EDAPHOS

D2.1 – Report of available methods and data

WP2 – Task 2.1

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Authors: Paula Madejón (CSIC), Engracia Madejón (CSIC),

Nicolas Manier (INERIS)

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12-08-24	2		Aleksandra Zgórska & Manhattan Lebrun	
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Table of contents

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List of figures

List of tables

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Summary

This deliverable aims at gathering and describing the pre-existing data and information for each case study (CS) selected in the EDAPHOS project. It also aims at identifying and harmonizing the methods used by each EDAPHOS partners for the determination of the soil parameters. Effort in harmonisation of sampling protocols and analytical methods through the EDPAHOS partner's labs should ensure the comparability of the data provided by the project.

The pre-existing data will serve as a basis for determining the analytical needs and the effort in methodological harmonisation needs for the WP2 and WP3 work. Because the clarity on data quality is crucial for the reuse of the data for downstream investigations, the task 2.1 set out a series of template to accurately (i) collect the data (raw data) and metadata (i.e. associated methods or protocol used, sampling information), (ii) harmonize the data collection and (iii) analyse the data and metadata availability/gaps. Moreover, these templates were built to ensure a high quality of data set along the project.

In the purpose of the Environmental Risk Assessment (ERA) planned in WP2, Task 2.3, and for further analytics in WP2 and WP3, the data availability and data gaps were identified.

This deliverable summarizes the knowledges and the data availability and reusability at the start of the EDAPHOS project for each CS. It also summarizes the methods used by each partner for the soil monitoring and describe the methods and protocols selected for further analyses on each CS which will be used for the ERA and soil monitoring along the project.

Keywords

Analytical methods, Data comparability, Method harmonization, Soil parameters.

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Abbreviations and acronyms

Acronym	Description
CEC	Cation Exchange Capacity
CS	Case Study
d.w.	Dry weight
DDT	Dichlorodiphényltrichloroéthane
ERA	Environmental risk assessment
HMTE	Heavy metalsTrace element
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
LoE	Line of evidence
LQ	Limit of Quantification
n.d.	No data
NBS	Nature based solution
PAHs	Poly Aromatic Hydrocarbons
PCBs	Polychlorinated biphenyls
SOC	Soil Organic Carbon
TE	Trace elements
TN	Total Nitrogen
WHC	Water Holding Capacity
WoE	Weight of evidence
WP	Work Package
TE	Trace Element

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Introduction

Soils are the keystone of healthy ecosystems, providing physical, chemical, and biological substrates and functions necessary to support life. However, soils are under constant threat from pollution, and contaminated sites in Europe (EU) are estimated to up to 3 million with an average of registered sites of 3.69 sites / km² and those which need of urgent remediation are around 250 000 (Pérez and Eugenio, 2018). Contaminated sites pose significant environmental sources of pollution and hazards resulting in deteriorated terrestrial and aquatic ecosystems quality (soil, water, fodder and food).

The European Union has established a set of actions to ensure the "soil protection and remediation strategies of contaminated sites" in the framework of the 8th Environment Action Programme (EAP), illustrating the extent of this challenge and the magnitude of the problem.

In this context and to contribute to the Mission "A Soil Deal for EU" and to its specific objective "Reduce soil pollution and enhance restoration", the overall objectives of the EDAPHOS project are to develop a holistic and innovative land management approach based on the phytoremediation. The project proposes the implementation and the demonstration of the effectiveness of such nature-based solution (NBS) on contaminated soils considering 7 Case Studies (CS) which cover different pedo-climatic, ecological, and social areas in the EU.

Within WP2 of the project, the deliverable D 2.1 comprises a comprehensive report detailing the pre-existing data and information at the beginning of the EDAPHOS project for each of the 7 CS selected. This deliverable aims at identifying the methods used by each EDAPHOS partners in the context of the soil monitoring and the ecological risk assessment (ERA). Given the project's objectives and their influence on soil monitoring and ERA results, the D2.1 aims at summarizing the methodology used for soil physico-chemical, biochemical, and ecotoxicological / ecological analyses. The pre-existing data and available methodologies will serve as a basis for determining the analytical needs and the effort to be done for both WP2 and WP3 work.

This deliverable addresses the following:

(i) Collecting pre-existing data and metadata (*i.e.* protocols, methods, uncertainties…)

The primary aim is to gather existing data for each CS in relation to the soil monitoring and the site-specific soil ecological risk assessment (ERA). For the ERA, the effort aligns with the so call "Triad approach", which will be applied throughout the project's Work Package 2, task 2.3. This task 2.1 ensures that all relevant historical data is compiled to provide a robust foundation for subsequent analyses. Because the clarity on data quality is crucial for the reuse of the data for downstream investigations, the task 2.1 set out a series of template to accurately collect the data (raw data) and metadata (i.e. associated methods or protocol used, sampling information) in a harmonized and structured way. These templates allow for the assessment of the completeness and reliability of the accessible data for each CS at the beginning of the project. Therefore, the first objective of this deliverable is to organize and analyse all the gathered information from each CS.

(ii) Analyzing the data/knowledge gaps regarding the needed data for ERA

This point refers to the process of examining and identifying areas where there is insufficient or missing data needed for conducting the ERA. Recognizing where there are deficiencies or gaps in the available data that hinder its reusability for further purposes.

(iii) Describing, comparing and harmonizing the available methodologies and protocols

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The third aim of D2.1 is to describe, compare and harmonize the methodologies employed by each project partner. Data harmonization, which involves combining different datasets to enhance their comparability and compatibility, has become an increasingly common method for addressing data challenges (Chen et al., 2024). This process is essential when conducting the same type of analysis in different laboratories, as multiple approaches can be used for the same variable.

This comparison focuses on the determination of basic physico-chemical and contamination parameters, ensuring consistency and reliability in data collection and analysis across the project. This harmonization process is crucial for the project's Work Package 3 (WP3), as it ensures that all partners utilize compatible methods, facilitating accurate and comparable results for the soil monitoring and the plant analyses.

1 Case studies description

CS 1: Carrières sous Poissy (FR) - Boosting the regeneration of an abandoned agricultural area contaminated by TE and PAH (lead: UBFC)

Located in the northwest region of Paris, the CS 1 site is part of a large agricultural area covering approximately 300 hectares, owned by the SYE (Seine & Yvelines Environnement) (Figure 1).

Historically, this area received untreated wastewater throughout the 20th century, leading to its current moderate level of contamination. The contaminants present include trace elements (TE) such as cadmium (Cd), lead (Pb), and zinc (Zn), as well as polycyclic aromatic hydrocarbons (PAHs) and pesticides commonly used in agricultural practices.

The **location of the area** corresponds to the coordinates 48°57'39.7"N 2°02'09.6"E.

The **total surface** area for the experimental study covers approximately 8000 m².

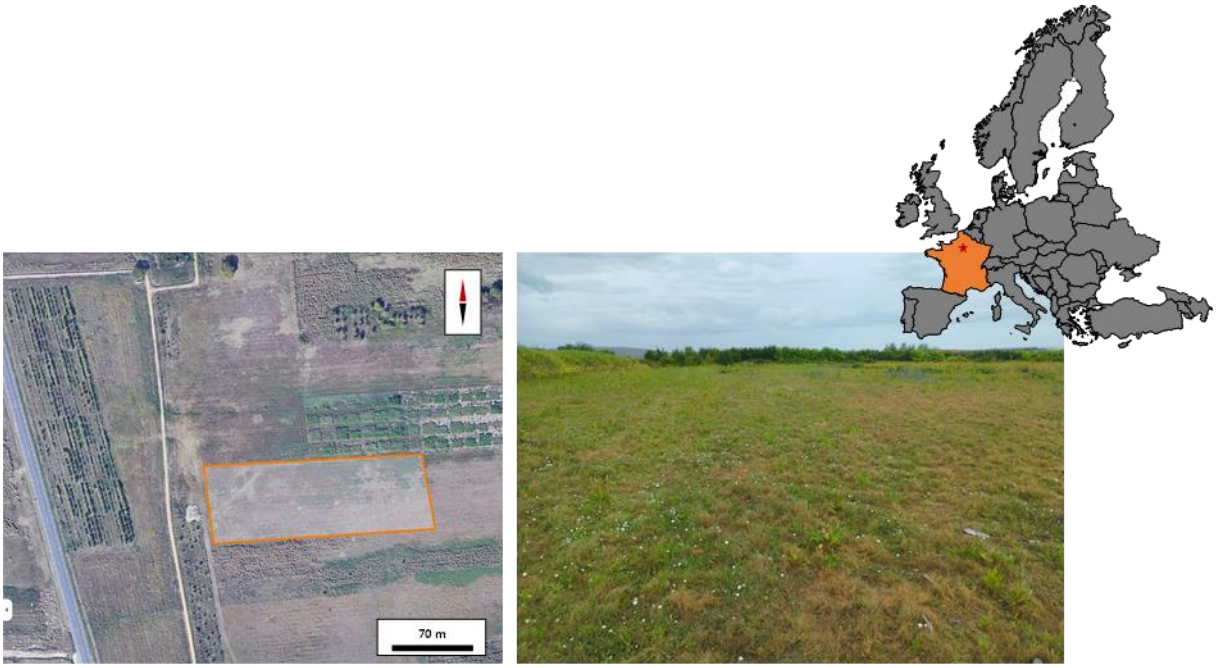

Figure 1. Localisation and picture of the CS 1

CS 2: Kozani (GR) - Applying agroforestry for Ni remediation at a lignin mining area (lead: CRES).

Located in the north of Greece, near Prosilio Kozani, the CS 2 site owned by the METE S.A. company (Mining – Technical – Trade S.A compagny). The main activities of the company are the exploitation of lignite and quartz deposits.

This area is characterized by exceptionally high concentrations of nickel (Ni), with levels measured at 20 times above the normal background levels. Recognizing both the environmental challenges and the potential opportunities, one hectare of this land has been designated for the EDAPHOS project (Figure 2). This site offers a unique opportunity for soil depollution efforts, especially in the context of Ni remediation.

The **location of the area** corresponds to the coordinates 40° 8'46.16"N: 21°55'56.67"E.

The **total surface** area for the experimental study covers approximately 2000 m².

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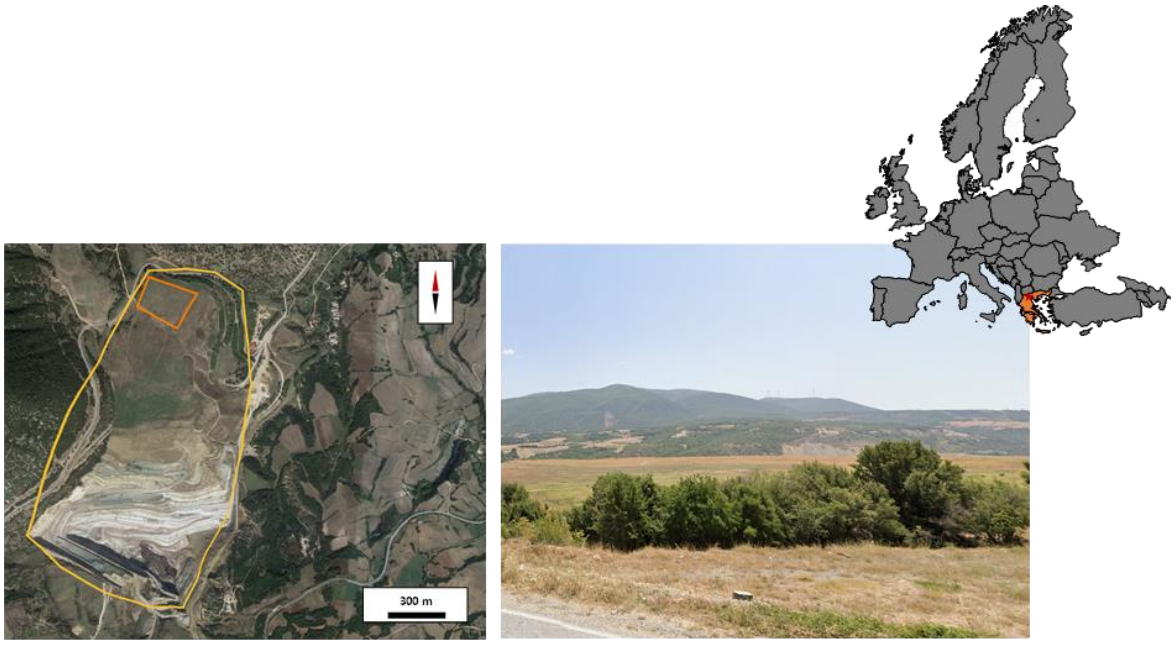

Figure 2. Localisation and images of the CS 2

CS 3: Odiel basin Area (SP) - Assisting the restoration of highly contaminated mining areas under Mediterranean climate (lead: CSIC)

The Iberian Peninsula lies one of the world's largest sulphide mining areas, spanning over 12,000 hectares, with a history of exploitation dating back to the third millennium BC. Centuries of mining activity have left behind numerous abandoned mine sites, notorious for generating severe metallic pollution through acid mine drainage. This has significantly degraded the ecological and chemical quality of the surrounding water bodies. The primary contaminants found in high concentrations include arsenic (As), lead (Pb), along with high contributions of copper (Cu) and mercury (Hg). The specific study area covers 2 hectares within the Atalaya Mine property, which holds active mining rights (Figure 3).

The **localization of the area** corresponds to the coordinates 37°41'26" N 6°34'3" W.

The **total surface** area for the experimental study covers approximately 5000 m^2.

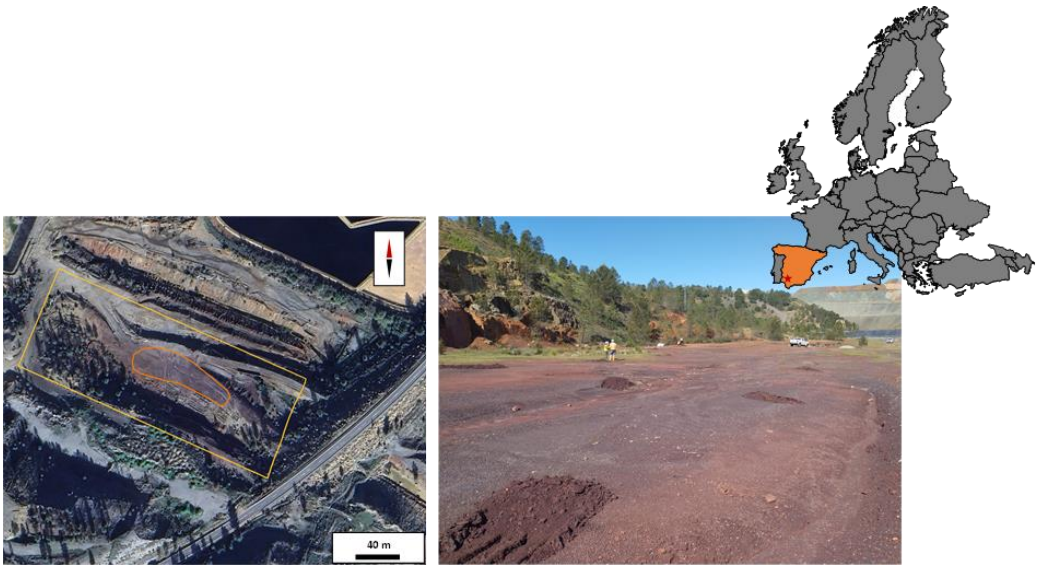

Figure 3 Localisation and images of the CS 3

CS 4: Upper Silesian Coal Basin, Silesian Voivodship (PL) - The use of phytoremediation techniques to restore the soil ecosystem of a post-mining area (lead: GIG-PIB)

The Upper Silesian Coal Basin (USCB) within the Silesian Voivodeship is located in southern Poland. The area of CS4 presents a significant challenge due to the high concentration of metals and metalloids contamination resulting from industrial activities conducted in the area. The CS4 is located within the Silesian Voivodeship, which is the most environmentally degraded region in Poland due to industrial activities, including heavy industry, mining and other related sectors (Figure 4). The importance of the problem is given by the fact that the total area of degraded and devastated land in Poland is approximately 3,463,374 ha (2017), which gives an area of 16.3 m² per Polish citizen (GUS 2017). In Silesia Voivodeship alone, the area of degraded and devastated terrains exceeded 11,300 ha, including more than 6000 ha in the central part of the region (Gasidło, 2019). However, the actual area occupied by brownfields in the region is much larger. The area has been heavily contaminated due to the proximity of industrial pollution sources, which has resulted in high concentration in, of arsenic (As), lead (Pb), zinc (Zn), and cadmium (Cd), as well as high contributions of copper (Cu) and manganese (Mn) in the soil. These pollutants have severely compromised the soil ecosystem, needing remediation efforts to restore ecological health and functionality. The total surface of the contaminated area is 36 300 m².

The **localization of the area** corresponds to the coordinates 50°30'11.14"N 18°54'46.87"E

The **total surface** area for the experimental study covers approximately 4000m²

Figure 4. Localisation and images of the CS 4

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CS 5: Galliera (IT) - Boosting the regeneration of an area contaminated with residues from the pesticide industry (lead: UNIBO)

Galliera site, within the Metropolitan area of Bologna, Italy, presents an environmental challenge related to industrial contamination, particularly from residues of the pesticide industry. This urban area, characterized by flat terrain, is significantly contaminated with various pollutants, mainly copper (Cu), lead (Pb), DDT, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). The t**otal surface of contaminated site** is approximately, 4.000 m² (Figure 5).

The **localization of the area** corresponds to the coordinates: 44°44'32"N 11°26'39"E

The **total surface** area for the experimental study covers approximately 500-1000 m²

Figure 5 Localisation and images of the CS 5

CS 6: Vieux-Charmont (FR) - Accelerating soil regeneration and involving the larger public (lead: UBFC).

The Bourgogne Franche Comté Region exemplifies a broader issue with industrial wastelands across the area, totaling 150 hectares (Figure 6). This specific site is heavily polluted with a mix of contaminants, including arsenic (As), cadmium (Cd), lead (Pb), zinc (Zn), and polycyclic aromatic hydrocarbons (PAHs). The contamination levels present significant environmental challenges, affecting soil quality and potentially posing risks to local ecosystems and human health.

The **localization of the area** corresponds to the coordinates 47°31'15.8"N, 6°50'23.8"E.

The **total surface** area for the experimental study covers approximately two hectares.

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Figure 6. Localisation and images of the CS 6

CS 7: Lavrio (GR) - Applying agroforestry for soil remediation at an old metallurgical (lead: CRES).

The lignite mining area in Lavrio, situated within a dense agricultural region, presents a complex environmental challenge due to extensive historical mining and metallurgical activities spanning millennia. Mining activities in Lavrio date back to ancient times (3000-200 B.C.) and continued through more recent periods (1864-1982 A.D.), leaving behind significant contamination of TE such as lead (Pb) and zinc (Zn). These metals have accumulated over time, creating localized hot spots of pollution within the area. The main contaminants in the area are Cd, Pb, Zn, Cu, Ni and As. The **total surface** of the contaminated area is 1 hectare (Figure 7).

The **localization of the area** corresponds to the coordinates 37°44'1.04"N 24° 2'40.68"E.

The **total surface area** for the experimental study covers approximately 2300 m^2

Figure 7. Localisation and images of the CS 7

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2 Information needs for the site-specific ERA

Site-specific ecological risk assessment (ERA) requires information to estimate the biological effects of a contaminated site not only by measuring the contamination level in soil but also through *in situ* ecological surveys, soil function analyses and soil ecotoxicological hazard analyses. Gathering information from different scientific fields following a Weight of Evidence (WoE) approach is necessary to determine the ecological risk reflecting realistic environment and to draw appropriate conclusions (Chapman, 1990; Critto et al. 2007). Based on the WoE approach, the Triad is considered as a comprehensive and well-structured method to identify an environmental risk associated with the presence of contaminants in soils (Grassi et al. 2022). The general procedure enables potential impacts on terrestrial ecosystems to be identified, assessed and managed, considering the level of contamination and site characteristics.

This method is described in ISO 19204:2017 entitled "Procedure for site-specific ecological risk assessment of soil contamination" and considers the measurement endpoints belonging to three major lines of evidence: environmental chemistry (Chem-LoE), ecotoxicology (Ecotox-LoE), and ecology (Eco-LoE) (ISO, 2017).

The chemical LoE consists in characterizing and quantifying the contaminants present in the soils. The aim is to characterize and measure the exposure of living organisms *via* total contaminant levels, or their mobility or bioavailability determination.

The ecotoxicological LoE consists in characterizing the potential hazard of contaminants on the living organisms present in the ecosystems concerned. It is mainly carried out using laboratory tests (bioassays). The organisms used are exposed either directly to the soil (direct exposure to identify the effect of the matrix) or to a soil eluate (indirect exposure to identify the effects of water-mobilizable pollutants). The aim of these tests is to determine the ecotoxicity of a soil for one or more species by characterizing the toxic effect.

The ecological LoE evaluates the ecology (*e.g.* soil biodiversity, ecological structure) as well as the soil ecological function (*e.g.* global metabolism, microbial respiration, enzyme activities). This approach focuses on understanding the complex interactions between living organisms, including plants, animals and microorganisms and the soil function and health.

Based on the recommendations of the standard procedure and on the proposal from previous works (Grassi et al. 2022, Kim et al. 2022, Son et al. 2019), a selection of relevant parameters was made for the purpose of the EDAPHOS project. These parameters (Table 1) were selected to characterize the source, the exposure and the effects of the pollution present in soil and to fit with the requirement of the ERA according to the WoE triad approach (covering chemical, ecotoxicological and ecological parameters), as well as being relevant as key parameters for monitoring the soil recovery using NBS and quantify the success and potential ecological benefits.

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Table 1. Type of data needed (toolbox) for the ERA according to the triad approach

Type of information needed for ERA	Description
Chem-LoE - Contamination level and contaminant behavior in soil and biota	Characterization of total concentration of contaminants Characterization of available contaminants Bioaccumulation of contaminants in plants and/or soil invertebrates
Chem LoE - Soil physico-chemical characterization (soil abiotic parameters)	Soil pH Soil texture Soil Organic Carbon (SOC) Soil Water Holding Capacity (WHC) Total nitrogen (N) Available phosphorus (P) Available potassium (K) Cationic exchangeable capacity (CEC)
Ecotox-LoE - Hazard of contaminants presents in the soil regarding different organisms	Acute and/or chronic effects of the soil on plant species Acute and/or chronic effects of the soil on invertebrates Acute and/or chronic effect of the soil leachates on aquatic species (transfer from soil to surface water)
Eco-LoE - Ecological and soil function analyses	Information on soil biodiversity (i.e. bacteria, microfauna, plant species…) Information on soil functionality (i.e. global metabolism, microbial respiration, enzyme activities…)

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3 Collecting and organizing the existing data and information of the EDAPHOS CS

3.1. Methods for the data and metadata collection

To facilitate this work, a specific template was developed which provide a structure to collect all relevant data and critical information that allow the assessment of the completeness and reliability of the accessible data regarding each CS at the beginning of the project.

This template was built to harmonize as much as possible the pre-existing data and metadata (method, sampling conditions, date of the analysis and any relevant remarks) coming from various sources according to the CS sites analytical background. Data entry templates were developed using the Microsoft Excel software (Figure 8) and contain 14 specific sheets allowing to collect both general information on the CS such as CS location, total surface area, area dedicated for the experiment during the project and specific information regarding the soil physico-chemical analyses, the soil pollution and any others biological information relevant for the project, especially for the soil monitoring and the ERA implementation. The template will be made available through the EDAPHOS web site (https://www.edaphos.eu/). The template is structured as follows:

Basic physico-chemical analytics:

- soil texture,
- soil pH,
- soil water holding capacity (WHC),
- soil Cation Exchange Capacity (CEC),
- soil Organic Carbon (SOC),
- soil Total Nitrogen, and other nitrogen forms (NH_4-N and NO_3-N),
- soil available P
- soil available K

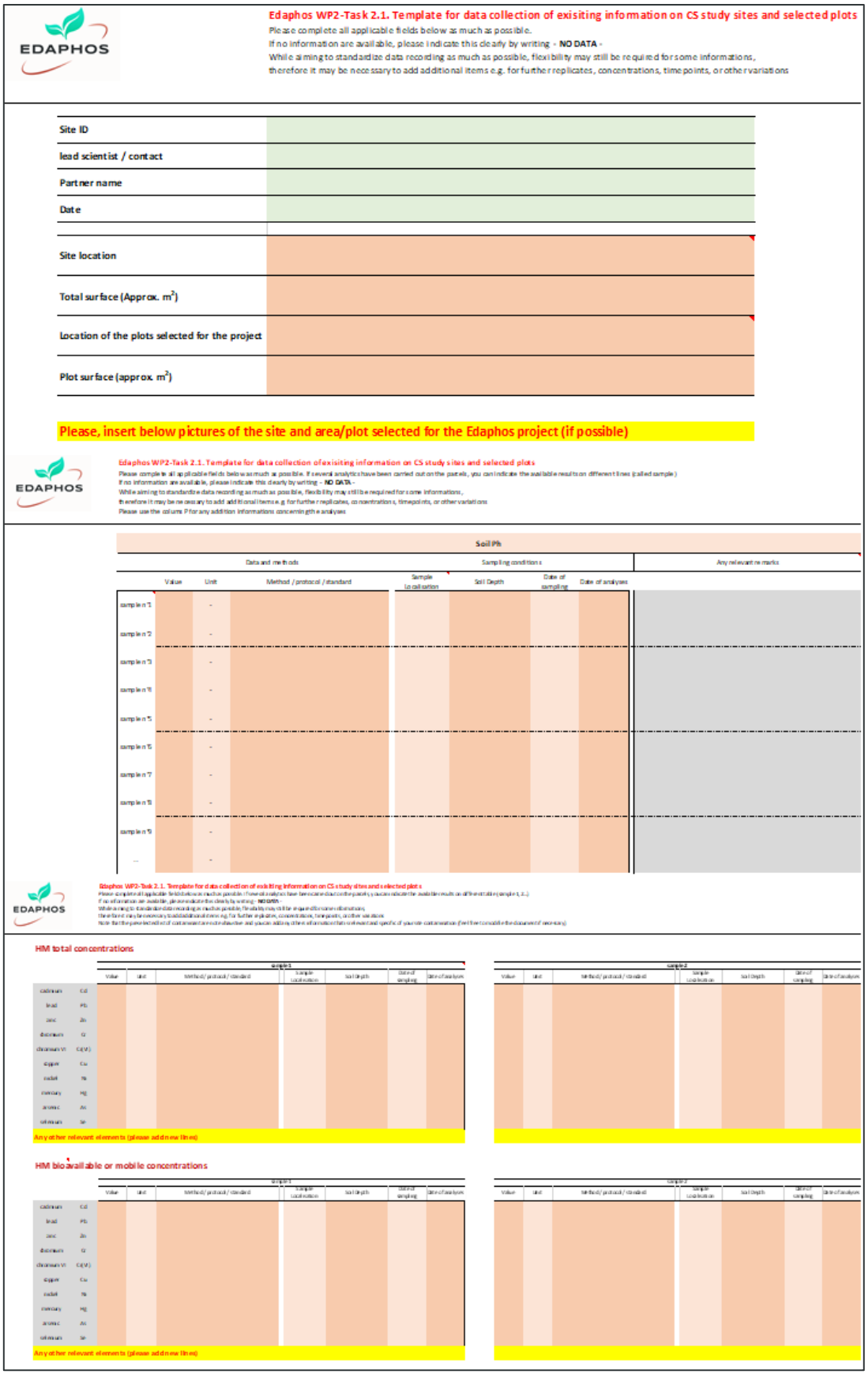
Soil contamination level:

- soil total TE contents (including all elements deemed significant in each CS study),
- available or mobile TE
- PAH, dioxine/PCB concentrations, other organic substances

Biological data (Ecotoxicological and ecological information)

- the land cover information,
- the ecotoxicological data,
- the ecological data (*i.e.* Shannon index, microbial diversity…)
- the vegetation biophysico-chemical measurements (i.e. pigments, water content…),
- the identification of major vegetation species or species assemblages,
- the bioaccumulation data
- the soil metabolism and functionality data (*i.e.* soil basal respiration, enzyme activities, organic matter, degradation capability, etc.).

The template was sent to each partner leading a CS at the beginning of 2024. The pre-existing data were collected the end of February 2024 to complete MS3 (due at month 6).



Edaphos WP2-Task 2.1. Template for data collection of exisiting information on CS study sites and selected plots

Please complete all applicable fields below as much as possible.
If no information are available, please indicate this clearly by writing - **NO DATA** -
While aiming to standardize data recording as much as possible, flexibility may still be required for some informations,
therefore it may be necessary to add additional items e.g. for further replicates, concentrations, time points, or other variations

Site ID	
lead scientist / contact	
Partner name	
Date	
Site location	
Total surface (Approx. m^2)	
Location of the plots selected for the project	
Plot surface (approx. m^2)	

Please, insert below pictures of the site and area/plot selected for the Edaphos project (if possible)

Edaphos WP2-Task 2.1. Template for data collection of exisiting information on CS study sites and selected plots

Please complete all applicable fields below as much as possible. If several analytics have been carried out on the parcels, you can indicate the available results on different lines (called sample)
If no information are available, please indicate this clearly by writing - **NO DATA** -
While aiming to standardize data recording as much as possible, flexibility may still be required for some informations,
therefore it may be necessary to add additional items e.g. for further replicates, concentrations, time points, or other variations
Please use the column P for any addition informations concerning the analyses

Soil Ph

Data and methods			Sampling conditions				Any relevant remarks
	Value	Unit	Method / protocol / standard	Sample Localisation	Soil Depth	Date of sampling	Date of analyses	
sample n°1		-					
sample n°2		-					
sample n°3		-					
sample n°4		-					
sample n°5		-					
sample n°6		-					
sample n°7		-					
sample n°8		-					
sample n°9		-					
...		-					

Edaphos WP2-Task 2.1. Template for data collection of exisiting information on CS study sites and selected plots

HM total concentrations

		sample 1						sample 2					
		Value	Unit	Method / protocol / standard	Sample Localisation	Soil Depth	Date of sampling	Value	Unit	Method / protocol / standard	Sample Localisation	Soil Depth	Date of sampling
cadmium	Cd												
lead	Pb												
zinc	Zn												
chromium	Cr												
chromium VI	Cr(VI)												
copper	Cu												
nickel	Ni												
mercury	Hg												
arsenic	As												
selenium	Se												
Any other relevant elements (please add new lines)													

HM bioavailable or mobile concentrations

		sample 1						sample 2					
		Value	Unit	Method / protocol / standard	Sample Localisation	Soil Depth	Date of sampling	Value	Unit	Method / protocol / standard	Sample Localisation	Soil Depth	Date of sampling
cadmium	Cd												
lead	Pb												
zinc	Zn												
chromium	Cr												
chromium VI	Cr(VI)												
copper	Cu												
nickel	Ni												
mercury	Hg												
arsenic	As												
selenium	Se												
Any other relevant elements (please add new lines)													

Figure 8. Example of an excel file to gather data from in each CS (general information, pH data and TE/chemical concentrations)

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3.2. Data collected

In this section, we summarize into different tables and sections all the pre-existing data collected from each CS. These data correspond to the available information of each CS at the beginning of the project.

3.2.1. Soil contamination level

Table 2 and 3 show a summary of CS contamination in soil (total and available concentration). The information about total soil contamination (Table 2) is the most complete data provided by each CS. TE content is the most important parameter to establish the pollution level of each area and the need for its recuperation. In Table 2 the different levels of pollution of each CS can be appreciated and the variety in level of the main contaminants. Apart from metals and metalloids, Table 2 also presents data about other types of contaminants. The only CS which presented some data about organic pollution are CS 1, CS 5 and CS 6. The other CS do not present these types of contamination (or have not been studied before).

Table 2. Total contents of TE and other contaminants at the EDAPHOS CS (mg kg^{-1})

	CS 1	CS 2	CS 3	CS 4	CS 5	CS 6	CS 7
Al	7716	n.d.	n.d	n.d	n.d.	n.d.	n.d.
As	n.d.	1.2	1050	15.3	n.d	35.5	296
Cd	6	<0.1	3.04	11.6	LQ	21.7	9.6
Cr	n.d	n.d.	4.08	4.02	<0.5	373	n.d
Cu	n.d.	12.8	2573	24.26	420	184	1711
Fe	12378	n.d.	n.d.	n.d	n.d	65467	n.d
Hg	n.d.	n.d.	21.81	0.1	n.d.	1.87	n.d
Mn	293	n.d.	n.d.	n.d	n.d	694	n.d
Ni	n.d.	1000	14.74	3.2	34	148	183
Pb	311	6.3	15076	436	55	15560	3588
Zn	685	43.0	468	577	394	45290	2789
Other known contaminants	PAH, pesticides	n.d.	n.d.	n.d	Organic pollutants, DDT, PAHs, PCBs.	PAH	n.d.

Table 3 shows the data collected regarding the availability of TE in soils. This information has been gathered from CS 1, CS 2, CS 6 and CS 7, for the rest of CS the information will be available during the project. As it can be seen, these concentrations are much lower than the total TE content in soil. However, the extraction methodology of available TE data is essential, depending on the extractants and procedure, the availability can differ significantly (see section 5. Methods).

Table 3. Available TE contents at the EDAPHOS CS (mg kg^{-1})

	CS 1	CS 2	CS 3	CS 4	CS 5	CS 6	CS 7
Al	0.07	<0.1	n.d.	n.d.	n.d.	n.d.	n.d
As	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
Cd	0.04	<0.1	n.d.	n.d.	n.d.	n.d.	3.1
Cr	n.d.	n.d.	n.d.	n.d.	n.d.	0.03	n.d
Cu	n.d.	0.88	n.d.	n.d.	n.d.	0.22	n.d
Fe	0.07	n.d.	n.d.	n.d.	n.d.	4.1	n.d
Hg	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
Mn	1.9	n.d.	n.d.	n.d.	n.d.	4.39	n.d
Ni	n.d.	6.6	n.d.	n.d.	n.d.	n.d.	1.9
Pb	<LQ	0.73	n.d.	n.d.	n.d.	n.d.	611
Zn	1.43	0.71	n.d.	n.d.	n.d.	n.d.	164

3.2.2. Soil physico-chemical analyses

Table 4 gathers the information about physical and chemical properties in the soil in each CS. For most CS there are data about the soil texture (% of Clay, Silt and sand) and pH. It can be observed the different texture and soil pH values of each CS. Data of soil fertility as Soil organic carbon (SOC) and Total Nitrogen (TN) were also reported for most CS, as well the nutrient availability as P and K in soil (see section 5. Methods). Data of Cationic Exchange Capacity (CEC) were also reported by some CS, although presented different extraction methods and units. In case of Water holding Capacity (WHC) no data were reported by any CS.

Table 4. Soil physico-chemical properties at the EDAPHOS CS

	CS 1	CS 2	CS 3	CS 4	CS 5	CS 6	CS 7
Clay %	6.4	25-36	6.41	60	20	n.d.	22-29
Silt %	1.8	30-37	36.9	3	50.5	n.d.	25-30
Sand %	82.6	30-45	56.6	25	29.5	n.d.	41-53
pH	7.2	7.8-8.0	3.34	n.d.	8.27	5.17-7.63	7.9-8.2
WHC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CEC	153 meq.kg^{-1}	24,3 meq Na/100g	1,62 cmol/kg	n.d.	n.d.	n.d.	12,8-19,4 meq Na/100g
SOC (mg kg^{-1})	39.6	9.7-28.2	1.76	n.d.	10.0	n.d.	15.7-27
Total N (g kg^{-1})	3.8	0.6-1.7	0.33	n.d.	1.00	n.d.	1.0-1.6
Available P (mg kg^{-1})	1.2	5.7-41.4	n.d.	n.d.	15.0	n.d.	7.5-13.6
Available K (mg kg^{-1})	n.d.	147-346	n.d.	n.d.	266	n.d.	304-695

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3.2.3. Soil biological analyses

With the aim to gather all the information available about each CS, biological data were also required. For these properties there is not much information, some data were obtained from previous projects for CS 1 and CS 6. This indicates that all new information is going to be obtained during the project according to the methodology that will be implemented.

Table 5. Soil biological properties at the EDAPHOS CS

	CS 1	CS 2	CS 3	CS 4	CS 5	CS 6	CS 7
Land cover information	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ecotoxicological data	Tree survival and height	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ecological data (shannon index…)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vegetation bio-physico-chemical measurements	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vegetation species and identification	n.d.	n.d.	n.d.	n.d.	n.d.	Quadrat method, herbaceous layer, species present and coverage	n.d.
Bioaccumulation data	Trees, leaf and stem TE concentrations	n.d.	n.d.	n.d.	n.d.	*In situ*, leaf TE concentrations	n.d.
Soil functionality data	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

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3.3. Summary of the data/knowledge gaps at the beginning of the project

The summary of all the data collected for each CS are in Table 6. This table help to identify for each parameter if we already have information ready to be used from the experimental plots for the project.

Table 6. Summary of the data gathered from each CS. For a given parameter, the green dot indicates that the data as well as the method for their analysis and additional information (*i.e.* sampling protocols, sampling period) were accurately reported, while red circle indicates the absence of data or missing information.

	CS 1	CS 2	CS 3	CS 4	CS 5	CS 6	CS 7
Soil physico-chemical characterization (soil abiotic parameters)							
Soil pH	🟢	🟢	🟢	🔴	🟢	🟢	🟢
Soil texture	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Soil Organic Carbon (SOC)	🟢	🟢	🟢	🔴	🟢	🟢	🟢
Soil Water Holding Capacity (WHC)	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Total nitrogen (N)	🟢	🟢	🟢	🔴	🟢	🟢	🟢
Available phosphorus (P)	🟢	🟢	🔴	🔴	🟢	🟢	🟢
Available potassium (K)	🟢	🟢	🔴	🔴	🟢	🟢	🟢
Cationic exchangeable capacity (CEC)	🔴	🟢	🟢	🔴	🟢	🔴	🟢
contamination level and contaminant behavior in soil and biota							
Characterization of total concentration of contaminants	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Characterization of available contaminants	🟢	🟢	🟢	🔴	🔴	🟢	🟢
Bioaccumulation of contaminants in plants and/or soil invertebrates	🟢	🔴	🔴	🔴	🔴	🔴	🔴
Ecotoxicological and Ecological information							
Land cover information	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Ecological data (shannon index, microbial diversity, alpha beta…)	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Vegetation bio-physico-chemical measurements (pigments, water content…)	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Vegetation major species or assemblage of species identification	🟢	🔴	🔴	🔴	🔴	🟢	🔴
Bioaccumulation on terrestrial species	🟢	🔴	🔴	🔴	🔴	🟢	🔴
Soil functionnality data (soil respiration, enzyme activies, OM biodegradation…)	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Ecooxicological data	🔴	🔴	🔴	🔴	🔴	🔴	🔴

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4 Methods and protocols used for the trace elements analyses in soil and in plant tissues

To ensure consistency in the methodology of the project, the methods used to obtain the data for each variable should be documented. This section aims to describe the methodologies applied by EDAPHOS partners for each CS. Specifically, this section describes the methods used for each abiotic variable, including any specific details relevant to each CS.

4.1. Total content of TE in soils

Usually, rather than assessing the complete TE content in the soil, a "pseudo-total" fraction is determined using robust acids or aqua regia in the digestion process. This method, which notably excludes the use of hydrofluoric acid (HF), typically omits TE that are firmly bonded to silicates. Consequently, the pseudo-total content indicates the maximum amount of TE that is potentially soluble and mobile within the soil matrix. This measurement is crucial as it highlights the upper limit of potential TE contamination that can occur in a specific soil sample. This approach provides valuable insight into the environmental impact and the risk associated with TE pollution in soils, offering a more practical assessment of contamination levels (González et al., 2009).

For the determination of total contents of TE in soil there are different steps to consider as follows:

4.1.1. Extraction protocols

Depending on partners, two main protocols were reported:

- The acid digestion using aqua regia and according to the ISO 54321:2020 standard protocol: The aqua regia solution consists in a solution 1 HNO_3 / 3 HCl (CS 1, CS 3, CS 4, CS 5 and CS 6).
- The use of HNO_3: H_2O_2: HCl according to the reference AOAC Official Method 990.08 (CS 2 and CS 7).

4.1.2. TE analyses

ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) and ICP-MS (Inductively Coupled Plasma Mass Spectrometry) are analytical techniques generally used to detect and quantify TE in various samples and depending on sample concentrations. Both were reported:

- **ICP-OES** (CS 1, CS 2, CS 4, CS 5, CS 6 and CS 7)
- **ICP-MS** (CS 3)

4.2. Available TE in soils (chemical extraction)

The presence of a contaminant in soil does not automatically result in its phytoavailability or bioavailability to other invertebrate species in soil or even disrupt the soil's functionality. For a TE to be absorbed by an organism, it must be in a form that could be "bioavailable". Bioavailability refers to the state in which the element is biologically accessible and can be taken up by an organism, allowing it to interact with the metabolic processes of the organism. In other words, the fraction of the total element concentration that can interact with the organism in question.

The bioavailability of TE plays a critical role in the soil-plant-food chain pathway. Various methods are employed to determine the bioavailability of TE, with neutral salts such as calcium chloride ($CaCl_2$), and ammonium nitrate (NH_4NO_3) being particularly used (Kabata-Pendias, 2004; Kumpiene et al. 2014). Extensive research has validated the efficacy of these salts, especially concerning their interaction with TE and plants. Recent findings have shown that these extractions accurately

represent the potential transfer of TE from soil to plants (Burgos et al., 2008). Chelating agents, including EDTA and DTPA, are also widely used to estimate the bioavailability of TE. These agents excel at extracting dissolved TE as well as those that are weakly adsorbed or exchangeable in the soil. However, it has been observed that these chelating agents may sometimes overestimate the availability of TE in soils.

Despite their prevalent use, it is important to recognize that the fractions identified in these procedures are entirely operational. Consequently, the information they provide is primarily qualitative, meaning the results should be interpreted as indicative rather than definitive. Additionally, the use of different methodologies can yield significantly varied results, underscoring the necessity for careful consideration when selecting and applying these techniques.

The methodology is based on the ISO 17402:2008 Soil Quality standard: Guidance on the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials.

4.2.1. Extraction protocols

Depending on the partner, different protocol for the extraction were reported:

- The extractions of TE using $CaCl_2$ at 0.01 M (1:10 soil solution ratio), according to Houba et al. (2000) (CS 1, CS 3 and C S6).
- The extraction of TE by buffered DTPA solution following the ISO 14870:2001 standard protocol (CS 2 and CS 7).
- The determination of TE after extraction with ammonium nitrate following the ISO 19730:2008 standard protocol (CS 4).

4.2.2. TE analyses

Like those described in section 5.1.2.

4.3. Determination of TE concentration in plant tissues

The analysis of TE concentration in an ERA is crucial for evaluating the potential toxicity to the environment. These TE can bioaccumulate in the ecosystem, affecting the food chain and causing long-term adverse effects. Identifying and quantifying their presence allows for the implementation of mitigation measures to protect biodiversity and the safety of the living organisms.

4.3.1 Extraction protocols

Acid digestion is a common method used for preparing plant tissues for TE analyses. This digestion successfully breaks down complex organic matrices in plant tissues, ensuring that TE are in a suitable form for precise and accurate determination. This process involves breaking down the organic matrix of plant materials using strong acids, resulting in a solution that contains the TE in a form that can be analyzed by various techniques. A mixture of concentrated acid is used. The commonly used acids include:

- Nitric acid (HNO_3): Oxidizes organic material and converts TE to soluble nitrates
- Perchloric acid ($HClO_4$): Strong oxidizing agent that helps break down tough organic matrices
- Hydrochloric acid (HCl): Helps in dissolving some TE compounds

4.3.2 Methods for the determination of TE

Similar to those described in section 5.1.2.

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4.4. Analytics validation for soil and plants TE analyses

As the project aimed at comparing results obtained through time and from a CS to another, it is crucial to maintain coherence and accuracy of the analyses throughout the project. Consequently, it is essential to validate and calibrate as much as possible the methods used by each partner for a set of basic physical and chemical parameters, mainly for the TE analyses in soil and plant tissues.

Reference materials are reliable quality assurance tools that improve confidence in test results obtained by laboratories. They play a key role in the calibration of laboratory instruments by providing precise reference values and data. For this purpose, reference certified material must be used to check the accuracy and reproducibility of the data.

For the soil analyses, these certified materials include:

- Loamy Clay 1 CRM052 (Supplied by LGC Promochem, Molsheim) - (CS 1 and CS 6)
- Loam Soil ERM-CC141 (ISO 17034; ISO/IEC 17025) (Supplied by LGC Promochem, Molsheim) - (CS 3)

Partners also reported other methods to check the accuracy and reproducibility of their analytical data:

- Interlaboratory Study of Inductively Coupled Plasma Atomic Emission Spectroscopy Method 6010 and Digest Method 3050, NTIS No. PB88-124318, National Technical Information Service, 5285 Port Royal Rd, Spring field, VA 22161, USA. JAOAC 73, 404(1990) - (CS 2 and CS 7).
- CEN EN 16174:2012 and ISO 11885:2009-(CS 4)
- Standard solution: Reference N: 111355.L1- Lot Nº 914014 - (CS 5)

In addition to the validation by reference material, exchange of soil and plant reference samples will be organized along the different partners involved in analytics to assess the accuracy of results and ensure agreement across the different laboratories. Partners who do not currently use certified reference samples will acquire the recommended ones.

For the TE in plant tissues, the certified reference based on tobacco leaves (INCT-OBTL-5), supplied by LGC Promochem, Molsheim, will be used.

5 Methods and protocols for abiotic soil parameters

5.1 Soil pH determination

The soil pH is a measure of the acidity or alkalinity of a soil. Soil pH is considered as a key parameter when characterizing a soil as it will determine the availability of certain nutrients in the soil and can regulate and control many chemical and biochemical reactions within the soil. Mainly, two methods are used to determine the soil pH:

- Determination of soil pH in water using a soil: water ratio of 1:2.5 (m/v) and after shaking for one hour. The pH determination is then made using a calibrated glass electrode. (CS 1, CS 4, and CS 6)
- Determination of soil pH using KCl (1M) using a soil: solution ration of 1:2.5 (m/v) after 30 mixing and 30 min standing. The pH determination is then made using a calibrated glass electrode. (CS 3 and CS 5)

Both approaches are covered by the standard protocol ISO 10390:2021 (soil, treated biowaste and sludge – Determination of pH). This document specifies an instrumental method for the routine determination of pH within the range pH 2 to pH 12 using a glass electrode in a 1:5 (volume fraction) suspension of soil, sludge and treated biowaste in either water (pH in H_2O), in 1 mol/l potassium chloride solution (pH in KCl) or in 0,01 mol/l calcium chloride solution (pH in $CaCl_2$).

5.2 Soil Organic Carbon determination

Soil organic carbon (SOC) represents the amount of carbon retained in the soil after the decomposition of the organic content. SOC only refers to the carbon component of organic compounds and is the major component of soil organic matter. SOC is extremely important in all soil processes and is considered as a vital indicator in soil health assessment. Several methods for its determination were reported by the different partners:

- Determination by dry combustion (elementary analyses) following the ISO 10694:1995 (CS 1, CS 3, CS 4 and CS 6)
- Determination using dichromate oxidation techniques following the Walkley-Black method and its derivates protocols: the modified Moebius procedure (CS 2 and CS 7) or the Springer-Klee protocol (CS 5).

The significant modification in the Modified Mebius Procedure is the extension of the oxidation time compared to the standard Walkley-Black method. This extended period allows for more thorough oxidation of organic matter in the soil sample, potentially improving accuracy, especially for soils with high organic carbon content or difficult-to-oxidize organic compounds. Sometimes, additives such as phosphoric acid (H_3PO_4) are included to help stabilize and control the oxidation process, ensuring complete oxidation and minimizing side reactions that could affect the accuracy of the results.

The Springer-Klee method, also known as the Springer-Klee modification of the Walkley-Black method. Like the Walkley-Black method, soil samples are initially mixed with a potassium dichromate ($K_2Cr_2O_7$) solution in the presence of sulfuric acid (H_2SO_4). However, in the Springer-Klee method, the oxidation time is extended beyond the standard 30 min used in the Walkley-Black method. The extended oxidation time (often up to 2 hr or more) is intended to ensure more thorough oxidation of organic carbon compounds present in the soil sample.

5.3 Soil Texture determination

The soil texture refers to the proportion of sand, silt and clay sized particles that constitute the mineral fraction of the soil. While several methods exist for determining the soil texture (*i.e.* sieving methods, sedimentation method by hydrometer, laser diffraction method).

The hydrometer methods were generally applied by the different partners to determine the soil texture for CS 2 and CS 7 (Bouyoukos protocol, 1951) and CS 3 (Gee and Bauder protocol, 1979).

The hydrometer method developed remains a fundamental technique in soil science for determining soil particle size distribution. By leveraging principles of sedimentation and particle settling, this method provides essential data for understanding soil texture and its implications for agricultural and engineering applications. The ISO 11277:2020 (Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation) provides recommendation for its determination.

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5.4 Soil Total Nitrogen (N) determination

Total nitrogen in soil (N) refers to the different available forms of N found in soil whether organic or inorganic (Nitrate, Ammonium). Most of the EDAPHOS CS presented data for the total N. Two methods were used for its determination:

- Dry combustion with elemental analyser following the ISO 13878:1998 standard (Determination of total nitrogen content by dry combustion ("elemental analysis") (CS 1, CS 3 and CS 6).
- Method of Kjendhal following the ISO 11261:1995 standard (Soil quality — Determination of total nitrogen — Modified Kjeldahl method) (CS 2 and CS 7).

5.5 Soil available Phosphorus (P) determination

The soil available P is the fraction of total P in soil that is readily available for absorption by plant roots. In the case of available P, different methodologies were reported:

- The determination of phosphorus soluble using a 0.1-mol. l^{-1} ammonium oxalate extraction solution ($(NH_4)_2C_2O_4$) following the Joret-Hébert method. This method is standardized (NFX31-161 - Soil quality - Determination of phosphorus soluble in a 0.1 mol.l^{-1} ammonium oxalate solution - Joret-Hébert method) (CS 1 and CS 6).

- The determination of phosphorus in the soil using a sodium bicarbonate extraction solution ($NaHCO_{3)}$ following the Olsen methods. This method is standardized (ISO 11263:1994- Determination of phosphorus — Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution) (CS 2, CS 3, CS 5 and CS 7).

5.6 Soil available Potassium (K) determination

The soil available K is the fraction of total K in soil that is readily available for absorption by plant roots. One method was reported by several partners of its determination on different CS (CS 2, CS 3 and CS 7).

In this method, the available K in soil is determined using the neutral 1N ammonium acetate (NH_4OAc) extraction method. This well-established protocol is widely used due to its reliability and effectiveness in extracting exchangeable potassium.

5.7 Cation exchange capacity (CEC)

The CEC is the ability of a soil to hold positively charged ions (cations). This property is important as it influences the soil structure stability and the nutrient availability to biota. As CEC increases, more nutrients are held tighter to the soil particles and less are available in the soil solution/soil water.

This parameter was not reported by any partner for any of the EDAHOS CS. However, several methods exist to measure the CEC of a soil, and some are standardized. Among them, the ISO 23470:2018 specifies a method for the determination of cation exchange capacity (CEC) and the content of exchangeable cations (Al, Ca, Fe, K, Mg Mn, Na) in soils using a hexamminecobalt(III)chloride solution as extractant. This protocol is applicable to all types of air-dry soil samples.

5.8 Water holding capacity (WHC)

The WHC determines the ability of a soil texture to physically hold water after all gravitational water drains out. The WHC is considered as the maximal amount of water that a soil can hold in this

situation. The ISO 16586:2003 (Determination of soil water content as a volume fraction based on known dry bulk density — Gravimetric method) is a relevant method to determine the WHC of a soil.

6 Methods and protocols for the ecotoxicological and ecological characterization

The following section describes the protocols which are relevant to characterize (i) the hazard of contaminants present in the soil (ecotoxicity of soil matrix) or soil leachates (potential effect of mobile substances to the aquatic environment) regarding different soil/ aquatic organisms, and (ii) the ecological and the soil function parameters. These protocols were selected to cover the different parameters identified in section 3 which are required for the ERA and which cover the Ecotox-LoE and Eco-LoE of the triad approach.

6.1. Methods and protocols for soil ecotoxicity assessment

6.1.1. Determination of the effects of pollutants on soil flora / Part 1: Method for the measurement of inhibition of root growth (ISO 11269-1:2012)

Scope: The ISO 11269-1 describes a method for the determination of the effects of contaminated soils or contaminated samples on the root elongation of terrestrial plants. The test method described in this part of ISO 11269 can be used to compare soils, to monitor changes in their activity or to determine the effect of added chemicals or materials (compost, sludge, waste).

Main endpoint: root elongation using *Avena sativa*

Test duration: 7 days

ERA LoE: Ecotoxicity

6.1.2 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)

Scope: The ISO 11269-2 describes a method to assess the quality of an unknown soil and the soil habitat function by determining the emergence and early growth response of at least two terrestrial plant species compared to reference or standard control soils. It is applicable to soils of unknown quality, e.g. from contaminated sites, amended soils or soils after.

Main endpoints: shoot elongation, shoot dry biomass production

Test duration: 16 to 19 days (depending on the test species). Two test species will be used *Avena sativa* (Monocotyledonae) and *Sinapis alba* (Dicotyledonae).

ERA LoE: Ecotoxicity

6.1.3 Contact test for solid samples using the dehydrogenase activity of Arthrobacter globiformis (ISO 18187:2024)

Scope: The ISO 18187:2024 specifies a rapid method for assessing solid samples in an aerobic suspension, by determining the inhibition of dehydrogenase activity of *Arthrobacter globiformis* using the redox dye resazurin. It is applicable for assessing the effect of water-soluble and solid matter bounded non-volatile contaminants in natural samples, such as soils and waste materials. This is an ecologically relevant assay as far as it uses a ubiquitous soil bacterial species with high affinity to surfaces whose dehydrogenases are involved in different biological mechanisms withstanding

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bacteria integrity (e.g. respiratory chains). Moreover, it has been noticed that this parameter (dehydrogenase activity inhibition) is quite sensitive to different toxic substances.

Main endpoints: dehydrogenase activity

Test duration: 2 hr

ERA LoE: Ecotoxicity

6.1.4 Effects of pollutants on earthworms / Part 1: Determination of acute toxicity to *Eisenia fetida/Eisenia andrei* (ISO 11268-1:2012)

Scope: The ISO 11268-1:2021 specifies one of the methods for evaluating the habitat function of soils and determining the acute toxicity (mortality) of soil contaminants and chemicals to *Eisenia fetida/Eisenia andrei* by dermal and alimentary uptake. It is applicable to soils and soil materials of unknown quality, e.g. from contaminated sites, amended soils, soils after remediation, agricultural or other sites concerned, and waste materials.

Main endpoint: worm mortality

Test duration: 14 days

ERA LoE: Ecotoxicity

6.1.5 Effects of pollutants on earthworms / Part 2: Determination of effects on reproduction of *Eisenia fetida/Eisenia andrei* and other earthworm species (ISO 11268-2:2023)

Scope: The ISO11268-2:2023 specifies one of the methods for evaluating the habitat function of soils and determining the effects of soil contaminants and chemicals on the reproduction of *Eisenia fetida/Eisenia andrei* by dermal and alimentary uptake. This chronic test is applicable to soils and soil materials of unknown quality, e.g. from contaminated sites, amended soils, soils after remediation, agricultural or other sites concerned, and waste materials.

Main endpoint: worm reproduction (number of juveniles)

Test duration: 2 months

ERA LoE: Ecotoxicity

6.1.6 Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of *Caenorhabditis elegans* (Nematoda) (ISO 10872:2020)

Scope: This document specifies a method for determining the toxicity of environmental samples on growth, fertility and reproduction of the nematod *Caenorhabditis elegans*. The method applies to contaminated whole freshwater sediment, soil and waste, as well as to pore water, elutriates and aqueous extracts that were obtained from contaminated sediment, soil and waste. Nematodes are one of the most abundant and species-rich metazoans in sediments and soils and possess key positions in benthic and soil food webs due to the evolution of various feeding types (bacterial, algal, fungal and plant feeders, omnivores, predators. Moreover, they are well acknowledged as environmental indicators for assessing the toxicity of chemicals and the quality of sediments and soils.

Main endpoints: nematod survival, nematod growth, nematod reproduction

Test duration: 4 days

ERA LoE: Ecotoxicity

6.2. Methods and protocols for the soil eluate ecotoxicity assessment

6.2.1 Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) — Acute toxicity test (ISO 6341:2012)

Scope: This document specifies a procedure for the determination of the acute toxicity of chemicals, waters and waste waters to the water flea *Daphnia magna* Straus. Crustaceans are of interest from the ecotoxicological point of view because they are primary consumers and a major component of the zooplankton in aquatic ecosystems.

Main endpoint: Daphnids mobility

Test duration: 2 days

ERA LoE: Ecotoxicity (soil eluates)

6.2.2 Fresh water algal growth inhibition test with unicellular green algae (ISO 8692:2012)

Scope: This document specifies a method for the determination of the growth inhibition of unicellular green algae by substances and mixtures contained in water or by wastewater. This method is applicable for substances that are easily soluble in water.

Main endpoint: Algal growth rate

Test duration: 3 days

ERA LoE: Ecotoxicity (soil eluates)

6.2.3 Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) (ISO 11348:2007)

Scope: This document specifies methods for determining the inhibition of the luminescence emitted by the bacterium *Vibrio fischeri*.

Main endpoint: bacterium luminescence

Test duration: 2 hours

ERA LoE: Ecotoxicity (soil eluates)

6.3 Methods and protocols for the ecological and soil functions characterization

6.3.1 Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil (ISO 17601:2016)

Scope: The ISO 17601:2016 specifies and methods to determine the microbial diversity by quantitative polymerase chain reaction (qPCR) amplification. This technic allows for the measurement of the abundance of specific microbial gene sequences from a soil DNA extract, thereby providing an estimate of the abundance of specific microbial groups. The microbial diversity in soil refers to the variety and abundance of microorganisms (i.e. bacteria, fungi) present in the soil ecosystem which is of importance for maintaining major ecological function as organic matter decomposition, nutrient cycling…

ERA LoE: Ecology / indicator of soil biodiversity

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6.3.2 Sampling of soil invertebrates - Part 4: Sampling, extraction and identification of soil-inhabiting nematodes (ISO 23611-4:2022)

Scope: This document specifies a method for sampling and handling free-living nematodes from terrestrial field soils as a prerequisite for using them as bio-indicators (e.g. to assess the quality of a soil as a habitat for organisms). This document applies to all terrestrial biotopes in which nematodes occur. Nematod community structure integrates information of the soil micro-food web (microbial compartment, microfauna and mesofauna) which is responsible for the decomposition and mineralization of nutrients through organic matter transformation. The abundance and diversity of nematodes provides insights into the soil biological functioning as they occupy different levels of the soil food web (Ekschmitt et al., 2001). The calculation of various indices, based on the abundance and composition of nematode communities, is here used to assess nutrient flows, environmental stability or the diversity of organisms in the soil.

Main endpoints: nematode abundance and diversity and community structure

ERA LoE: Ecology / indicator of soil biodiversity and soil process

6.3.3 Test for estimating organic matter decomposition in contaminated soil (ISO 23265:2022)

This document specifies a test procedure for the evaluation of the habitat function of soils by determining effects of soil contaminants and substances on organic matter decomposition. This test is applicable to natural soils and soil materials of unknown quality (e.g. contaminated sites, amended soils, soils after remediation, agricultural or other sites under concern). This document also specifies how to use this method for testing substances under temperate conditions. The ability of soil microorganisms to decompose lignin cellulosic material provides evidence that the microbial population in soil is active in organic matter decomposition and carbon cycling.

Main endpoint: kinetic of the degradation of cellulosic materials

Test duration: up to 60 days

ERA LoE: Ecology / indicator of soil process

6.3.4 Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates (ISO 20130:2018)

Scope: This document specifies a method for the measurement of several hydrolase activities (arylamidase, arylsulfatase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-glucosaminidase, acid, alkaline and global phosphatases, urease) in soil samples, using colorimetric substrates. Enzymes are responsible for the degradation of organic molecules and their mineralization. Extracellular enzymes in soil play key roles in the biodegradation of organic macromolecules. The monitoring of several enzyme activities important in the biodegradation of organic compounds and mineralization of carbon, nitrogen, phosphorus and sulphur in soil may reveal harmful effects caused by chemicals and other anthropogenic impacts.

Main endpoints: Hydrolase activities

ERA LoE: Ecology / indicator of soil process

6.3.5 Laboratory methods for determination of microbial soil respiration (ISO 16072:2002)

Scope: this document describes methods for the determination of soil microbial respiration of aerobic, unsaturated soils. The methods are suitable for the determination of O_2 uptake or CO_2

release, either after addition of a substrate (substrate-induced respiration), or without substrate addition (basal respiration). This process reflects the overall metabolic activity of the soil microbial community, and a high respiration rate generally indicates a high level of organic matter decomposition. This method is applicable to the measurement of soil respiration to determine the microbial activity in soil, establish the effect of additives (nutrients, pollutants, soil improvers, etc.) on the metabolic performance of microorganisms and determine the microbial biomass.

Main Endpoint: soil respiration rate

ERA LoE: Ecology / indicator of soil process

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Conclusions

This deliverable compiled the pre-existing data and information at the beginning of the EDAPHOS project for the different CS. It describes the relevant parameters needed for the soil monitoring and the ecological risk assessment according to the holistic "Triad" approach.

The parameters as well as the methods/protocols reported and applied by the different partners cover information on (i) the soil contamination level and contaminant behaviour in biota (bioavailability, bioaccumulation in plant and invertebrates), (ii) the soil physico-chemical characterization (abiotic parameters), (iii) the ecotoxicological assessment of the contaminated soil regarding soil organisms (direct exposure) or aquatic organisms (indirect exposure by soil eluates) and (iv) the ecological and soil function characterization.

These methods and protocols will then be applied to the different CS of the project to (i) performed the initial ERA on each CS, before the implementation of nature-based solution (NBS) (WP2, Task 2.2 and 2.3), and (ii) to monitor the success of NBS implemented on the different CS along the project (WP3).

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