



Soil Health Evaluation Manual

Soils Cross Cutting Project
East/Southern Africa Community of Practice
McKnight Foundation
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Manual of Protocols: Soil Health Assessment

McKnight Cross-Cutting Soils Project

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Section 1: Introduction and overview of soil sampling and handling

This manual seeks to provide methods for assessing soil health in smallholder contexts. The methods presented range in complexity, but the idea is that they can all be done either by farmers or by organizations that work with them in research and innovation networks. There is other literature which explains the concepts of soil health and how they relate to the measurements we present here. In this manual we will focus narrowly on the technical aspects of the tests and some guidelines for their interpretation. The first two sections of the manual provide guidance on soil sampling and the equipment and materials needed to conduct the tests in this manual. Then each method for assessing soil is described in detail in section three.

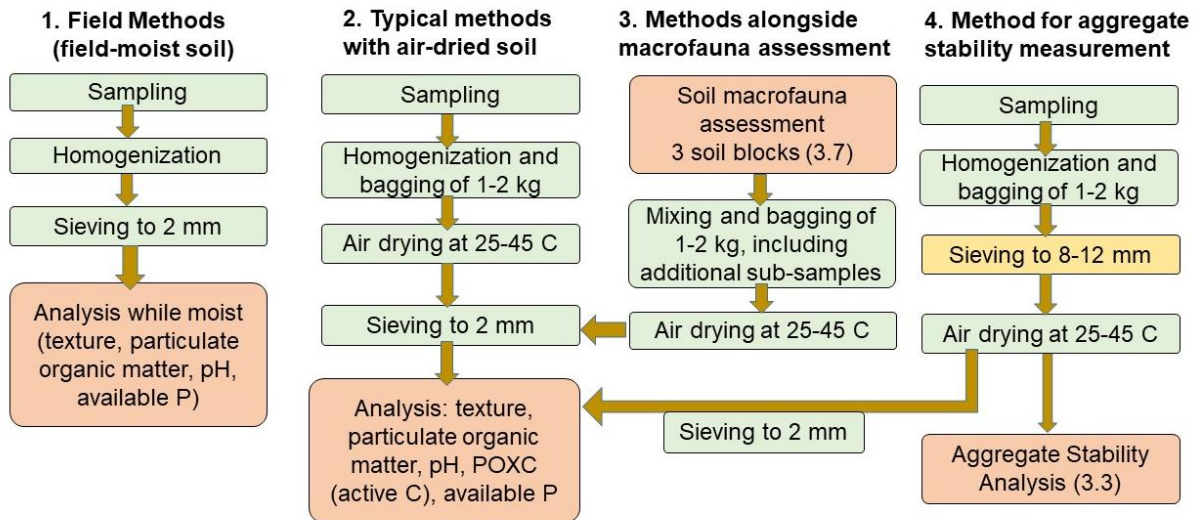
1.1 Sampling overview: The objective of soil sampling is to accurately represent a layer (or layers) of soil at a particular site (e.g. field) and then prepare it adequately for the next steps in analysis. For farm fields in smallholder agriculture, we are usually interested in the top layer of soil where crop roots are most active; and we often are dealing with relatively small fields. This guide presents relatively simple soil sampling and analysis methods where soil is only air-dried and does not need other special handling. For other more complex analyses not in this guide, like analyzing soil microbial communities, types of nematodes, or soluble soil nitrogen, we would need more specific and careful handling of soil, which would be defined in other protocols regarding these measurements.

1.2 Sampling methods: To generate a sample that represents soil at different depths equally, a soil probe is often used, such as a **sharpened tube** or an **auger** that enters the ground to remove a cylinder of soil (Fig. 1). It is often necessary to replace this probe with a shovel plus a knife or machete to cut a slice from the edge of a hole in the soil, followed by trimming this slice with a knife to create a square section (Fig. 1). This shovel sampling method is slower, but not less rigorous than a soil probe (which can cost hundreds of dollars). Another alternative is that if soil macrofaunal assessment will be carried out (see section 3.7 below), blocks of soil will be generated and hand sorted to complete the assessment. These blocks become a way to also collect a sample. If fewer blocks are excavated or if greater coverage and representation of soil variability is desired, it may be necessary to collect a few additional samples using a shovel to add to soil from these three blocks (see number of subsamples in 1.4 below). Fig. 3 shows different pathways for sampling and processing samples.



Figure 1.1. Two methods to sample soil: **Left:** a soil sampling tube or probe; **Right:** using a shovel to cut a slice and then trim it into a square column with an even mix of depths.

soil can sometimes be directly sieved and analyzed, even in a partly moist state (**flow 1** in Fig. 3). More generally this sample is taken and then air-dried in order to sieve it to 2mm size (**flow 2**). This typical flow can also be combined with the macrofaunal sampling (**flow 3**). **Note:** if aggregate stability testing is to be carried out (method 3.3 further on in this manual), it is important to sieve at least part of the sample to a larger size, so that larger aggregates are not destroyed (e.g. with an 8-12 mm sieve or grate, see **flow 4** to the right of Fig. 3 and the method 3.3 further on in the manual, on aggregate stability)



1.3. Different flows for soil sample handling depending on the types of analysis to be performed: **Flow 1.** some analyses can be done in the field (or nearby village site) on the same day of sampling after sieving but without air-drying. **Flow 2.** more typically, bagged soil samples are taken from the field and air-dried to be sieved and analyzed; **Flow 3.** if macrofaunal assessment (method 3.8) is performed in the field, this will generate a sample that can be air-dried, sieved, and analyzed (additional subsamples should be added to assure at least 5 subsamples); **Flow 4.** for aggregate stability analysis at least a part of the sample should be sieved only to 8-12 mm size rather than 2 mm (see method 3.3 below)

Air drying of samples: After taking the soil sample and while it is still moist, it is best to store it in an open bag in a shaded area at cool to medium temperature (e.g. 5-15°C). This will prevent accumulation of condensation and CO₂ that could alter future results. For almost all analyses here, we emphasize that **it is important to air dry as soon as possible** (for example, in closed paper bags or in open dishes or bowls, under cover). Unless we are interested in measuring soil moisture, soil should never be dried in a hot oven (no more than 45 or 50 degrees C, for example). If we want to determine soil moisture, it is best to take only small portions of the total homogenized sample that was taken from the field and dry only this part of the soil at 105°C, weighing these small subsamples before and after drying to determine the moisture content (based on the change of weight with drying). You can also see appendix A with a visual guide to soil moisture, for a more approximate measurement of soil water content.

1.6. Sample homogeneity: An important principle of soil sample handling is to mix the sample well before analyzing, so that when a result of the soil is obtained, it represents as well as possible the sample that was collected in the field and in turn the mean properties of the agricultural plot. Note: this *obscures* micro-level variation across the field; sometimes we are interested in this micro-level and we should sample differently for that purpose; mixing everything from one field to an average value is often a practical starting point and that is what we assume in this manual. Sieving the soil will remove non-soil components that can have a strong biasing effect on results, such as larger roots or macrofauna. Sieving also creates homogeneity since it fractures and mixes small grains, creating a homogenous mass that guarantees the representativeness of any subsample or analysis.

Section 2. Equipment, materials, and reagents

The following section contains description of equipment, materials and reagents that will be used in the methods of soil analysis presented further below. Where possible, we indicate some possible web links for purchasing these items. There are other sellers and other models of the items we note below and you should be able to find acceptable substitutes.

2.1 Equipment

2.1.1 Field pH meter, ExTech stick model



This is a direct reading device, with a flat surface electrode for pH measurements of solutions and soil slurries like those in the pH method.

You can search “Extech pH110” or “Extech pH100” on the internet, or on the site www.testequipmentdepot.com

There are other similar portable pH meters for similar prices between US\$70 and \$150. Three are shown below from different sites and country origins. One should read reviews to assess whether the meter is easy and precise in its calibration. We have had relatively consistent and good results with this the Extech stick model.



Apera Instruments AI311 PH60
(Amazon.com, U.S. site)



Hanna pHep+ meter, HI-98108, Hanna Instruments,
UK online store



Zerodis “3 en 1 testeur”
(French Amazon.fr website)

2.1.2 Portable Colorimeter, Hanna checker high range phosphate model



This is a field model and is the same colorimeter for the test of soil available P (Olsen method) and the test for active soil carbon (carbon oxidizable by KMnO_4 , or POXC)

The model number from Hanna Instruments is HI-717:

The model number is the Hanna Instruments model number HI-717: <http://hannainst.com/hi717-phosphate-hr.html>

2.2 Materials (some other standard lab or home items may also be needed, see each test)

2.2.1 pH paper: This may be a viable alternative to evaluate pH, but the costs of paper and a pH meter may be similar over time. We have also had problems getting pH paper results to agree with pH meter measurements (see the pH method, 3.2).

2.2.2 Extra vials for use in the colorimeter (11 mL vials with diameter 0.75 inch). It is convenient to have a set of about 10 to 30 or more vials per kit, to allow tests to be done in larger batches. These are sold by Hanna, the maker of the colorimeter:

<https://hannainst.com/hi731315-glass-cuvettes-and-caps-for-checker-hc-colorimeters.html>

However they can also be ordered much more cheaply with catalog number CT15196525-C-F217-N (and a Teflon seal closure for use with these test solutions) from discount vials in the United States, or other suppliers:

<https://www.discountvials.com/3-dram-glass-vial-w-cap-pkg-of-25/>

A larger package of 144 vials may also be available.

2.2.3 Filter Paper: For many relatively low clay soils, fine grade cone coffee filters may be enough to filter soil extracts (Fig. 4; see method 3.6 below regarding the available P method for a technique to use these). However, these can be clogged by soils high in clay. In this case, laboratory filters may be needed such as Whatman's grade #5 filters with a fine pore size (2.5 microns) and a sufficient flow rate so that they do not become clogged during filtering. One provider of these is Cole-Parmer:

<https://www.coleparmer.com/i/whatman-1005-090-qualitative-filter-papers-9-0-cm-dia-pore-size-2-5-100-box/0664822>; These cost approximately US \$20 for a box of 100 filters, with a diameter of 9 cm. To be sure of the type note the Whatman catalog number for these: # 1005-090. These 9 cm large circular filters can be cut to smaller circles (for example, 2.7 cm diameter, Fig. 4) in order to filter soil suspensions with a plastic bottle (see section 3.5). In this way four samples per large circle can be filtered so that 400 samples can be filtered with a box of 100 filters. We also note that many users of the tool kit have relied on lab methods for filtering soil extracts already in use by labs, and any of these will work, if they produce an extract that is not cloudy.



Fig. 4. Left: cone coffee filters; Right: lab-quality filters, Whatman type #5 with 2.5 μm pores. Circles with diameter $\sim 3\text{cm}$ are cut to use in a filtering rig, or the entire filter folded into a funnel.

2.2.4 Sieves for general soil sieving, the aggregate stability test and the particulate organic matter (POM) test:

1. **2mm (2000 micron) sieves:** This sieve is fundamental for soil analysis since 2mm is a size threshold for defining soils. Therefore it may be desirable to invest in a high quality metal sieve (brass or stainless steel, 15 or 20 cm in diameter). There are also low-cost plastic sieves with stainless mesh that have been found and work well as described below:
 - A. **All-metal sieve, 2mm or #10 mesh:** There are many businesses that sell such a sieve. You may be able to find them online via these sites:
 - www.zoro.com, product number G3842894, \sim US\$60
 - www.forestrysuppliers.com, stock number 53301, \sim US\$60
 - www.retsch.com, European supplier of sieves and testing equipment, see the links at www.retsch.com/products/sieving/test-sieves/
 - www.amazon.com, www.amazon.co.uk, www.alibaba.com, and other large retail sites; various sieve options from US\$20 to US\$70 but make sure that the sieve purchased will be robust to satisfy expectations; you can use “2mm test sieve” as a search term.
 - B. **Low-cost 2 mm sieve with stainless mesh** (Fig. 5, below): these can be found at www.forestrysuppliers.com for US\$ 9, search product number 53935. This sieve works well if it is mostly used for wet sieving where it will not experience heavy use.
 - C. **Home-made 2 mm sieve:** If sieve, screening, or perforated plate with 2mm holes can be found or manufactured, a sieve can be made with pipe, or the bottom of a bucket. Sieve material can sometimes be found where materials are sold for mining or from larger supply houses and hardware stores, though

it may be not be cost-effective in comparison to buying a sieve. In east Africa some of the heavy grades of mesh sold as window mosquito netting in hardware stores have a mesh size of 2mm (unlike standard western mosquito mesh which has a ~1mm mesh size), and this can make a good sieve.



Figure 5. Left: low-cost plastic sieve with 2mm hole size; Right: Home-made 250-micron sieve using #60 mesh from the silk-screening industry, made with a large-diameter plastic tube as a frame. Rulers shown on enlarged insets use cm units, with small millimeter ticks.

- 2. 250 micron (0.25 mm) sieve.** For this sieve it is possible to purchase a sieve or make a home-made model, as described below:
- A.** A 0.25 sieve can be purchased on the internet, for example the #60 sieve (250 microns) available from Forestry Suppliers, or the other suppliers listed above for 2mm sieves. For example, the sieve is item number 53650 at www.forestry-suppliers.com.



Figure 6. Creation of a 250 micron sieve from the top section and lid of a 5-liter paint bucket with a heavy snap-on lid. Mesh used for screen printing with a hole size of 250 microns is sandwiched between the top rim of the bucket and the snap-on lid. When the lid is snapped on, the mesh is tensioned to provide a very effective sieving surface.

- B.** A 250-micron sieve can be also be made with mesh fabric fastened into a round frame made from a large plastic tube or a bucket (Fig. 5, right side, above). Another easy way to make such a sieve is also to cut the top of a medium-size paint bucket with a strong snap-on lid, and fasten the mesh underneath the lid

to create the sieve surface (Fig. 6 above). This mesh is sold for use in the screen-printing industry; it has a mesh size designated as #60 in the United States and 24 or 24T, with 24 threads per cm in UK and European systems. Such mesh can sometimes be found in larger cities from businesses that screen print on fabric, or supply houses for the screen printing industry. In the United States, mesh can be ordered from Holden Screen Supply in New York City. <http://www.standardscreen.com/mesh.aspx> (international shipping is offered). In the United Kingdom two sources are screen-stretch at <http://www.screenstretch.co.uk/> and screen colour systems at www.screencolourssystem.co.uk/.

2.3. Reagents

Finding chemical reagents will be a challenge to address in many regions. Starting early and identifying potential suppliers in larger cities is important. Here are descriptions of the major chemicals required for the P and active C tests.

2.3.1 pH buffers for calibrating a pH meter, with pH values of pH 4 y pH 7 (sometimes pH 4.01 and 7.01 are sold, these are equivalent). pH buffers are usually found in lab supply stores.

2.3.2 For the test of permanganate-oxidizable carbon or POXC (“active carbon”):

1. **Potassium Permanganate (KMnO₄)**: not much is needed per test: 64 mg per test, or 20 g for 300 tests. This means that if necessary, a small plastic container of KMnO₄ can be flown in with travelers to the region. In the Andes, KMnO₄ can be restricted as a drug manufacture precursor, but can be found in small quantities in lab supply stores.
2. **Calcium Chloride (CaCl₂; magnesium chloride may be acceptable as well)** – Calcium chloride is needed in the largest amounts for the POXC test: 300 mg (0.3 g) per test, so that finding it locally is preferable. In this test the ion Ca⁺⁺ from CaCl₂ acts as a flocculent for clays to help them settle from the test solution. The Mg⁺⁺ ions from MgCl₂ will also flocculate clays, though a little less well, so that Magnesium Chloride may also work. About 90 g of CaCl₂ is needed for 300 tests.
3. **Citric acid (or just lemon juice)**: this is used to clean the containers used with KMnO₄ for the test, which will become stained over time from the permanganate. This is not really a reagent so the purity can be quite low, and simple lemon juice will also work.

2.3.3 For the available phosphorus (P) test: Olsen P solution and colorimeter reagents.

1. **Sodium bicarbonate (NaHCO₃)**: Clean baking soda from a supermarket may work well. If this common baking soda is used it needs to be tested to see what level of P it contains as an impurity, by analyzing the Olsen bicarbonate solution without reacting it with soil and using the colorimetric test outlined in section 3.5. If analytic or reagent grade sodium bicarbonate is available from a laboratory supply house, this is an excellent option. 1.05 grams are needed for each soil analysis (i.e. 42 g for each liter of Olsen analysis solution, which can be used to test 40 samples). A soil kit for 300 samples thus requires about 350 grams of

NaHCO₃, rounding up a bit to cover blanks and testing of the method which can require some reagent.

2. **Sodium Hydroxide (NaOH)**. Common lye from a hardware store can usually be used, and impurities are not terribly important since this reagent is only used in small quantities to adjust the pH of the Olsen solution to 8.5. However, this reagent is very commonly found in laboratory supply houses and it may be just as easy to find it there. Between 1 and 2 g per liter of solution is used, or about 10 grams for a soil kit to test 300 samples.
3. **Sodium Bisulfate (NaHSO₄)**. This is a much safer and easier to dose alternative to sulfuric acid or hydrochloric acid. It is sold relatively cheaply as a swimming pool chemical in the U.S., and can be found either as a pool chemical, or in laboratory supply houses in capital cities like Nairobi in Africa, for example. Each test uses 450 mg (0.45 g; i.e. 150 g for 300 tests), so it is not out of the question to import it, but could also be found locally. Sulfuric acid purchased as battery acid and then diluted by half for safety is also an alternative and may be easier to find in many locations, see the test instructions below in section 3.5 for this substitution.
4. **Reagent packs** for the analysis of P in water solutions: LOW RANGE reagent pack from Hanna Instruments, for example at the following link: <http://hannainst.com/hi93713-03-phosphate-low-range-reagents-300-tests.html> . Note that this is the **low** range reagent pack, but the colorimeter we use is for **high** range (this is on purpose). One reagent pack per test is needed. In the United Kingdom and Kenya, we have found that only the 100-pack of the reagents is available at supply houses.

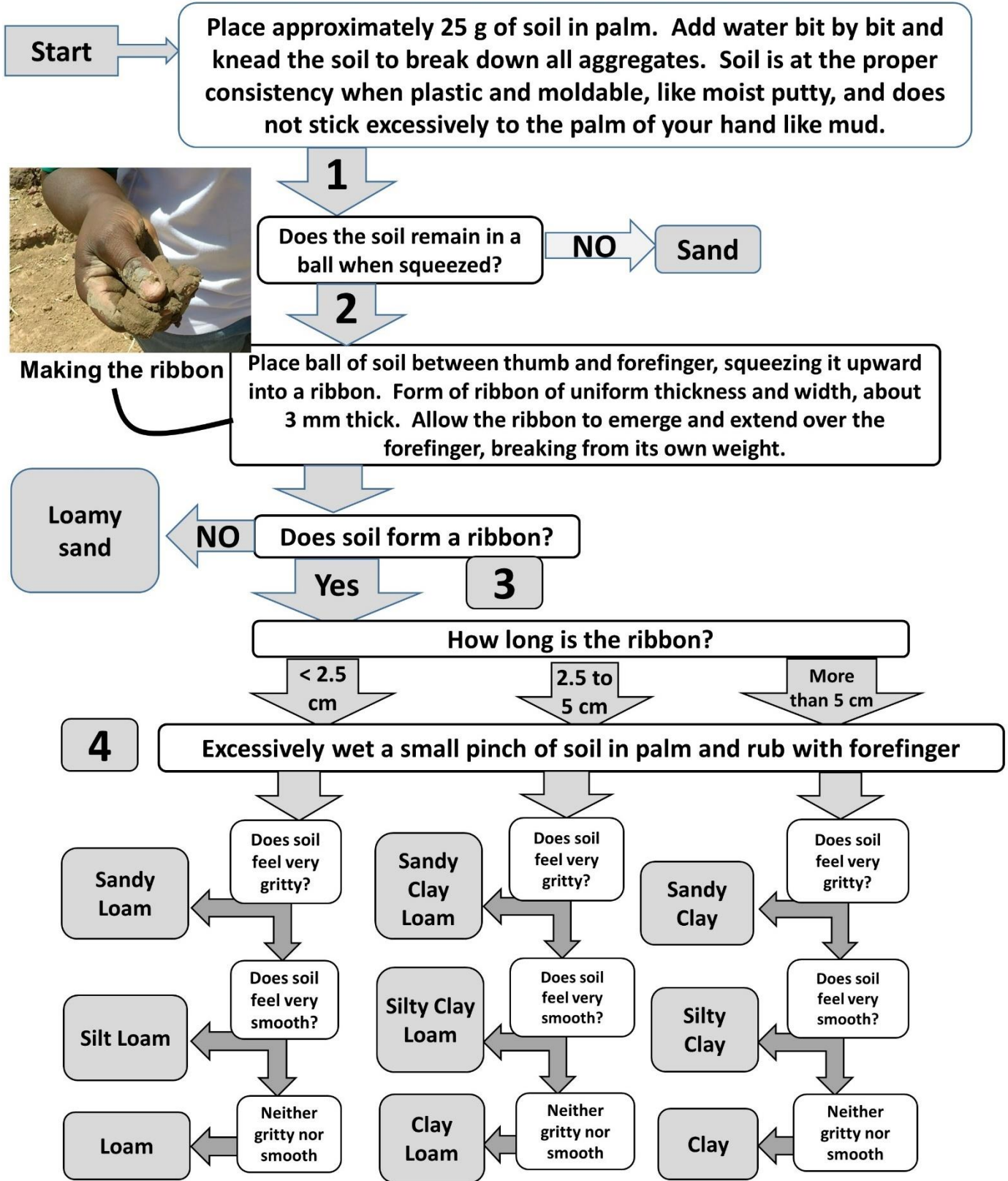
2.3.4 Alcohol (Ethanol or Propanol). This may be necessary to preserve soil macrofauna (section 3.7), in those cases where it is desirable to preserve insects or worms from the field for identification in the lab, rather than just rough classification and counting in the field, which may be sufficient. Alcohol may also be helpful in cleaning the colorimeter vials from the phosphorus and POXC analyses. Alcohol can usually be purchased in any pharmacy.

2.3.5 Hydrogen Peroxide (H₂O₂): this is the normal type of peroxide (~3% H₂O₂) that is sold in pharmacies and is used for the simple qualitative test / demonstration for organic matter in section 3.4.1.

Section 3: Analysis Methods for Soils

- 3.1. Soil Texture:** proportions of sand, silt and clay in the soil. Soils high in clay are considered "fine-textured" and can have problems with compaction and drainage. However with good management, clayey soils can store large amounts of organic matter and have good water retention. Sandy soils drain well, but are thus vulnerable to drought and difficult for building their organic matter content. In the middle of these extremes are the loamy soils that tend to combine the good properties of clay and sandy soils (see Fig. 7).
- 3.1.1. The feel method:** this method was developed by the US Department of (USDA); see the similar alternative method below in 3.1.2, developed by the United Nations Food and Agriculture Organization (FAO). Feel testing is quite rapid and practical and produces results at a precision of +/- 5% to 10% of proportions of sand, silt, and clay. To practice the USDA method, consult Fig. 6 on the following page and the following steps:
1. It is best to use sieved soil (2mm) or remove the stones by hand that are felt in the ball as you begin to knead the wetted soil. Add water to form a putty-like consistency. To wet easily you can use a small bottle of water or a rinse bottle. It is important to be patient in forming a uniform mass without stones, which is plastic but does not stick too much to your hand. If, when the soil has adequate moisture, a ball can still not be formed, the soil is classified as sand (Fig. 6).
 2. After ensuring that the ball has the right level of moisture, try to form a "ribbon" of moist soil with your thumb on the index finger, draped over the index finger (Fig. 6). The length of the ribbon that can be made before it is broken by its own weight can distinguish loam, loamy clays, and clay soils (Fig.6, the three "columns" of alternatives under step 4). Pay attention to the humidity of the soil, since if it is too dry, it will be weak and break only due to lack of water; if it is too wet it will stick on the hand instead of being moldable into a ribbon. The initial level of moisture in the ball requires practice to produce consistency of results.
 3. Finally, pinch off a piece of the ball and wet into a paste in order to evaluate the proportions of sand versus silt in the soil (step 5 in Fig. 6). These can be used to add "sandy" or "silty" descriptive words to the main soil types from the previous step (loams, loamy clays, and clays; Fig. 6). This step may take the most amount of practice to estimate correctly, especially regarding the adjective "silty" where it may be difficult to distinguish the feel of clay versus silt between the fingers. Some testing with obviously clayey versus silty soils should help to develop this skill.
 4. After this procedure, it should be possible to estimate the percentage of sand, silt and clay by matching the soil texture name in the textural triangle (Fig. 7) to the range of percentages for that type. For example, if the name is determined as clay loam, an approximate level of these fractions would be 35% sand, 30% silt, and 35% clay. For borderline types (based on how the soil feels or the length of the ribbon) you can place the percentages on the border of the two types.

Figure 6 (next page): Flow diagram for determining soil texture classes using the USDA feel method with a ball of moistened soil.



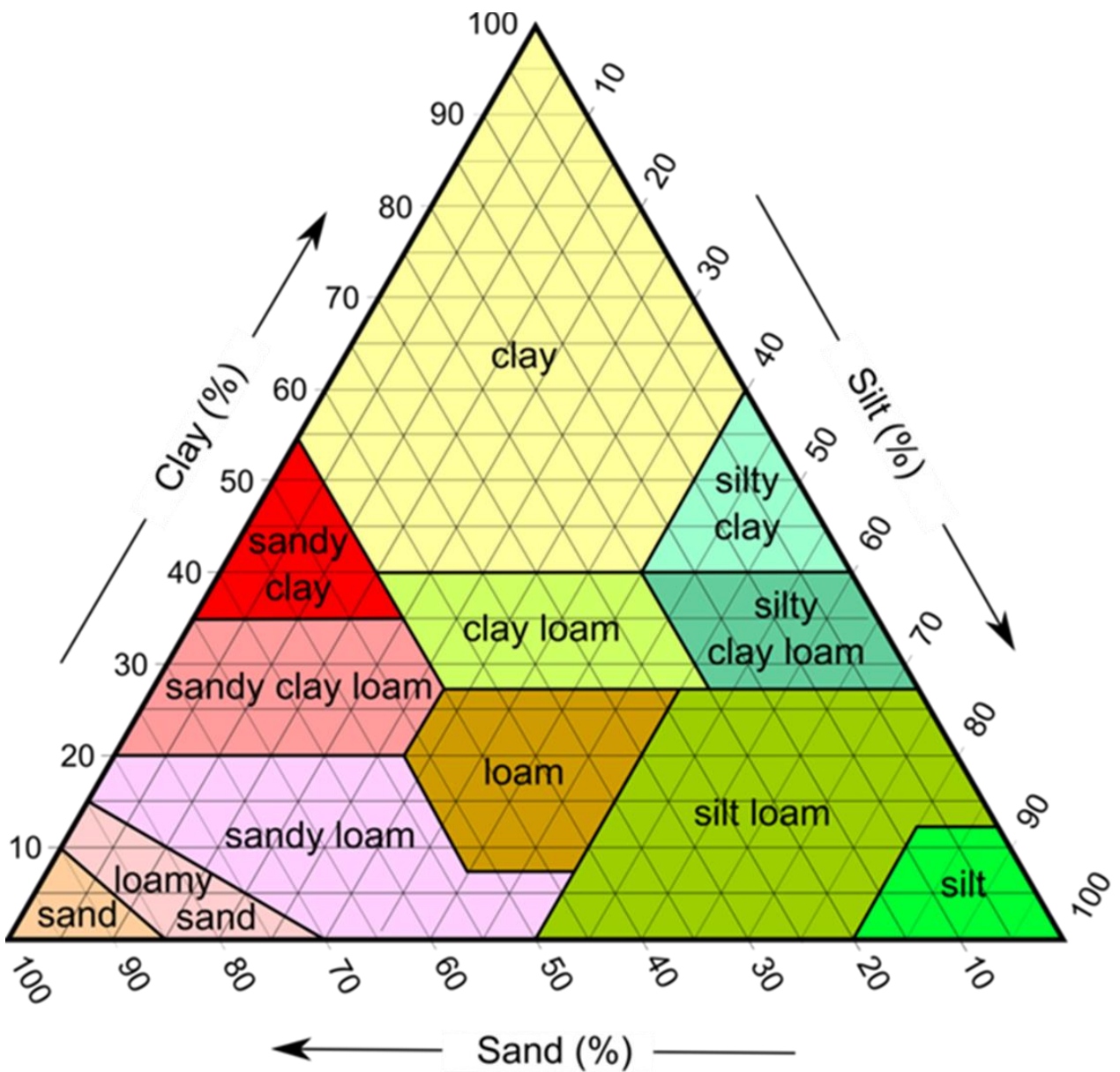


Figure 7. Triangular graph of soil texture (the soil *textural triangle*) Different regions of the graph are named as soil textural types according to their percentages of sand, silt, and clay. Pay attention to the labels on each axis and the orientation of these number labels (horizontal and diagonal) which indicate which set of lines within the triangle describes the proportion of each of the three components.

3.1.2. U.N. Food and Agriculture Organization (FAO) feel method: This method serves as a complement to the USDA feel method in section 3.1.1, which can be used to confirm your findings or as an alternative. Its advantage is that the steps are easy to conceptualize (Fig. 8 below) and is often preferred by beginners. However, it does not categorize soils into all the types in the textural triangle of Fig. 7. Some double checking with the USDA method is helpful to determine these finer gradations in textural class.

3.1.2.1. Procedure:

1. In the same way as the USDA feel method, start by forming a ball of diameter ~ 3cm, like a soil putty with water, without stones that can interfere with the test (using 2mm sifted soil is optimal). The putty-like ball must have just the amount of water to be moldable without sticking too much to the hand, and it is worth kneading with patience until mixing all the dry soil with water. We've noticed that often beginners end up adding too much water so that the putty smears rather than rolling into a sausage in the next step. If so, add a little bit more soil. Regardless, if this ball cannot be formed, it is classified as **sand** (Fig. 8).
2. If a ball can be formed, next you should try rolling the ball into a sausage, about 6-7 cm long. If the "sausage" falls apart as it is rolled, it is classified as a **loamy sand**
3. If a 6-7 mm sausage can be formed, try to roll the sausage further into a "pencil" about 15-16 cm long. If the pencil cannot be formed but falls apart, the soil is a **sandy loam**.
2. If the pencil can be formed, try to bend it into a half circle. If the half-circle cannot be formed or falls apart, the soil is a simple **loam**.
3. If the half circle can be formed without breaking, try to continue bending the "pencil" into a complete circular ring with an approximate diameter of 5 cm.
4. If this ring cannot be formed without breaking, the soil can be classified as a **silt loam** or a **silt** soil.
5. If the ring can be formed but some cracks appear as it is bent, the soil may be a number of types that tend to be clayey without having enough clay to be formally called clays, such as a **clay loam**, a **silty clay** or **sandy clay**. These are all the types that border the "clay" type in the textural triangle (Fig. 7), as well as the **sandy clay loam** type. By following the same strategy of feeling a wet pinch of soil in step 5 of the USDA feel method (Fig. 6), these types may also be distinguished.
6. Finally if the ring can be formed with very few cracks, and tends to look more like potter's clay rather than a soil, it is likely the **clay** type of soil.

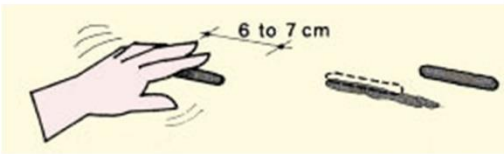
The FAO feel test for soil texture



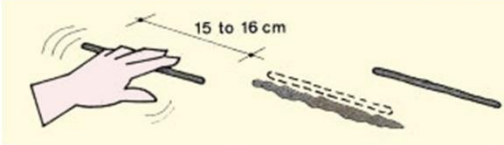
1. Wet and knead the soil



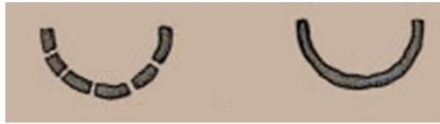
2. Can you form a **ball**?



3. Can you form a **“sausage”**?



4. Can you form a **“pencil”**?



5. Can the pencil be turned into a **half circle**?



6. Can the half circle be further bent into a **circle**?

Figure 8. The FAO feel test for soil texture: a sequence of tests performed on a kneaded ball of moistened soil, which can be used to classify soil textural type in the FAO “feel method”

Image credit and original source:

http://www.fao.org/fishery/static/FAO_Training/FAO_Training/General/x6706e/x6706e06.htm

3.1.3. Jar or bottle settling demonstration for understanding size fractions: In contrast to the above feel method, this is not really a rigorous test of texture but does allow farmers and others to better visualize the different sizes of sand, silt, and clay particles by observing their settling times in a water column. The sand (size 50 microns - 2 mm) settles first within 40-60 seconds. Silt particles (2 microns - 50 microns) settle for up to about 12 hours, and the clay takes longer to settle. However, these times apply only to fully dispersed suspensions of soil, which are difficult to achieve just by shaking a jar and even with a blender. Clays are aggregated into larger size particles in most soils, even with many minutes of blending, and so chemical dispersion of the clays is required, at which point the settling times of clays can reach to days, making the test a bit less convenient. Meanwhile, even very small aggregates of clays settle as if they were silt or sand, leading to an **underestimate** of the clay fraction. For this reason, this method is better used to teach about the different particle sizes that exist in the soil than it is as an accurate test of soil texture or clay content. It should therefore be combined with the other tactile methods that can estimate the proportions of sand, silt, and clay more accurately.

3.1.3.1. Materials

7. Bottle or jar (glass or plastic)
8. Small graduated cylinder or accurate measuring device for liquids to the nearest 10 mL (a balance can also be used to measure 10 mL, i.e. 10 gram, increments).
9. 100 to 200 mL of soil approximately, depending on the total size of the jar or bottle.

3.1.3.2. Procedure

1. 1. Prepare a jar or bottle with graduations to measure volume, marking the bottle every 10 mL. A thinner bottle or jar will provide more precision to distinguish the percentages of the fractions when they settle in the water column. Sieve the soil to 2mm
2. Add the soil to the jar and then 2-3 times the volume of water. Mix the soil and water well with shaking or a blender, to try to break up the aggregates (in practice it is very difficult to completely destroy microaggregates without chemical dispersant like sodium metaphosphate, which is one reason why this method tends to **underestimate** the percentage of clay in the soil and **overestimate** the percentage of silt.)
3. Leave the soil to settle between 4 and 24 hours. You will notice different layers settling, first the sand, then silt, and finally clays, from the cloudy part of the suspension. At the end of this, the supernatant or upper part of the soil suspension will be transparent or translucent.
4. Identify visually the different layers, where possible, between larger, visible sand particles, silt that settled earlier, and a fine clay that settled last. At this point, the purpose of the jar test is more to talk about the different fractions rather than estimate them accurately, where we suggest that the best strategy is to use the feel test.

3.2. Soil pH

3.2.1 Materials and reagents

1. **Portable pH meter** for field type, pen-type or similar, with electrode placed in its storage solution if necessary (see the equipment section, 2.1.1).
2. **Buffers or calibration solutions** for the pH meter. Calibration should be performed with pH 7 and pH 4 buffers, which gives information on the most important pH range for soils.
3. **Another alternative:** pH paper with a precision of at least one pH units and better if it can indicate pH with gradations of 0.5 pH units. In any case pH paper tends to be less accurate than a calibrated pH meter.
4. **Small plastic cups** or containers for between 50 and 100 mL.
5. **A balance** (1 g or 0.1 g precision) to weigh soils and water.
6. **Distilled water**, or bottled water tested for impact on pH measurement. In order not to interfere with the pH measurement, water with either no or low total mineral content should be used, that is, with a mineral content such as calcium or magnesium below 50 ppm (mg / kg) or even better, less than 10 ppm. The total dissolved solids (TDS) reading can be checked on the label. In some countries reverse osmosis water is sold as bottled water and this water works well. Rainwater can also be collected in a clean container (glass or plastic) to use. If necessary, the readings can be validated in about 4 or 5 soils, using a "candidate" water, in comparison with known distilled water, to verify if the use of bottled water makes a difference for the pH reading. Small differences of ~0.1 pH unit are not a problem.

3.2.2. Procedure:

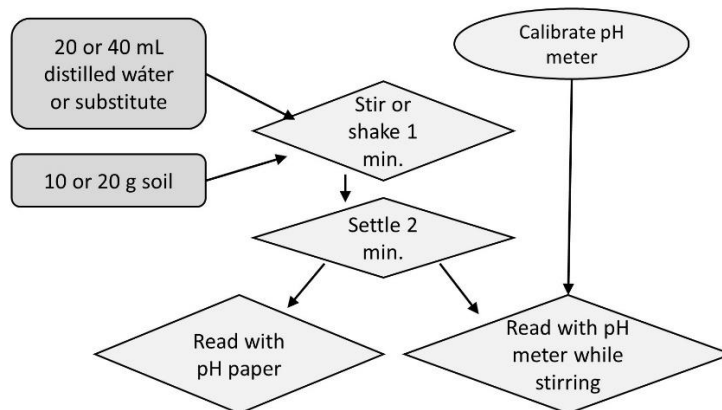


Figure 9. Flow diagram for the measurement of soil pH with pH paper or a portable pH meter

1. **Weigh 20 +/- 0.5 g** of soil in a small glass (or only 10g if you want to economize on the use of the soil sample). If there is no balance, it can also be estimated that a

volume of 14 to 16 mL of soil will weigh approximately 20 g, or another measure of volume can be used based on an approximate density that may be known for soils in the area where the sampling was done. This adjustment to the method works because soil pH measurement is less sensitive to the soil: water ratio than other soil chemical measurements. In any case, when a balance is available it is better to use the indicated weight and not the volume.

2. **Add 40 ml of distilled water**, rainwater, or alternative of low mineral content; see materials list above (or only 20 mL if only 10 g of soil was weighed, in every case, 2x the weight of the soil)
3. **Mix the soil and water** and stir quickly or shake for 1 minute. Shaking with water for one minute in a closed container can be very effective. In this case it is then transferred to the small cup or beaker.
4. **Let stand 2 minutes** or more, mixing from time to time.
5. **Measure with the pH meter**: Place the pH electrode in the cup and stir slowly during the measurement, keeping the pH electrode in the supernatant or top suspension in the cup. Record the pH after the reading stabilizes. The goal is to maintain a stable reading that does not change more than 0.1 pH unit in about 30 seconds. Variation on a finer scale is not important. However, if the meter changes continuously up or down, without stabilizing (e.g. > 0.1 unit in 10 seconds), it is possible that the electrode must be maintained since time electrodes can become dirty and blocked or occluded.
6. **Measuring with pH paper**: after allowing the solution to settle for a few minutes (so as not to stain or color the paper strips excessively with the soil's color, the top liquid or supernatant of the soil suspension can be measured with pH color test strips of the appropriate interval (e.g. pH paper strips with pH range 0 to 14, 4 to 7 or 5 to 8). This paper is then compared with a color chart. We are conducting tests to see how reliable this is, and so far it seems to lead to readings that are 0.5 to 1.5 pH units different (usually lower) than with a pH meter that may be unacceptable. However, if the difference between paper strips and the pH meter is relatively stable for a set of soils from a given zone or region, it may be acceptable to apply this difference or conversion to the paper strip readings and use these as a way to measure pH. Any pH paper alternative should be tested against a calibrated pH meter for the best results.

3.3. Soil aggregate stability

3.3.1. Materials

1. Large size mesh with a hole size of 8 to 12 mm to perform pre-screening of soil aggregates and gently break large soil clods and remove larger stones (Fig. 10).



Fig. 10. Examples of different types of mesh that can be used for pre-screening dry soil, with a hole size between 8 and 12 mm.

2. Soil sieves with mesh size 2 mm (10 mesh) and 0.25 mm (250 microns, 60 mesh), with a sieve diameter of at least 6 "(150 mm) (see section 2.2 and Figs. 5 and 6). The diameter is important so that the screen does not become clogged with too much material during wet-sieving. As detailed in section 2.2.3, the 250-micron sieves can be made of wide-diameter plastic tubing or the bottom of a plastic bucket, combined with 0.25-millimeter (size #60) plastic mesh that is used in screen printing of fabric (Fig. 5).
3. Water: clean tap drinking water is perfectly sufficient, and any village water supply will work as well.
4. Small basins or tubs that the sieves fit into comfortably for sieving in water (for example 25 cm diameter x 8 cm of height, see photos below).
5. Balance to weigh soils and aggregates (precision 1g or preferably 0.1g).
6. Metronome app on a cell phone, or an audio file that can mark a 50 beats per minute tempo for the timed washing steps.
7. Rinse bottles that allow the rinsing of sieves to move and capture soil and aggregates. A good (perhaps even superior) substitute is a ~500 mL disposable plastic bottle (from water or other) with small holes drilled or poked in the lid to allow a small shower-like stream of water to flow from the bottle when squeezed. The holes in the lid can be made with a pen tip, safety pin, or drill bit (to make approximately 1 mm diameter holes).
8. A medium-sized funnel, 10 to 20 cm in diameter.
9. Squares of cloth (fabric from bedsheets, used T-shirt etc.) or paper filters to capture, visualize, and weigh the stable aggregates from the analysis. It is helpful to weigh these fabrics or filters in advance and write the tare weight on each one with an indelible marker (to 0.1g precision), to facilitate the weighing of the dry aggregates at the end.

3.3.2. Video and other considerations

1. **Video:** a video that shows this method is available at:
<https://www.youtube.com/watch?v=DucBmQBpX6Q>

To see a more complete version of this test, and some of the theory behind the test and why aggregates are stable in water, see this video (note: the method performed here is more complex than the adaptation in this manual):

<https://www.youtube.com/watch?v=VOaae2bDDCY>

2. **Soil aggregation or soil structure also depends on soil texture** – for example a silty clay will tend always towards higher levels of aggregation than a sandy loam. Therefore, aggregation ideally has to be compared in two soils that are similar in texture if we want to evaluate the management impacts on soil structure. For example, if we compare the aggregation in a sandy loam soil (lower amount of clay) compared to the silty clay (more clay), the differences we observe may be more related to the differences in sand and clay content, and not soil management, so the comparison may be invalid.
3. **Other related tests:** there are other possible structure tests that can be performed, for example the "soil porosity" and "soil structure and consistency" visual evaluations described in the FAO Soil Visual Evaluation Guide by Shepherd et al. (see bibliography). There is also a soil stability analysis developed by the United States Department of Agriculture Natural Resource Conservation Service (NRCS) similar to the procedure below, which produces a stability rating with small "baskets" of window screening mesh. This "tackle box" analysis method appears in the soil quality kit guide of the US soil conservation service: page 20 of the following document:

https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb1044790.pdf

3.3.3. Procedure

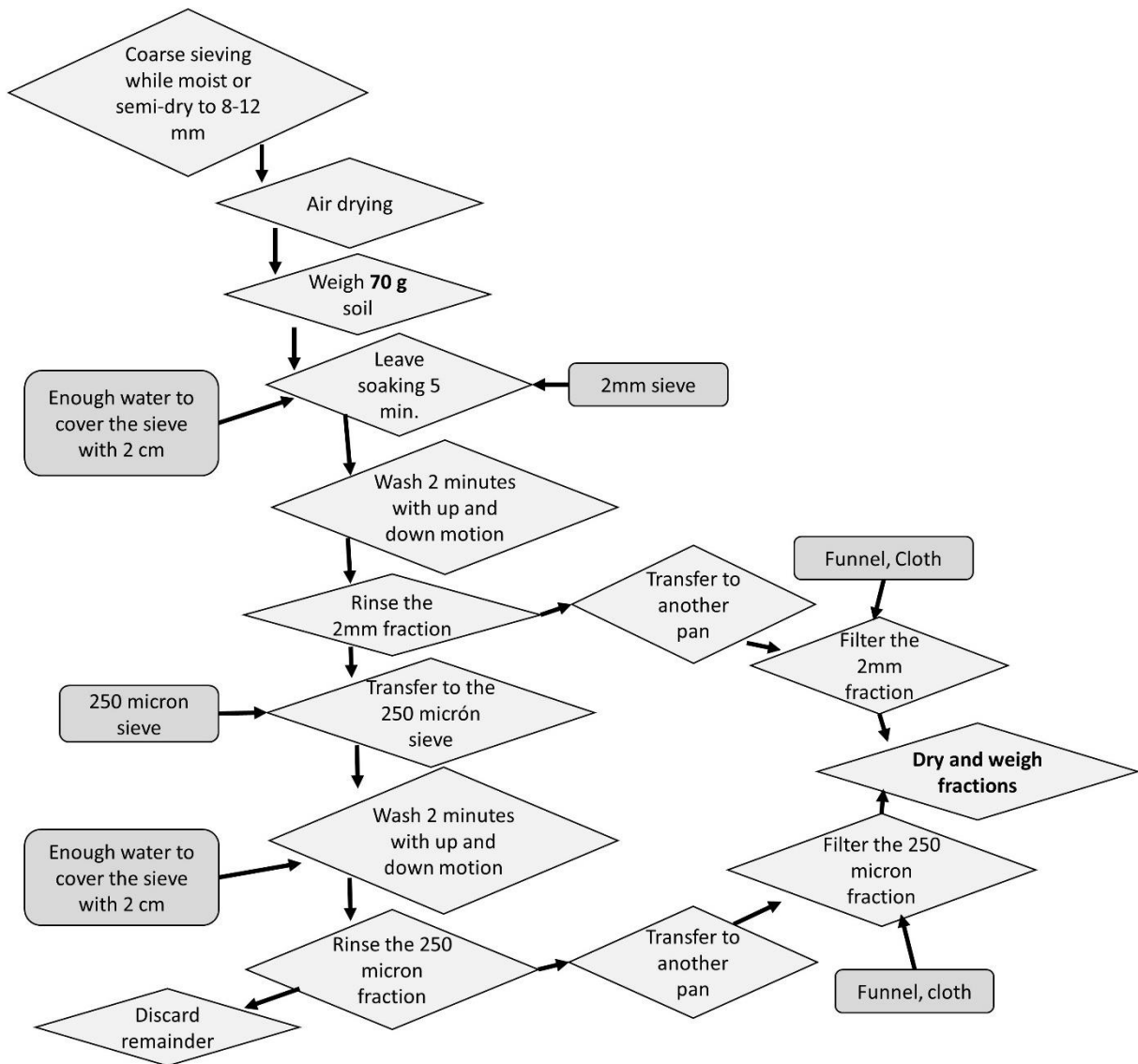


Fig. 11. Flow diagram for the aggregate stability test.

1. Prepare 70 g of air-dried soil (this can be dried in oven, but no more than 45° C) sieved to a size between 8 mm and 12 mm. For most soils it is easiest if the soil is coarse-sieved when partially dry and then fully dried afterwards. To perform the sieving a mesh of 8 to 12 mm size can be used. If no mesh is available, aggregates larger than 8-10 mm diameter can be broken and rocks removed by eye. During this process you should in every case remove stones that do not pass the mesh. Also, when soil is broken by hand, natural planes of weakness should be found in the clods and large aggregates, rather than forcing the soil through the mesh. In this way, at least 300g of moist soil should

be sieved (to maintain a representative subsample) to then take a 70 g as a representative part for analysis. However if there is very little soil in the sample, as little as 40 or 50 g can be used.

2. Immerse the 2mm sieve with a depth of at least 2 cm water above the mesh, and then gently pour the 70 g of soil onto the submerged mesh (Fig. 12).
3. Leave the soil in the water to wet up and slake (break apart) the aggregates naturally for 5 minutes.



Figure 12. Adding coarse sieved soil (sieved to ~10 mm) to the 2mm sieve in a basin of water.

4. Move the sieve in and out of the water slowly, 50 times in 2 minutes (Fig. 13). This can be done using a metronome app on a smart phone, or an audio file (available at www.smallholder-sha.org) to create a 50 beat per minute rhythm, and then raising the sieve out of the water in one beat and lowering it back into the water on the next, repeating this cycle 50 times in the two minutes. Many metronome apps will also measure the time since beginning the rhythm, or you can also use a stopwatch to measure out the two minutes. Make sure you are not doing this action at twice the pace: you should NOT lower and raise the screen on each beat of the rhythm, which would be 100 times in two minutes.

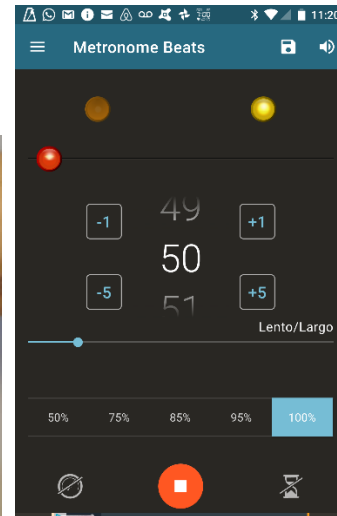


Figure 13. Left, Lowering and raising the sieve 50 times in two minutes. At right, a metronome app for cell phone to regulate the pace of dipping the sieve into the water.

5. After this washing action with the sieve for 2 minutes, a rinse bottle is used to wash the sides of the sieve and wash any residue of organic matter or small particles of clay through the sieve. The aggregates should not be rinsed so strongly that additional aggregates are destroyed, since these aggregates were already defined as stable during washing; for example do not spray them directly with the stream of the bottle. Then put the bottom pan with water and all the material <2mm on one side for the next wash in step 8. If you want, you can also proceed directly to step 8 and come back to capture and dry the 2mm fraction later, which may be more efficient with time.
6. Next, empty the stable 2mm aggregates (particles > 2mm left in the sieve), to a new, dry pan or basin, and then capture them in a cloth or filter. This is easily done by just turning over the 2mm sieve, over a wider pan, and washing all the contents from the back of the sieve out the front into this pan. You can then do a final rinse with a wash bottle to completely empty the sieve, working from the back and the front. As you do this, you can remove any large organic residues such as sticks and long root segments from the sample (these are not aggregates).
7. When all the 2mm stable aggregate material (and likely some stones) have been moved to the second basin, use the wash bottle to move this material to the pre-weighed cloth sitting within a funnel. The funnel is placed in a cup or other container to catch the water passing through, and leaving the stable aggregates in the cloth (see Fig. 15 for an example of this funnel/cloth combination for the smaller aggregate fraction). This cloth is set aside for drying while we turn attention to the smaller 250 micron fraction.



Figure 14. Rinsing all the material <2mm into the 250 micron sieve to perform the next wash of stable aggregates at the 250 micron size.

8. Next we consider the fraction that passed the 2mm sieve, i.e. aggregates <2 mm and any smaller soil components. This material is poured gently into the 250 micron (0.25 mm) sieve sitting in another small pan or basin similar to the others (Fig. 14). Any remaining soil is then washed from the first basin into the sieve. In addition, water can be added if necessary so that the 250 micron sieve sits with about 2cm water above the mesh, just like in the first washing step with the 2mm sieve.
9. Repeat the washing movement of the 250 micron sieve in the new basin, 50 times in 2 minutes. After this step, what remains in the sieve will be a mixture of sand and aggregates that are between 0.25 and 2 mm in size. As in the first step, this fraction should be gently rinsed with a wash bottle before the next step.
10. Wash the sieve contents (aggregates and sand) with the wash bottle onto a filter or cloth. This can be done first into another basin to make it easier, washing from the back of the sieve, and then into the funnel. The fraction can also be transferred directly to the funnel with the cloth (Fig. 15), though this may result in more water spillage.



Figure 15. Washing a stable aggregate fraction from the sieve to a funnel with a cloth to retain the soil for drying and weighing.

11. Next, the two aggregate fractions (> 2mm plus stones and the fraction between 250 microns and 2mm) are dried in a hot place or drying oven before weighing and calculation of quantitative results (up to 105°C, since we only want the dry weight and are not interested in chemical properties). However, for a simpler, qualitative comparison, such as during an educational program in the field, you can visually compare the amount of aggregates among different management practices or experimental treatments, without weighing, or take pictures to compare afterwards.
12. After this, first we consider the >2mm fraction, where you will need to measure two weights to a precision of 0.1g or 1g:
 - a. After picking out or sieving out the small stones >2mm from the soil on the cloth, The dry weight of these stones >2mm, (note that some soils may not contain such stones) **and**
 - b. The dry weight of the cloth plus soil, without stones, after picking or sieving these out. The weight of the cloth taken previously should also be noted.

The stones are separated to correct the weight of aggregates as well as the total soil weight, since stones >2mm are not considered aggregates or part of the soil that can be aggregated (see 3.3.4. below for calculations).
13. For the small-sized aggregates between 250 microns and 2mm in size, just weigh the cloth with its dry soil; no separation of stones is needed. The tare weight of the cloth taken previously should also be noted.

3.3.4. Calculations to obtain results: As indicated above, for an approximate assessment it is possible to simply compare the qualitatively stable aggregates (both sizes, > 2mm and > 250 microns) between two fields or management practices according to the approximate volume of stable aggregates which are seen in the cloth. In every case keep in mind that it is most valid to compare the stability of aggregates in two soils that have similar textures. For a more rigorous result you can get the percentage of dry stable aggregates that were left in the two cloths or filters:

1. Percentage of soil in large macro-aggregates (> 2mm) is calculated with the following equation, with all the weights in g. (Note that for this calculation the tare weight of the cloth or filter is needed in advance):

% aggregates > 2mm =

$$\frac{[(\text{Weight of } > 2\text{mm fraction without stones} + \text{cloth or filter}) - \text{weight of filter or cloth}]}{70 \text{ g} - (\text{weight of stones } > 2\text{mm})}$$

Referring to the weights taken in the procedure step 12 above, this is:

$$\frac{\left[\begin{array}{l} \text{(weight from step 12b)} \\ -\text{weight of filter or cloth} \end{array} \right]}{70 \text{ g} - \text{(weight from step 12a)}}$$

As noted above, if there are stones > 2mm in this fraction, these should be removed to find only the weight of the soil in this fraction. Optionally, the weight of these stones as a proportion of the 70 g soil can also be used to characterize the small stone content in the soil.

2. Then consider the cloth or filter with aggregates of size between 250 microns and 2 mm. In this case you do not need to remove small stones from the smaller aggregates, but we still take the proportion out of the stone-free mass of soil as above for the >2mm fraction.

% aggregates from 250 microns to 2 mm =

$$\frac{\left[\begin{array}{l} \text{(Weight of the 250 } \mu\text{m to 2mm plus the cloth or filter)} \\ -\text{weight of the cloth or filter} \end{array} \right]}{70 \text{ g} - \text{(weight of stones > 2mm)}}$$

Or referring to the procedure steps above in 3.3.3.,

$$\frac{\left[\begin{array}{l} \text{(weight from step 13)} \\ -\text{weight of filter or cloth} \end{array} \right]}{70 \text{ g} - \text{(weight from step 12a)}}$$

3. The value of 70 g is placed because we used 70 g of soil initially. If the initial soil weight was changed, different weight should be used in place of 70 g.
4. Interpreting the results: The following table gives rough guidelines for interpreting the results, expressed as the sum of percentages between the two fractions, 250 microns at 2 mm and > 2 mm. The table is separated into three different categories of soil textural types. We emphasize again that the degree to which a soil can develop and maintain structure by the activity of roots, microbes, and macrofauna has a lot to do with its texture and other factors, so it is best to compare between plots that have the same type of soil and different types of management, or try to measure the impact of management on the structure over time. In general, more water stable aggregation is better.

	Qualitative score, based on % 250 μm + % 2mm water stable aggregates			
Soil textural type	Very low	Low	Medium	High

Very coarse soils: Sands and loamy sands	Aggregate stability is of limited usefulness: look for aggregation in a dry soil, but this structure is not expected to be water-stable			
Coarse and Medium-textured soils (sandy loams, loams, silt loams, silt, < 35% clay)	<15%	15% - 30%	30% - 45%	>45%
Fine- textured soils: (clays, sandy clays, silty clay loams, or >35% clay)	<20%	20% - 40%	40% - 55%	>55%

3.4. Soil Organic Matter (SOM): three tests are presented below that visualize soil organic matter in different ways. The three methods have different levels of rigor and refer to different fractions or processes in the soil. However, they can all be used to foster learning about soil organic matter.

3.4.1. Demonstration/test with hydrogen peroxide: This test is based on the reaction obtained between hydrogen peroxide (H_2O_2) and microbial life and its enzymes in the soil. It generates bubbles which form a foam. In theory, the reaction of the peroxide is proportional to biological phenomena in the soil. Therefore, it is not a direct test on the amount of organic residues or stabilized organic matter in soil (forms of **SOM**) but it is likely proportional to organic matter **provided** there are active microbes in the soil. When soils are dry, for example, we have noticed that this demonstration does not work very well and gives a lower impression of SOM than from more rigorous analyses or what can be observed regarding color of the soil. Nevertheless, the demonstration can be used in learning activities to demonstrate that the soil contains "living beings" with the analogy to a wound of a human being that also forms bubbles with peroxide. It is a quick way to demonstrate biological aspects of soil and soil health in the field, without any more complicated procedure. We are validating whether a rough relation exists between this peroxide test and the permanganate test below, which is already established as a measurement of available SOM (and thus soil organic carbon).

3.4.1.1. Materials and reagents

1. **Hydrogen peroxide** (type purchased in a pharmacy for wound cleaning; best to have it inside a bottle with a dropper)
2. **A plastic bottle cap** from a drink bottle or bottled water (~2 cm in diameter)
3. **Field-moist soil** is used, as this is a field demonstration

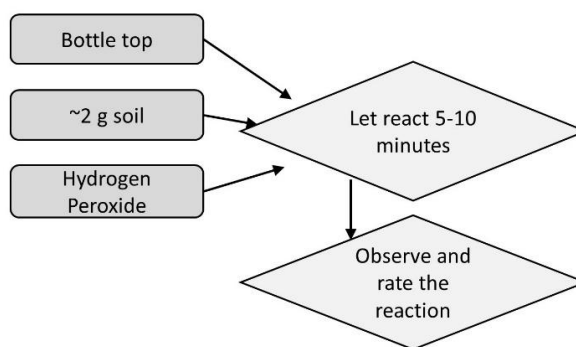


Figure 16. Flow diagram for the simple demonstration and test of soil biological activity with hydrogen peroxide (H_2O_2).

3.4.1.2. Procedure:

1. Place a depth of 4 to 5 mm of soil at the bottom of the bottle cap. If the soil is not sieved to homogenize it and remove stones, then stones should be removed by hand since they do not contribute organic matter (SOM) or microbial biomass to

the sample. Before taking this small amount for testing, be sure to mix the soil well to homogenize it and represent the plot adequately.

2. With the dropper, add enough hydrogen peroxide just to soak the soil, or just until a liquid surface shines on the surface of the soil inside the lid.
3. Over time bubbles will appear. Often they will form a foam that rises inside the bottle cap.
4. After 5 minutes, you can rate the amount of bubbles and the speed of the reaction, the speed of reaction being an important parameter in this test. A sample of moist compost or damp manure from a pen can be used for comparison as a "positive control" that represents a very high value, which will also validate that the hydrogen peroxide is still fresh enough to use. Although this is a test or demonstration still in testing, a scale of 5 levels (0 to 4) is proposed, which can be adjusted with experience, as follows:

Scoring table for the hydrogen peroxide soil test

Score	Description of bubbling behavior of hydrogen peroxide
0	Very little reaction, none or almost no bubbles (like clean sand, for example)
1	Bubbles only on the soil surface or very slow reaction
2	Layer of bubbles with 1-2 mm depth, or slow reaction with a thicker layer of bubbles, but only at the end of 5 minutes
3	Layer of bubbles with 5-10 mm depth, appreciable reaction after only 30 seconds
4	Froth of bubbles with depth > 10 cm, and a quick reaction within 30 seconds, close to the reaction of a wet farmyard manure or moist compost.

3.4.2. Particulate Organic Matter (POM)

This test can be used as a demonstration to show what soil organic matter is, in a very visual way, for farmers or other audiences. POM is also a measurement of recent contributions of organic inputs to soils, such as manure, crop stems and roots, compost, and biochar. POM gives an idea of what will decompose in the near future and release nutrients to crops. The streamlined version of the test is presented here. A somewhat longer laboratory version can also be done with 2mm and 250 micron sieves instead of the mesh bag and bottle shown below, and will give the same results.

See the YouTube video at: https://youtu.be/cL_nWS_xWAw

3.4.2.1. Materials



Figure 17. The mesh bag from 250-micron mesh and a bottle big enough for sieving 100g of soil, with 2mm holes drilled into the side of the bottle with a drill or hot nail.

1. Plastic bottle of size 200 to 400 mL approximately, with holes of size 2mm drilled (with a 2mm drill) or melted using heated nails slightly smaller than 2mm, for example 1.8 mm. About 200 holes should be drilled or melted into the bottle side near the bottom (see Fig. 17 at left).

2. A bag sewn out of 250 micron (or #60 mesh), large enough for the bottle (1) above to enter it. A 13x20cm bag is generally large enough to accommodate most bottles.

3. Buckets of size 5 to 8 litres (larger is alright too). The buckets should be deep enough to allow shaking the bottle/bag combination vigorously while immersed.

4. Water: clean tap drinking water or a village water supply is sufficient.

5. Balance to weigh soil and POM. To weigh the initial soil amount, only a precision of 1 g to 0.1 g is necessary. See the protocol below for options when there is no balance to use

in the field. To accurately weigh the light POM separated from soil, a more accurate scale (0.001 g or 1 mg) is usually used, therefore a visual rating may be easier and better if such a balance is not available (see the scoring table scale at the end, Fig. 23)

Nevertheless the POM can be saved as small samples in small envelopes or other such for later weighing if desired.

- 6. Rinse bottles** that allow rinsing of sieves to transfer and capture soil and aggregates. These wash bottles can be made from a common flexible plastic water or soda bottle (500 mL) by opening gaps in the lid with a thick needle, thumbtack, or a fine drill bit (~1 mm diameter).
- 7. A beaker, measuring cup, or other cup** with a spout (~500 mL) that can be used to decant floating organic matter from water (see Fig. 22 below).
- 8. A short tube of plastic** where a filter or cloth (next item) can be stretched for receiving particles of organic matter, diameter between 8 and 15 cm approximately. This can be made by cutting a short section of a plastic bottle or plastic jar.

9. **Pieces of cloth or filters** to capture, visualize, and dry the particles of organic matter at the end of the evaluation, just like those used in the aggregate stability test (section 3.3.1). Cloth can be bedsheet fabric or part of an old T-shirt.

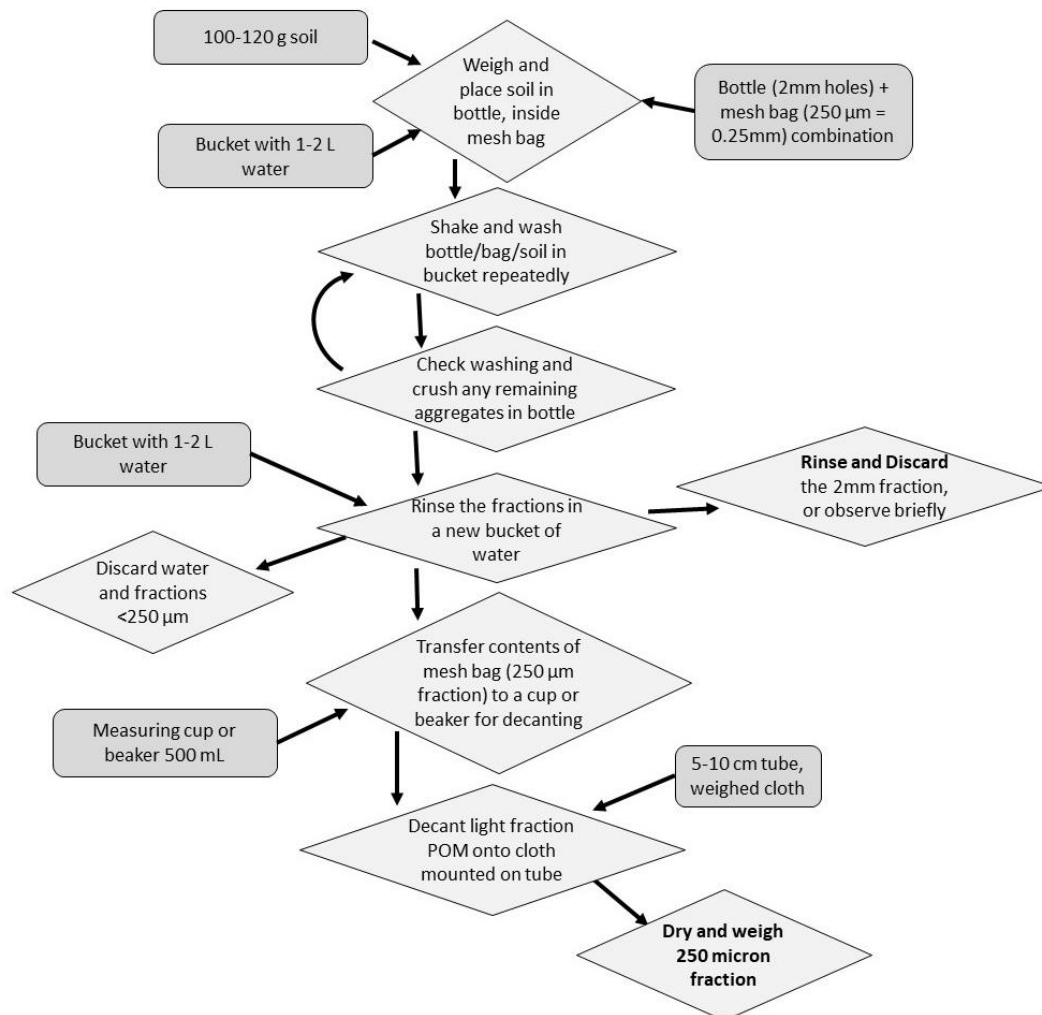


Figure 18. Flowchart for the streamlined POM method, see steps R1 through R8 below.

3.4.2.2. Procedure

A video of this procedure is available at: https://youtu.be/cL_nWS_xWAw

1. Place the bottle with the 2mm holes in the 250-micron mesh bag to form two "layers" of screen material through which the soil must pass (Fig. 19).
2. Working above the bucket of water, or another place where soil can be spilled without issue, weigh out and add 100g of soil to the bottle with holes. If the soil was not sieved previously and there are large stones (> 5mm) it is preferable to remove them so that they do not present a bias in the starting weight of the soil. While pouring the soil into the bottle, it could leak a little through the holes and the mesh, this is not a problem, since we do not care about any particles small enough to leave the bag.



Figure 19. Washing the soil in the bottle and bag combination. After a minute or so, clays and silt will have moved outside that bag and the water will become quite cloudy (right-hand image)

3. Holding the bottle inside the mesh, immerse and shake the bottle / mesh / soil vigorously in the bucket of water (Fig. 19). Silt and clay will start to come out of mesh bag. Take care that no soil leaves through the mouth of the bottle.
4. After approximately 3 minutes, examine the material inside the bottle. This should contain only large roots and / or stones > 2mm to continue with the next step (Fig. 20). If there are still large aggregates or lumps, it is necessary to break them gently by breaking up clods by inserting a stick into the bottle, or simply continue shaking the bottle in the bucket. Because the soil weight has already been taken, at this point gravel-sized stones could also be inserted to break up the clods when shaking.

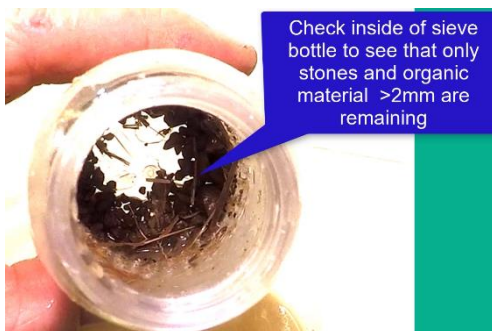


Figure 20. Checking the bottle to see that only stones and large organic debris remain there.

5. Once there are only small stones, sticks, and large roots in the bottle, remove it from the bag and rinse the bottle with the bottle or other water, into the mesh bag, so that any material between 250 microns and 2 mm is kept in the bag and not lost.
6. Some soils may contain very strong aggregates of soil that will hold together in the mesh bag, even after shaking in the bottle, and act like sand. If you notice this, you should gently massage the bag with your hand to break up these soil crumbs and free any organic matter and clays that will be then rinsed in the next step.

7. Rinse the mesh bag in a new bucket with just enough clean water to cover the bag (the bucket does not need to be full, see Fig. 21).



Figure 21. Washing the mesh bag a last time in clean water (left) before washing the material inside the bag to a beaker or other cup with a spout for decanting (right).

8. Wash the contents of the mesh bag into a beaker, measuring cup, or other container for decanting off the organic matter or POM (cup of size 300-500 mL, see Fig. 21).
9. Mount the small piece of cloth or filter on the end of the plastic cylinder (cut-open jar or similar), using rubber bands.
10. Decant the floating POM repeatedly into the plastic cylinder so that it remains on the cloth, refilling the decanting cup with water as many times as is needed to separate the less dense POM from the sand. See Fig. 21 and the YouTube video (link above) for more details.



Figure 22. Decanting the floating POM into a cloth that is mounted on a plastic cylinder made by cutting a plastic jar or bottle.

11. When you finish decanting each round, you may see particles that look organic (darker), but that are left at the top of the sand layer (their density is between the density of the sand and the density of POM). These are usually organic matter complexed with clay and other mixed forms of organic matter, perhaps with a little charcoal. You should try to capture these on the cloth or filter by shaking the decanting cup a little as you pour. However, it is usually impossible to capture every last grain and at some point you will need to call the evaluation finished.
12. Continue this decanting process until the water above the sand washed in the beaker is free of almost all particles, and nearly 100% of the POM has been captured in the sieve.

Alternative method: The somewhat longer **lab-based method** uses laboratory soil sieves instead of the bottle/bag combination above for wet-sieving. You can see a video describing this method at: <https://www.youtube.com/watch?v=zOrG3Ma2ceA>

3.4.2.2. Scoring and Calculation of POM Results:

1. To generate a particulate organic matter (POM) data, one strategy is simply to score the amount of POM visually, using a visual guide based on experience and comparison with other soils (e.g. Fig. 23):

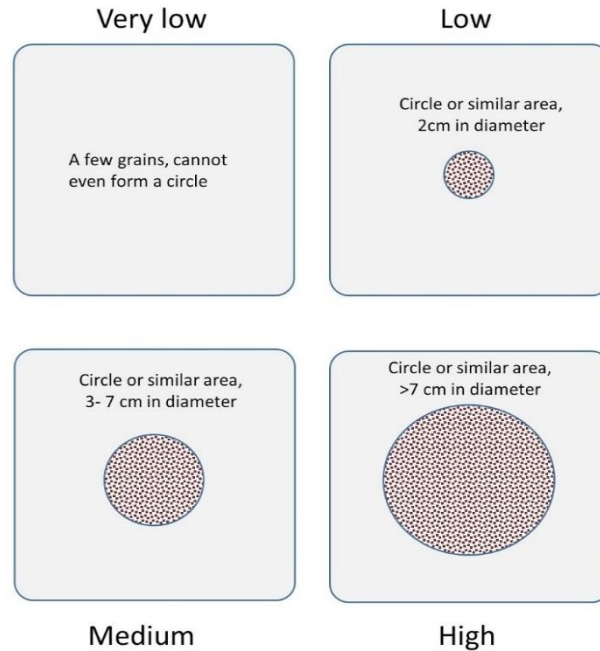


Figure 23. Guide for visual scoring of POM density in soil. This guide is for the suggested 100g of soil in the method above, if you use more soil you will need to adjust the diameter guidelines to larger circles.

2. In addition to this visual scoring, you can store the small samples of POM particles in the cloths or filters and then dry them in the air or in an oven (40° to 60° C), and weigh them on a precision scale (0.001 g or more accurate) which will produce a more precise quantitative result.
3. POM samples can be carefully scraped off the dry cloths or filtered and weighed in milligrams. Then the fraction (%) of particulate organic matter is calculated as:

$$\frac{[\textit{Weight of the dried decanted POM}] \textit{(mg)}}{\textit{initial weight of soil (g)(e.g. 100 g)} \times 10}$$

This result will have units of percent. (%) and can be compared between agricultural plots or practices in an experiment.

4. Another alternative is to measure the volume of dry POM, and to develop a conversion factor between the volume of POM and the dry weights of a set of samples. Then the volume can be used directly as a measure of the amount of POM. To do this, a consistent method of measuring a volume of POM must be used.

3.4.3. Permanganate-oxidizable ('active') carbon (POXC)

3.4.3.1. Materials and Reagents.

- 1. Water:** Unlike concerns about phosphorus-free water in the available phosphorus test (section 3.5 below), there are usually no substantial impurities of organic carbon in public water supplies, and tap water can be used. However, the additional cost of using bottled water (for the $\text{KMnO}_4/\text{CaCl}_2$ solution below) that is low in salts and organic matter will not increase costs greatly and is recommended.**Potassium permanganate (KMnO_4)** – this is purchased locally or can be shipped in small quantities. It is important to test KMnO_4 that is purchased in pharmacies or other less formal settings to make sure it behaves in the manner indicated in this method, since KMnO_4 deteriorates (under exposure to light and other environmental factors) to other forms of K-Mn oxides (with a greenish color, which is a way to see if the reagent is expired) and this will not lead to a reliable measurement. One way to check the source of KMnO_4 in practice is to do the analysis on the same sample with a "pure" or reliable lot, compared to a lot of KMnO_4 to be tested.**Calcium chloride (CaCl_2)** - This is sometimes easier to find than other reagents (for example, in supply houses for the local food industry). This is only used in the solution as a soil flocculent and it is possible that magnesium chloride will also work (it is the divalent Ca^{++} ion, or on the contrary Mg^{++} ion, which is important)**Citric acid or lemon juice:** citric acid is not necessary for the analysis, but it is useful to clean brown permanganate staining from the containers used in the method, by soaking them for a few hours followed by normal washing.**Centrifuge tubes** (50 mL) or other small containers of 50 to 100 mL in which to shake the digestion solution with the soil and then allow the suspension to settle. A tall, narrower-style container is desirable to allow pipetting from the top of the suspension.
- 6. Soil** sieved to 2 mm and air dried (if it is necessary to use moist soil, a correction for the water content must be applied, see end of method)
- 7. Digestion solution:** 0.015 M potassium permanganate (KMnO_4) + 0.1 M calcium chloride (CaCl_2) in the SAME solution. See the recipe below
- 8. Transparent glass vials** with volume 11 ml and a diameter of 0.75 inches, to perform the color reading with the Hanna colorimeter (see photos below and equipment section 2.1.3).
- 9. Centrifuge tubes** or other small container (volume about 50 ml), to dilute the digested KMnO_4 solution before reading.
- 10. Graduated dropper** or graduated transfer pipette with volume measurements. These can be purchased with graduations of 0.5 mL. You can also make a dropper graduated to a volume of 0.5 mL with a precision balance to mark the level at which the dropper contains 0.5 mL (equal to a weight of water of 0.5 g).

3.4.3.1. Recipe for the KMnO_4 / CaCl_2 digestion solution

This is a solution of 0.015 KMnO_4 and 0.1 M CaCl_2 in the same solution. The original method published by Weil et al. (available [here](#)) uses a 0.02 M solution of KMnO_4 , but we are trying to save on reagents and, therefore, use a slightly more dilute solution.

For each 100 mL of solution (multiply in case of larger volumes):

1. Measure with a graduated cylinder, or weigh, 100 mL (which is equal to 100 g water) in a transparent bottle or beaker (to be able to see that the reagents are completely dissolved).
2. To each 100 mL of water, add 1.11 g of CaCl_2 . For volumes greater than 100 mL, multiply the amount of CaCl_2 proportionally to the volume, e.g. for 1000 mL or 1 L, multiply by 10 = 11.1 g CaCl_2 .
3. Mix the solution well until all the calcium chloride is dissolved. If the solution is made inside a bottle, you can cover the bottle and shake or swirl to dissolve it quickly.
4. *To this same solution*, add 0.237 g KMnO_4 (potassium permanganate) per 100 mL of solution (or 0.24 g if there is only one precision balance 0.01 g). Mix well again, until completely mixing the KMnO_4 particles. This amount (0.24g) is a small amount for many non-precision balances, and it may be better to make a larger volume, for example 500 mL (with 1.185 g KMnO_4), to achieve greater precision in weighing the permanganate. Other amounts of solution are also possible.
5. It is best to make just enough of this solution, plus a small margin, to analyze a batch of soils, calculating 20 mL for each analysis. If the solution is to be stored between evaluations (less than a week), the bottle must be covered against light (with tape or aluminum foil for example) so that the KMnO_4 does not decompose.
6. If you want to store this solution for a few weeks to a month, you can add a small amount of NaOH to adjust its pH to 7.2 (just after mixing, and assuming distilled or very pure water, it will have a pH of around 5.7). This will help to preserve KMnO_4 in solution. The solution can be stored for a few weeks in a refrigerator, in a wrapped bottle to prevent light from entering. However, it is a better idea to mix the solution in small batches and use the entire solution within a week.

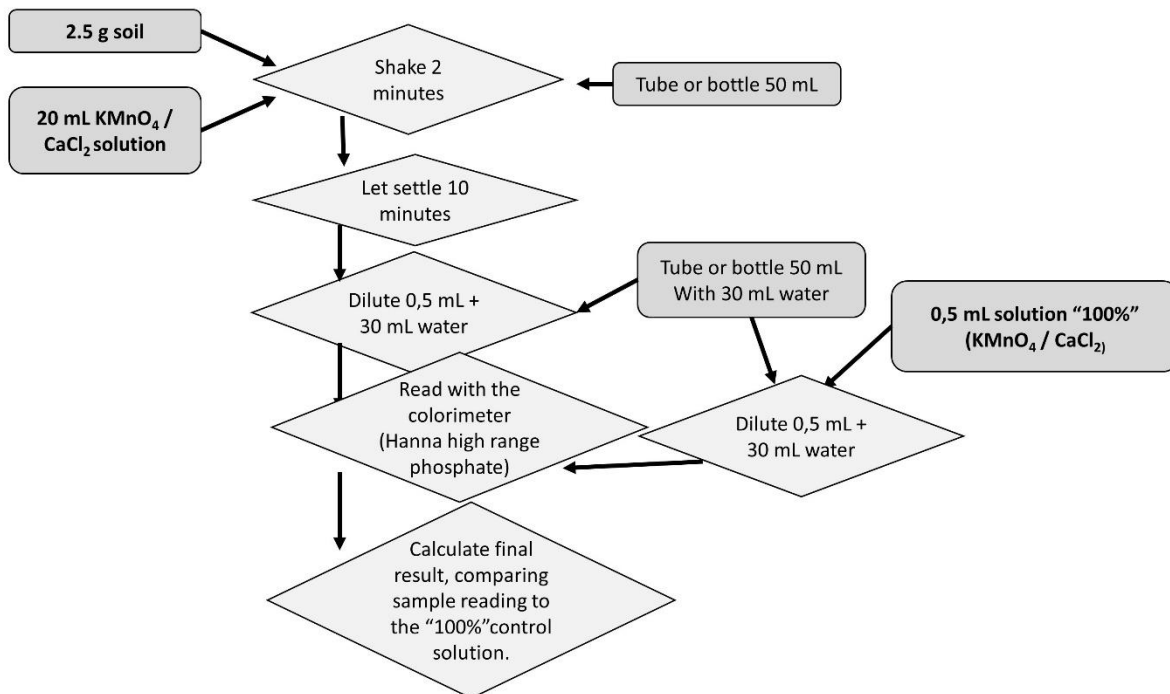


Fig. 24. Flowchart for the test of KMnO_4 -oxidizable carbon ("active carbon" or POXC).

3.4.3.2. Procedure (see flowchart in Fig. 24)

1. A video for this method is available at:
<https://www.youtube.com/watch?v=89Bn5P4y3n4&t=601s>
2. Mix 2.5 g +/- 0.3 g of soil in 20 mL of digestion solution (recipe above). Record the exact weight for the calculations at described below, e.g. "2.61 g". More soil can be weighed out (e.g. 3.5 g) if it is estimated at the outset that the soil contains very low levels of SOM, or less soil for soils very high in SOM.
3. Shake 2 minutes, shaking with your hand or with a shaking machine.
4. Let stand 10 minutes. The CaCl_2 will cause the clay to flocculate and settle, to leave a clear solution except for the color of KMnO_4 . The timing of these two steps is relatively important, e.g. not more than 20 seconds imprecision in shaking and not more than one minute imprecision in settling.
5. During this time, if it has not been prepared in advance, you must fill a second centrifuge tube or bottle with 30 mL or 30 g of water, to prepare a dilution of 0.5 mL KMnO_4 + 30 mL water. This dilution will allow reading of the color in the colorimeter.
6. Dilute the settled solution of KMnO_4 in the tube with 30 mL water (the KMnO_4 solution without dilution is too dark to read). Take 0.5 ml of this settled solution with a graduated dropper or transfer pipette from the top layer of liquid in the vial, being careful to make sure that there is exactly 0.5 mL in the vial. Add this 0.5 mL to the 30 ml of water in the tube and then rinse the pipette or dropper with the 30 mL (sucking and expelling) to transfer all the color to the tube.
7. A "control" tube containing 100% KMnO_4 solution, direct from the bottle and without having reacted with soil is also needed for comparison to the soil sample. To make this, dilute 0.5 mL of the solution directly from the reagent bottle where it was prepared and dilute by adding to 30 mL water.
8. The final step of the measurement is to read the color of the diluted solution (step 5) as compared to the color of the 100% diluted solution (step 6). The measurement with the colorimeter is done in the following way (See Figs. 25 and 26):
 - a. Insert a vial in the colorimeter with clean water as a blank value.
 - b. Push the button to turn on and wait for "C1" on the screen (Fig. 25)

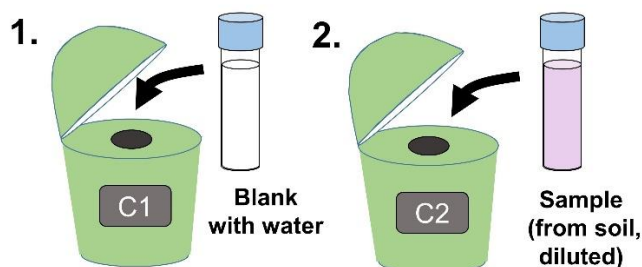


Figure 25. Measuring the sample value for permanganate solution reacted with soil.

- c. Re-push the button to measure the blank vial, and wait for "C2" on the screen
- d. Pour approximately 10 mL of the solution diluted with soil and diluted (step 5) into another clean vial to read in the colorimeter. Push the button.
- e. Wait for the value of the result on the screen, which will usually read between 0 and 22.
- f. To measure the 100% control value, repeat steps (a) through (e), repeating the blank as C1 and inserting a vial with the 100% diluted solution (from step 6) instead of the solution reacted with soil as C2; see Fig. 26.

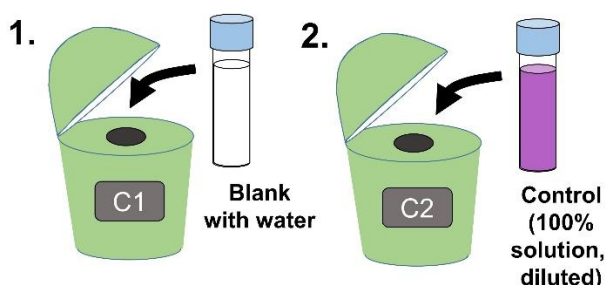


Figure 26. Measuring the 100% control value of the unreacted KMnO_4 solution.

- g. It is not necessary to repeat the measurement of the 100% solution for each soil sample. The 100% control solution can be re-measured only every 3 or 4 samples. In the calculations below, for the 100% value, the two closest 100% measurements are used.

3.4.3.3. Calculations to determine permanganate-oxidizable carbon (POXC) or active carbon:

1. Three pieces of information come from each sample and are used together to calculate the final POXC result: the weight of the soil introduced to the test (about 2.5 g, for example), the reading of the sample reacted with soil in the colorimeter, and the reading of the 100% KMnO_4 solution (without reacting with soil). Note that if the sample solution loses a lot of its color and the sample reading on the colorimeter is low, this means there was a lot of oxidation of carbon in the soil and the value of activated carbon will be high. When there is not much change in color, the level of active carbon is low.
2. Example: Let's say that exactly 2.50 g of soil was weighed to analyze, that the reading of the 100% solution is 17.6, and that the reading of the soil sample is 13.2. The result of active carbon or POXC would be calculated in this way:
 - a. First, the change in the concentration of the KMnO_4 solution is calculated:

$$\text{Change in concentration } \text{KMnO}_4 = \left(1 - \frac{\text{sample reading}}{100\% \text{ solution reading}}\right) \times 0,015 \text{ M}$$

- b. For the example we get Change in concentration= $[1-13.7/17.6] \times 0.015 = 0.00375 \text{ M}$, where M stands for moles per litre.
- c. In the next step we use the volume of permanganate solution to figure out the change in the actual amount of permanganate based on this change:

$$\text{Change in quantity } KMnO_4 = \text{change in concentration} \times 0,02 L$$

Since the 20 mL solution used is equal to 0.02 litres.

- d. For the example we get: Change in amount $KMnO_4 = 0.00375 M \times 0.02 L = 0.000075 \text{ moles } KMnO_4$
- e. To convert this amount in moles to mg of activated carbon in the soil oxidized by $KMnO_4$, the authors of this test determined that a conversion factor of 9000 can be used:

$$\text{Soil carbon oxidized (mg)} = \text{change in the amount of } KMnO_4 \times 9000$$

- f. In the example, Soil carbon oxidized = $0.000075 \times 9000 = 0.675 \text{ mg}$
- g. This amount of soil carbon oxidized (in mg) is then divided by the initial amount of soil used (in kg) to yield a result in mg/kg or parts per million (ppm):

$$\text{POXC or "active" carbon (mg/kg)} = \frac{\text{Soil C oxidized (mg)}}{\text{initial soil mass (kg)}}$$

- h. In the example, $POXC = 0.675 \text{ mg} / 2.50 \text{ g soil} = 0.675 \text{ mg} / 0.0025 \text{ kg} = 270 \text{ mg/kg soil}$.
- i. Comparing with the histogram of values in figure 27 and the scoring chart in scoring table below, we can see that this represents a very low to low level of POXC.

In Fig. 27, a range of typical values of POXC or active C of the soil is indicated, in a graph that shows the distribution of values from highland Bolivia as well as Western Kenya.

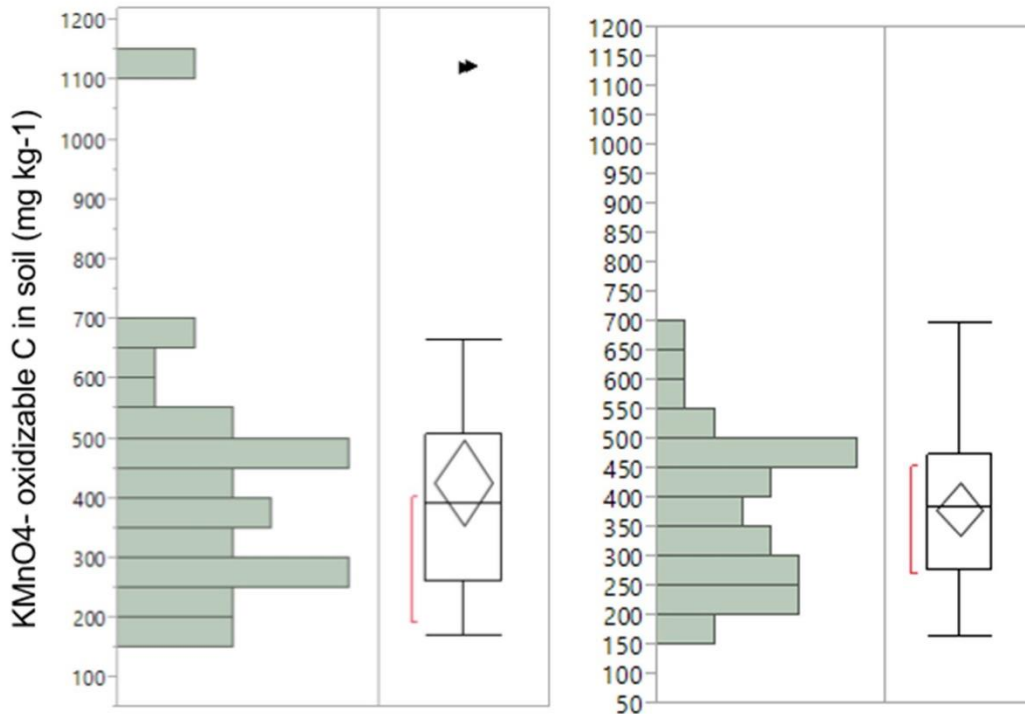


Figure 27. The frequency distribution of values for active carbon measured with this method, from 17 smallholder fields in a mountainous region of Bolivia at left, and 36 fields from Western Kenya at right (Nandi and Vihiga counties) with a box and whiskers plot at the right of each plot. The range of the data represents the full range of the POXC test outlined here (0-1200 ppm). In the data from Bolivia, the two high outlier values at top are from high-elevation peat soils, however, these values could also occur in soils that are frequently amended with compost and manure. Kenyan data courtesy of the CCRP Multipurpose Legumes project and Blessing Magonziwa.

Scoring table for interpreting the results of the “active carbon” or permanganate-oxidizable carbon test (POXC):

Ranges of POXC (mg/kg)	Score or qualifier	Description
<250	Very Low	Indicates soils that have not received substantial organic inputs for many years, or where subsoil is present due to erosion. These values will also occur more frequently in warm, light textured (sandy) soils where organic residues break down quickly and where there are abundant and dense sand particles that dilute the organic matter reading.
250-400	Low	Indicates a soil that may still need work in building organic matter to better support microbes, water holding, and building of soil structure.
400-600	Medium	Likely available organic matter is supporting good function of microbial nutrient cycling, water-holding capacity, etc. These values may also occur in heavy-textured soils that store more carbon, even when soil organic matter is suboptimal. By contrast, in a sandy soil this range of values may already indicate very good levels of organic carbon.
600-1000	High	Soil either has high levels of remnant organic matter from forest conversion, and/or substantial effort has been made to supply crop residues and manures to these soils.
>1000	Very high	These values are found in intensively manured or composted home gardens, in soils converted recently from forest, or in highland peat soils. They are good for many types of crops, but may rarely be attained in more extensive cereal fields where limited organic inputs are available.

3.4.3.4. Example data table and program to perform evaluations of several samples in sequence:

1. Often several samples need to be analyzed in sequence (10 or more samples). For this it is important to realize the importance of maintaining the exact times of the analysis (shake 2 minutes, let sit 10 minutes in the same way for all samples). The following table shows an example of a way to record the active carbon data and also gives cues for the suggested times for each sample, for example the "start time" when the soil is added to the solution, the "settling time" two minutes later when the tubes are left to stand after shaking, and the "measuring time" when they should be read with the colorimeter.
2. As shown in the table, if two people are working together, 5.5 minutes between the sequential samples allows adequate time for all the different steps needed to shake

and read the samples, and so evaluate the samples in series one after the other. However this sequence time can be adjusted once a team has experience. It is especially important that the samples be read 10 minutes after they have been allowed to settle. It may take practice to accomplish this, but try not to be more than 1 minute late (or early) as this will provide the best data. If it is data that has to be rigorous and comparable as in the case of an experiment, it is preferable to practice with samples in sequence that are not important (e.g. batch of 3-4 "expendable" samples) before starting with samples where the data is important.

Table of example data for the POXC (active carbon) test, including times in which the different steps for multiple samples have to be performed.

Sample ID	Weight soil in (g)	Start time	Settling time	Measuring time	Reading in the colorimeter
101	2.52	0	2	12	13.7
102	2.47	5.5	7.5	17.5	5.2
100% solution control	--				17.4
etc.	2.54	11	13	21	etc.

3.5. Extractable Soil Phosphorus (Olsen method)

Video on YouTube : <https://www.youtube.com/watch?v=R1lFrMjoraE>

3.5.1. Materials y Reagents

1. **"Phosphorus-free" water.** In general, tap water (public water supply) will have too much phosphorus (P) to be useful. We have tested different brands of bottled water in several countries and generally there are one or two brands that have a P level below 0.5 ppm (mg / L) that makes it acceptable for this method (there are some brands that have no detectable P, which is even better. Some companies publish their chemical analysis value on the bottle label and if the content of phosphorus (P) is 0.5 ppm or less, the water can be used for this test.

Phosphorus (P) content test for water:

In case you want to test the level of phosphorus (P) in the water, it can be done with the following method and the same reagents and colorimeter that is used in the soil test, as follows:

- Place 10 mL of the water to be tested in a vial for the colorimeter, and 10 mL in another vial for a blank measurement without color.
 - To one of the vials, add a reagent packet as detailed in the method below (step 14); **Note:** it is not necessary to neutralize the water sample with sodium bisulfate as in the soil method, you can directly add the reagent.
 - Cover the vial with reagent, shake well, and wait 10 minutes. In general the water will turn either a very weak color of blue, or no obvious color at all.
 - Read the color of the vial with the colorimeter as described in step 17 below, using the water without reagent added as the C1 blank. The number that appears in the colorimeter must be subtracted from the phosphorus reading in the soil extract before calculating the concentration of P in the soil (see the calculations section in 3.5.5). If the number that is read is zero, no correction is applied.
2. **Sodium bicarbonate** (NaHCO_3): This must also be low enough in P to avoid introducing errors in the method, as described above. Tests in Malawi, for example, showed that sodium bicarbonate purchased in supermarkets had low levels of P and could be used, so local options may work if they can be tested, by preparing the Olsen solution and testing it with the procedure below but without reacting it with soil. Reagent-quality bicarbonate will generally be pure enough without testing.
 3. **Sodium hydroxide** (NaOH), this is not necessary in large quantities in the solution - so it will not generally contribute large amounts of impurities of phosphorus.
 4. **Sodium bisulfate** (NaHSO_4) to acidify the soil extract in preparation for the color reaction; approximately 0.45 g per sample. It is possible to substitute battery acid for this reagent, see the recipe below for sodium bisulfate solution and the instructions below for acidifying the soil extract (step 8) for more details.
 5. **Hanna low-range phosphate reagent** packet (Hanna product number 93713-03, see the materials and reagents at the beginning of this manual); one packet per analysis.
 6. **Balance**, best with at least 0.01 g precision

7. **Olsen extraction solution** (See recipe below). This solution does not maintain its properties and it is better to prepare shortly before the analysis (maximum 1 week if stored in fridge).
8. **High-range Hanna field colorimeter** (see the materials section at the beginning): Note that we INTENTIONALLY use the low range reagents with the high range colorimeter.
9. **Graduated cylinder**, 25 mL with at least 1 mL graduations (see photos below in manual)
10. **A bottle or tube** to shake and extract the soil with the Olsen solution, which can be a well-washed 250 ml plastic bottle (<300 mL, for example) or a 50 mL centrifuge tube.
11. **A second filter bottle**, with a wider lid, for example 4 cm in diameter, which can have 300 to 500 mL of volume, e.g. from milk or liquid-style yogurt. This second bottle is modified with holes drilled in the lid with a sewing needle. If the inside of the lid is not completely flat and has a ring raised inside to seal with the mouth of the bottle, it is necessary to remove this ring. See a video that describes the entire soil extract filtering rig at: https://www.youtube.com/watch?v=FEcQOSA_ur4
12. **Important:** Both bottles must be thoroughly washed with water and rinsed with clean bottle water or distilled water, so as not to contaminate the sample with soluble P.
13. **Paper filters:** cone-shaped coffee filters for cutting circles, or laboratory filters (Whatman # 5, see the equipment at the beginning of the manual). For very clayey soils, the coffee filters will be clogged and are too slow, so in this situation the laboratory # 5 filters are required.
14. **Transparent 11 ml, 0.75 inch diameter vials** that are used with the Hanna colorimeter to read the blue color of the phosphate reaction; the same vials as for the POXC test above, section 3.4.3.
15. **Small plastic cups** to capture the filtered extract and to acidify the extract (2 cups per test, about 6 cm wide 8 cm high)
16. **A rinse bottle** with a nozzle will facilitate rinsing and precise addition of water to bring dilutions up to volume.

3.5.2. Preparing Olsen Solution:

1. The standard definition of Olsen extraction solution a 0.5 M (moles per liter) solution of NaHCO_3 adjusted to pH 8.5 with the required amount of NaOH. As follows:
2. For each 100 mL of solution, put 100 mL of "P-free" water in a clean bottle (rinsed with P-free water) and add 4.20 +/- 0.01 g of NaHCO_3 (0.05 moles) It is better to make just enough solution plus a small margin for each analysis batch, to use within a few days. Each analysis uses 25 ml of solution.
3. Swirl or shake the solution until all NaHCO_3 dissolves. This can take 5 to 10 minutes, it is not a very soluble salt.
4. The pH of this solution will be approximately 7.7 or 7.8. After measuring the pH of the solution with a calibrated pH meter or pH paper strips, add small amounts of NaOH (e.g. 0.1 g if it is 100 mL, 0.2 to 0.5 g if there is more solution) and stir with a spatula or small spoon, or by swirling the bottle. Measure the pH and add more NaOH until the solution has a pH of 8.5 +/- 0.05 (that is, any value between 8.45 and 8.55 is acceptable).

3.5.3. Preparing sodium bisulfate neutralizing / acidifying solution

1. Mix 15.0 g (+/- 0.2 g) Sodium bisulfate (NaHSO_4) into 100 mL water, measuring the water either by weighing or with a graduated cylinder
2. Store in a plastic bottle and avoid contact with skin and eyes. This is an acidic solution and should keep quite well, and can be used for several months. You will need 3 mL for each analysis and therefore may want to mix 100 mL at each time for up to 33 samples.
3. **TO USE DILUTED BATTERY ACID AS A SUBSTITUTE:** carefully and with gloves and goggles, and in a well ventilated space or outdoors, accurately dilute battery acid v:v 3+1 with water (add 3 parts “phosphorus-free” water considered above in the materials to one part battery acid). This concentration of acid (~7.5%) can now be handled in a plastic bottle and with a dropper, without representing an inhalation hazard. Care should still be taken since it will destroy clothing and burn skin if not rinsed quickly with water. The appendix in section 3.5.6 describes testing this diluted battery acid to establish how much volume should be used to neutralize the sample extracts.

3.5.4. Procedure (Fig. 28):

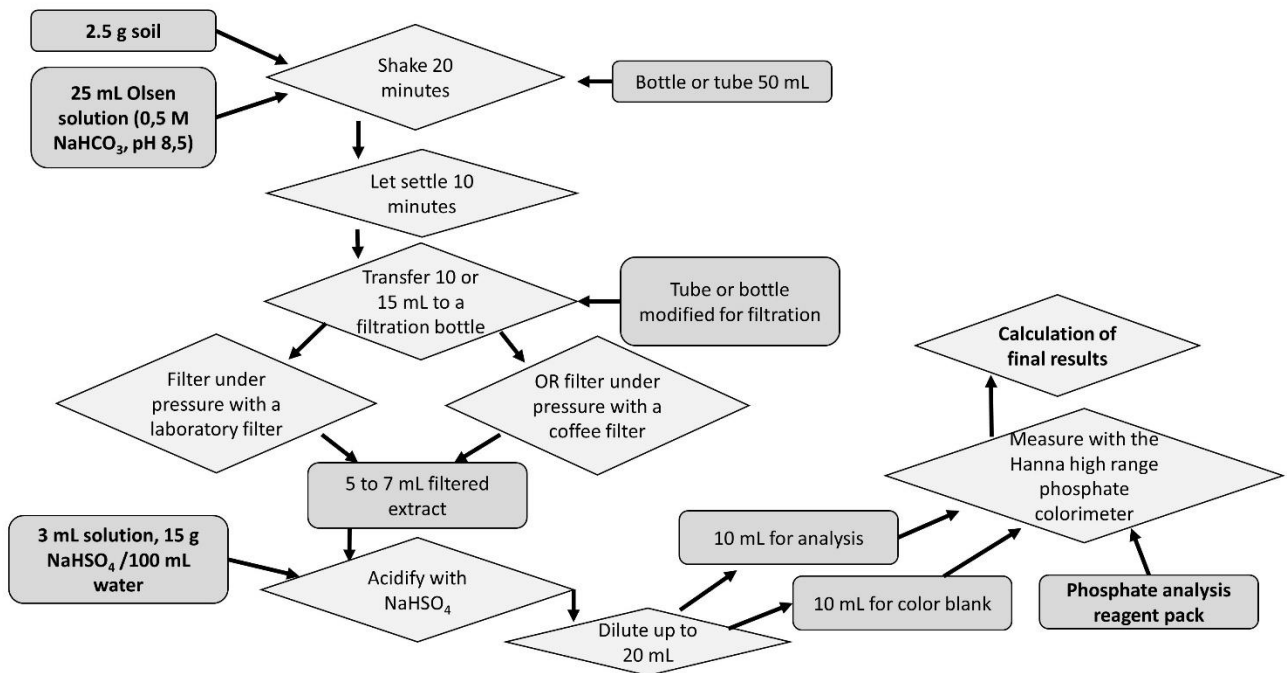


Figure 28. Flow chart diagram for the analysis of soil extractable phosphorus (P).

1. Weigh 2.5 g of sieved soil (2mm) in the centrifuge tube or other bottle.
2. Using a graduated cylinder add 25 mL of Olsen solution to the sample of soil in the bottle or centrifuge tube (Fig. 29). When many samples are analyzed, Olsen solution can also be pre-measured or weighed in bottles before adding soils, to save time)



Figure 29. Adding 25 mL Olsen solution to the soil in a re-used juice bottle to start the extraction.

3. Cover the bottle with the lid closed and shake 10 minutes. If by hand, it is acceptable to alternate between shaking the bottle and putting down briefly to tend to other tasks.
4. Let the suspension sit 10 minutes. Some of the clays will settle during this time, but a transparent solution is not necessary.
5. There are now a number of alternatives for filtration: (1) traditional filtering with filter paper folded into a cone; (2) using laboratory filter paper and a filter press to speed filtering of each sample; and (3) using a filter press and filter paper from cone coffee filters, which is usually successful for light textured and loamy soils. To create a filter press, a bottle is used to perform the filtration under pressure, with a filter inserted into the bottle lid with holes in it to allow the filtrate to pass. . See a video that describes the entire soil extract filtering rig at: https://www.youtube.com/watch?v=FEcQOSA_ur4 . In every case It is important to practice this step to gain confidence in producing a filtered extract without turbidity (suspended clays) which will invalidate the measurement of blue color to estimate phosphorus content:

In the case of traditional lab filter paper folded into a funnel:

- i. Fold Whatman's #5 filter paper in quarters or other means to produce a cone-shaped filter. Some workers make a very wide, shallow funnel which can be set easily into a plastic cup and drops filtrate directly into the cup.
- ii. Using a transfer pipet or pouring the extract, transfer settled Olsen extract from the top of the filter paper funnel.
- iii. Wait 20 or 30 minutes for 5 to 7 mL of filter to pass through (ideally, 7 mL)



Figure 30.

In the case of coffee filter paper and a filter press:

- i. Transfer the supernatant (clay suspension in the upper part of the bottle) from the extraction bottle to another filtration bottle. This bottle must have a lid with a flat surface inside to place a filter in the shape of a circle, and small holes in the lid (<1mm diameter) to let the filtered liquid flow. See the YouTube video referenced above.
- ii. A double layer of coffee filter paper cut into a circle is inserted into the perforated lid of this bottle. Then, by hand or with a wooden press (see Figs. 31 to 33), press the bottle until you see drops coming out.
- iii. Do not initially save these drops as they will come out with some clay and turbidity. Over time the drops should become more transparent, although there will still be a light brown color of the transparent solution, and this is fine.
- iv. After discarding the first drops if these are cloudy, start collecting the clear drops in a new clean glass until you have a volume between 5 and 7 mL. 7 mL is indicated if there are no problems in collecting this solution. This process can take up to 10 minutes, so it is recommended to use the wooden press (Fig. 32) to be able to free your hands for other work, especially if there are many samples to analyze.



Figure 31. Placing a double layer of coffee filter paper below the lid of the bottle.



Figure 32. Pressing the bottle by hand to create a filtered soil extract.

In the case of laboratory filter paper and a filter press (Whatmans grade #5 filter with 2.5 micron por size, see materials in section two of this manual)

- i. Leave the extraction bottle without moving it a lot so as not to disturb the settled clays.
- ii. Using the transfer pipette or a dropper, transfer the upper 10 to 15 mL of the soil suspension in the extraction bottle to the new bottle for filtration. This suspension will still be cloudy, but the idea is to minimize its level of clay so as not to obstruct the filter paper too much. See the filter rig video referenced above for more details on the filtration method.

- iii. Put a single circle (not double) of filter paper inside the lid with holes and place carefully, but firmly, on the filtration bottle. Overtightening may rip the filter paper, but it does need to be quite tight. Trial and error will indicate the correct tightness.
- iv. Turn over the bottle and press it by hand or with a filtration press that can be made for the purpose (Fig. 30 below)
- v. The drops that come out of the filter should be clear and can be collected in a clean glass directly (unlike the first drops with the coffee filter option above). If they are cloudy, you should check for rips or cracks in the filter.
- vi. Keep collecting drops until you have between 5 and 7 mL for the next steps (7 mL is best)



Figure 33. A bottle press to help with filtration using bottles and filter paper in the lids, which allows maintaining pressure on the bottles and use a laboratory filter or a coffee filter to extract the clays from the extract. This approach saves time because while pressing the bottles one can proceed with other tasks.

6. **Regardless of the filtering method above:** Continue filtering clear drops in the cup until you have 7 mL or a little more. If filtration is very difficult, you can reduce this amount by something between 5 and 7 ml, and you will need to reduce the amount of sodium bisulfate solution proportionally in the acidification step below (see table below)
7. Empty exactly 7 ml of filtrate (or less if the filtrate was difficult) into a clean or recently rinse graduated cylinder (shake out any excess water if rinsed).

mL filtered soil extract	mL of sodium bisulfate solution to add for acidification of solution	Alternative: volume of diluted battery acid (3+1) to add (mL)
5	2.25	1.1
6	2.75	1.3
7	3.10	1.5

8. Pour the 7 mL soil solution into a clean plastic cup, and then using a clean transfer pipet or dropper with graduations, add 3.1 mL of the 15g/100 mL sodium bisulfate solution prepared previously (step 3.5.3 at beginning of this protocol).
9. **TO SUBSTITUTE DILUTED BATTERY ACID:** instead of the 3.1 mL of bisulphate solution, add 1.5 mL dilute battery acid for each 7 mL of filtered extract. You should add less bisulfate solution or diluted battery acid if less filtrate was obtained (see the table above for bisulfate and diluted battery acid amounts, assuming battery acid diluted 1+3 acid+water) With soil samples from very calcareous (neutral to high pH) soils, you can add a few extra drops of bisulfate solution or battery acid to neutralize extra carbonates in the solution.
10. The solution will bubble as the bicarbonate ions are neutralized (Fig. 33). The acid bisulfate (or diluted battery acid) lowers the pH of the extract from approximately pH 8.5 to pH 6, so that when the reagents are added then they can lower the pH to approximately pH 1 or 2 where the development of the blue color of the molybdate-ascorbic acid complex can occur. Without this low pH the blue color (proportional to phosphate concentration) cannot be developed.



Figure 34. Mixing 3 mL solution of sodium bisulfate (15g NaHSO₄ per 100 mL) to acidify 7 mL of filtered extract. Note the bubbling of the soil extract as bicarbonate reacts with the acidic bisulfate.

11. While allowing the solution to bubble off CO₂ from the bicarbonate, in order to save time, a reagent pack can already be added to a clean, dry colorimeter vial for step 14 below (Fig. 35).
12. After bubbling subsides, pour the acidified extract from the cup back to the 25 mL graduated cylinder. Rinse the cup with a little water (<4 mL for each rinse) with the wash bottle and add this rinsing water to the cylinder as well. Then fill the cylinder up to a final volume of 20 mL +/- 0.2 mL (Fig. 34). This will give you 10 ml diluted extract for the vial with the reagent, and 10 ml as a control without added reagent to be placed into the colorimeter. (to explain: the extract without reagent will be yellow to brown colored, so we want to correct for this color by using it as a blank in the colorimeter).



Figure 35. The extracted, filtered solution has been acidified and brought to a standard volume of 20 mL. It is ready for reacting with the color reagent to develop the blue color and then read with the colorimeter.

13. **Important:** Mix the sample well before placing it in the vial to perform the colorimetric reaction, by pouring it back and forth between the graduated cylinder and the cup where you acidified it.
14. Add the contents of the phosphate reagent packet to a clean, dry vial (Fig. 35). You can cut the package straight on the top, then open the top of the package in a square or diamond shape, and pour a few times, to ensure that all the reagent enters the vial.

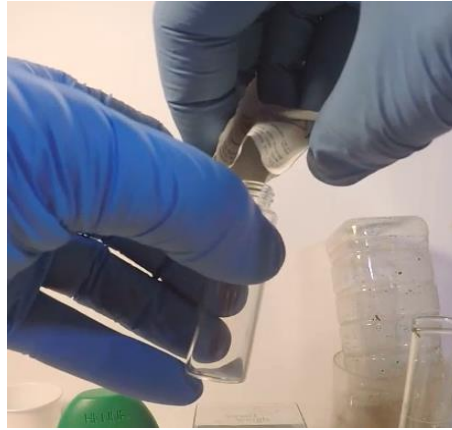


Figure 36. Adding the phosphate reagent packet to a clean, dry vial.

15. Add 10 ml of the 20 mL extract solution in the graduated cylinder to the vial with the reagents. Mark a 10 ml line in advance or pour until the space between the meniscus and the lid is the same as the thickness of the lid, which is equivalent to the 10 mL level.
16. Add the remaining solution in the test tube to a 'control' vial or blank without added reagent. For samples of the same approximate type of soil, the same control solution can be used for several samples because the yellow or brown color of the extract will be quite similar. When large differences in organic matter occur, the control vial will be darker for soils with more organic matter and a different control vial should be used for these different soils.
17. Cap and shake the bottle with the reagent. You may have to unscrew the vial once or twice to release bubbles. If, when adding the reagent, it releases many bubbles as in the acidification step above, it is a sign that not enough bisulfate was used to acidify, perhaps because it is a very calcareous soil, and it can take another few drops of bisulfate solution (up to 0,2 mL) before reading the result. However, note that it is normal for the solution to release some bubbles with the reagent.
18. A blue color should develop in the reagent vial (Fig. 36). Read the blue color after about 10 to 15 minutes in the high-range Hanna phosphate colorimeter:
 - Turn on the colorimeter
 - When C1 appears, put the control vial (clear or light brown color, no reagent) and press the button.
 - When C2 appears, change the control bottle for the vial with added reagent (blue color, if there was phosphate present) and press the button.
 - Record the reading in ppm of phosphate. Remember that this reading is **not** the final result because you have to perform the calibration steps and the final calculations



Figure 37. A blue color will develop in the extract with the reagent packet added. The other yellow colored vial serves as the color blank to be measured in the colorimeter.

19. It is possible that, when the test is performed the first few times, you want to repeat the readings after 20, 25 and 30 minutes, to test if the color development continues. The blue color should reach a maximum after 15 to 20 minutes but does not change much after 15 minutes.
20. In many soils, after approximately 30 minutes the blue color is combined with organic matter (MOS) dissolved by Olsen extraction and produces blue particles and a precipitate. It should not be read after this point because the blue color will begin to diminish. In very high soils in organic matter, this process of precipitation can complicate the reading already from the 20 minutes, and it is necessary to make a conjecture as far as the best moment of the reading, and to register this in the observations.
21. In case the level exceeds the calibration limit below, which would be greater than 20 ppm reading in the colorimeter, it is advisable to use less soil in the analysis (eg 1.5 g instead of 2.5 g) to reduce the final level of phosphate that needs to be read by the colorimeter.
22. **Handling reaction wastes:** Placing diluted reagents in an infertile soil or compost will likely not cause any adverse effects or toxicity, and molybdenum may even act as a nutrient for plants. It can also be disposed of in a public sanitation system. The extracts read in the colorimeter (which are acidic) can be neutralized with a little bit of kitchen or fire-pit ash.

3.5.5. Calculation of Olsen available phosphorus in the soil:

1. First, the raw concentration of phosphorus in the final solution that was placed in the colorimeter is calculated by means of a previously developed calibration curve:

$$\text{Raw Conc. of P} = P_{\text{raw}} = 0.0559 \times \text{colorimeter reading} - 0.0052$$

2. In the event that a blank value was recorded when the reagents were checked while preparing to do this test, reflecting slight contamination of the water or sodium bicarbonate used (see section 3.5.1, section on “**Phosphorus (P) content test for water**”), this equation is modified slightly to reflect the blank value (C_{blank}):

$$\text{Raw Conc. of P} = P_{raw} = 0.0559 \times (\text{colorimeter reading} - C_{blank}) - 0.0052$$

3. Where C_{blank} is the value of the blank measured with the colorimeter for a sample of clean water. In the event that bicarbonate purchased from a supermarket was used, it is preferable to assume that there is a certain level of contamination and use a value of $C_{blank} = 0.2$
4. Then the concentration of P in the original extract is calculated after shaking the Olsen solution with the soil:

$$\begin{aligned} \text{Conc. P in extract} &= [P_{extract}] \\ &= P_{raw} \times \frac{20 \text{ mL}}{\text{mL solution used for neutralizing}} \text{ (mg/L)} \end{aligned}$$

5. Where the "mL solution used for neutralizing" generally refers to 7 mL measured to neutralize with sodium bisulfate, and may vary between 5 and 7 (see step 6 above)
6. Next, $[P_{extract}]$ is the concentration (mg/L) of phosphorus in the extract and from this we can calculate the **amount** (mg) of phosphorus in the extract, in this way:

$$\text{Amount P in extract (mg)} = P_{extract}(\text{mg}) = [P_{extract}] \times 0.025 \text{ L}$$

Remembering that there we created a total of 25 mL of extract from the soil, equivalent to 0.025 L.

7. Finally, to calculate the amount of phosphorus available in the soil, we divide this amount in mg by the initial weight of the soil in kg:

$$P_{available \text{ in soil}} \text{ (mg/kg)} = \frac{P_{extract}}{\text{initial weight of soil (kg)}}$$

8. Take into account that the weight in grams of the soil must be divided by 1000 to find the weight in kg: for example, 0.0025 kg for 2.50 g or 0.00243 kg, for example if we use 2.43 grams of dry soil.
9. In case we use moist soil we have to adjust the results for soil moisture, which would be to divide the result up by [1 - water content in the sample]. Moisture must be measured separately, or can be approximately rated with a visual rating such as that in [appendix A](#).

Example of calculating the result for available P:

10. 2.63 g of soil is initially weighed. Then 7 mL of extract was filtered for neutralization, and when analyzing the color with the colorimeter an intensity of 14.5 units is read. Distilled water and a bicarbonate reagent have been used that

have no detectable phosphorus content. Then the level of available P is calculated as follows:

- a. $P_{\text{raw}} = 0.0559 \times 14.5 - 0.0052$ (no correction for a blank value)
= 0.8503 mg / L
- b. Then, $[P_{\text{extract}}] = 0.8503 \times (20/7) = \mathbf{2.429 \text{ mg / L}}$
- c. And the quantity extracted will be: $2.429 \text{ mg / L} \times 0.025 \text{ L} = \mathbf{0.06074 \text{ mg}}$
- d. Then the level of $P_{\text{available}}$ in the soil would be: $P_{\text{available}} = 0.06074 \text{ mg} / 0.00263 \text{ kg} = \mathbf{23.1 \text{ mg / kg}}$
- e. This corresponds to a high level of P available according to the table below for the interpretation of results. Keep in mind that in reality this result is “extractable P” according to a certain method (the Olsen method). It is an estimation of available P that allows us to compare different soils and rate their P fertility, rather than an absolute definition of plant-available P, which depends on many other factors.

Scoring table of qualitative ranges for extractable P with the Olsen method:

Value of Olsen available P (mg/kg)	Score	Description
0 to 5	Very Low	Biomass, vigor, and maturity of most crops will be severely limited and deficiency symptoms may occur, especially when P is the only limited nutrient; if crops are multiply limited, growth may just be limited in an overall way (low biomass)
5 to 10	Low	Crops may exhibit maximal P response since some P, but not entirely enough, is present to fuel growth and vigor.
10-20	Medium	Many crops will still respond to additional inputs of manure, compost, or P fertilizer, especially legumes and many flowering/fruited vegetables. Some P-efficient cereals may already attain sufficiency.
20 and above	High	Most crops will not be limited by P fertility. Some vegetables and weeds may however continue to respond better at ever higher levels e.g. 30 to 50. Values greater than 50 indicate inefficient, over-allocation of phosphorus to these fields, and nutrient pollution of soils and potentially, watersheds.

3.5.6. Appendix: verifying the amount of diluted battery acid needed to neutralized Olsen extract solutions:

1. If you are interested in substituting dilute battery acid for the Sodium bisulphate solution (see materials section 3.5.3) you may be using 1.5 mL of the diluted battery acid as suggested in step 9 of the procedure, but you may also want to verify that this amount is correctly neutralizing the soil extract. You will have some battery acid that you have diluted in the relation of 3 parts "P-free" water plus one part battery acid. Have this on hand as well as a transfer pipet or small graduated cylinder that will allow you to add measured amounts of the diluted acid to some Olsen solution, also prepared according to the materials section.
2. You will also need a pH meter or pH test strips to verify the pH of the Olsen Solution
3. Place 70 mL of Olsen solution (without adding any soil or other materia) into a beaker or jar. Measure the pH of this solution, which will be around 8.5.

4. Add small amounts of the diluted battery acid to the beaker or jar with Olsen solution, starting with 1 to 2 mL, and verify that the pH is dropping.
5. Measure the amount of the diluted battery acid required to lower the pH of the Olsen solution to between pH 6.0 and 6.2. This will probably be around 15 mL of diluted acid.
6. Since 70 mL of Olsen solution was used for this calibration test and only 7 mL is neutralized in the main procedure (see steps 8 and 9 of the main procedure) , you should divide the amount you measured in this exercise by 10. So if 17 mL were needed, you would add 1.7 mL in step 9, instead of the 1.5 mL suggested.

3.6. Amino-Sugar N test for medium-term available soil nitrogen



Figure 37. view of the test jar before closing the jar and incubation, note dark soil + NaOH solution at bottom of jar and small cup of boric acid color indicator suspended above.

Method in brief - N in amino sugars (Amino Sugar Test, Khan et al., 2001^{*1}),)

3.6.1. Materials:

- Heating plate that can maintain between 45 to 50 C, (48 to 50 °C is ideal)
- Modified glass jars to suspend a small cup inside which can easily hold 5 mL
- Distilled water for the indicator solution (10 mL total per test)
- Boric acid (HBO_3)
- Digital balance with 0.01 g precision (10 mg): some substitution of this balance with graduated transfer pipets is possible, but the balance will be needed in any case for weighing an exact amount of soil, and is also a very easy way to perform the titration at the end, therefore it is very helpful to have such a balance.
- Methyl red and bromocresol green - pH indicators
- Sodium hydroxide (for the digestion solution to react with soil, also a small amount for adjusting the boric acid solution)
- Sulfuric acid (a diluted solution is prepared for titration)
- Dry soil sieved to 2mm



Figure 38. Design of a 500 mL jar with a small plastic cup suspended inside containing the boric acid trap solution. Soil being digested by 2M sodium hydroxide solution at 48°C is placed on the bottom of the jar below the cup.

¹ See: Khan, S.A., Mulvaney, R.L. and Hoeft, R.G., 2001. A simple soil test for detecting sites that are nonresponsive to nitrogen fertilization. Soil Science Society of America Journal, 65(6), pp.1751-1760.

3.6.2. Procedure Overview

First, consider the experimental setup for this test (Fig. 3.6.1):

- Inside a sealed jar is suspended a small cup of boric acid indicator solution with two pH color indicators. Below about pH 4.2, the solution is a bright red; above this pH, it turns purple and then green-blue (Fig. 41).
- Below at the bottom of the jar is one gram of soil mixed with a strong basic solution (2M NaOH). When placed on a hot plate at 48-50°C the basic solution will hydrolyze organic nitrogen in the soil and create ammonia (inorganic ammonium in the soil will also be released).
- This ammonia is then trapped by the boric acid indicator solution. The pH and color of the indicator solution will change, and by returning it to its initial pH and color, we can know how much ammonia was produced.
- This amount of ammonia released is an indicator of soil medium-term available N.
- Note that the hot plate supporting the jar, and the fact that the top of the jar (and the trap solution) hanging from the lid) are cooler than the bottom of the jar and soil/NaOH mixture is key to performing the measurement. It will not work if placed in an oven so that the entire jar heats to 50 C, for example.

3.6.3. Making Reagents

7. Prepare the trap / indicator solution for ammonia which is 40 g / L of boric acid, with two pH indicators, bromocresol green and methyl red (Fig. 39). The amounts of the pH indicators are so small that it is best to make a more concentrated stock solution of these indicators, and then dilute these into water before making the boric acid trap solution. This is done as follows:



Figure 39. The color indicators Methyl Red and Bromocresol Green. Very tiny amounts of each are needed to make the indicator solutions.

1. Make a stock solution of 0.35 g per litre in water of methyl red and 0.50 g/L of bromocresol green (or equivalent concentration, for example 0.17g and 0.25 g of each color indicator, respectively, in 500 mL). Note that these are already very small amounts and that these will be diluted 1:50 in the final solution, i.e. 500 mL of stock solution will last a very long time, and should be frozen if possible, to preserve it.

2. Now, to make 200 mL of boric acid indicator solution, **first** add 4 mL of color indicator stock solution to 196 mL of distilled water (Fig. 40). This will dilute the indicators to their final concentration.



Figure 40. Adding 4 mL indicated stock solution to 196 mL water to create the final concentration of pH indicator, before adding boric acid.

3. To this 200 mL of water with color indicator, add 8 g boric acid and mix well. Mixing this in a glass bottle will allow you to shake the solution and heat with a hot water bath to dissolve the boric acid more quickly. This proportion of boric acid to water is close to the limit of solubility for boric acid, and heating this solution slightly (to 50 C, say) will help markedly to dissolve the boric acid.

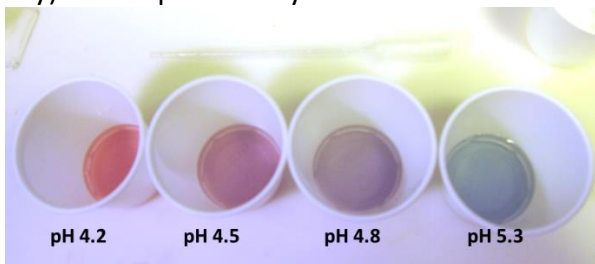


Figure 41. The color of the Boric Acid indicator solution at different pH levels.

4. Adjust this solution with drops of 0.1 M H_2SO_4 or NaOH solution (with a concentration of 0.1M of either, say) to pH 4.2. Note that it may already have a pH very close to this value so it may not be necessary to adjust very much. When this solution is below pH 4.2 it has an orange hue because of the combination of yellow from the bromocresol and red from the methyl, at higher pH the solution turns purple and then blue as the methyl red disappears and bromocresol change colors (Fig. 41).
5. Cool and store this solution before use (within a few days or a week, say). 200 mL is enough for 40 tests, so make less if you do not need as much.
8. Make 2M NaOH solution (10 mL needed per measurement). For each 100 mL solution, add 8g NaOH (sodium hydroxide) to clean water. This does not need to be distilled water, clean bottled water is sufficient. Mix well and allow to cool. Do not make more than is needed of this solution since it is quite caustic and best not to store large quantities of it.
9. 0.01 M H_2SO_4 is needed for titration. It is important that this be of the most precise concentration possible. This can be prepared from commercially bought 1M or other concentration standard sulfuric acid (if 1M, q1dilute this 100x with distilled water). It can also be prepared from concentrated sulfuric acid, which should be handled with great care

(chemical-resistant gloves, protective eyewear, add the acid to water and not the reverse, work outside to avoid exposure to fumes, or in a fume hood). To make 0.01 M H₂SO₄, first make a small amount of 1 M stock solution: place 30 mL of distilled water in a glass container-- either a graduated cylinder, volumetric flask, or narrow bottle marked previously with a line to contain exactly 50 mL. Then add 2.7 mL of concentrated sulfuric acid with a transfer pipet (you can also add this by weight, it will weigh exactly 5 grams). Add additional water to the line until you have a volume of 50 mL and mix thoroughly, for example by pouring back and forth into a clean storage bottle. You should handle the acid with care and wear rubber gloves and safety glasses for this procedure. Now that you have a 1 M stock solution, add exactly 1.0 mL with a transfer pipet to 99 mL of water to make 0.01M sulfuric acid. **This standard solution can also be prepared from *commercial battery acid*** after measuring its concentration and then making the 0.01 M solution based on this concentration, see the section at the end of this method.

10. Distilled water is also needed at the end of the procedure to dilute the trap solutions before titration. It is important that this water be as pure as possible since any effect on the pH of the trap solution from this water will be interpreted as nitrogen in samples, causing errors.

3.6.4. Procedure:

1. Preheat a hot plate to 48°C +/- 2°C (the surface of the hot plate can be checked for this temperature with a traditional glass or digital thermometer)
2. To the small plastic cup that will be suspended in the jar, add 5.00 mL indicator solution. One way to do this precisely is to weigh out 5.00g (+/- 0.03g) of solution for each test. However, a graduated transfer pipet can also work if care is taken with filling the pipet exactly; if a precision pipettor is available, this is also a good method.
3. This small cup can either be stored temporarily on the table or lab bench, or already suspended and "stored" in the jar by clamping it to the bent screw (or other arrangement) on the bottom of the lid and placing it temporarily on top of a jar.
4. Add 1 g soil to the bottom of a clean jar. To perform many tests in parallel, jars can be prepared all at once without adding the 2M NaOH solution yet. Be sure to label each jar with the identification of the sample.
5. At the moment of beginning the test, add 10 mL of the basic NaOH solution to the 1g soil in the jar, and swirl gently to suspend the soil.
6. Immediately cap the jar with the lid and the small cup with indicator solution suspended below, and tighten the lid to seal the jar. Place the lid on carefully to not spill the indicator solution inside the jar.
7. Heat the jar or jars on the hot plate for 4 hours. If there are more than one jars, these should be carefully exchanged in position on the hotplate every hour or so, to correct for unevenness in the temperature of the plate.
8. In the meantime, a 0.01 M H₂SO₄ solution can be prepared, by diluting a 1 M H₂SO₄ solution (prepared previously) exactly in the relation 1:100 with distilled water. Mixing 1 mL 1 M H₂SO₄ with 99 mL distilled water will accomplish this.

9. Just before the end of the incubation of four hours, prepare a “blank” cup of indicator solution for color comparison of the indicator solutions from the jars:
 - a. Weigh out or pipet 5.00 mL indicator solution into a small, clean plastic cup of the same type you are using in the jars. This will allow more precise comparison of the indicator color across all the cups.
 - b. Add 5.00 mL distilled water; weighing 5.00 g of water +/- 0.04 g is a good way to do this. The solution will change color slightly to a more purple color as the pH rises from adding water.
 - c. Re-tare the balance with the cup and mixed indicator solution/water, and add drops of 0.01 M H₂SO₄, weighing what is added, until the original color of the solution is restored (red with a slight orange tinge, see the pH 4.2 cup in Fig. NNNN). This will fix the endpoint color for the titration, and you will know the grams of dilute acid (H₂SO₄) it took to establish a “blank” reading, without a soil incubation. You should record this amount as T_{blank} or the “blank titrant” and you will need to subtract this “blank” amount of acid added from the readings of samples, see step 11 below. Usually this blank value is quite small, only a few drops.
10. Then when the incubation is finished, remove the jars from the heat and place them carefully to one side. One by one you will open these jars and titrate the indicator solution which will have likely changed color. Titration means to add enough 0.01 M H₂SO₄ solution to restore its original pH, measured by examining its color. This is done as follows for each jar, basically repeating what was done for the blank solution above in step 9:
 - a. Carefully remove the small cup with indicator solution from the jar, lifting it out with the lid and unclamping the small cup. Be careful as you do this to not allow condensation from the lid of the jar to fall into the cup with the indicator solution. To avoid this problem, you can suspend a rag or paper towel firmly to allow blotting of the lid as you move it to where you will un-clip the plastic cup.
 - b. Next, add an additional 5 mL of distilled water to the cup with indicator solution (this will fix the amount of ammonia dissolved and keep any more from escaping). There may be a change in color. This can be done by placing the cup on the scale, taring it, and then adding 5.00 g of water.
 - c. Next tare the scale again and begin to add drops of 0.01 M H₂SO₄ using a transfer pipet or dropper and watching the weight of acid added (here grams of solution = mL, since this is a very dilute acid solution). You will also be gently swirling the cup after every few additions of the dilute acid and observing the colour change of the solution which needs to eventually resemble the blank solution from step 11.
 - d. As the color of the solution begins to resemble the blank solution from step 9, it is important to swirl the cup every few drops to ensure that the acid has been fully mixed in so that the color has stabilized. Usually the correct color can be achieved to about 1 or 2 drops.
 - e. Record the weight of dilute acid added from the digital balance as the titration value for the sample, T_{sample}, in mL – you will need this for the calculations in step 11 below.

3.6.5. Calculation of Amino-Sugar or hydrolysable N in soil:

11. The amount of labile N in the soil is estimated using the calculations:

$$\text{Quantity N released in hydrolysis (micrograms)} = 280 \times (T_{\text{sample}} - T_{\text{blank}})$$

where T_{sample} is the quantity of 0.01 H_2SO_4 added in step 10 above to titrate the cup incubated with the soil, and T_{blank} is the quantity of 0.01 H_2SO_4 added in step 9 to titrate the blank cup.

12. From this quantity N released and knowing the amount of dry soil that entered the test, the concentration of this fraction of N is calculated:

Concentration of N = Quantity N released (micrograms) / soil used (g) which also has units of ppm or parts per million, or mg/kg. Usually the amount soil used is just 1 g, which makes this calculation especially easy. However, you may have precisely weighed the weight of soil as 1.05 g, for example, and this more precise weight should be used.

13. **Rating of Available N using this test:** there is limited experience with the use of this test in smallholder agriculture systems. Nevertheless, research done by those who developed the test a critical value which established about the levels of Amino-sugar N at which crop growth no longer responds to extra added fertility of Nitrogen is about 230 mg/kg (Khan and Mulvaney, 2001²), and this may be a convenient dividing line to rate soils which are low (<230 mg/kg) versus high (>230 mg/kg) in nitrogen that is likely to become available to crops over the next three to six months.

3.6.6. Appendix: Finding the molarity of battery acid in order to prepare a 1 M stock solution of H_2SO_4 for titration:

Materials:

- Sodium Hydroxide (NaOH)
- Battery acid for testing (generally this is sulfuric acid at about a 30% concentration)
- Beaker and graduated transfer pipet for precisely transferring 1 mL quantities
- Graduated cylinder for measuring liquid amounts to dilute solutions
- Gloves and safety glasses are recommended for this entire procedure, especially the dilution of battery acid at the beginning.

1. Prepare a small amount of 4 M Sodium Hydroxide for neutralizing the battery acid, by diluting 8 g of NaOH into 50 mL of water. This can be mixed in a small glass bottle to swirl or shake it to dissolve the NaOH. The solution will warm up and needs to be cooled to

² See: Khan, S.A., Mulvaney, R.L. and Hoeft, R.G., 2001. A simple soil test for detecting sites that are nonresponsive to nitrogen fertilization. Soil Science Society of America Journal, 65(6), pp.1751-1760.

room temperature to ensure its density is correct, before finding the concentration of the battery acid via neutralization (titration) as shown below

2. Add 1 mL of the 4M NaOH solution to 39 mL of water in a separate bottle, to create some 0.1 M NaOH that can be used for final neutralization of the battery acid once it is above pH 2 or so.
3. Be sure to have a calibrated pH meter, or a supply of pH test strips on hand that can measure both below and above pH 7.
4. Add 10 mL of battery acid to 20 mL in a beaker (3x dilution), to avoid exposure to fumes from full-strength battery acid. You will now find the concentration or molarity of this diluted battery acid by neutralizing its pH to around 7.
5. Add 15 mL of the 4 M NaOH solution to the beaker with the diluted battery acid. This will start to get the solution from strongly acidic towards neutral, although its pH will likely still below 1. Measure the pH of the solution at this point. Do not leave the probe for long in the solution, just check if it is less than 1 and then put it quickly back into rinse water. If it has already exceeded 7, the battery acid was not very strong. Start again and add only 10 mL before measuring the pH and preceding to the following step.
6. If the pH measured in step 4 above is still less than 1, add 5 mL more of 4M NaOH.
7. Then, add 4M NaOH 1 mL at a time with the graduated transfer pipet, as precisely as possible. Check the pH after every mL addition. When the pH starts to rise noticeably above 1 or 1.5, say, record the milliliters of 4M NaOH added, and switch to the 0.1 M NaOH created in step 2 to neutralize, adding this 1 mL at a time, until you achieve a pH between 6 and 8 (more precision is not necessary and will not create greater accuracy for the method)
8. If you overshoot and the pH rises well above 8, either estimate the milliliters of 0.1 M NaOH at which the pH between 6 and 8 occurred, or add 0.5 mL of similarly diluted battery acid to what you began with (1 H₂SO₄ + 2 distilled water)
9. Now you can calculate the total moles of NaOH added which is the same as the moles of OH⁻ added to neutralize the H⁺ ions from the sulfuric acid: Because molarity is moles per litre, you can find this as:

$$\text{Moles NaOH} = (\text{mL 4M solution} / 1000) * 4 + (\text{mL 0.1M solution} / 1000) * 0.1$$

Then, since each mole of H₂SO₄ will produce 2 moles H⁺, we should divide this amount of OH⁻ ions by 2 to find out approximately how many moles of sulfuric acid were in the original acid, and the molarity, knowing that 10 mL of sulfuric acid were in the original 3x dilution of battery acid. If you had to correct the procedure for overshooting (step 8) by adding an additional 0.5 mL of diluted battery acid, be sure to correct for this by using (30.5/3) or 0.01017 L as the volume to divide by below, instead of 0.01.

$$\text{Concentration of original battery acid} = (\text{Moles NaOH from above} / 2) / 0.01 \text{ L,}$$

Where 0.01 L is the equivalent of 10 mL expressed as litres.

10. **Example:** Using the procedure above for battery acid purchased in the United States, it was found that we needed 24 mL 4M NaOH to bring the pH to the point where it was rising towards 7; and then 7 mL additional of 0.1M NaOH to bring the pH to pH 6.5, where we stopped.

So, following the calculations in 9. Above, we would find:

$$\text{Moles NaOH} = 24/1000 \times 4 + 7/1000 * 0.1 = 0.0967 \text{ Moles}$$

$$\text{Then, the molarity of the 10 mL battery acid is } 0.0967 / 2 / 0.01 = 4.835$$

(divide by 2 for the difference between one OH on NaOH vs. two H+ in H₂SO₄, 0.01 = 10 mL)

This molarity is then 4.835 or just 4.8 (we can see why it was only important to neutralize pH to between 6 and 8, not more precisely)

So, to make the initial 1M stock solution in the main method, for later dilution to 0.01 M to use in titration of the color indicator solution of boric acid (step 8 of the main procedure above), we would want to dilute this solution by 4.8x, say 100 mL of the battery acid + 380 mL of distilled water for a total volume of 480 total mL.

3.7. Soil Macrofauna Evaluation:

Evaluation of soil invertebrate communities offers a simple and low-tech option for studying soil biology, and this method offers a number of key advantages. Soil macrofauna are sensitive to changes in their environment and shifts in their community structure offer an integrative assessment (i.e., combining changes in multiple soil properties into a single measure) of ecosystem impacts over time. Also, soil macrofauna, particularly ecosystem engineers (e.g. ants, earthworms), can have significant influences on soil and ecosystem functioning and thus their populations reflect key ecological processes within soils and ecosystems. Finally, large soil invertebrates are relatively simple to measure, ubiquitous, and familiar to land managers, as they are frequently encountered during soil management activities.

This procedure takes more time than some other evaluations. It is good to perform the evaluation as a team, to share the work with a group and foster group learning and observations about life in the soil. The evaluation requires the soil to be in a productive state, generally during the rainy season and with growing plants and crops present. Although it appears at the end of this manual, it can be an excellent starting point for observing and learning about a soil because soil life can be observed and because the soil is extensively handled, leading to a general sense of its tangible qualities before pursuing the other chemical and physical tests. In addition, as described in the introduction of the manual (see Fig. 3) this procedure will generate a sample of "clean" soil to be used in the chemical tests such as pH, activated carbon, and available P, and can be thought of as a sampling method. As noted earlier however, this procedure should not be used to create a sample for aggregate stability (section 3.3), because in the macrofauna evaluation we deliberately destroy natural aggregates looking for macrofauna, invalidating the aggregate stability method.

3.7.1. Materials

2. **Shovel** with square tip for digging a regular, square hole.
3. **Knife or machete** for trimming the edges of a hole
4. **Ruler**, at least 20 cm long, to measure the dimensions of the hole. Sometimes two 20-cm rulers can be taped to form a right angle, which makes it easy to measure the hole precisely.
5. Feed sacks or other similar strong sack to collect soil before looking for macrofauna
6. Trays to use for distributing and searching soil for macrofauna
7. Vials, tubes, or bottles (50 to 100 mL size) to store macrofauna samples (if you want to perform a detailed classification)
8. Tweezers or forceps to transfer macrofauna to vials or bottles.
9. Alcohol to conserve the macrofauna, and possibly formaldehyde for long-term storage, but the latter only as part of the lab work to avoid carrying this toxic substance in the field

3.7.2. Procedure:

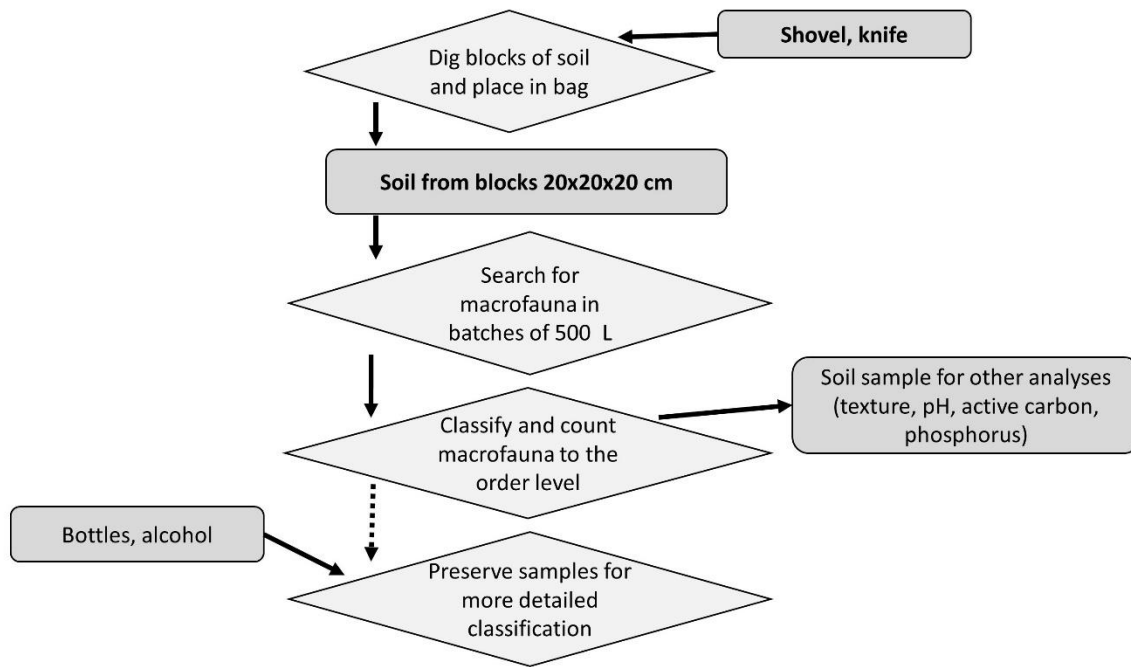


Fig. 42. Flowchart diagram for the soil macrofauna evaluation.

1. Using a ruler or small quadrat, mark out an area of 25 x 25 cm (square) using flags or similar marker. Try to avoid heavy footsteps, as some macrofauna (especially large anecic earthworms, with deep vertical burrows) can escape fairly quickly. In general macrofauna are heterogeneous in their spatial distribution, so it is good to collect at least 3 samples per treatment plot being evaluated.
2. Using a flat edge spade or shovel, excavate the soil quickly into a large woven plastic sack (Fig. 43 below). Try to ensure from the start that the walls of the pit are vertical and excavate the pit in as few shovel loads as possible to avoid damaging macrofauna. It is possible to reduce the volume of the sample slightly for demonstration purposes or when labor or time is in short supply (e.g., 20 x 20 cm), but it is important to recognize that this introduces greater error and can result in damaging a greater proportion of macrofauna (this can make tallying more difficult in later steps).



Figure 43a. Excavating a square hole (20x20x20cm deep) to assess the macrofauna in this volume.



Figure 43b. Soil is rapidly transferred from the hole to the bag to keep macrofauna from escaping.

- 3. Hand-sorting of macrofauna from soil (Fig. 44):** Find a comfortable place to work with adequate light, and with shelter from the sun, rain and wind. Scoop handfuls of about 500 mL, onto trays to sort through the soil, use tweezers to gently pick out any 'critters' that are > 2mm in size (in practice, most anything that is easily visible will be included, such as earthworms, ants, termites, beetles, spiders, and insect larvae). Fill two specimen cups (~120ml) about ¼ full with 70% ethanol and label them according to the sample being collected (Fig. 45). Place soft-bodied organisms (these are organisms without an exoskeleton; generally limited to earthworms, slugs, and earthworm cocoons) in one specimen cup. Everything else goes into another specimen cup (these are all arthropods, and have legs). If there are lots of small ants or termites (more common for warm tropical soils), it can be helpful to use a small paintbrush dipped in ethanol to collect the rapidly moving macrofauna (as they generally stick to the brush when wet). A team of at least 3-4 people handling each sample bag should be able to finish sorting a sample in 20 to 40 minutes, depending on the number of organisms encountered. One challenge is to not undercount (i.e., become rushed or sloppy) as one grows tired of sorting.
- 4.** Back in the lab, macrofauna can be sorted and counted. Typically, sorting to the level or order (i.e., beetles, ants, spiders, etc.) is sufficient for understanding the functional composition of soil macrofauna communities, but further identification is useful for better understanding impacts on diversity depending on interest and expertise. Sorting to the level or order can often be done with the naked eye, but it is useful to have a dissecting microscope or good hand lens for some specimens. Also, for longer-term storage (>2 weeks) it is important to dump off the old ethanol and replace it with clean 70% ethanol for the arthropods. Soft-bodied organisms can be stored in formalin if long-term preservation is desired. Earthworms can become difficult to identify if stored in ethanol for more than a few weeks; replacing the dirty ethanol with clean ethanol and

storing samples in the refrigerator can extend this time substantially, but one must use air-tight vials to avoid possible fire risk.



Figure 44: Hand-sorting of macrofauna from a tray. Note collection vials for macrofauna at right, if this is desired for precise identification and archiving of samples.



Figure 45. Arthropods collected in 70% ethanol

5. It is important to note that macrofauna data can often be quite “noisy” and conclusions are not always absolute or clear-cut. For this reason, analyses are often best conducted at the level of orders and/or with the most abundant taxonomic groups (often earthworms, ants, and beetles). Results are typically reported on a basis of individuals per square meter (so multiplying abundance numbers by 16 is necessary if using a 25 x 25 cm pit).
6. An excellent field key which is simplified to capture most orders of soil macrofauna is available from resources prepared by IRD / FAO. This guide is reproduced as [appendix B of this manual](#) and is also available at (<http://www.fao.org/3/a-i0211e.pdf> ; or also <http://ftp.fao.org/docrep/fao/011/i0211e/i0211e.pdf>).

3.7.3. Results calculations:

After sorting into either orders or genera, which can include morphotypes common at a site with a photo to help log them, each count of an organism or group can be expressed as a number per square meter. Note that if a 20x20x20 cm block were excavated, then this means multiplying all the raw counts in the data by 25 = 5 x 5 since each side of the block has a length of one-fifth of a meter (20 cm) so that 25 such blocks would fit in a square meter. If a 25x25cm block is excavated then the numbers should be multiplied by 16, not 25.

3.8. Water Infiltration using a simplified Beerkan method

This method is adapted from a simplified 'Beerkan' method (Bagarello et al. 2014)³. Its advantage is that it is relatively simple and can give a relative comparison between fields (of similar soil texture), and indicate the presence of compaction or crusting in the soil. The method attempts to estimate the saturated hydraulic conductivity of a soil, which is the rate of infiltration once the soil has been wet up, or 'saturated' during a long period of rain. When rainfall can no longer soak in, ponding can occur and movement over the soil surface, along with soil erosion, will be greatly accentuated.

By looking at the initial few points of data taken here, this infiltration test may give the user insight into the rate of infiltration during the initial part of a rain event.

Other related tests: See the evaluations "waterlogging in the soil", "identification of a plow pan", and "surface crusting of the soil" in the FAO visual soil evaluation guide by Shepherd et al. (see bibliography)

3.8.1. Materials

1. **Metal ring of 12 to 20 cm diameter and 10 to 20 cm in height.** A large metal can is acceptable. For a more durable ring, cut the bottom off a small aluminum or steel pot to create a ring with straight sides. Rings can also be fabricated by a sheet metal workshop. A wider ring (say, 15 to 20 cm) makes the method more accurate.
2. **Stopwatch or a stopwatch app** on a smart phone
3. **Reasonably clean water** of any type, from a stream or river is acceptable.
4. **4 to 5 containers of the same size** or marked at the exact same volume, with volume between 200 and 300 mL. This volume should be adapted to the diameter of the ring so that it fills the ring to a depth of roughly 1.5 to 2 cm when placed on the soil surface. A larger diameter ring will need more water to generate the same depth.
5. **Scissors or knife** to trim vegetation at the soil surface.
6. **Larger hammer** (small sledge) or a medium-sized stone
7. **Board** to distribute the force of the hammer or stone when installing the ring.

3.8.2. Procedure

a video of the method was created by di Prima et al. and shows the steps clearly, at

<https://youtu.be/vDrpUBuwvwY>

1. Cut plants to leave a bare soil surface in an area to fit the ring. Try not to alter the soil surface at all when cutting.
2. Insert the metal ring about 3 cm into the ground, using the board on top of the ring and pushing firmly or hammering the ring. If there are large roots that obstruct the ring from entering the ground, a new site should be selected. This should be at least

³ Bagarello, V., Di Prima, S., Iovino, M. and Provenzano, G., 2014. Estimating field-saturated soil hydraulic conductivity by a simplified Beerkan infiltration experiment. *Hydrological Processes*, 28(3), pp.1095-1103.

one meter away, ideally, so that the work of installing the first ring has not compacted the soil in the new site.

3. In some soils you may need to use your fingers to gently press and fill the outside edges of the ring with soil to prevent leakage under the ring.
4. Fill the containers all to the same volume of water. The volume should be enough to fill the ring to a depth of 1.5 to 2 cm.
5. Examine the table of water additions and recorded times below and be prepared to add each successive container while simultaneously logging the time elapsed since the beginning on the stopwatch. Be sure to read the rest of these instructions and practice to understand clearly the data recording. Some stopwatch applications on a smartphone, and many stopwatches, will automatically record 'Lap times' at the end of each volume infiltrated as a list or table of times, by pressing the 'Lap' function with each water addition. This is very helpful.
6. Pouring from just above the soil surface, add the first addition of water and simultaneously start the stopwatch.
7. Record the time necessary for the water to infiltrate into the soil, using the highest point inside the ring is just exposed. A good guideline for being "exposed" is that the soil surface on this highest point begins to glisten without any standing water. Infiltration rates will vary greatly on this first addition, and may be anywhere from 5 seconds to two minutes.
8. As soon as this time is recorded (minutes and seconds), pour another of the same volume of water onto the soil. Record the time for this second volume to infiltrate, and then repeat this step for a third, fourth, fifth volume, and so on.
9. Continue pouring volumes of water until there is a stable interval of time for each successive volume, to within a second or two of the previous volume added. This will usually take between 10 and 15 successive volumes of water.

Table of data for the infiltration test at one location of the infiltration ring.

Volume additons	Time in mm:ss	comments
1 (example)	0: 15	
2 (example)	0: 52	
1 (first)		
2 (second)		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		

10. Use the excel sheet to find an estimate of the saturated infiltration rate K_{sat} based on the simplified beerkan method of Bagarello et al. 2014 (see citation on first page), as follows. More detailed instructions are shown on the excel sheet and below in Fig. 46.
 - a. Open the spreadsheet and copy/rename/clear a new sheet to use for your data.
 - b. In the upper left, fill in the diameter of the ring you are using in cm, to the nearest 0.1 cm, and the volume added each time you refill the ring (the identical volume that you poured each time into the ring)
 - c. In the table of data, fill in time in minutes and seconds that ended each infiltration. For example if the first volume took 21 seconds, and the second one ended at 45 seconds (24 second later), and a third volume ended at 1 minute 11 seconds, enter 0 minutes and 21 seconds in the two cells on the first row, then 0 minutes 45 seconds on the second row, 1 minute and 11 seconds in the two cells on the third row, and so on for the cumulative time logged at the end of each volume infiltrated.
 - d. There are two graphs to the right which will plot the data automatically. In the one at the farthest right, you should click on the box outlines of the points that define the green line being fit to the data, then adjust the range of data being fit from the spreadsheet, so that the line has the best possible fit to the points, ideally with an $R^2 > 0.97$.
 - e. Enter the slope value from this fitted line, shown in a window on the furthest right graph, into the form below the graph with "B Value from line fit in figure two" indicated. For example if the equation on the graph reads " $y = 0.2444x + 5.4608$ " you should enter '0.2444' into this part of the form.
 - f. You will also need to choose a value for a^* below this 'B-value' in the excel sheet. For most surface soils with agricultural management, a value of $a^* = 0.012$ is acceptable. For very sandy soils or those with large macropores, a^* can be set to 0.036 and for heavy clay subsoils, set to 0.004.
 - g. An estimate of the saturated hydraulic conductivity of the soil then appears to the right of the slope value you entered in e., both as mm per second and as mm of rainfall per hour, which the soil can pass when in a saturated state.
11. **Interpretation of the infiltration test:** for most soils, rapid infiltration is a sign of good aggregation and overall soil health, but comparisons are best made among soils of the same texture with different management, somewhat like the aggregate stability test (section 3.3). For very sandy soils, infiltration will always be rapid unless there is an impermeable layer near the surface underneath the sandy layer. Clay soils may have slower rates of infiltration than loams or sands. In very general terms, infiltration rates below about 50 mm/hour may signify problems with compacted clay in the soil.

time between refills, sec	Cum. time minutes	Cum. time seconds	cumulative time	volume of water added	mm water equivalent	cum time	cum infiltr. (mm)	square root time
35	0	35	35	300	12.5	35	12	5.92
45	1	20	80	300	12.5	80	25	8.94
50	2	10	130	300	12.5	130	37	11.40
60	3	10	190	300	12.5	190	50	13.78
65	4	15	255	300	12.5	255	62	15.97
67	5	22	322	300	12.5	322	75	17.94
69	6	31	391	300	12.5	391	87	19.77
69	7	40	460	300	12.5	460	100	21.45
70	8	50	530	300	12.5	530	112	23.02
70	10		600	300	12.5	600	125	24.49
71	11	1	671	300	12.5	671	137	25.90
72	12	23	743	300	12.5	743	150	27.26
70	13	33	813	300	12.5	813	162	28.51
71	14	44	884	300	12.5	884	175	29.73

One: Raw infiltration Data - just for reference and checking

Figure Two: Processed data for curve fitting - modified Beerkan

How to use this form:

- Enter the diameter of the infiltration ring in cell A3. Enter the volume of the small cup you are using to add water to the ring in cell A4, which will generally be the same each time each time you add water to the ring.
- You will see in Figure One in the middle of the sheet that the raw infiltration curve will appear, showing mm of water depth infiltrated over time. This is for information and for you to check that the data makes sense or to see any typical or atypical patterns, and is not used for the final calculation.

More instructions here

- Enter up to 16 cumulative times of finishing infiltration of the same volume of water
- Enter up to 16 cumulative times of finishing infiltration of the same volume of water
- Change the range of the green line in Figure two at right to create a good fit of data points to the estimated line
- Slope from green line in Figure Two entered here
- Final estimate of soil infiltration rate in mm/hour shown here

Key equation for the simplified beerkan infiltration model:

$$K_{fs} = b / (0.467 * (2.92 / r * a * + 1))$$

Estimated value of the slope from the fitted line above:
 B Value from line fit in figure two: **0.1431**

Final result: **292 mm/hour estimated saturated infiltration**

Figure 46. Guide to the predefined excel sheet / form used to calculate an estimated saturated infiltration rate or K_{fs} from the infiltration data. The four steps are illustrated by the yellow callouts: 1. Enter the ring diameter and volume of each water addition; 2. Enter up to 16 cumulative infiltration times; 3. Change the range of the green line in Figure two at right to create a good fit of data points to the estimated line; 4. Enter the slope of this line from Figure Two to the light green square at lower right; and 5. record the calculated infiltration rate at lower right in the orange box.

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APPENDICES

Appendix A: Visual and feel guide for soil moisture:

Here estimates are presented for moisture based on how typical soils look and feel. These estimates can be used to adjust measurements of nutrient or carbon content to what the measurement would have been in fully air dried soil, which is the best, standardized way to express these measurements. The adjustment to air dried soil can be calculated by multiplying by the factor $[1/(1-\%moisture)]$. For example if a moisture content of 9% is estimated, chemical results using this soil moisture would be multiplied by $[1/(1-0.09)] = 1/0.91 = 1.10$ which uses the factors in the table below. If many samples are to be evaluated in a single region it is probably best to create a local guide by associating how local soils look and feel with their real moisture content.

Table 1. Moisture contents of different textured soils at different stages (% moisture given)

	I. Very moist	II. Moist	III. most crumbs Moist	IV. most crumbs dry	V. air dry	VI. Air dry ⁴ , many days or heated (~45 C)
loamy sands and sands	9%	7%	5%	3%	2%	1%
Sandy loams	13%	10%	8%	5%	3%	2%
sandy clay loams, loams, silt loams, silts	18%	14%	10%	6%	3%	2%
Sandy clays, clay loams, silty clay loams, silty clays, clays	23%	18%	14%	7%	4%	2%
High organic matter soils (e.g. > 5% SOM; see note below)	+3%	+2%	+2%	+2%	+1%	+1%

Moisture levels (see corresponding images below):

- I. **Very moist.** the soil is wet enough that when handled, clumps form that are larger than the normal crumb size aggregates from sieving (5-15 mm), and is very difficult to sieve at 2 mm without clogging the screen. With just a little more water we would start to see glistening soil (free water not held in the aggregates); when pressed with the fingers and thumb, the soil almost forms a smeared together clod, but still shows some grain structure of the individual crumbs.
- II. **Moist.** Larger clumps (5-15 mm) are no longer forming from soil being handled, but all crumbs are still visibly moist. Sieving is still difficult from moisture, but doable. When pressed with the fingers and thumb, the soil holds together loosely but does not smear together, and the clump formed is relatively easily broken. In the sandiest soil the clump with fingers and thumb does already not quite hold together.

⁴ For air dried soils, in most cases the moisture content is assumed to be zero and no adjustment is made to any chemical analysis results.

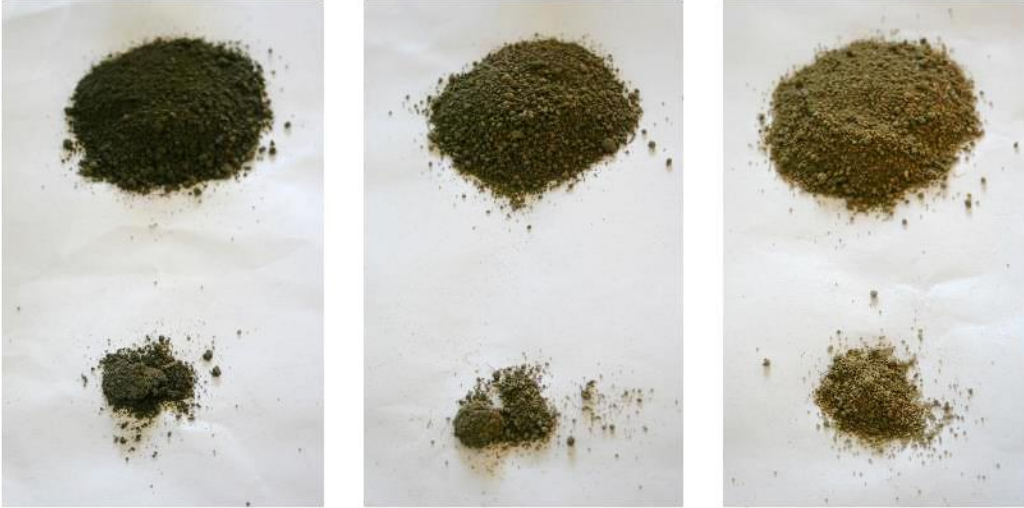
- III. **Most crumbs moist.** The loamy sand no longer adheres when pressed, while the clay loam and high organic matter soil still adhere a little bit, about half sticks together. Sieving is now relatively easy.
- IV. **Most crumbs dry:** Aggregates are still moist inside. When pressed together, there is no adhesion between sieved crumbs. Sieving is now very easy without soil clogging the sieve.
- V. **Air dry:** Samples have dried until they appear completely dry, though many days or weeks have not passed, and the soil has not been dried at higher temperatures.
- VI. **Air dried, many days or heated:** The soil has been in dry conditions for many days to months, or has been heated for drying, at 40 to 45 degrees C for example. Usually no adjustment is made for moisture.

Addition for high organic matter soils: for soils that are visible very high in organic matter, with a notable dark color or organic matter higher than 5%, the amounts given in the table based on texture should be augmented by a few percentage points, as shown in the last line of the table.

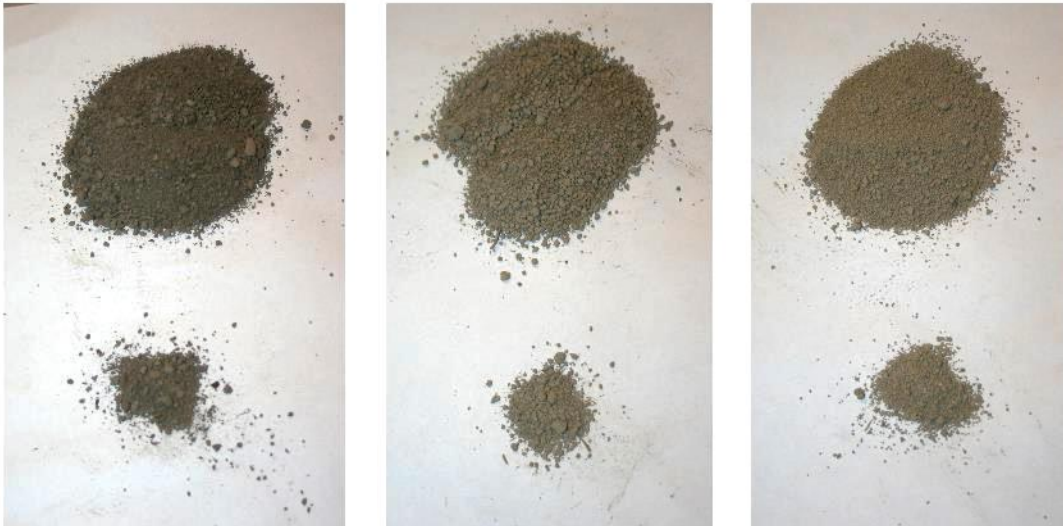
Pictures of the different stages:



III. Most crumbs moist



IV. Most crumbs dry



V. Air dry:



Appendix B: Simplified Key to Macrofauna.

Adapted from the FAO/IRD macrofauna field manual, check the link for the original:
<http://ftp.Fao.org/docrep/fao/011/i0211e/i0211e.pdf>

The key identifies most macrofauna to the level of order:

WITHOUT LEGS:

1. **WITHOUT LEGS, NON-SEGMENTED**, clear head with antennae; **MOLLUSCA**

- a) With Shell: **Snails** (Fig. 1)
- b) Without a shell: **Slugs** (Fig. 2)



Fig. 1. Snails



Fig. 2. Slugs

2. **WITHOUT LEGS, SEGMENTED**

a) *WORM - LIKE*,

- More than 15 body segments, pigmented:
Earthworms (most >20 mm long) – (Fig. 3)
- Suckers at both ends of a flattened body:
Hirudínea (leeches) (Fig. 4)



Fig.3: Earthworm

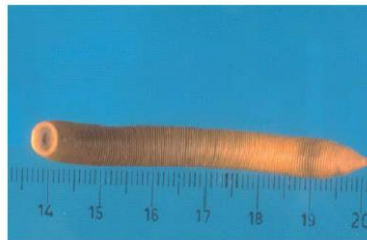


Fig.4: Hirudinea (leeches)

b) *Not worm-like*, fewer than 15 segments

- Beetle Larvae (**Coleoptera**), generally with strongly developed head capsule (well developed coronal structure). Often U-shaped and more or less swollen. (Fig. 5)
- Fly larvae (**Diptera**), often without strongly developed head capsule. Long and thin, not U-shaped (Fig. 6)



Fig.5: Beetle Larvae (coleóptera)



Fig.6: Fly Larvae (diptera)

WITH LEGS:

1. WITHOUT WINGS:

3 Pairs of Legs (INSECTS).

A. Caterpillar-Like, Soft bodied

- With Pseudo-legs (four pairs or fewer):

Larvae of **Lepidoptera** (larvae of butterflies and moths, Fig. 7) these lepidopteran caterpillars have both true legs found on the thorax and pseudolegs (prolegs) found on the abdominal segments:

- Without pseudo-legs, and 3 pairs of legs on thorax:

Larvae of **Coleoptera** (beetles; frequently U-shaped “grubs”) - (Fig. 8)



Fig.7: Lepidoptera Larvae (butterflies and moths)



Fig.8: Beetle Larvae, “grubs”

B. Abdomen > 6 segments and > 4 segmented antennae

- Conspicuous pronotum (the upper dorsal plate of the first segment of thorax):
 - i. Pronotum saddle-shaped, not projecting forward:

Orthoptera (grasshoppers) (Fig. 9)

ii. Pronotum not saddle-shaped, projecting forward over head :

Blattaria (cockroaches) (Fig. 10)



Fig.9: Orthoptera



Fig.10: Blattaria

➤ Pronotum not conspicuous (the upper dorsal plate of the first segment of thorax):

i. Mouthparts formed into sucking tube held under body, no palps:

Hemiptera (such as lace bugs, aphids and woodlice (Fig. 11)



Fig.11: Hemiptera, note sucking tube

ii. No sucking tube, palps: Abdomen ends in a **certain number of cerci** (paired appendages on the rear-most segments of many arthropods)

Cerci either:

a) **2 cerci**

❖ Curved into pincers: **Dermaptera** (earwigs) (Fig.12)

❖ Long and thin, at least 1/3 length of abdomen, projecting from tip, Antenna short: < 2 x head width: **Coleoptera larvae** (Fig. 13)



Fig. 12. Dermaptera (earwigs)

- ❖ Short cerci and may be located forward of the tip of the abdomen:
 - Antennae long, 8 segments:
Isoptera (changed to Blattaria, recently) blind poorly pigmented, sometimes with large mandibles [soldiers], legs fully developed, tropics and subtropics) (Fig. 14)
 - **Antennae short**, <6 segments: beetle larvae, flat, short antennae (<8 segments) (Fig. 15)



Fig.13: Coleoptera Larvae



Fig.14: Isoptera (now Blattaria)



Fig.15: Coleóptera larvae – cerci (beetles)

b. **Without cerci**; with a certain number of antennal segments: (see number of segments below):

- ❖ <6 antennal segments, with 3 clear thoracic segments :
Larvae of coleoptera (beetles; Fig. 16)



Fig.16: Coleoptera (beetle) thorax (three clearly defined segments behind the head)

- ❖ More than 10 segments in antennae, with a wasp-type waist (very narrow):
 - ❖ Waist with 1 to 2 petioles: **Ants** (Fig. 17)
 - ❖ Waist without petioles: other **Hymenoptera** (bees y wasps)
- ❖ More than 10 segments in the antennae, without narrow wasp-type waist
 - ❖ Long and thin: **Phasmida** (walking sticks and leaf insects) (Fig. 18)
 - ❖ Small insects, with relatively long antennae: **Psocoptera** (bark lice) (Fig. 19)
 - ❖ With short antennae: beetle larvae or wingless adults (Fig.20)



Fig.17: Ants; at right, one or two petioles connect the thorax and abdomen



Fig.18: Phasmida



Fig.19: Psocoptera



Fig.20: Beetle larvae or wingless adults

4 pairs of legs: Arachnida

(sometimes pedipalps –the second pair of appendages of the head and thorax section– look like an extra pair of legs)

A. Thorax and abdomen separated by a constricted waist, pedipalps without claws:

Spiders (Fig. 21)

B. Thorax and abdomen fused into one, without pedipalps-

➤ Body clearly segmented, with ocularium (eye-area tubercle):

Opilions (very similar to spiders) (Fig. 22)

➤ Body not segmented, without ocularium: **Acarina** (mites and ticks) (Fig. 23)



Fig.21: Spiders



Fig.22: Opilions



Fig.23: Acarina (mites y ticks)

C. Pedipalps with claws or pincers

➤ Large claws, telson (sting): **Scorpions** (Fig. 24)

➤ Small claws, without telson (sting): Pseudoscorpions (Fig. 25)



Fig.24: Scorpions



Fig.25: Pseudoscorpions

6 or 7 pairs of legs: Isopoda (Fig. 26)



Fig.26: Isopoda

More than 15 pairs of legs:

- A. One leg pair per body segment: **Chilopoda** (centipedes, generally flattened body) (Fig. 27)



Fig.27: Chilopoda



- B. Two leg pairs per body segment: **Diplopoda (millipedes)**, generally the body is more round than flat, usually >30 pairs of legs (Fig. 28)



Fig.28: Diplopoda

2. WITH WINGS

A. Only two wings, no appendage: **Diptera adults (flies)**

(with halteres –small knobbed paired structures near wings) (Fig. 29)



Fig.29: Diptera

B. 4 wings

➤ Mouthparts modified into sucking tube, no palps: **Hemiptera** (Fig. 30)



Fig.30: Hemiptera

➤ Biting mouthparts, palps:

❖ Forewings hardened to form a wing case:

○ Hind legs long:

i. Hind legs modified for jumping, head not partially covered by pronotum: **Orthoptera** (Fig. 31)

ii. Hind legs not modified for jumping, head partially covering pronotum: **Blattaria** (Fig. 32)



Fig.31 Orthoptera



Fig.32: Blattaria

- o Hind legs short:
 - i. Abdomen with terminal pincers : **Dermaptera** (Fig. 33)
 - ii. Without terminal pincers: **Coleoptera** (beetles) (Fig. 34)



Fig.33: Dermaptera



Fig.34: Coleoptera (both left and right image))

- ❖ Forewings not hardened - hind legs modified for jumping ; pronotum saddle shaped : **Orthoptera** (Fig. 31)

- Other winged groups are rarely found in hand-sorted soil samples, but example is shown: **Hymenoptera**: bees and wasps (Fig. 35) and **Lepidoptera** (moths and butterflies).



Fig.35: Hymenoptera (Not commonly found in soils)