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FIELD PROTOCOLS

ENGLISH

ECHO

SOIL HEALTH ASSESSMENT

In ECHO, you will assess soil health by focusing on eight key indicators described in the Misson Soil Implementation Plan, helping you to better understand the condition of your soil. This section provides a step-by-step guide for proper sample collection, which will be used to evaluate all eight soil health indicators. Specifically, you will collect information both directly in the field and through laboratory analysis to evaluate:

1. **Presence of pollutants and soil nutrients**
2. **Soil organic matter**
3. **Soil structure and soil texture**
4. **Soil biodiversity**
5. **Soil pH**
6. **Vegetation cover**
7. **Landscape heterogeneity**
8. **Forest cover**

As a citizen scientist, you will receive the ECHO toolkit from your ECHO Ambassador, with each kit designed to collect a single soil sample, and containing all the materials needed for soil sampling, including containers and clear instructions.

The toolkit is designed to include everything necessary for accurately analysing the soil health indicators evaluated in ECHO, except for a container with a small amount of tap water, which you will need to bring on the sampling day for the soil texture assessment. Videos showing all the procedure are available on our ECHO YouTube channel (**https://www.youtube.com/@ECHOsoilproject**). Additionally, you can explore detailed information evaluated using the ECHO kit by consulting factsheets for each indicator, which are available for download through the ECHO app.

Detailed content of the ECHO toolkit:

1. Field instructions;
2. Protective gloves;
3. A metal trowel;
4. A wooden spoon;
5. A 15 mL plastic tube containing distilled water;
6. A paper strip;
7. A 5 mL plastic tube containing preservation solution*;
8. A small biodegradable plastic bag;
9. A large biodegradable plastic bag with a QR code.

(*) The preservation solution is not harmful, and a datasheet from the company provides full details about it. However, handle the tube carefully, wear gloves for safety, and avoid drinking the solution. Keep the kit out of reach of unsupervised children to prevent accidental ingestion.

FIELD PROTOCOLS

Although various methods could be considered for soil analysis based on the indicators outlined in the Mission Soil Implementation Plan, ECHO activities have been simplified and tailored for citizen use. This approach emphasizes its primary role in citizen science and engaging also younger participants, who, with adult support, can follow and understand the activities.

In ECHO, citizens will collect data both directly through on-site field activities and indirectly via laboratory-based (off-site) analyses to evaluate the indicators described earlier.

The detailed protocols in the following sections are designed to guide citizens in assessing soil health at their chosen location. The field protocols are a supplement to the handbook derived from D2.3, providing a deeper understanding of the theory behind each protocol for assessing the eight soil health indicators.

ON-SITE ACTIVITIES

1. SELECTING THE SAMPLING SITE AND TIME

As a citizen science project, ECHO allows each participant to independently choose their sampling location. If you have any doubts about the area you've selected, feel free to contact the ECHO team or your designated ECHO Ambassador for guidance.

Choosing the right sampling location is crucial to ensure accurate and meaningful results. A well-chosen site helps capture the true characteristics of the soil, reflects local conditions, and provides valuable data to assess soil health effectively.

It is best to avoid sampling when the soil is too wet, such as after heavy rainfall, or too dry, such asduring summer heat waves. In colder regions, don't sample when soil is frozen and covered with snow in winter and saturated by water in spring. Rather, it is recommended to collect samples in the summer months for more representative results.

Please note that one ECHO kit is designed for a single complete sampling. The materials inside are limited to one sampling.

If collecting soil samples as a group of citizen scientists, please ensure that:

1. You complete one sampling, following all the required steps through the ECHO App, before opening another kit.
2. It is important to ensure that the distance between sampling sites is at least 50 meters.

PRACTICAL ADVICE

Before collecting any soil samples, make sure you have the necessary authorization to sample the chosen site, especially if it is not your own property. If the sampling site is inaccessible (e.g., due to barriers or restricted zones) do not take any risks, and instead, choose an alternative site. Also avoid sampling in fragile environments (e.g. legally protected areas) which are sensitive to disturbances and vulnerable to degradation.

2. GPS COORDINATES

Once you have selected your sampling location, it is important to record the GPS coordinates, as this helps us identify the area's specific context and characteristics, complementing the data you collect. Additionally, it enables better mapping of soil health across regions, facilitates comparisons between different locations, and ensures the reproducibility of the study, contributing to the creation of a citizen science-generated soil map.

The GPS coordinates saved on the map will indicate an area, not an exact point. If you are in an area without an internet connection, you can save the coordinates offline. If you need help with recording your GPS coordinates, contact us in advance or reach out to your ECHO Ambassador.

Your coordinates will only be shared once you have given authorization through the ECHO App, and they will only be used for the duration of the sampling.

3. VEGETATION COVER, FOREST COVER, LANDSCAPE HETEROGENEITY

Describing vegetation, forest cover, and landscape heterogeneity is essential as it provides context for understanding soil health and its interaction with the surrounding environment. Such information helps assess how land use and biodiversity influence soil conditions. Additional data collected through the ECHO app supports this step, offering valuable insights to enrich the analysis.

Try to take clear, high-quality pictures that really capture the surroundings. If you are unsure about anything, feel free to reach out to us or your ECHO Ambassador for help.

The ECHO App will ask for permission to access your camera so you can take the photos. Once you give permission, the app will only use the camera for sampling, and your photos will be shared for that purpose only.

Conduct a thorough walk around the site to identify any potential sources of contamination or disruptions, such as nearby roads, traffic, and industrial facilities. Additionally, if you have any prior knowledge of the area, include relevant details about waste disposal sites, agricultural activities, pesticide use, standing water, or indications of recent flooding. Please provide any other pertinent information that can help describe the surrounding landscape in the comments. The more comprehensive the information, the better.

Photos will be used to support environmental assessments. They help assess the density and health of plant species, and document signs of soil degradation or erosion. Photos also aid in identifying visible biodiversity, analysing land use patterns, and observing human activities in the area. Additionally, they contribute to creating visual maps of the landscape and are useful for communicating findings through reports and presentations.

Figure 1 gives you some examples of photos to upload on the ECHO App.

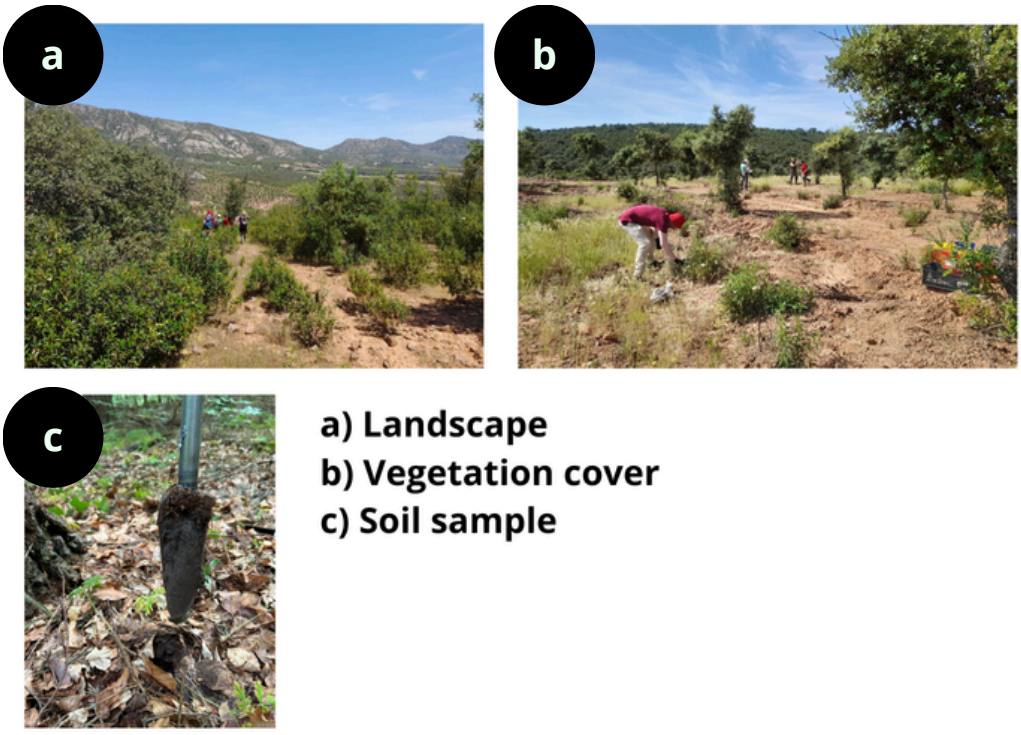

***Figure 1**: Examples of photos to upload on the ECHO App*

4. SOIL DIGGING PROCEDURE

Various guidelines exist for soil sampling. For instance, the LUCAS guidelines recommend creating a composite soil sample by mixing several subsamples collected from different locations within a defined area to create a single, averaged sample, providing a general representation of the area. In contrast, ECHO adopts the discrete soil sampling approach, which involves collecting soil from a single, specific location without mixing it with other samples, representing the unique characteristics of that exact spot and useful for identifying localised contamination or assessing variability across an area.

For ECHO, it is important to carefully follow this procedure to prepare the sampling site and to collect the exact volume of soil needed for analysis.
Here is how to proceed with soil sampling (**Figure 2**):

1. Use the trowel to carefully rake away the litter and/or the upper soil layer, including dense grass, roots, vegetation residues, and stones. If you encounter roots or rocks in the topsoil that make digging difficult, feel free to move a few meters away* and collect the soil sample from a new location.
2. Use the trowel to dig a 30x30x30 cm soil pit. The length of the trowel blade (excluding the handle) is 15 cm, so dig to a depth of 30 cm by measuring two times the length of the blade. Remove soil from the pit and begin assessing the different soil health indicators.
3. Leave all the soil you collect during digging on one side, without mixing it. Try to keep the soil structure intact as you collect it, for the first analyse of soil structure.

(*) Always keep in mind that if collecting soil samples for ECHO as a group of citizen scientists, the distance between sampling sites must be at least 50 meters.

***Figure 2**: Soil digging*

5. SOIL STRUCTURE

Using the soil you have just collected from the 30x30x30 cm soil pit, you will conduct a Visual Evaluation of Soil Structure (Ball et al., 2007) (VESS). The VESS method has been simplified in this project to ensure that you can carry out the procedure in the easiest and most accurate way possible, helping you assess the soil's structure effectively without requiring advanced expertise.

How to perform the VESS method (**Figure 3**):

1. From the soil sample left on one side (section), gently open the soil block;
2. Break the soil into smaller aggregates;
3. Evaluate the aggregates using your hands:
    a. Do they crumble easily?
    b. Can you break them with one hand?
    c. Does it take strength to break them with one hand?
    d. Does it require considerable effort to break larger aggregates?
4. Assess soil structure according to the VESS grid available on the ECHO App;
5. Record soil structure on the ECHO App.

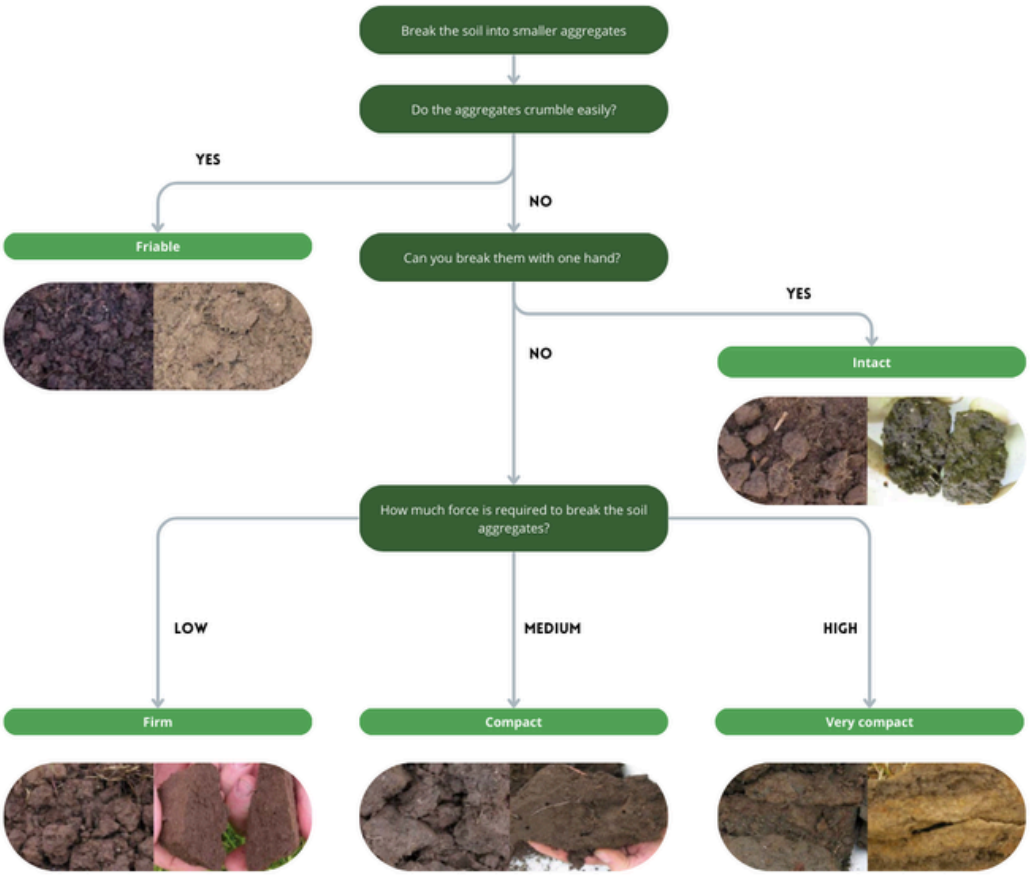

***Figure 3**: Reference grid to use for the visual evaluation of soil structure (adapted from Agriculture and Horticulture Development Board)*

6. SOIL BIODIVERSITY IN TERMS OF EARTHWORMS

In ECHO, you will assess soil biodiversity on-site by counting earthworms, as their presence can reveal much about the soil structure and quality. However, numbers of earthworms are only relevant when you identify the species present. Following the process detailed below helps ensure an accurate earthworm count while preserving the soil for further testing.

Here is a clear step-by-step guide to help you with the earthworm count:

1. Take the soil sample that you have already left on one side and carefully break it apart by hand.
2. Place any earthworms you find on the soil surface next to the pit. *Be aware that some earthworms will react to light and try to move away (surprisingly quickly!).*
3. Count the earthworms and record the number in the ECHO App.
4. Gently return the earthworms to the soil.
5. Leave aside the soil sample you removed from the pit for further analysis.

Additionally, check for the presence of other animals such as slugs, snails, spiders, woodlice, millipedes, centipedes, beetles, ants and moles, and add this information to the “Observation box”.

7. PRESENCE OF POLLUTANTS

Visually inspect the sampling site for plastic, metal debris and litter. Look for visible fragments that may be on the surface or embedded in the soil. These observations can provide valuable context for understanding soil characteristics, since these pollutants can significantly impact soil health and ecosystem function and highlight potential contamination that may affect the results of the analysis of your soil sample.

How to observe the presence of pollutants:

1. Use the hole you dug for the soil sample to check for fragments, like large pieces of plastic or metal, within the soil.
2. Observe the visible debris and record the number and the size in the ECHO App.
3. Add further comments in the ECHO App that you think may be useful to understand possible sourcesof contamination.

8. SOIL ORGANIC MATTER

In ECHO, you will evaluate the content of soil organic matter (SOM) by comparing soil colour to a colour chart provided through the ECHO App (**Figure 4**).
This colour chart is used because soil colour is a reliable indicator of organic matter content, with darker soils typically containing more organic material.

Steps to assess SOM content:

1. Take a spoonful of the mixed soil sample.
2. Compare the soil sample to the colour chart available on the ECHO App.
3. Select the corresponding SOM content value on the ECHO App.
4. At some sampling sites, soil organic matter can be much higher than 5% with no mineral soil at 30 cm depth (e.g., peatland, or former peatland). Organic soil can be recognized by the intense dark colour, comprising of decomposing vegetation and lack of sand, clay or other mineral soils. Organic soils differ from mineral soils in their biological and structural features and defining soil texture is not possible. Thus, select the "Other" box on the ECHO App instead of selecting a value.

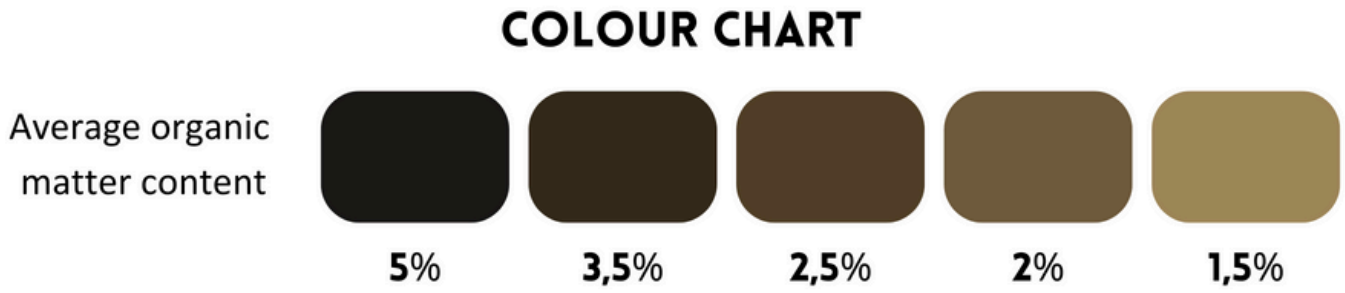

***Figure 4**: Soil colour chart to assess soil organic matter content*

9. SOIL TEXTURE

In ECHO, you will assess soil texture by conducting the “texture-by-feel method” (**Figure 5**), adapted from USDA soil quality guide. This method allows you to estimate the soil texture based on its feel and consistency when handled in your hands.

By testing how the soil feels when you moisten and manipulate it, you can classify the soil as sand, silt, clay, or a combination of these, which provides important information about soil texture and its ability to retainwater and nutrients.

How to perform the “texture-by-feel method”:

1. Remove any plants and roots, then disaggregate and mix the soil sample previously taken and well-mixed from the 30x30x30 cm pit, breaking it into smaller pieces to ensure it is evenly mixed.
2. Follow the decision-making flowchart provided for the next steps, also available in the ECHO App.
3. Record soil texture on the ECHO App.

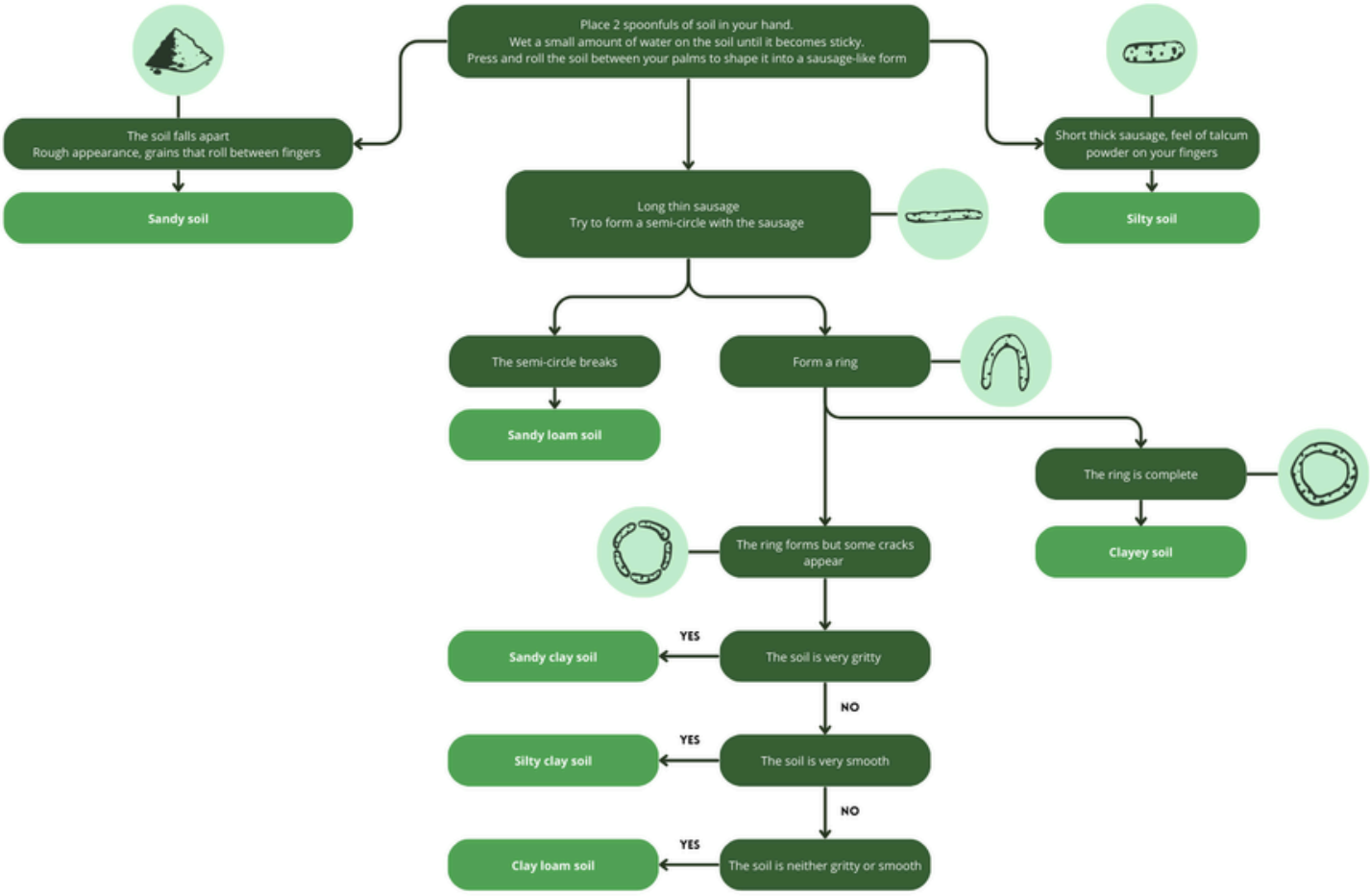

Figure 5: *Decision-making flowchart to determine soil type according to the “texture-by-feel method” (modified after USDA soil quality guide)*

10. SOIL pH

To measure the pH of your soil sample with the ECHO toolkit, you will use a simplified method, which involves using a pH paper strip (**Figure 6**). This approach is cost-effective, easy to use, and accessible for citizen scientists, ensuring reliable results without the need for complex equipment.

How to measure soil pH using the paper strip method:

1. Using the wooden spoon, collect the mixed soil sample and add it to the plastic tube that is pre-filled with distilled water, until the mixture reaches 14 mL.
2. Securely close the tube and shake it gently for 30 seconds to ensure the soil and distilled water are well mixed.
3. Place the tube upright, ensuring it is not lying flat, and allow the mixture to settle for 5 minutes.
4. Dip the coloured end of the pH paper strip into the solution for 30 seconds.
5. Remove the paper strip and compare its colour to the pH colour chart available on the ECHO App.
6. Select the corresponding pH value in the ECHO App.

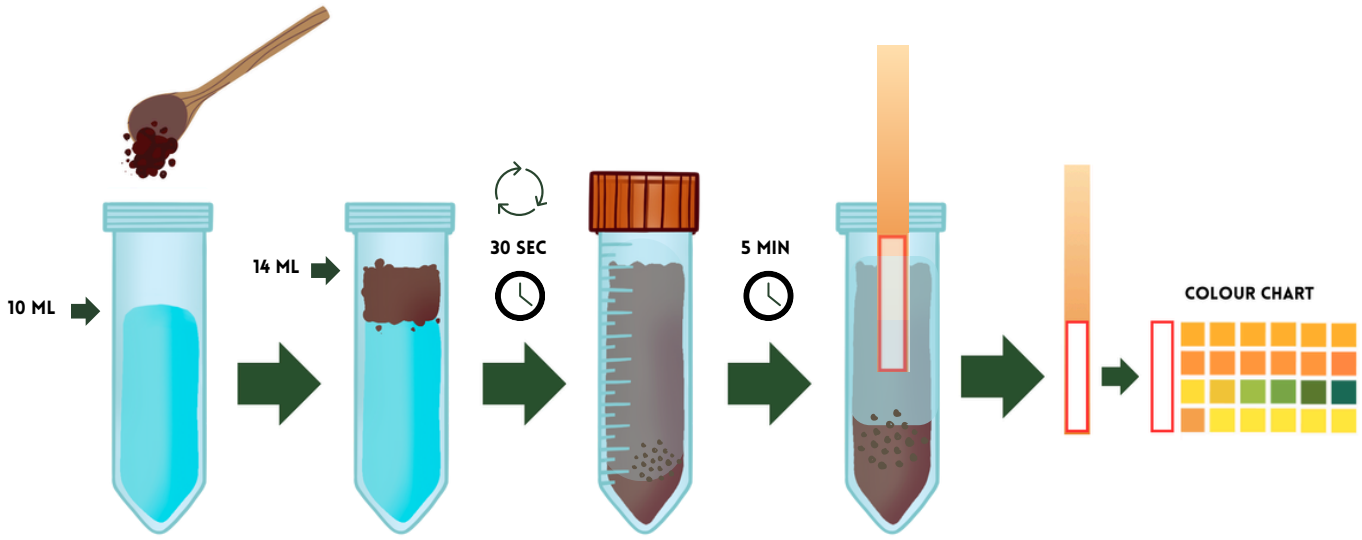

Figure 6: *Step-by-step process for soil pH measurement*

OFF-SITE ACTIVITIES

Not all indicators from the Mission Soil Implementation Plan can be simplified for on-site analysis. Therefore, we will need you to send soil samples to the laboratories of ECHO scientists (UNIBZ lab), allowing us to conduct more accurate analyses using laboratory equipment and expertise. Specifically, the laboratory will analyse your soil samples for two indicators: soil biodiversity (bacteria and fungi) and heavy metals. For soil biodiversity (bacteria and fungi), soil should be placed in a plastic container with a preservation solution (see 1). For heavy metal analysis, it is sufficient for the soil to be field-moist, with a spoonful placed in a plastic bag (see 2). Your role in collecting and sending the samples is essential to the success of the overall site evaluation.

1. SOIL BIODIVERSITY IN TERMS OF BACTERIA AND FUNGI

The soil sample for biodiversity analysis (bacteria and fungi) must be stored in a preservation solution to prevent degradation during shipping to the unibz laboratories, where DNA will be extracted and microbial diversity sequenced. This solution ensures that the DNA remains intact and viable for accurate analysis. The provided plastic tube already contains this solution and is ready to be filled with soil. **The preservation solution is not harmful, and a datasheet from the company provides full details about it (available via the ECHO App). However, handle the tube carefully, wear gloves for safety, and avoid drinking the solution. Keep the kit out of reach of unsupervised children to prevent accidental ingestion.**

How to collect a soil sample for off-site soil biodiversity assessment (**Figure 7**):

1. Put on gloves and keep them on throughout the entire procedure for your safety when using the preservation solution and to prevent contamination of the sample.
2. Take the small plastic tube that contains the preservation solution.
3. Open the tube and use the wooden spoon to collect the mixed soil, filling the tube to the top, until the mixture reaches 5 mL.
4. Close the tube carefully and shake it gently for 15 seconds to mix the soil with the preservation solution, ensuring that all the soil is in contact with the solution.
5. Place the small plastic tube in the plastic bag marked with the QR code from your toolkit.

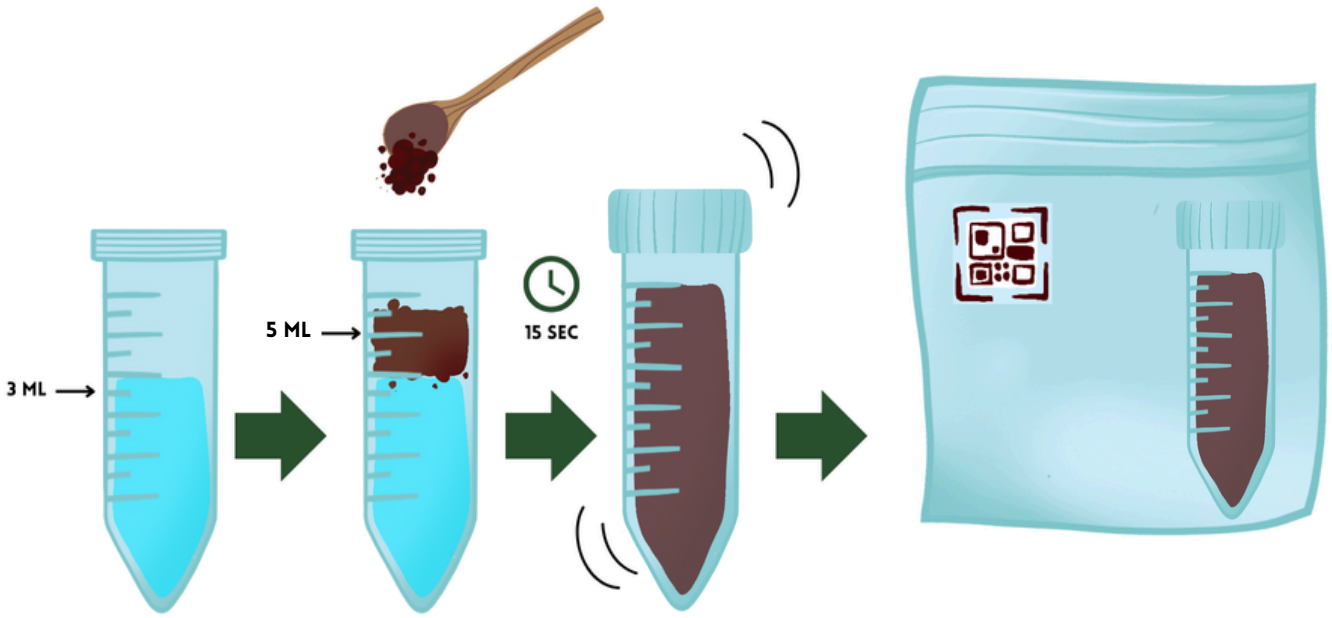

Figure 7: *Procedure to collect a soil sample for off-site biodiveristy assessment*

2. HEAVY METALS AND SOIL NUTRIENTS

As ECHO follows a citizen science approach, there is no simple method available for on-site assessment of soil nutrients and heavy metals. Heavy metals and essential plant nutrients are both elements that play significant roles in the environment and plant growth, but they differ in their functions and potential impacts on living organisms.

Essential plant nutrients are elements required by plants for growth and development. These nutrients are divided into macronutrients (N, P, K, Ca, Mg, S) and micronutrients (Fe, Mn, Zn, Cu, B, Mo, Cl, Ni). While macronutrients are required in large quantities, micronutrients are needed in trace amounts. These nutrients support essential plant functions, including photosynthesis, cell division, nutrient uptake, and overall metabolism. They are typically non-toxic when present in appropriate amounts.

On the other hand, heavy metals are a group of naturally occurring elements that have high atomic weight and density. As heavy metals we can include the following elements: Arsenic (As), Cadmium (Cd),Cobalt (Co), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Zinc (Zn). Excessive levels of heavy metals in soils can hinder plant growth and accumulate in the food chain, posing risks to human and animal health.

This is why we need to analyse these elements in our laboratory, where ECHO scientists will use a micro-X-Ray Fluorescence (μXRF) technique. Note that with the μXRF, the nutrients B and N cannot be measured. The citizen science approach means that participants help collect data, but more complex analyses require specialised equipment and expertise. Please use the small plastic bag (the one without QR code) for collecting the soil sample for this analysis (**Figure 8**).

How to collect the soil sample for off-site heavy metals and soil nutrients assessment:

1. Open the small plastic bag and start collecting soil with the wooden spoon;
2. Fill the plastic bag completely (6 full spoonfuls of soil) and close it carefully;
3. Place the plastic bag containing the soil sample into the larger plastic bag already containing the sample tube for biodiveristy analysis, and marked with the QR code, and seal the bag.

Once the sample arrives for analyses, the soil will be oven dried at 105°C until constant weight is reached before being analysed for total heavy metals and nutrients concentration by µ-X-ray fluorescence.

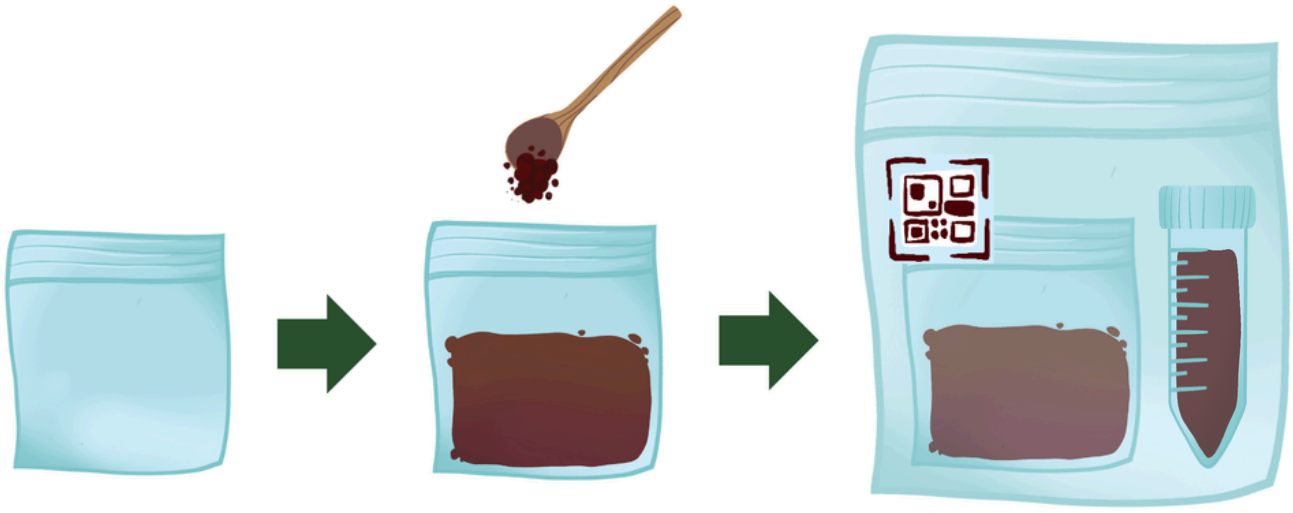

Figure 8: *Procedure to collect a soil sample for off-site heavy metals and nutrients assessment*

3. SITE CLEANUP AND SAMPLE SHIPMENT

Once you have collected the two soil samples for off-site indicator analysis (the tube for soil microbial diversity and the plastic bag for heavy metals and nutrients) and placed inside the larger plastic bag marked with the QR code, they are ready to be sent to the laboratory for analysis (**Figure 9**).

To do this, schedule an appointment with your nearest ECHO Ambassador to hand over the bag containing both samples. To find your nearest ECHO Ambassador, simply search on the ECHO App through the list of Ambassadors available in your area.

The ECHO Ambassador will then scan the QR code on the plastic bag and will plan the shipment to the UNIBZ laboratory with the ECHO partners. Upon arrival, ECHO scientists will scan the QR code to confirm receipt and proceed with the next steps. Your sample will be assigned a specific code that allows you to access your results through ECHOREPO. The results will be available once the analysis is complete. Using this code, you can access your data in ECHOREPO. Once the results are ready, you will receive detailed instructions on how to retrieve them.

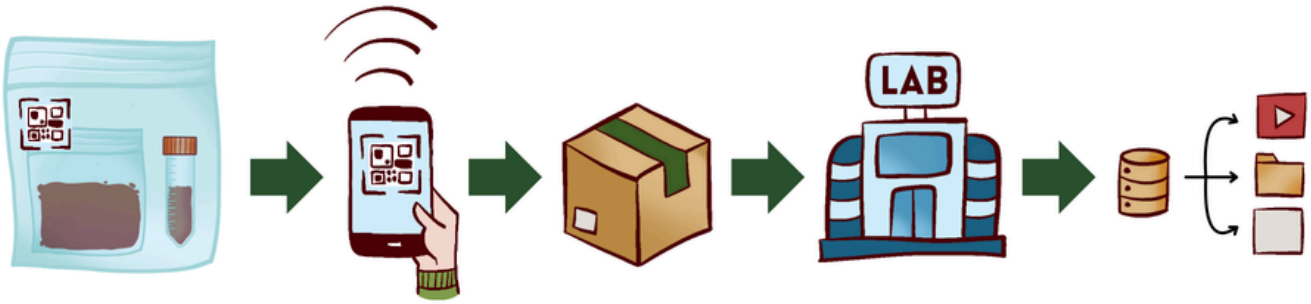

Figure 9: *Sample shipment process*