle density, enhancing root development and water movement. Soils with lower particle densities tend to have higher porosity, facilitating better water infiltration and root growth. Additionally, analysing particle density provides insights into the soil's structural characteristics, such as its ability to form aggregates and overall stability (Marakkala Manage et al., 2019; Rabot et al., 2018).

Measurement is performed using the pycnometer method (ISO 11508:2017). Soil samples are first air-dried and sieved to remove large debris. The sample is then weighed and placed in a pycnometer, which is initially filled with a known volume of liquid (usually distilled water or kerosene) of known density, up to about half the volume. The pycnometer is then subjected to a vacuum to remove air from the soil, until no further air escapes, and is then filled completely with the same liquid. If water is used, it must be de-aerated by boiling and cooling. The change in volume of the liquid is used to calculate the particle density using the formula:

$$\rho = \frac{m_s}{V}$$

where ρ is the particle density, m_s is the dry mass of the soil, and V is the volume of the displaced liquid.

3.2.7 Porosity

Soil porosity indicates the proportion of the soil volume that is not occupied by solid material, which directly affects air and water movement through the soil (Nimmo, 2004). In phytoremediation, porosity is crucial for soil health and plant root penetration. High porosity enhances water infiltration, root development, and provides ample space for microbial habitats, thereby supporting effective contaminant degradation (Lucas et al., 2023).

Porosity is derived from bulk density and particle density using the formula:

$$\textbf{Porosity (\%)} = \frac{(\text{Particle Density} - \text{Bulk Density})}{\text{Particle Density}}\ x\ 100$$

Soil samples are collected using a core sampler, and both bulk density and particle density are measured. Once these two parameters are determined, the soil porosity can be calculated.

3.2.8 Soil Type/Texture

Soil texture, determined by the proportions of fine, medium, and coarse particles, significantly influences various soil functions, including nutrient availability, root system development, aeration, water retention, and microbial communities. The particle sizes in the soil determine whether it is classified as clay, loam, or sandy, and these properties dictate soil functions, ultimately affecting plant growth and health (Adeniji et al., 2024; Yim et al., 2022).

Table 3. Influence of Soil Texture on Soil Functions.

Soil Type	Nutrient Availability	Root System Development	Aeration	Water Retention	Microbial Communities	Soil Reactivity (Acidity & Buffering)
Clay	High nutrient retention due to fine particles' large surface area.	Shallow root systems due to dense structure and limited aeration.	Limited aeration can restrict root growth and oxygen availability.	High water retention but poor drainage; prone to waterlogging.	Supports stable microbial communities, including beneficial microbes.	High surface area, leading to higher CEC and buffering capacity; may lead to more acidic soils.
Loam	Balanced nutrient availability.	Deep and extensive root systems facilitated by optimal soil structure.	Good aeration supports healthy root development.	Balanced water retention and drainage; ideal for most plants.	Supports a wide range of beneficial microbes, promoting soil health.	Moderate CEC and buffering; pH tends to be near neutral.

Sand	Low nutrient retention; requires frequent fertilization.	Deep root systems due to good aeration but limited nutrient and water retention.	Excellent aeration but may lack moisture for root sustenance.	Low water retention; requires regular watering.	Supports drought-tolerant microbial species but may lack diversity.	Low surface area, leading to lower CEC and buffering capacity; more prone to nutrient leaching and pH fluctuations.

Soil texture is assessed through particle size distribution using sieving and sedimentation with the hydrometer method (ISO 11277:2020). The results are classified based on the European Soil Map (HYPRES) texture triangle (Figure 3). This analysis will be conducted once at the project's start, as soil texture is a permanent characteristic.

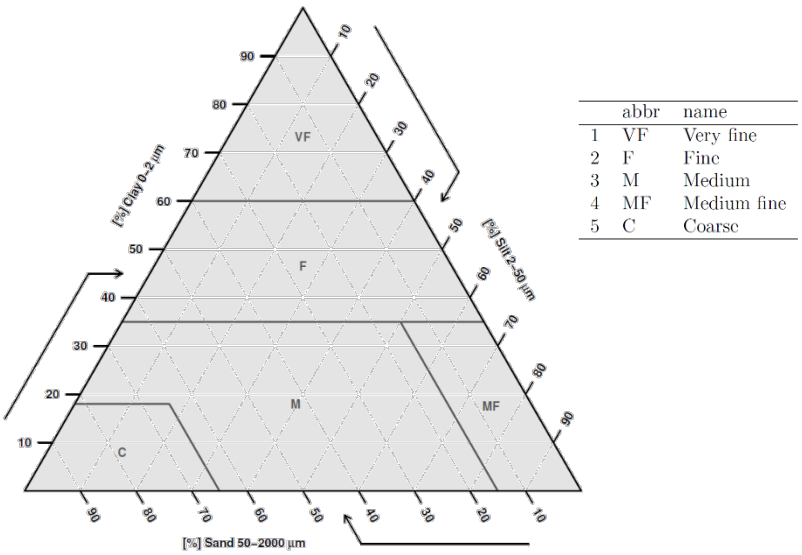


Figure 3. *European Soil Map (HYPRES) texture triangle. Reprinted from Moeys (2024).*

3.2.9 Electrical Conductivity (EC)

Electrical conductivity (EC) is a crucial indicator of soil salinity (Table 4), reflecting the concentration of soluble salts within the soil. These salts significantly influence plant growth and overall soil health. EC consists of two primary components: the soil solution (the liquid portion containing dissolved ions) and the solid phase, which includes soil particles, organic matter, and minerals, primarily associated with exchangeable cations that contribute to the ionic content in the solution (Heiniger et al., 2003).

EC generally increases with higher clay content due to its high surface area, as well as with higher porosity and moisture content, as these factors enhance the soil's ability to conduct electrical currents (Klein & Santamarina, 2003). Elevated EC levels, which are often associated with high soil salinity, lead to osmotic stress, impairing water and nutrient uptake in plants (Rengasamy, 2010). This stress also reduces microbial biomass—especially fungi—disrupting their ability to perform essential functions like substrate diffusion and affecting their structure and activity (Chowdhury et al., 2011). In contrast lower EC is typically linked to low organic matter content

in the soil, which can negatively affect soil structure and water retention. Reduced organic matter can decrease the soil's ability to retain nutrients and water, leading to poor infiltration rates (Aboukarima et al., 2018; Franzluebbers, 2002). This, in turn, can create unfavourable conditions for plant growth, often resulting in stress symptoms such as stunted growth, chlorosis, and poor overall health.

Table 4. Types of soil salinity based on EC. Adapted from FAO (2021)

ECe (dS/m)	Salinity Intensity	Effect on Crop Growth
< 0.75	None	None
0.75 - 2	Slight	None
2 - 4	Moderate	Yields of sensitive crops may be restricted
4 - 8	Strong	Yields of many crops are limited
8 - 15	Very strong	Only tolerant crops yield satisfactorily
> 15	Extreme	Only a few very tolerant crops yield satisfactorily

Regular monitoring of EC helps assess the soil's nutrient status, ensuring that plants receive the necessary nutrients for optimal growth. This indicator is crucial for identifying areas where amendments may be needed to reduce salinity. Electrical conductivity is measured using a conductivity meter, following ISO 11265:1994. Soil samples are mixed with distilled water at a 1:5 ratio (m/V), allowed to stand for 30 minutes, then filtered through a fine filter paper (e.g., Whatman), and the EC of the filtered solution is measured. Additionally, for Case Study (CS) 5, an EC-salinity map will be created to visualise both the level and spatial distribution of salinity across the site.

3.2.10 Cation Exchange Capacity (CEC)

Soil nutrient supply depends on its ability to buffer nutrient loss. Mineralization of soil organic matter provides limited supplies of nitrogen (N), sulfur (S), and micronutrients, while mineral dissolution and surface exchange reactions replenish phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and other micronutrients (Havlin, 2020). Cation exchange capacity (CEC) is a key indicator of a soil's ability to retain and exchange essential cations, i.e., positively charged ions like calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), and potassium (K^{+}), which are vital for plant nutrition. High CEC soils, typically associated with alkaline pH, are often rich in clay or organic matter, which adsorb and retain these cations through electrostatic forces, thereby reducing nutrient leaching. Consequently, high CEC soils tend to be more fertile. In contrast, soils with low CEC, usually found under acidic conditions where cations are replaced by H^{+} ions, have a reduced ability to retain nutrients, making them more prone to leaching and requiring more frequent fertilization to sustain plant growth (Sharma et al., 2015).

CEC measurements typically involve the replacement of exchangeable cations with a saturating solution, using procedures such as the barium chloride solution (ISO 11260:2018) or the ammonium acetate method (ISO/TS 22171:2023). Soil samples are first air-dried and sieved to remove debris. For the barium chloride method, the soil is saturated with a 0.1 mol/L barium chloride ($BaCl_2$) solution, and after equilibrating with a 0.01 mol/L $BaCl_2$ solution, the soil is leached with a 1 mol/L $BaCl_2$ solution to displace exchangeable cations. For the ammonium acetate method, the soil is saturated with a molar ammonium acetate (NH_4OAc) solution buffered at pH 7. In both methods, the leachate is filtered to remove soil particles, and the concentration of exchangeable cations is measured using Inductively Coupled Plasma Optical

Emission Spectrophotometry (ICP-OES). Alternatively, flame photometry can be used for sodium (Na) and potassium (K), and flame atomic absorption spectrophotometry (FAAS) for calcium (Ca) and magnesium (Mg).

As plants can influence CEC, particularly through changes in organic matter content and soil structure, CEC will be assessed three times during the project. The first measurement will establish a baseline, followed by assessments around planting to capture any early changes before the phytomanagement strategy is fully implemented, and finally, at the end of the project to evaluate any changes in soil fertility and nutrient availability due to the proposed phytomanagement practices.

3.2.11 Water Retention and Holding Capacity

Water retention capacity is an important indicator for evaluating soil's ability to retain water and make it available to plants. This is critical for understanding how the soil will perform under different moisture conditions, especially in phytoremediation and ecological restoration projects, where plant survival and growth depend heavily on adequate water supply.

Total Water Holding Capacity (WHC) measures the total amount of water held in the soil, including both water that is available to plants and water that is not accessible. This is typically evaluated by measuring the soil's saturation point, followed by draining under controlled tension via suction to simulate natural drainage. The remaining water content is then measured at different tensions to construct the water retention curve, providing insights into the soil's overall water holding capacity. Therefore, the water retention capacity is measured in two stages (de Oliveira et al., 2015) (Figure 4):

a) **Field Capacity (FC)** measures the amount of water retained in the soil after the gravitational water has drained away, and the soil has reached a point where plants can begin to access the moisture. This is typically measured at a soil water potential of – 10 kPa in sandy soils or -33 kPa in clay soils.

b) **Permanent Wilting Point (PWP)** represents the soil's water potential at which plants can no longer extract water, typically defined at -1500 kPa. Beyond this point, water is bound too tightly in the soil for plant roots to access, and the plants begin to wilt permanently.

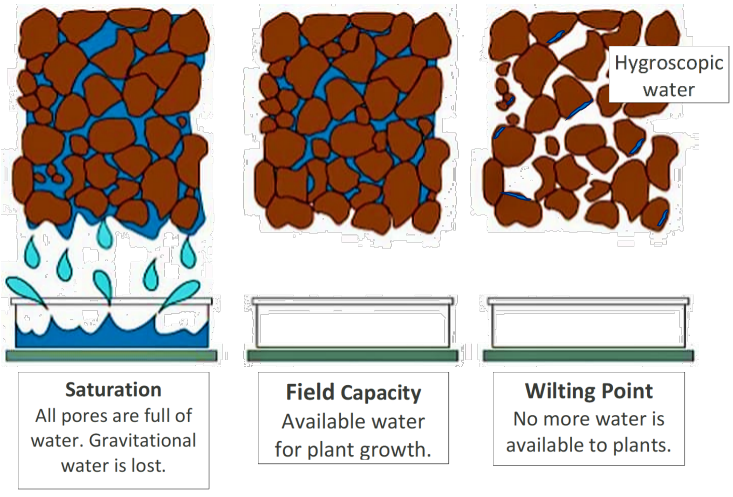


Figure 4. *Stages of water holding capacity, adapted from Futo & Bodnar (2021).*

The water available to plants is defined as the difference between field capacity (FC) and permanent wilting point (PWP). These parameters can be determined using ISO 11274:2019, which describes methods for establishing soil water retention characteristics through equilibration at defined matric potentials using suction tables or pressure plate apparatus. Water content is then measured gravimetrically at each pressure step to derive soil water retention curves and estimate plant-available water.

When laboratory pressure systems are not available, gravimetric determination of soil water content can be performed following FAO-recommended procedures based on oven-dry mass measurements (gravimetric soil water content determination).

3.2.12 Pore Water Pressure

Pore water pressure is a key indicator of a soil's ability to retain and transmit water, offering insights into the current state of soil moisture. It reflects whether water is under tension (negative pressure) or positive pressure, which influences soil structure, plant root growth, and microbial activity. Understanding this property helps assess how tightly water is held within the soil and predicts its movement. For example, negative pore water pressure can cause capillary rise, drawing water upward, while positive pressure may promote downward percolation. These dynamics are especially important in the root zone, where water availability directly impacts plant health.

High pore water pressure, commonly seen in saturated soils, can lead to waterlogging, as the soil becomes overly saturated with water. This can result in reduced soil strength, compaction, and limited oxygen availability to plant roots. In contrast, negative or low pore water pressure, typically found in unsaturated soils, indicates that water is held loosely and available for plant uptake. During drought or dry conditions, low pore water pressure reflects a lack of available water, making it difficult for plants to access moisture. Pore water pressure is also closely linked to other soil properties like texture and permeability, which influence water movement. For example, soils with high clay content often exhibit high pore water pressure under saturation, restricting water movement, while sandy soils, which retain less water, typically show lower negative pore water pressure, allowing water to move more freely through the soil.

To measure pore water pressure, a piezometer or tensiometer (following ISO 11276:1995) is used to monitor the pressure within the soil pores. These instruments measure the tension or pressure of water in the soil matrix, helping to determine the soil's capacity to retain water at various depths. Data is collected at several soil depths to capture vertical variations. This measurement is essential for assessing how soil moisture is distributed and available for plant roots.

3.2.13 Permeability

Soil permeability, or hydraulic conductivity, is a critical indicator of how easily water and air can move through the soil, directly influencing drainage, infiltration, and aquifer recharge. It quantifies the rate at which water flows through a unit area of soil under a unit hydraulic gradient, with sandy soils typically exhibiting higher permeability than clayey soils.

In phytomanagement projects, understanding soil permeability is essential as it affects plant health, the movement of nutrients, and the spread of contaminants, thereby influencing the risk of leaching. High permeability facilitates rapid water movement, while low permeability may

restrict water and nutrient availability to plants, potentially leading to waterlogging. This property is closely linked to pore water pressure; for instance, high permeability allows water to flow through the soil more easily, but under negative pore water pressure (such as during drought), water movement can still be limited due to reduced soil moisture availability.

To measure permeability, the constant head or falling head permeameter methods are commonly used (ISO 22282-2-2:2012). In these methods, a soil sample is placed in a permeameter, and water is allowed to flow through it under controlled conditions. The time required for a known volume of water to pass through the sample is recorded, and the permeability coefficient (k) is calculated based on the soil's texture, moisture content, and the observed flow rate. This measurement is essential for understanding soil structure and the potential for sustaining plant growth.

3.2.14 Organic Matter Content

Organic matter (OM) content is a key indicator of soil fertility, microbial activity, and carbon cycling. It offers valuable insights into soil health, plant growth potential, and microbial communities. OM content is estimated by measuring soil organic carbon (SOC), which is then converted to OM by a factor of 2 (since organic matter is approximately 50% carbon, 1% SOC equals 2% SOM).

OM is composed of two primary fractions: **particulate organic matter (POM)** and **mineral-associated organic matter (MAOM)**. POM (labile) consists primarily of decomposing plant and animal residues that are physically protected within soil aggregates, making it more susceptible to microbial decomposition. In contrast, MAOM (stable) is composed of organic materials bound to soil minerals, which offers greater protection against decomposition.

The relative abundance of these fractions in soil has significant implications for soil health and carbon sequestration. High POM content is associated with increased soil fertility and microbial activity, enhancing nutrient cycling and soil structure. However, because POM is more readily decomposed, soils rich in POM may be more susceptible to carbon loss under certain conditions. In contrast, soils with higher MAOM content tend to have more stable organic carbon pools, contributing to long-term carbon sequestration (Lavallee et al., 2020).

For determination, these fractions are separated using density fractionation, where POM (lighter and less dense) is separated from MAOM (denser and bound to clay and silt particles). The MAOM fraction undergoes further chemical fractionation, particularly isolating the non-hydrolysable (recalcitrant) fraction via acid hydrolysis with a strong acid like 6M HCl. This process removes labile (easily decomposed) organic matter, leaving behind more stable, resistant fractions. The carbon content of both hydrolysed (labile) and non-hydrolysed (recalcitrant) fractions is quantified using techniques like elemental combustion or carbon content analysis, providing a detailed profile of the organic matter composition.

Soil organic matter (SOM) can be further categorized into **humin, humic acids**, and **fulvic acids**, each playing distinct roles in soil health. These fractions are components of humus, the stable, decayed organic material formed over time from plant and animal residues. Humus serves as a long-term carbon store in the soil, contributing to both soil fertility and structure (Frank et al., 2023).

Humin is the most recalcitrant fraction of SOM, being largely insoluble and tightly bound to soil minerals. Due to its resistance to decomposition, humin plays a crucial role in long-term carbon

sequestration and the stabilization of soil structure. Humic acids, which are soluble in alkaline conditions, are important for nutrient retention and cycling, as they form complexes with minerals and other organic matter, enhancing soil fertility. Fulvic acids, the most soluble and reactive of the three, are involved in nutrient cycling and microbial activity. Their high reactivity facilitates nutrient uptake by plants and accelerates carbon cycling within the soil (Sherrod et al., 2019). Together, these organic fractions regulate soil structure, fertility, and carbon sequestration. Their balance within the soil is essential for maintaining soil health and supporting ecosystem functioning.

Following (ISO 19822:2018), a progressive extraction using a strong alkaline solution under anoxic conditions, followed by acidification, separates soil into these three fractions. The ash-free content of each fraction is determined gravimetrically, providing a detailed profile of the organic matter composition. To track temporal changes in OM, soil samples will be collected at various depths and analysed three times throughout the project. This will help monitor shifts in OM distribution and composition over time.

3.2.15 Potential of Hydrogen

Soil potential of Hydrogen (pH) is an important parameter for understanding soil fertility, microbial activity, and nutrient availability. It affects the solubility and mobility of minerals, salts, metals, trace elements, and other nutrients, influencing how they interact with organic matter or soil components. This, in turn, has a significant impact on plant growth. Monitoring soil pH is crucial for evaluating how phytoremediation and soil restoration techniques influence the soil's acidity or alkalinity, which can directly affect the bioavailability of contaminants and essential nutrients.

Soil pH plays a vital role in determining nutrient availability and the mobility of trace elements and metals. In acidic soils (pH < 6), the solubility of many essential nutrients, including iron, manganese, and zinc, increases, potentially leading to toxicity in plants. Conversely, in alkaline soils (pH > 7), the availability of these micronutrients decreases, causing deficiencies that can impair plant growth and productivity (Rahman et al., 2018).

The pH of the soil also significantly affects the behaviour of toxic metals and metalloids. In acidic conditions, trace elements like cadmium, lead, and arsenic become more soluble and bioavailable, increasing the risk of plant uptake. In alkaline soils, the solubility of these metals decreases, reducing their immediate bioavailability and potentially leading to leaching (Adamczyk-Szabela & Wolf, 2022). Thus, maintaining an optimal soil pH is critical not only for improving nutrient availability but also for managing the risks associated with pollutant toxicity. Proper pH management supports healthier soil ecosystems, promotes plant growth, and enhances the effectiveness of phytoremediation techniques in contaminated soils.

To measure soil pH, a soil sample is mixed with distilled water in a 1:5 (soil:water) ratio, and the pH is determined using a pH meter (ISO 10390:2021). This method involves placing the soil-water mixture in a container and measuring the electrical potential difference between two electrodes, which correlates to the soil's pH. This indicator will be measured three times during the project to monitor any changes in acidity or alkalinity resulting from the phytoremediation efforts.

3.2.16 Redox Potential

Redox potential (Eh) serves as a crucial indicator of soil oxidative and reductive conditions, significantly influencing nutrient availability, microbial activity, and contaminant dynamics. Under reducing conditions (low or negative Eh), the soil has a tendency to donate electrons, promoting reduction reactions, where substances gain electrons. This can increase the solubility of certain nutrients and trace elements, enhancing their bioavailability but potentially leading to toxicity. Conversely, in oxidizing conditions (high positive Eh), the soil has a tendency to accept electrons, which encourages oxidation reactions, where substances lose electrons. This can immobilize these substances, reducing their availability and altering microbial interactions (Mattila, 2024). Therefore, monitoring Eh is essential in phytoremediation to assess soil suitability for specific plant species and to understand microbial support mechanisms.

Integrating Eh measurements with other soil health indicators provides a comprehensive understanding of soil conditions, aiding in the development of effective soil management and remediation strategies. Soil pH and redox potential are interconnected. For example, in highly acidic soils (low pH), reducing conditions are often favoured, leading to the solubility of toxic metals like iron, manganese, and heavy metals. In alkaline soils (high pH), oxidizing conditions may predominate, causing metals like iron to precipitate and become unavailable.

To measure redox potential (ISO 11271:2022), a redox electrode (typically a platinum electrode) is inserted into the soil, and the electrical potential is measured relative to a reference electrode (often a silver/silver chloride electrode), with the resulting voltage reflecting the soil's oxidation-reduction status. This measurement is usually taken in the field, under specific moisture conditions, and can be adjusted for temperature to provide accurate readings of the soil's redox potential. This provides an indication of the soil's oxidation-reduction state. Redox potential is influenced by factors such as oxygen availability, soil moisture, and microbial activity, all of which are relevant to the success of phytoremediation efforts.

3.2.17 Buffer Index

The buffer index of soil reflects its ability to resist pH changes when acidic or alkaline substances are added. Soils with a high buffer index contain a substantial reserve of basic materials, such as calcium, magnesium, and potassium, that neutralize added acids. This reserve helps maintain a stable pH, even when organic acids are released from plant roots during nutrient uptake, supporting healthier plant growth and more efficient contaminant uptake. In contrast, soils with a low buffer index are more susceptible to pH fluctuations, which can lead to nutrient deficiencies or toxicities, such as aluminium toxicity in acidic conditions.

Buffering capacity is influenced by soil texture and organic matter content (Wei et al., 2022). Clayey soils, with their fine particles and higher cation-exchange capacity, typically have higher buffer indices, while sandy soils, with lower cation-exchange capacity, are less able to resist pH changes. Organic matter contributes to buffering by donating or accepting protons, further stabilizing pH which can fluctuate in response to plant root activity and other environmental changes.

To determine the buffer index, the soil pH (ISO 10390:2005) is first measured, followed by titration using a suitable acid or base to alter the soil's pH. The amount of acid or base required to change the pH by a fixed amount is used to calculate the buffer index, which provides an estimate of the soil's resistance to pH changes.

Table 5. Soil Structure and Physicochemical Properties Indicators.

Category	Indicator	Methodology/ Source	Recommended Frequency	Notes/ Remarks
Soil Structure	Total Carbon	ISO 10694:1995: Dry Combustion. DOC: Extraction followed by TOC analysis	Three times	
	SOC Stock	Based on formula, considering % SOC, Soil Bulk Density and Depth	Three times	
	Erosion	Documented if observed. Calculated using USLE model	Single time	USLE model: Rainfall, Soil Type, Slope, Vegetation, Management
	Herbaceous Layer Presence & Composition	Field Survey, Remote Sensing	Three times	
	Soil Map	GIS-based, UAV imagery	Single time	Depth layers showing the spatial distribution of soil properties
	Bulk Density	ISO 11272:2017: Core sampling, clod or excavation method	Two times	Different soil depths
Soil Properties (Topsoil and Subsoil)	Particle Density	ISO 11508:2017: pycnometer method	Single time	
	Porosity	Based on formula derived from Bulk Density & Particle Density	Single time	
	Soil Type/ Texture	ISO 11277:2020: hydrometer method	Single time	Classification based particle size according to European Soil Map texture triangle
	Electrical Conductivity	ISO 11265:1994: conductivity meter	Three times	Indicator of salinity. CS4 will use Veris sensor.
	Cation Exchange Capacity	ISO 11260:2018: Barium chloride *OR* ISO/TS 22171:2023: Ammonium acetate methods	Three times	

	Water Retention Capacity	ISO 11274:2019: Tension table technique	Single time	Measures water available to plants, from FC to PWP
	Water Holding Capacity	ISO 11274:2019: Tension table technique	Single time	Measures total water held in soil, including unavailable to plants
	Pore Water Pressure	ISO 11276:1995: Piezometer or tensiometer method	Single time	
	Permeability	ISO 22282-2:2012: Constant head or falling head permeameter method	Single time	
	Organic Matter Content	ISO 19822:2018: Alkaline-Acid extraction method. Density and Chemical Fractionation.	Three times	ISO is for Humin and Humic Substances. Fractionation for POM & MAOM
	pH	ISO 10390:2005: pHmeter method	Three times	
	Redox Potential	ISO 11271:2022: Redox electrode method	Three times	
	Buffer Index	Titration to alter pH	Three times	

3.3 MICROBIAL HEALTH AND TOXICITY

3.3.1 Soil Basal Respiration

Soil basal respiration is a crucial indicator of microbial activity and overall soil health. It measures the amount of carbon dioxide (CO_2) released from soil as a result of microbial respiration, which is directly linked to microbial metabolic processes and organic matter decomposition. Basal respiration is commonly used to assess the microbial activity of soils, offering insights into soil biological function, nutrient cycling, and potential responses to phytoremediation efforts.

The methodology for determining soil basal respiration follows the guidelines outlined in ISO 16072: 2002, where soil samples are incubated under controlled conditions, and the O_2 uptake or CO_2 released during microbial respiration is measured. This process provides an estimate of the soil's microbial activity, which is a key parameter when assessing how phytomanagement and phytoremediation influence soil biota.

Soil basal respiration (without substrate addition) will be measured three times throughout the project, ensuring that temporal variations in microbial activity are accurately captured. This

indicator will help monitor the soil's microbial health and the overall impact of the phytoremediation treatments on the soil ecosystem.

3.3.2 Microbial Biomass Carbon (MBC)

Microbial Biomass Carbon (MBC) is an important indicator that reflects the quantity of living microbial biomass in the soil, which is directly related to microbial health and nutrient cycling. MBC is often considered a sensitive measure of soil microbial activity and is crucial in understanding how soil microorganisms contribute to organic matter decomposition and nutrient availability.

The MBC is determined using the method outlined in ISO 14140-2:1997, which involves extracting microbial biomass from soil samples through fumigation-extraction techniques. Total microbial carbon is quantified using standard methods such as elemental combustion or carbon content analysis. This indicator provides a snapshot of the microbial community's capacity to process nutrients and contribute to soil fertility.

For this project, MBC will be measured three times to evaluate changes in microbial biomass throughout the phytomanagement process. This frequency will allow for an accurate assessment of how the phytoremediation treatments influence soil microbial communities.

3.3.3 Enzymatic Activity

Enzymatic activity in soil is an important indicator of microbial function, as enzymes are critical for breaking down organic matter and facilitating nutrient cycling. The activity of various soil enzymes can reflect the health and diversity of microbial communities, as well as the soil's capacity to support essential ecological processes. Enzymatic activities are particularly important in understanding the degradation of contaminants during phytoremediation.

The enzymatic activity will be measured using either colorimetric or fluorogenic substrates, as per ISO 20130:2018 and ISO/TS 22939:2019. The method involves incubating soil samples with specific substrates that produce a measurable change in colour or fluorescence when broken down by microbial enzymes. This approach allows for the quantification of various enzymes, including arylsulfatase, β-glucosidase, alkaline and global phosphatases, and urease, which are key players in the main biogeochemical cycles.

Dehydrogenase activity will also be measured as an indicator of microbial respiration and soil health. ISO 23753-1:2019 outlines a method using triphenyltetrazolium chloride (TTC), which is reduced by microbial dehydrogenases to form a coloured formazan product. Alternatively, ISO 23753-2:2019 incorporates the INT-based method for measuring soil dehydrogenase activity, as established by Von Mersi & Schinner (1991). In this method, iodonitrotetrazolium chloride (INT) serves as the electron acceptor, and dimethylformamide (DMF) is used to dissolve INT, producing a quantifiable formazan product.

Enzymatic activity will be measured three times during the project to monitor changes in microbial functionality as the soil undergoes phytoremediation. This indicator is critical for evaluating microbial performance and soil nutrient cycling dynamics.

3.3.4 Microbial Profile

Understanding the microbial profile of soil is essential for evaluating its ecological health, and one of the most important indicators is the fungi-to-bacteria ratio. This ratio provides critical insights into the soil's microbial community structure and ecological health. A higher ratio typically reflects organic, complex environments dominated by fungi, which is common in more mature and complex ecosystems. In contrast, a lower ratio suggests bacterial dominance, often seen in environments with less organic material. This balance is essential for understanding soil health, nutrient availability, and microbial processes critical to phytoremediation.

Phospholipid Fatty Acid Analysis (PLFA) will be used to assess the fungi-to-bacteria ratio. This technique leverages gas chromatography to identify and quantify specific microbial biomarkers based on the fatty acid profiles present in soil phospholipids. Key fatty acids allow the identification of distinct microbial groups, based on their carbon content and their degree of saturation (ω). By analysing these markers, PLFA can distinguish between fungi, bacteria, and specific bacterial types such as Gram-positive and Gram-negative organisms (Quideau et al., 2016).

Key Fatty Acids for Microbial Group Identification:

- **Fungi**: Fungal biomarkers are primarily identified by the 18:2ω6,9 (linoleic acid) fatty acid, which is specific to the fungal community.
- **Bacteria**: Bacterial groups are identified by a range of fatty acids, including:
    - **Gram-positive bacteria**: Characterized by i15:0, a15:0, and i17:0 (iso- and anteiso-branched fatty acids).
    - **Gram-negative bacteria**: Identified by 16:1ω7 and 18:1ω7 (monounsaturated fatty acids), which are commonly found in their phospholipids.
- **Actinomycetes**: These are particularly identified by the fatty acid 10Me16:0 (10-methylhexadecanoic acid), which is a hallmark of this group, linked to organic matter degradation and bioactive compound production.

The degree of saturation of fatty acids (ω) provides additional information about microbial stress and adaptation to environmental conditions, which is useful for tracking shifts in microbial populations over time.

These microbial markers will be measured at the beginning and end of the project to track the evolution of microbial community composition and assess the impact of phytomanagement treatments.

3.3.5 Metagenomics - Metabarcoding

Metagenomics is a powerful tool for assessing the microbial diversity, species count, and functional potential of soil microbial communities. Unlike traditional methods that focus on culturing or identifying a limited number of species, metagenomics allows for a comprehensive and untargeted analysis of the entire microbial community, including bacteria, fungi, and eukaryotes. This approach provides taxonomic profiling of the soil's microbial species and

information on functional genes of interest, such as those involved in nutrient cycling or contaminant degradation.

For pHYBi, metagenomics will be conducted using shotgun sequencing with hybrid short-read (Illumina) and long-read sequencing technologies (PacBio/Nanopore). This method provides high-resolution data on microbial species composition and functional gene diversity. It offers valuable insights into how the microbial community is structured, how it evolves over time, and how it may contribute to the success of phytomanagement strategies.

In line with the specific sites' phytomanagement approach, metagenomic analyses can be conducted twice during the project (at the first and last monitoring campaigns) to align with their progress and needs, capturing temporal changes in microbial diversity and functional capabilities. By combining the results from microbial profiling and enzymatic activity assays, microbial heterogeneity at each site can be assessed. This evaluation will help determine whether samples can be pooled for metagenomic analysis or if intermediate time points should be monitored throughout the experiments. These indicators will assess shifts in microbial communities in response to phytoremediation treatments and provide deeper insights into the functional genes involved in soil health and pollutant degradation.

3.3.6 Phytotoxicity

Toxicity bioassays are essential for evaluating the impact of soil contaminants on seed germination, seedling emergence, early plant growth, and overall plant health. These assays serve as direct indicators of soil toxicity and are crucial for assessing the effectiveness of phytoremediation treatments in mitigating soil contamination.

For the pHYBi project, bioassays will be conducted following ISO 18763:2016, which measures:

- **Germination Percentage**: The proportion of seeds that successfully sprout under controlled conditions.
- **Survival Rate**: The percentage of seedlings that remain viable after exposure to contaminated soil.
- **Early growth**: Typically assessed by measuring root and shoot length.

The plant species selected for this assay include one monocot, *Sorghum saccharatum*, and two dicots, *Lepidium sativum* and *Sinapis alba*. These species are chosen for their sensitivity to pollutants and relevance to the local ecosystem. This bioassay will assess the toxicity levels in soil and their impact on the early stages of plant development, which is particularly important for evaluating the phytotoxicity of the soil, contaminants, or changes in soil properties resulting from phytoremediation efforts.

In parallel, ISO 11269-1:2012 can be employed to assess the emergence and early growth responses of at least two terrestrial plant species such as barley, wheat and oat when exposed to contaminated soils. This involves comparing the performance of these species in test soils against reference or standard control soils, providing insights into the impact of soil pollutants on plant development.

This indicator will be measured only once during the project to capture the baseline effects of soil toxicity on plant growth, with the results providing crucial insights into the success of the phytomanagement interventions.

3.3.7 Carbon Transformation Test

The Carbon Transformation Test measures the inhibitory effect of pollutants on soil microbial activity by assessing glucose-induced respiration rates in the microbial community exposed to polluted soil. Compared to a control sample, a reduction in respiration indicates decreased microbial activity, reflecting the impact of contaminants on soil health.

Following OECD Test No. 217 (OECD, 2000), soil samples containing pollutants are incubated in a closed respirometer. Glucose is added to stimulate microbial activity, and oxygen consumption is monitored to assess microbial respiration. The control sample serves as a baseline for comparison.

This test will be conducted at the beginning of the project to evaluate the impact of pollutants on microbial activity and soil toxicity. It provides valuable insights into the health of the microbial community and the potential for phytoremediation efforts to restore soil function.

Table 6. Microbial and Toxicity Indicators.

Category	Indicator	Methodology/ Source	Recommended Frequency	Notes/ Remarks
Microbio-logical Indicators	Soil Basal Respiration	ISO 16072:2002: CO_2 titration, GC or IRGA methods	Three times	
	Microbial Biomass	ISO 14140-2:1997: Fumigation-Extraction method	Three times	
	Enzymatic Activity	ISO 20130:2018: colorimetric *OR* ISO/TS 22939:2019: fluorogenic substrates. ISO 23753-1:2019: formazan quantification	Three times	At least arylsulfatase, β-glucosidase, alkaline and global phosphatases, urease *AND* Dehydrogenase
	Microbial Profile	PLFA: Gas Chromatography method	Two times	Fungi: Bacteria Ratio, Gram negative and positive bacteria & Actinomycetes
	Metagenomics/ Metabarcoding	Shotgun sequencing (hybrid short read – Illumina and long - read – PacBio/ Nanopore)	Two times	Functional and taxonomic diversity. Rhizosphere and plant tissues considered.

Toxicity Indicators	Phytotoxicity Bioassay	ISO 18763:2016: Germination and early growth ISO 11269:2012: emergence, early growth and root inhibition	Single time	Measures the effect of soil toxicity on seed germination and early growth of higher plants
	Carbon Transformation Test	OECD Test No. 217: Respirometer method	Single time	Glucose-induced respiration rates in the microbial community exposed to polluted soil

3.4 NUTRIENT AND CONTAMINATION PROFILE

3.4.1 Nutrient Profile

Nutrient indicators (Ca, K, Mg, Na, N, P) are essential for evaluating soil fertility and microbial activity, which are key factors in the success of phytoremediation. These nutrients influence plant growth and soil biological processes, impacting nutrient cycling and microbial health.

To analyse phosphorus in soil, samples will be tested for total and extractable phosphorus following ISO 11263:1994. This method extracts phosphorus using a sodium bicarbonate solution at pH 8.5. The phosphorus is then quantified using colorimetry, where a phosphate-molybdate complex is reduced with ascorbic acid to form a blue-coloured complex. This method measures both plant-available (extractable) inorganic phosphorus and total phosphorus, including organic and inorganic forms. Extractable phosphorus indicates immediate availability for plant uptake, while total phosphorus reflects the soil's overall phosphorus content, including forms that may become available over time through mineralization.

Nitrogen analysis will be performed using the Kjeldahl method as outlined in ISO 11261:1995, which quantifies total nitrogen content—including ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and organic nitrogen forms. This method involves digesting soil samples with concentrated sulfuric acid, followed by distillation and titration. It effectively measures amino nitrogen and ammonia but does not account for certain nitrogen forms, such as those in nitro, azo, and some heterocyclic compounds (e.g., pyridine).

For assessing Dissolved Organic Nitrogen (DON), important for microbial activity, the persulfate oxidation method described in ISO 14255:1998 is recommended. This technique involves extracting soil with a 0.01 mol/L calcium chloride solution, followed by oxidation with potassium persulfate ($K_2S_2O_8$) to convert all nitrogen forms to nitrate (NO_3^-). The resulting nitrate concentration is then measured colorimetrically. DON is calculated by subtracting the inorganic nitrogen content from the total soluble nitrogen pool.

It's important to note that total nitrogen methods employing strong oxidants convert all nitrogen forms into nitrate ions, measuring both reduced and oxidized species. In contrast, the Kjeldahl method focuses on quantifying organic nitrogen and ammonia, excluding certain oxidized nitrogen forms. Regarding Total Oxidized Nitrogen, this fraction includes nitrate and

nitrite, representing the most oxidized forms of nitrogen. Nitrate is readily taken up by plants and can be reduced enzymatically to amino forms or lost through denitrification under anaerobic conditions. Ammonia, the most reduced nitrogen form, originates from the decomposition of organic matter and urea hydrolysis.

This nutrient analysis will be conducted three times throughout the project to monitor changes in soil nutrient availability and assess the impact of phytoremediation efforts on soil fertility. The data will provide insights into the success of phytomanagement strategies in improving nutrient status and microbial function.

3.4.2 Soil Contamination – Trace Elements (Total or Phytoavailable)

Heavy metal contamination significantly impacts soil health and ecosystem functionality. The pHYBi project aims to assess and remediate these pollutants using phytomanagement strategies. By establishing a baseline of existing contamination, pHYBi examines how trace elements and heavy metals are translocated into plants or bio-stabilized, particularly in contaminated sites like CS1 and CS2.

The presence of metals such as Aluminium (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), boron (B), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), phosphorus (P), potassium (K), selenium (Se), silver (Ag), sodium (Na), strontium (Sr), sulfur (S), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V), and zinc (Zn) can hinder plant growth and microbial function, making their analysis essential for the pHYBi project.

To extract these elements from soil samples, the ISO 54321:2020 standard is typically applied. This method involves digesting soil samples using a mixture of nitric acid (HNO_3) and hydrochloric acid (HCl), commonly referred to as aqua regia. Aqua regia is effective because it breaks down the soil matrix and releases metals into solution. The resulting solution is suitable for the determination of trace elements using atomic spectrometric techniques. Common methods include Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), ICP-MS (with Mass Spectrometry detector) and Atomic Absorption Spectroscopy (AAS). This provides a comprehensive measure of all metals present in the soil, regardless of their chemical form or bioavailability.

However, the presence of a contaminant in soil does not automatically indicate its phytoavailability or bioavailability to organisms such as plants or microbes, or even that it will disrupt soil functionality. For a trace element (TE) to be absorbed by an organism, it must be in a form that is bioavailable. As a result, metals firmly bonded to silicates, clay minerals, or other stable soil components such as OM, are often excluded from being bioavailable. Thus, an indication of the maximum amount of metal that is potentially soluble and mobile within the soil matrix, highlighting the upper limit of potential TE contamination, is often more useful.

ISO 17402:2008 provides guidance on selecting and applying methods to assess bioavailability for the characterization of contaminated soils and materials. While it doesn't specify the best methods, it outlines the boundary conditions, principles, and minimum requirements for developing such methods.

For assessing the bioavailable fraction of heavy metals, i.e., the fraction accessible to plants and microorganisms, methods such as ISO 14870:2001 can be used. This standard describes the extraction of trace elements using a buffered DTPA solution, primarily targeting plant-available

micronutrients such as Cu, Fe, Mn, and Zn, and is commonly applied in neutral to alkaline soils. It can also provide information on the extractable fraction of potentially toxic elements such as Cd, Ni, and Pb. Alternatively, the labile fraction can be estimated using 0.01 M $CaCl_2$ extraction, a widely used operational method that targets the readily soluble and exchangeable pool of metals in soil. Although not formalised under a specific ISO standard for bioavailability, it is broadly accepted in soil science as a proxy for the soil solution–accessible fraction.

In addition, in pots or when conditions allow, soil pore water can be obtained following the general principle described in ISO 17586:2016, which is based on extraction of soil solution via suction or physical separation techniques. In practice, this approach can be implemented using tools such as Rhizon samplers or similar suction lysimeters, which allow minimally disruptive collection of pore water for chemical analysis.

Finally, depending on the context and specific requirements of the CS, trace element fractionation by sequential extraction can be employed. This method partitions metals into different operationally defined fractions based on their chemical forms and associations within the soil. The procedure typically involves multiple steps, each using specific reagents to isolate different soil components. Various protocols have been developed, including those by the European Commission's Standards, Measurements and Testing Programme, formerly known as the Community Bureau of Reference (BCR) (Ure et al., 1993) and Van Herck & Vandecasteele (2001). These methods are adaptations of the original procedure established by Tessier et al. (1979), which outlines the following general steps:

1. **Step 1: Exchangeable fraction**

    a. Reagent: 1 M Magnesium chloride ($MgCl_2$) pH 7.0 or Sodium acetate (NaOAc) pH 8.2
    b. Purpose: Extract metals that are weakly bound to soil particles and are readily available for plant uptake.

2. **Step 2: Carbonate-bound fraction**

    a. Reagent: 0.1 M Sodium acetate (NaOAc) at pH 5.0
    b. Purpose: Extract metals that are bound to carbonates, which can be released by changes in soil pH (e.g., through acid rain).

All samples will be characterized up to this point to determine the labile, readily available fraction (bioavailable). Due to time and cost constraints, only selected samples will undergo further extraction to investigate mobilization under reducing conditions, trace elements (TE) that can be released through decomposition or microbial activity, and the fraction considered not bioavailable, as outlined below:

3. **Step 3: Iron and Manganese oxide-bound fraction**

    a. Reagent: 0.04 M Ammonium oxalate ($NH_4C_2O_4$) in 25% (v/v) Acetic acid (HOAc)
    b. Purpose: Extract metals bound to iron and manganese oxides, which are important for metal adsorption in the soil.

4. **Step 4: Organic matter-bound fraction**

a. Reagents: 0.02 M Nitric acid (HNO_3) and 30% Hydrogen peroxide (H_2O_2) (adjusted to pH ~2 with HNO_3).
   Ammonium acetate (NH_4OAc) in 3.2 M in 20% HNO_3
b. Purpose: Extract metals associated with organic matter, which can affect metal mobility and bioavailability.

5. **Step 5: Residual fraction**

   a. Reagent: Hydrofluoric acid (HF) and Perchloric acid ($HClO_4$)
   b. Purpose: Extract metals that are tightly bound within the soil's mineral matrix, typically requiring harsh acid digestion.

After each extraction step, the solutions are filtered and analysed using ICP to determine the concentration of metals in each fraction. This provides valuable insight into the mobility and bioavailability of metals in the soil, which is critical for understanding their potential uptake by plants.

The analyses will be performed three times during the project to monitor temporal variations and the impact of phytoremediation on soil metal content. These indicators will provide valuable insights into how phytomanagement strategies are influencing soil metal concentrations and their potential availability to plants.

3.4.3 Organic Pollutants

Organic pollutants, including total petroleum hydrocarbons (TPHs) and polycyclic aromatic hydrocarbons (PAHs), pose significant risks to soil health and plant growth. These contaminants can hinder plant development and disrupt microbial activity. Sites like CS3a and CS3b are contaminated due to the history of oil spills and petroleum exploitation, while other sites have been impacted by TPHs from the use of agrochemicals. To establish a reference point and track the progress and effectiveness of phytomanagement strategies, the initial levels of these contaminants in the soil will be assessed.

The methodology for analysing organic pollutants follows advanced chromatographic techniques such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS). These methods can detect and quantify a wide range of organic pollutants. Specifically, Pyrolysis-GC-MS (Py-GC-MS) will be employed for complex soil samples, offering high sensitivity in detecting compounds like PAHs, which include naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(k)fluoranthene, benzo(b) fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-c,d)pyrene, benzo(g,h,i) perylene. These compounds are separated and identified based on their unique mass spectra.

The analysis of organic pollutants involves extracting soil samples using a dichloromethane: acetone (1:1) mixture in a Soxtherm apparatus. This extraction efficiently isolates hydrocarbons, which are then analysed using a GC-MS system. For Polycyclic Aromatic Hydrocarbons (PAHs), the analysis follows EPA Method 8272 (U. S. Environmental Protection Agency, 2007), which employs Pyrolysis-GC-MS (Py-GC-MS) to detect and quantify compounds such as naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(k)fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-c,d)pyrene, and benzo(g,h,i)perylene. This method is particularly effective for analysing complex soil samples and provides high sensitivity for PAH

detection. For Total Petroleum Hydrocarbons (TPH), EPA Method 8270 (U.S. Environmental Protection Agency, 2018) is utilized, which involves extracting samples with dichloromethane and analysing the extracts using GC-MS. This method is suitable for detecting a broad range of semi-volatile organic compounds in environmental samples. Both methods rely on the unique mass spectra of the target compounds for accurate identification and quantification.

The analysis will be performed three times during the project to evaluate the success of phytoremediation treatments in mitigating the presence of these contaminants. The results will provide critical insights into how organic pollutants impact soil health and plant growth, and how phytoremediation strategies can reduce or eliminate these pollutants from the soil.

3.4.4 Salt content

Salinity is a significant environmental stressor in areas affected by historical mining activities, e.g., CS4. High concentrations of soluble salts can severely impact soil structure, reduce plant growth and productivity, and negatively affect microbial communities essential for soil remediation and ecosystem recovery.

To accurately assess the extent and impact of salt contamination, comprehensive characterization of soil salinity and specific salt composition will be performed. Soil EC and pH will initially be determined as primary indicators of overall salinity and soil alkalinity. Further analytical methods, including ion chromatography (IC) and ICP-OES, will be employed to quantify major ions commonly associated with mining-impacted soils. Specifically, analyses will focus on CEC cations as well as anions including chloride (Cl^-), sulfate (SO_4^{2-}), nitrate (NO_3^-), and bicarbonate (HCO_3^-). These ions are selected due to their frequent occurrence in mining-affected regions and their known phytotoxic effects, influencing soil osmotic balance, nutrient availability, and microbial community function. Salt characterization will be performed at three stages during the pHYBi project: initial site assessment, mid-project monitoring, and final evaluation. This will allow a clear understanding of how effectively phytomanagement practices can reduce soil salinity and mitigate its harmful impacts.

Table 7. Soil Nutrient and Contaminant Profile Assessment.

Category	Indicator	Methodology/ Source	Recommended Frequency	Notes/ Remarks
Nutrient Indicators	Nutrients	ISO 11263:1994: Sodium Bicarbonate for P. ISO 11261:1995: Kjeldahl Method for Total Nitrogen. ISO 14255; 1998: Persulfate Oxidation for DON	Three times	Measures essential nutrient levels in soil, including Ca, K, Mg, Na (previously determined during CEC assessment) N, and P.
Soil Contamination	Trace Elements (Total and Phytoavailable)	ISO 17586:2016: Aqua regia method for total heavy metals. ISO 14870:2001: DTPA extraction	Three times	Includes metals like Ag, Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Ti, V, Zn. ISO 17402:2008

		method for bioavailable fraction. Sequential extraction		provides guidance for selecting method.
	Organic Pollutants	EPA 8272 for PAHs; EPA 8270 for TPH (Extraction followed by GC-MS)	Single time	Comprehensive list of total petroleum hydrocarbons (TPHs) and polycyclic aromatic hydrocarbons (PAHs)
	Salt content (anions and cations)	EC meter, pH meter, IC, ICP-OES	Three times	Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, NO_3^-, HCO_3^-

4. PLANT AND BIOMASS CHARACTERIZATION

Phytomanagement relies heavily on the ability of plants to grow, thrive, and remediate polluted soils, as they are the primary agents of pollutant removal and ecosystem restoration. Monitoring plant health and biomass yield is essential for evaluating the success of phytoremediation efforts. Plant responses to stressors, such as pollutants and salinity, are key indicators of plant resilience and the potential for pollutant uptake or immobilisation. In the pHYBi project, a wide range of plant and biomass indicators are employed to assess plant performance and evaluate the success of phytoremediation efforts. This includes tracking plant growth rates, health indices and stress indicators, as well as determining biomass yield and evaluating its chemical composition, considering pollutant accumulation and particularly its lignocellulosic content, which has direct implications for industrial applications

This section outlines the plant and biomass indicators to be monitored throughout the pHYBi project's lifecycle, ensuring comprehensive data collection on both plant physiology and biomass quality. Key metrics, such as transfer, bioconcentration, and translocation factors, will be used to assess how effectively plants uptake, translocate, and store contaminants, and how this may impact the quality of biomass for organosolv valorisation. By evaluating plant resilience and biomass quality, the pHYBi project aims to generate valuable insights into the effectiveness of various phytomanagement strategies, ultimately contributing to the development of more sustainable remediation techniques within a circular economy framework.

4.1 PLANT HEALTH AND BIOCHEMICAL STRESS INDICATORS

In the pHYBi project, assessing plant health and stress is essential for understanding how plants respond to pollutants and environmental factors, as well as how these responses affect biomass production and feedstock quality. These indicators help evaluate the effectiveness of phytomanagement strategies, providing insights into plant resilience and their ability to grow in contaminated soils. Ultimately, this supports the extraction of organosolv fractions from biomass, enabling their use in the textile industry within a circular production framework.

Visual assessments of **plant condition** provide a snapshot of health, categorizing plants as “Good,” “Fair,” “Poor,” or “Dead/Dying.” This simple method captures signs of stress or vitality,

offering quick, informative data. Age is another important indicator, determined through planting data or dendrochronology. Knowing a **plant's age** helps track its growth and biomass accumulation over time. **Genotype** identification, based on variety or cultivar, is crucial for understanding how genetic factors influence plant responses to environmental stressors. It also helps identify specific hybrids with traits beneficial for pollutant tolerance and high-quality feedstock production. This data will be collected early in the project, following plant selection based on lab experiment performance. The goal is to establish a database of suitable plants for phytomanagement, emphasizing both phytoremediation capacity and high yield, which will serve as a baseline for future comparisons.

Growth characteristics such as **height and diameter** (dbh, diameter at breast height) are direct measures of plant vigor. Height can be measured using traditional dendrometry or remote sensing with UAVs, while diameter is typically measured using a tape or caliper. These indicators will be measured at a single point in the project but serve as ongoing references for plant health. **Growth rate**, estimated from height and diameter changes, will be tracked annually to assess plant maturation and adaptability to stressors.

Growth stage or developmental phase, including dormancy, leaf development, and flowering, provides key insights into a plant's physiological state and response to environmental stress. Regular visual observations will help track these stages, which are crucial for interpreting fluctuations in other indicators like growth rate and biochemical stress markers. Understanding plant activity is essential for explaining variations in these metrics and assessing overall plant health and resilience.

Biochemical stress indicators offer deeper insights into plant health. **Hormones** like abscisic acid (ABA) and other stress-related compounds will be measured using qPCR, offering a molecular-level view of how plants manage environmental stress. **Protein content**, measured using Bradford or BCA assays, also signals how plants are coping with stress at the cellular level.

Antioxidant enzymes, such as SOD, CAT, and POD, play a crucial role in defending plants against oxidative damage. Their activity will be measured using spectrophotometric assays, providing clear data on plant resilience. Similarly, **antioxidants** like vitamins C and E, flavonoids, and polyphenols will be quantified using spectrophotometry and mass spectrometry, indicating the plant's ability to neutralize free radicals and protect against cellular damage.

Markers of oxidative stress, including ROS and MDA levels, will be tracked using spectrophotometric assays. Elevated levels of these markers signal potential damage to plant cells and stress-related issues, while lower levels may suggest better health and recovery.

Finally, **volatile organic compounds (VOCs)** emitted by plants under stress, such as isoprenes and terpenoids, will be analyzed using Gas Chromatography-Time of Flight Mass Spectrometry (GC-ToFMS). VOCs are early indicators of plant stress responses and can provide real-time insights into plant health.

Together, these indicators offer a comprehensive picture of how plants in the pHYBi project are reacting to environmental stress and contaminants. By monitoring these markers, the project can assess phytoremediation success and refine strategies for more resilient ecosystems.

Table 8. Plant Health, Fitness and Stress Indicators.

Category	Indicator	Methodology	Recommended Frequency	Notes/ Remarks
Plant Sample	Overall Plant Condition	Visual assessment	Single time	Classify: Good, Fair, Poor, Dead/ Dying
	Age	Planting data; Dendro-chronology	Single time	
	Genotype	Source-Based Identification	Single time	Name and classification: Variety, Cultivar, Hybrid, Clone
	Height	Dendrometry, Remote Sensing UAV	Single time	To note also at harvest
	Diameter (dbh)	Caliper or tape measurement	Single time	
	Growth Rate	Estimated with formula	Three times	Yearly basis, based on height and diameter.
	Growth Stage	Visual assessment	Single time	Observe signs of dormancy, leaf development, vegetative growth, flowering or fruiting.
Plant Biochemical Stress Indicators	Hormones	qPCR	Single time (vs control) or three times	Hormone related gene expression (ABA, SA, CK, and Auxins)
	Total protein content	Extraction followed by Bradford assay, BCA assay	Single time (vs control) or three times	Phytochelatins for metal polluted sites; acquaporines for high salinity
	Antioxidant Enzymes	Spectrophoto metric assays	Single time (vs control) or three times	SOD, CAT, APx, and POD

	Antioxidants	Spectrophotometry (e.g.: UV-Vis adsorption + fluorescence), mass spectroscopy	Single time (vs control) or three times	Vitamin C, Vitamin E, Flavonoids, Polyphenols, Carotenoids, Glutathione, Gallic acid, Catechins
	Stress Biomarkers	Spectrophotometric assays	Single time (vs control) or three times	Measure reactive oxygen species (ROS), H_2O_2, and lipid peroxidation (MDA) and proline
	Volatile Organic Compounds	GC- ToFMS	Beginning and end of project	Full scan or selection of 20-30 major VOCs (e.g., isoprenes, terpenoids, acetone, ethylene, GLVs, JA)

4.2 BIOMASS YIELD AND POLLUTANT FATE

The pHYBi project proposes a comprehensive approach to phytoremediation by cultivating both woody and herbaceous industrial crops on polluted soils. This strategy aims to restore soil health while producing high-quality lignocellulosic biomass suitable for bio-based applications, particularly in the textile industry.

In this context, assessing biomass production is essential for evaluating plant growth and its potential for feedstock production. Both **above-ground and below-ground biomass** are key indicators, with above-ground biomass measured by dry weight and moisture content determined through oven-heating or thermogravimetric methods. For poplar, below-ground biomass will not be measured, as these plants will not be harvested for root analysis.

For the analysis of plant parameters at the existing sites, groups of trees will be formed, and material (branches, leaves, and roots, when possible) will be collected from a representative number of trees, or from all of them, depending on the plot size and tree density. These samples will be pooled to create a representative sample, which will undergo analysis and be prepared for organosolv fractionation by CETIM. For the new sites or pot experiments, we propose collecting the entire plant and analyzing it individually, as these plants are expected to be smaller.

Trace elements, including heavy metals, are critical for understanding how pollutants move through plants. The fate of these metals is tracked through ICP techniques following a total or

sequential extraction procedure. This approach will allow us to calculate the Translocation Factor (TF), Geoaccumulation Index (Igeo), and Bioconcentration Factor (BCF), helping to quantify how much metal is absorbed by the plants, translocated to different parts or accumulated in the roots. These calculations are applied to the same heavy metals detected in soil, linking soil contamination levels with plant uptake and accumulation.

Organic Pollutants are similarly tracked through GC-MS, which analyse compounds in plant tissue in the same way they are analysed in soil. As with heavy metals, the Translocation Factor, Geoaccumulation Index, and Bioconcentration Factor are calculated to evaluate the movement and concentration of these pollutants in plants. This helps understand their environmental persistence and the plant's role in accumulating or remedying these substances.

By integrating these assessments, the pHYBi project aims to optimize phytoremediation strategies that restore soil health and produce high-quality biomass for sustainable, bio-based applications.

Table 9. Biomass Production and Contaminant Fate.

Category	Indicator	Technique	Notes/Remarks
Biomass	Above Ground	Dry weight measurement	
	Below Ground	Dry weight measurement	Not in the case of poplar
	Moisture Content	Oven- heating, Thermogravimetric	
Derived ecosystem-scale indicators	Above-ground biomass	Calculated from biomass × tree density	
	Below-ground biomass	Calculated	Not in the case of poplar
	Total biomass	Calculated	
Contaminant Fate	Heavy Metals	ICP or Sequential extraction	Same elements detected in soil Calculate Traslocation Factor and/ or Geoaccumulation Index (Igeo) and Bioconcentration Factor
	Organic Pollutants	GC-MS	Same compounds detected in soil Calculate Traslocation Factor and/ or Geoaccumulation Index (Igeo) and Bioconcentration Factor

4.3 FRACTIONATION COMPOSITION AND QUALITY

4.3.1 Ratio C/N

The Carbon to Nitrogen (C/N) ratio is a critical indicator in the analysis of biomass, providing valuable insights into its decomposition potential and nutrient balance. In the context of the pHYBi project, this ratio is particularly important as it impacts the suitability of biomass fractions for textile applications. A balanced C/N ratio promotes efficient biomass processing and influences the quality of the cellulose, hemicellulose, and lignin fractions extracted during organosolv treatment. A higher C/N ratio may indicate a more stable material, while a lower ratio can lead to quicker degradation, which could affect the fractionation process.

The C/N ratio is determined through elemental analysis using a FlashEA112 elemental analyser, which allows for the precise measurement of carbon, nitrogen, hydrogen, and sulfur. This method provides rapid results and offers a reliable measure of biomass composition. The data helps refine the fractionation process, ensuring that the lignocellulosic biomass is optimised for subsequent use in textile production.

4.3.2 Ash Content

Ash content refers to the inorganic residue left after the combustion of biomass. It is a key indicator of the mineral content in the biomass, which can influence both the fractionation process and the final product quality in the textile industry. High ash content can interfere with the processing of biomass by potentially contaminating the extracted fractions or requiring additional purification steps.

To determine ash content, the biomass is subjected to a calcination process as outlined by the NREL/TP-510-42622 method (Sluiter et al., 2005). The procedure involves heating a known weight of dry biomass at 575°C for 24 hours. After cooling in a desiccator, the sample is weighed to determine the percentage of ash present. This method provides an accurate and straightforward way to quantify the mineral content of the biomass, which is essential for ensuring the quality of the lignocellulosic fractions to be used in bio-based textile finishing applications.

4.3.3 Lignin

Lignin is one of the three main components of lignocellulosic biomass, alongside cellulose and hemicellulose. Its presence in biomass affects the texture, strength, and biodegradability of the material. In the context of the pHYBi project, understanding lignin content is crucial for the efficient extraction of cellulose for textile applications, as high lignin content can complicate the process by increasing resistance to chemical treatments.

Lignin content is typically quantified using quantitative acid hydrolysis (HAC), following the procedure described by NREL/TP-510-42618 (Sluiter et al., 2008). This method involves breaking down lignin into soluble acidic materials through a two-step acid hydrolysis process. The resulting fractions are analysed using HPLC to determine the quantity and composition of lignin present. By accurately measuring lignin content, the extraction process can be optimised to ensure that this fraction is of high quality for textile coatings production.

4.3.4 Cellulose

Cellulose, primarily composed of glucan, is the most abundant polymer in plant biomass and serves as the primary component for producing fibres in the textile industry. The pHYBi project focuses on extracting high-quality cellulose fractions, which are integral to creating sustainable, bio-based textile finishes. The amount and quality of cellulose determine the efficiency and viability of the fractionation process.

Cellulose content is measured through the same quantitative HAC method, which involves breaking down the cellulose into its monomeric form, glucan. The hydrolysis process is followed by HPLC analysis, where the sample is processed to identify and quantify glucose. This approach ensures that the cellulose fraction is accurately measured, providing the necessary data to optimise the use of cellulose in textile applications.

4.3.5 Hemicellulose

Hemicellulose is a complex carbohydrate found in plant cell walls and is the second major component of lignocellulosic biomass. In the context of textile applications, hemicellulose fractions offer unique properties for specialized textile finishes or treatments. For example, within the pHYBi framework, the extracted fraction will be used for carotene production, which can then be utilised as dyes. Along with the coatings from the other lignocellulosic fractions, these components form the sustainable products that underpin the project's objectives, offering opportunities in the fashion industry to enhance textile properties through bio-based solutions.

Hemicellulose content is also quantified using the HAC method, which allows for the hydrolysis of hemicellulose into its monomeric sugars, such as xylose, mannose, galactose, and arabinose. These sugars are then separated and quantified using HPLC, which provides precise measurements of the hemicellulose components. Understanding the hemicellulose content ensures the effective separation of this fraction, contributing to the creation of diverse textile products with sustainable properties.

4.3.6 Extractives

Extractives refer to the soluble compounds found in biomass that are not part of the cellulose, hemicellulose, or lignin structures. These include fats, waxes, resins, and other organic compounds that may interfere with the extraction of pure lignocellulosic fractions. In the textile industry, removing these extractives is crucial to ensure the quality and purity of the fractions that are proposed for use bio-based textile finishing production.

Soxhlet extraction with absolute ethanol is employed to determine extractive content, following the ASTM E1690-08(2016) standard method. This technique ensures that all soluble compounds, including waxes and fats, are removed from the biomass, leaving behind the more desirable lignocellulosic components. The extracted material is weighed to calculate the total extractive content, which is expressed as a percentage of the sample. This analysis ensures that the fractions used in textile applications are free from contaminants that could affect their processing and final properties.

4.3.7 Fibre Quality: Purity and Length

The quality of the extracted lignocellulosic fibres plays a significant role in determining their suitability for textile applications, particularly for the development of textile coatings. Purity is a key parameter that defines the performance of the final textile finishing products. For the pHYBi

project, ensuring that the fibre fractions meet the necessary quality standards is essential for their successful use as bio-based finishes for textiles.

Fibre purity is assessed by measuring the ratios of lignin, hemicellulose, and cellulose in the different fractions obtained from biomass. During HAC, it is also possible to determine the presence of residual components such as formic acid, hydroxymethylfurfural (HMF), and furfural. These compounds are degradation products of cellulose and hemicellulose, formed during biomass breakdown, and can be considered undesirable for the production of textile finishes.

Fibre length will be assessed following the Italian regulation UNI 5751 ('Determination of the length of fibres – Method of measurement of single fibre') (Ente Nazionale Italiano di Unificazione (UNI), 1990). However, if the fractions are produced as a fine powder with lengths of less than 1 mm, characterization can be performed using optical microscopy, coupled with SEM analysis if needed. Also, the presence of metals can be determined in the different fractions if they are presents in the biomass, using the same technique previously explained (ICP). These properties are assessed to ensure that the biomass fractions are suitable for high-performance, sustainable textile finishes.

Table 10. Lignocellulosic Biomass Fractionation and Quality Assessment.

Category	Indicator	Methodology	Notes/ Remarks
Composition	Ratio C/N	Elementary analysis CHNS with FlashEA112 elemental analyser	
	Ash Content	NREL /TP-510-42622: Calcination method followed by ICP-MS	Ash percentage content and composition
Fractionation components	Lignin	NREL/TP-510-42618: Quantitative acid hydrolysis	Klason's lignin
	Cellulose		Glucan
	Hemicellulose		Xylan, Mannan, Galactan, Arabinan, Uronic acids, Acetyl groups
Other components	Extractives	ASTM E1690-08(2016): HAC and Sohxlet extraction	Waxes, fats, etc. that may interfere with the determination of lignin
Fibre Quality	Purity	HAC to determine the quantity of lignin/cellulose or hemicellulose	
	Length	UNI 5751: Method of measurement of single fibres	If needed optical microscopy, coupled with SEM analysis is possible

5. CONCLUSION

The comprehensive monitoring and characterisation framework outlined in Deliverable D1.1 provides a robust foundation for assessing the impact of phytomanagement strategies throughout the pHYBi project. By systematically measuring soil, plant, and biomass indicators, we ensure a detailed understanding of the environmental and biological processes involved in phytoremediation. The integration of physicochemical, microbiological, and ecological indicators allows for a holistic evaluation of soil health, plant growth, and biomass quality, enabling us to track and optimise remediation interventions effectively.

Through continuous data collection, including the use of advanced remote sensing technologies, the project will not only contribute to the development of more efficient and sustainable phytomanagement strategies but also provide valuable insights into contaminant transfer, plant resilience, and feedstock suitability for industrial applications. By aligning our methods with European regulations and leveraging stakeholder expertise, pHYBi aims to drive innovative solutions for soil restoration, pollutant management, and circular economy practices.

The data and insights gained from this monitoring strategy will serve as a critical resource for the optimisation of phytomanagement techniques, ultimately contributing to the long-term environmental and economic goals of the project. As the project progresses, ongoing monitoring and evaluation will ensure that interventions remain adaptive, scalable, and aligned with the overall objectives of sustainable land management and bioremediation.

6. BIBLIOGRAPHY

Aboukarima, A. M., Al-Sulaiman, M. A., & Marazky, M. S. E. (2018). Effect of sodium adsorption ratio and electric conductivity of the applied water on infiltration in a sandy-loam soil. *Water SA*, *44*(1 January), Article 1 January. https://doi.org/10.4314/wsa.v44i1.12

Adamczyk-Szabela, D., & Wolf, W. M. (2022). The Impact of Soil pH on Heavy Metals Uptake and Photosynthesis Efficiency in Melissa officinalis, Taraxacum officinalis, Ocimum basilicum. *Molecules*, *27*(15), 4671. https://doi.org/10.3390/molecules27154671

Adeniji, A., Huang, J., Li, S., Lu, X., & Guo, R. (2024). Hot viewpoint on how soil texture, soil nutrient availability, and root exudates interact to shape microbial dynamics and plant health. *Plant and Soil*. https://doi.org/10.1007/s11104-024-07020-y

Çellek, S. (2022). Effect of the slope angle and its classification on landslides. *Himalayan Geology*, *43*, 85–95.

Certini, G., & Scalenghe, R. (2023). The crucial interactions between climate and soil. *Science of The Total Environment*, *856*, 159169. https://doi.org/10.1016/j.scitotenv.2022.159169

de Oliveira, R. A., Ramos, M. M., & de Aquino, L. A. (2015). Chapter 8—Irrigation Management. In F. Santos, A. Borém, & C. Caldas (Eds.), *Sugarcane* (pp. 161–183). Academic Press. https://doi.org/10.1016/B978-0-12-802239-9.00008-6

Ente Nazionale Italiano di Unificazione (UNI). (1990). *UNI 5751: Textiles. Determination of Length of Fibres. Method of Measurement of Single Fibres*. https://www.intertekinform.com/en-gb/standards/uni-5751-1990-1086264_saig_uni_uni_2529387/

FAO. (2021). *Global map of salt-affected soils*. FAO. https://openknowledge.fao.org/handle/20.500.14283/cb7247en

Fernández-Ugalde, O., Orgiazzi, A., Jones, A., Lugato, E., & Panagos, P. (2017). *LUCAS 2018—Soil component : sampling instructions for surveyors.* https://data.europa.eu/doi/10.2760/023673

Frank, T., Brauckmann, H.-J., & Broll, G. (2023). Humus Forms of Moist and Wet Forest Stands. A Review. *International Journal of Plant Biology*, *14*(3), Article 3. https://doi.org/10.3390/ijpb14030058

Franzluebbers, A. J. (2002). Water infiltration and soil structure related to organic matter and its stratification with depth. *Soil and Tillage Research*, *66*(2), 197–205. https://doi.org/10.1016/S0167-1987(02)00027-2

Frene, J. P., Pandey, B. K., & Castrillo, G. (2024). Under pressure: Elucidating soil compaction and its effect on soil functions. *Plant and Soil*, *502*(1), 267–278. https://doi.org/10.1007/s11104-024-06573-2

Futo, Z., & Bodnar, K. (2021). Soil Hydrology. In A. Gadomski (Ed.), *Water in Biomechanical and Related Systems* (pp. 277–302). Springer International Publishing. https://doi.org/10.1007/978-3-030-67227-0_14

Gholami, H., Mohammadifar, A., Song, Y., Li, Y., Rahmani, P., Kaskaoutis, D. G., Panagos, P., & Borrelli, P. (2024). An assessment of global land susceptibility to wind erosion based on deep-active learning modelling and interpretation techniques. *Scientific Reports*, *14*(1), 18951. https://doi.org/10.1038/s41598-024-70125-y

Gomes, M. P., Pereira, E. G., Qiu, B.-S., & Juneau, P. (2021). Editorial: Coping With Pollution – the Effects of Environmental Contaminants on Plant Growth and Physiology. *Frontiers in Plant Science*, *12*, 740802. https://doi.org/10.3389/fpls.2021.740802

Hamidov, A., Helming, K., Bellocchi, G., Bojar, W., Dalgaard, T., Ghaley, B. B., Hoffmann, C., Holman, I., Holzkämper, A., Krzeminska, D., Kværnø, S. H., Lehtonen, H., Niedrist, G., Øygarden, L., Reidsma, P., Roggero, P. P., Rusu, T., Santos, C., Seddaiu, G., … Schönhart, M. (2018). Impacts of climate change adaptation options on soil functions: A review of European case-studies. *Land Degradation & Development*, *29*(8), 2378–2389. https://doi.org/10.1002/ldr.3006

Havlin, J. L. (2020). Soil: Fertility and Nutrient Management. In *Landscape and Land Capacity* (2nd ed.). CRC Press.

Heiniger, R. W., McBride, R. G., & Clay, D. E. (2003). Using Soil Electrical Conductivity to Improve Nutrient Management. *Agronomy Journal*, *95*(3), 508–519. https://doi.org/10.2134/agronj2003.5080

International Organization for Standardization. (1994). *Soil quality—Determination of the specific electrical conductivity (ISO 11265:1994)*. https://www.iso.org/standard/19243.html

International Organization for Standardization. (1995). *Soil quality—Determination of organic and total carbon after dry combustion (ISO 10694:1995)*. https://www.iso.org/standard/18782.html

International Organization for Standardization. (1995a). *Soil quality—Determination of pore water pressure—Tensiometer method (ISO 11276:1995)*. https://www.iso.org/standard/19254.html

International Organization for Standardization. (1995b). *Soil quality—Determination of total nitrogen—Modified Kjeldahl method (ISO 11261:1995)*. https://www.iso.org/standard/19239.html

International Organization for Standardization. (1997). *Soil quality—Determination of soil microbial biomass. Part 2: Fumigation-extraction method (ISO 14240-2:1997)*. https://www.iso.org/standard/23951.html

International Organization for Standardization. (1998). *Soil quality—Determination of nitrate nitrogen, ammonium nitrogen and total soluble nitrogen in air-dry soils using calcium chloride solution as extractant (ISO 14255:1998)*. https://www.iso.org/standard/23081.html

International Organization for Standardization. (2002). *Soil quality—Laboratory methods for determination of microbial soil respiration (ISO 16072:2002)*. https://www.iso.org/standard/32096.html

International Organization for Standardization. (2012a). *Geotechnical investigation and testing—Geohydraulic testing. Part 2: Water permeability tests in a borehole using open systems (ISO 22282-2:2012)*. https://www.iso.org/standard/57723.html

International Organization for Standardization. (2012b). *Soil quality—Determination of the effects of pollutants on soil flora. Part 1: Method for the measurement of inhibition of root growth (ISO 11269-1:2012)*. https://www.iso.org/standard/51388.html

International Organization for Standardization. (2012c). *Soil quality—Determination of the effects of pollutants on soil flora. Part 2: Effects of contaminated soil on the emergence and early growth of higher plants (ISO 11269-2:2012)*. https://www.iso.org/standard/51382.html

International Organization for Standardization. (2016). *Soil quality—Determination of the toxic effects of pollutants on germination and early growth of higher plants (ISO 18763:2016)*. https://www.iso.org/standard/63317.html

International Organization for Standardization. (2017). *Soil quality—Determination of dry bulk density (ISO 11272:2017)*. https://www.iso.org/obp/ui/en/#iso:std:iso:11272:ed-2:v1:en

International Organization for Standardization. (2017). *Soil quality—Determination of particle density (ISO 11508:2017)*. https://www.iso.org/standard/68257.html

International Organization for Standardization. (2018a). *Fertilizers and soil conditioners—Determination of humic and hydrophobic fulvic acids concentrations in fertilizer materials (ISO 19822:2018)*. https://www.iso.org/standard/66271.html

International Organization for Standardization. (2018b). *Soil quality—Determination of effective cation exchange capacity and base saturation level using barium chloride solution (ISO 11260:2018)*. https://www.iso.org/standard/60566.html

International Organization for Standardization. (2018c). *Soil quality—Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates (ISO 20130:2018)*. https://www.iso.org/standard/67074.html

International Organization for Standardization. (2019a). *Soil quality—Determination of the water-retention characteristic—Laboratory methods (ISO 11274:2019)*. https://www.iso.org/standard/68256.html

International Organization for Standardization. (2019b). *Soil quality—Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates (ISO/TS 22939:2019)*. https://www.iso.org/standard/74739.html

International Organization for Standardization. (2020). *Soil quality—Determination of particle size distribution in mineral soil material—Method by sieving and sedimentation (ISO 11277:2020)*. https://www.iso.org/standard/69496.html

International Organization for Standardization. (2021). *Soil, treated biowaste and sludge – Determination of pH (ISO 10390:2021)*. https://www.iso.org/standard/75243.html

International Organization for Standardization. (2022). *Soil quality—Determination of redox potential—Field method (ISO 11271:2022)*. https://www.iso.org/standard/83962.html

International Organization for Standardization. (2023). *Soil quality—Determination of potential cation exchange capacity (CEC) and exchangeable cations buffered at pH 7, using a molar ammonium acetate solution (ISO/TS 22171:2023)*. https://www.iso.org/standard/80891.html

Jakovac, C. C., Junqueira, A. B., Crouzeilles, R., Peña-Claros, M., Mesquita, R. C. G., & Bongers, F. (2021). The role of land-use history in driving successional pathways and its implications for the restoration of tropical forests. *Biological Reviews of the Cambridge Philosophical Society*, *96*(4), 1114–1134. https://doi.org/10.1111/brv.12694

Jendoubi, D., Liniger, H., & Ifejika Speranza, C. (2019). Impacts of land use and topography on soil organic carbon in a Mediterranean landscape (north-western Tunisia). *SOIL*, *5*(2), 239–251. https://doi.org/10.5194/soil-5-239-2019

Jiang, F., Huang, Y., Wang, M., Lin, J., Zhao, G., & Ge, H. (2014). Effects of Rainfall Intensity and Slope Gradient on Steep Colluvial Deposit Erosion in Southeast China. *Soil Science Society of America Journal*, *78*(5), 1741–1752. https://doi.org/10.2136/sssaj2014.04.0132

Kervroëdan, L., Armand, R., Rey, F., & Faucon, M.-P. (2021). Trait-based sediment retention and runoff control by herbaceous vegetation in agricultural catchments: A review. *Land Degradation & Development*, *32*(3), 1077–1089. https://doi.org/10.1002/ldr.3812

Klein, K. A., & Santamarina, J. C. (2003). Electrical Conductivity in Soils: Underlying Phenomena. *Journal of Environmental and Engineering Geophysics*, *8*(4).

Kong, J., Liu, H., Wang, R., Wang, J., Zhai, P., Ma, R., Kang, P., & Liu, B. (2024). Influence of herbaceous litter thickness on bacterial community structure and physicochemical properties of aeolian sand. *Canadian Journal of Microbiology*, *70*(10), 433–445. https://doi.org/10.1139/cjm-2023-0219

Korkalainen, T., & Laurén, A. (2006). Using phytogeomorphology, cartography and GIS to explain forest site productivity expressed as tree height in southern and central Finland. *Geomorphology*, *74*(1–4), 271–284. https://doi.org/10.1016/j.geomorph.2005.09.001

Kumar, N., Kumar, A., Jeena, N., Singh, R., & Singh, H. (2020). Factors Influencing Soil Ecosystem and Agricultural Productivity at Higher Altitudes. In R. Goel, R. Soni, & D. C. Suyal (Eds.), *Microbiological Advancements for Higher Altitude Agro-Ecosystems & Sustainability* (pp. 55–70). Springer. https://doi.org/10.1007/978-981-15-1902-4_4

Lavallee, J. M., Soong, J. L., & Cotrufo, M. F. (2020). Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global Change Biology*, *26*(1), 261–273. https://doi.org/10.1111/gcb.14859

Lucas, M., Santiago, J. P., Chen, J., Guber, A., & Kravchenko, A. (2023). The soil pore structure encountered by roots affects plant-derived carbon inputs and fate. *New Phytologist*, *240*(2), 515–528. https://doi.org/10.1111/nph.19159

Marakkala Manage, L. P., Katuwal, S., Norgaard, T., Knadel, M., Moldrup, P., De Jonge, L. W., & Greve, M. H. (2019). Estimating Soil Particle Density using Visible Near-infrared Spectroscopy and a Simple, Two-compartment Pedotransfer Function. *Soil Science Society of America Journal*, *83*(1), 37–47. https://doi.org/10.2136/sssaj2018.06.0217

Marquis, B., Bergeron, Y., Simard, M., & Tremblay, F. (2021). Disentangling the effect of topography and microtopography on near-ground growing-season frosts at the boreal-temperate forest ecotone (Québec, Canada). *New Forests*, *52*(6), 1079–1098. https://doi.org/10.1007/s11056-021-09840-7

Mattila, T. J. (2024). Redox potential as a soil health indicator – how does it compare to microbial activity and soil structure? *Plant and Soil*, *494*(1), 617–625. https://doi.org/10.1007/s11104-023-06305-y

Moeys, J. (2024). *The soil texture wizard: R functions for plotting, classifying, transforming and exploring soil texture data* [Computer software]. https://cran.r-project.org/web/packages/soiltexture/vignettes/soiltexture_vignette.pdf

Muscarella, R., Kolyaie, S., Morton, D. C., Zimmerman, J. K., & Uriarte, M. (2020). Effects of topography on tropical forest structure depend on climate context. *Journal of Ecology*, *108*(1), 145–159. https://doi.org/10.1111/1365-2745.13261

Nayak, A. K., Rahman, M. M., Naidu, R., Dhal, B., Swain, C. K., Nayak, A. D., Tripathi, R., Shahid, M., Islam, M. R., & Pathak, H. (2019). Current and emerging methodologies for estimating carbon sequestration in agricultural soils: A review. *The Science of the Total Environment*, *665*, 890–912. https://doi.org/10.1016/j.scitotenv.2019.02.125

Nimmo, J. R. (2004). Porosity and pore size distribution. *Encyclopedia of Soils in the Environment*, *3*(1), 295–303.

OECD. (2000). *Test No. 217: Soil Microorganisms: Carbon Transformation Test* (No. OECD Guidelines for the Testing of Chemicals, Section 2). OECD Publishing. https://doi.org/10.1787/9789264070240-en.

Pandey, V. C., Gajić, G., Sharma, P., & Roy, M. (2022). 1—Phytoremediation in a changing climate. In V. C. Pandey, G. Gajić, P. Sharma, & M. Roy (Eds.), *Adaptive Phytoremediation Practices* (pp. 1–23). Elsevier. https://doi.org/10.1016/B978-0-12-823831-8.00004-9

Quideau, S. A., McIntosh, A. C. S., Norris, C. E., Lloret, E., Swallow, M. J. B., & Hannam, K. (2016). Extraction and Analysis of Microbial Phospholipid Fatty Acids in Soils. *Journal of Visualized Experiments : JoVE*, *114*, 54360. https://doi.org/10.3791/54360

Rabot, E., Wiesmeier, M., Schlüter, S., & Vogel, H.-J. (2018). Soil structure as an indicator of soil functions: A review. *Geoderma*, *314*, 122–137. https://doi.org/10.1016/j.geoderma.2017.11.009

Rahman, M. A., Lee, S.-H., Ji, H. C., Kabir, A. H., Jones, C. S., & Lee, K.-W. (2018). Importance of Mineral Nutrition for Mitigating Aluminum Toxicity in Plants on Acidic Soils: Current Status and Opportunities. *International Journal of Molecular Sciences*, *19*(10), Article 10. https://doi.org/10.3390/ijms19103073

Raupp, P., Carrillo, Y., & Nielsen, U. N. (2024). Soil Health to Enhance Ecological Restoration and Conservation. *Journal of Sustainable Agriculture and Environment*, *3*(4), e70022. https://doi.org/10.1002/sae2.70022

Sharma, A., Weindorf, D. C., Wang, D., & Chakraborty, S. (2015). Characterizing soils via portable X-ray fluorescence spectrometer: 4. Cation exchange capacity (CEC).

Geoderma, *239–240*, 130–134. https://doi.org/10.1016/j.geoderma.2014.10.001

Sherrod, L. A., Vigil, M. F., & Stewart, C. E. (2019). Do Fulvic, Humic, and Humin Carbon Fractions Represent Meaningful Biological, Physical, and Chemical Carbon Pools? *Journal of Environmental Quality*, *48*(6), 1587–1593. https://doi.org/10.2134/jeq2019.03.0104

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2005). Determination of ash in biomass (NREL/TP-510-42622). *National Renewable Energy Laboratory, Golden*, *19*.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). Determination of structural carbohydrates and lignin in biomass. National Renewable Energy Laboratory. *National Renewable Energy Laboratory (NREL), Golden CO*.

Stefanowicz, A. M., Kapusta, P., Stanek, M., Rola, K., & Zubek, S. (2022). Herbaceous plant species support soil microbial performance in deciduous temperate forests. *The Science of the Total Environment*, *810*, 151313. https://doi.org/10.1016/j.scitotenv.2021.151313

Tessier, A., Campbell, P. G. C., & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry*, *51*(7), 844–851. https://doi.org/10.1021/ac50043a017

Tian, P., Tian, X., Geng, R., Zhao, G., Yang, L., Mu, X., Gao, P., Sun, W., & Liu, Y. (2023). Response of soil erosion to vegetation restoration and terracing on the Loess Plateau. *CATENA*, *227*, 107103. https://doi.org/10.1016/j.catena.2023.107103

U. S. Environmental Protection Agency. (2007). *Validated Test Method 8272: Parent and Alkyl Polycyclic Aromatics in Sediment Pore Water by Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry in Selected Ion Monitoring Mode*. https://www.epa.gov/sites/default/files/2015-12/documents/8272.pdf

Ure, A. M., Quevauviller ,Ph., Muntau ,H., & and Griepink, B. (1993). Speciation of Heavy Metals in Soils and Sediments. An Account of the Improvement and Harmonization of Extraction Techniques Undertaken Under the Auspices of the BCR of the Commission of the European Communities. *International Journal of Environmental Analytical Chemistry*, *51*(1–4), 135–151. https://doi.org/10.1080/03067319308027619

U.S. Environmental Protection Agency. (2018). *SW-846 Test Method 8270E: Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)*. https://www.epa.gov/sites/default/files/2019-01/documents/8270e_revised_6_june_2018.pdf

Van Herck, P., & Vandecasteele, C. (2001). Evaluation of the use of a sequential extraction procedure for the characterization and treatment of metal containing

solid waste. *Waste Management*, *21*(8), 685–694. https://doi.org/10.1016/S0956-053X(01)00011-3

Večeřová, K., Oravec, M., Puranik, S., Findurová, H., Veselá, B., Opoku, E., Ofori-Amanfo, K. K., Klem, K., Urban, O., & Sahu, P. P. (2022). Single and interactive effects of variables associated with climate change on wheat metabolome. *Frontiers in Plant Science*, *13*, 1002561. https://doi.org/10.3389/fpls.2022.1002561

Von Mersi, W., & Schinner, F. (1991). An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. *Biology and Fertility of Soils*, *11*(3), 216–220. https://doi.org/10.1007/BF00335770

Wei, H., Yang, J., Liu, Z., & Zhang, J. (2022). Data Integration Analysis Indicates That Soil Texture and pH Greatly Influence the Acid Buffering Capacity of Global Surface Soils. *Sustainability*, *14*(5), Article 5. https://doi.org/10.3390/su14053017

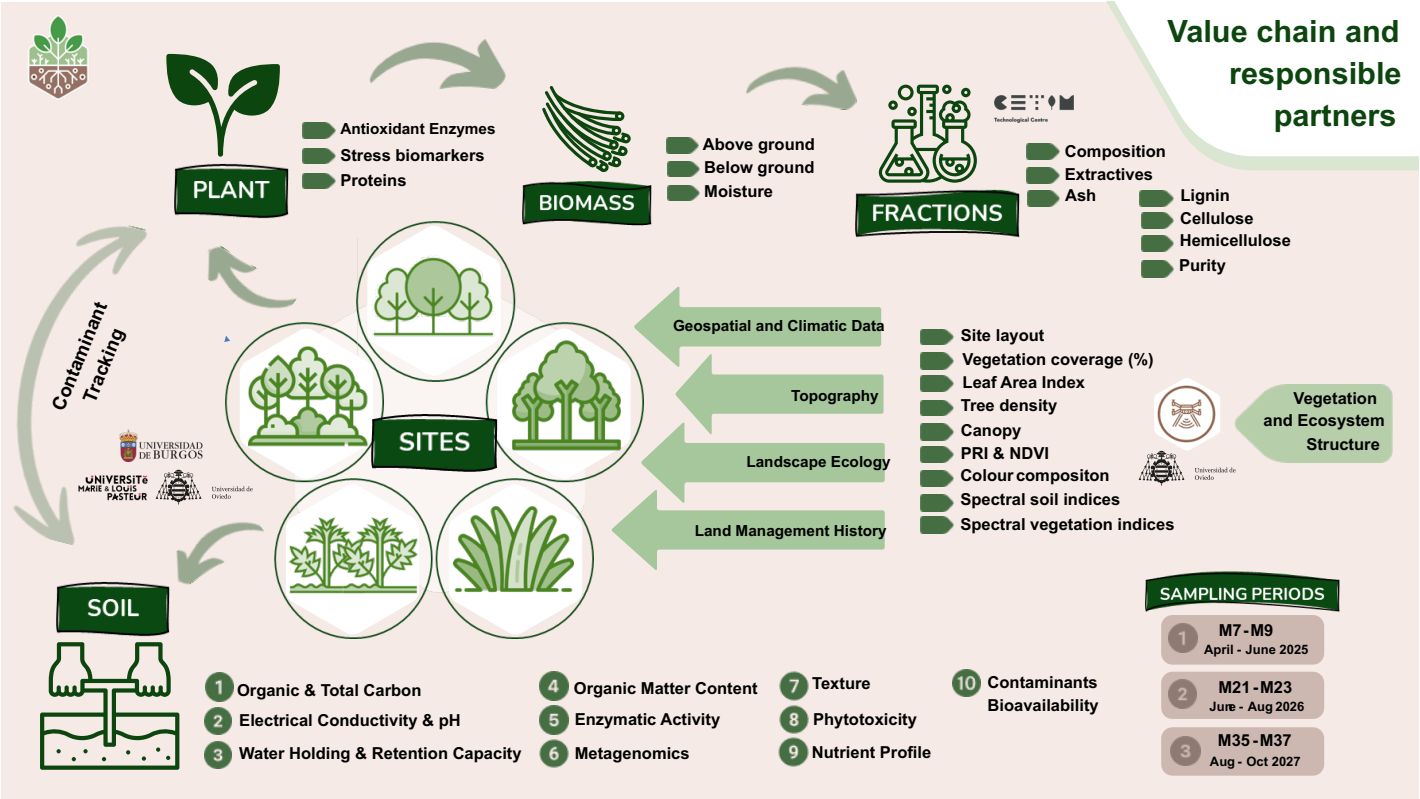
Xing, Y., Wang, X., & Mustafa, A. (2025). Exploring the link between soil health and crop productivity. *Ecotoxicology and Environmental Safety*, *289*, 117703. https://doi.org/10.1016/j.ecoenv.2025.117703

Yim, B., Ibrahim, Z., Rüger, L., Ganther, M., Maccario, L., Sørensen, S. J., Heintz-Buschart, A., Tarkka, M. T., Vetterlein, D., Bonkowski, M., Blagodatskaya, E., & Smalla, K. (2022). Soil texture is a stronger driver of the maize rhizosphere microbiome and extracellular enzyme activities than soil depth or the presence of root hairs. *Plant and Soil*, *478*(1), 229–251. https://doi.org/10.1007/s11104-022-05618-8

Zhang, N., Nunan, N., Hirsch, P. R., Sun, B., Zhou, J., & Liang, Y. (2021). Theory of microbial coexistence in promoting soil–plant ecosystem health. *Biology and Fertility of Soils*, *57*(7), 897–911. https://doi.org/10.1007/s00374-021-01586-w

Zhang, Y., Hartemink, A. E., Vanwalleghem, T., Bonfatti, B. R., & Moen, S. (2024). Climate and land use changes explain variation in the A horizon and soil thickness in the United States. *Communications Earth & Environment*, *5*(1), 1–11. https://doi.org/10.1038/s43247-024-01299-6

7. SUPPLEMENTARY INFORMATION



Supplemental Figure S1. Value chain and responsible partners for activities related to WP1.

SOIL COMPOSITE SAMPLING SHEET			
1. General Information			
Date & Time		Weather Conditions	
Sampler's Name		Recent Weather Events	
Site Name/Location		Sampling Objective	
2. Sample Characteristics			
Color		Presence of fragments (e.g., stones, debris, roots)	
Texture (e.g., smooth, gritty)		Structure (e.g., compacted, crumbly)	
Moisture (e.g., dry, moist, saturated)		Ribbon Test (e.g., no ribbon, length)	
3. Sampling Conditions			
Method (e.g., manual, mechanical)		Tool (e.g., shavel, spade, auger, scoop, probe)	
Depth (cm)		Number of subsamples	
Stratified sampling (yes/no)		Layer (bulk, rhizosphere, topsoil, subsoil)	
Cooling required (yes/ no)		Storage conditions (refrigerated, room temperature)	
Container (e.g., plastic, paper, glass)		Processing (air dried, sieved, intact)	
4. Field & Other Observations			
Presence of wildlife or insects? (yes/no & notes)		Deviations from standard sampling protocol	
Erosion (yes/ no & type)		Proximity to vegetation or roads	
Photo documentation		Additional observations	

CODE: CSX_DDMMYY_PX_LPX_XS

Supplemental Figure S2. Soil composite sampling sheet.

PLANT SAMPLING SHEET

1. General Information			
Date & Time		Weather Conditions	
Sampler's Name		Stress (e.g., light, temperature)	
Site Name/Location		Sampling Objective	

2. Sample Description			
Plant species		Genotype (e.g., hybrid, variety, cultivar)	
Age		Water Conditions (e.g., irrigated, rain-fed, drought)	
Growth stage (e.g., vegetative, flowering)		Signs of stress/disease (e.g., wilting, pests, lesions)	

3. Sample Characteristics			
Height (cm/m)		Diameter [dbh] (mm/cm)	
Root condition		Leaf condition (e.g., healthy, chlorotic, necrotic)	
Fresh above ground (g)		Fresh below ground (g)	

4. Sampling Conditions			
Design (e.g., random, stratified, systematic, cluster)		Sampling type (i.e., destructive or non-destructive)	
Sampling fraction		Number of subsamples	
Type (e.g., manual, mechanical)		Tool (e.g., pruner, saw, knife)	
Tissue (e.g., whole plant, leaves, stems, branches, bark, wood cores)		Processing (e.g., immediate, dry, cut, crush, freeze)	
Container		Cooling required (yes/ no)	
Preservation required (yes/no & method)		Handling instructions	
Photo documentation		Additional observations	

CODE: CSX_DDMMYY_PLANT_PX_TX

Supplemental Figure S3. Plant sampling sheet.