Chapter 9

Soil and litter sampling, including MSS

by

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Abstract

Soil is defined, and its components, structure, types, pedogenic regimes and classifications are briefly described. Rhizosphere and MSS (mesocavernous shallow stratum) are summarized and their importance emphasized. A diagnosis of the soil fauna is given and its representative groups recorded, these being divided for practical reasons into microfauna and macrofauna. Conservation issues are briefly addressed. Sampling methods are reviewed in a comprehensive treatment, and divided into field procedures and laboratory techniques. The field methods described are: direct sampling, sifting, pitfall traps, MSS traps, slope boring and hand collecting. Laboratory methods treated are: filtering, flotation, decantation, elutriation, flotation-centrifugation, and use of Berlese-Tullgren funnels, Moczarski eclectors and Baermann funnels. Recommendations on the construction of different traps, conservation, transport and preservative preparation are also given.

Key words: mesocavernous shallow stratum, trapping, field methods, laboratory methods, conservation

1. Introduction

1.1. Definition and components

Soil, at a global scale, is a complex natural film discontinuously covering the Earth's surface that is not underwater. As with most living things, soils start, develop, mature, age and either disappear or fossilize. The peculiar chemistry of the constant presence of large quantities of water impede the formation of soils, although moderate to high quantities of water do not stop the formation of particular kinds of soils, but soil formation is never completely finished under water. Soil can also be defined as an interface between the four main components: minerals, water, air and organic matter (either living or dead), or in other words, the lithosphere, the hydrosphere, the atmosphere and the biosphere.

This mixture of gas, liquid and solid (a three-phase system) has a structure, which varies depending upon several factors: the nature of the original rock(s); their mineral component(s); the porosity of the structure and the ability of these pores to absorb gas and liquid components; the climate where the soil is developing; the biota inhabiting it; and the time all these factors have been interacting. Or in other words, its history (*pedogenesis*).

Soils are absent in some parts of the terrestrial environment: ice caps and perpetually frozen peaks. Even on bare rocks, bacteria, lichens and mosses start the gradual transformation into soil: this is called *primary succession*. Sooner or later, depending upon these factors, a complex community will develop in this growing soil (Lomolino *et al.*, 2006).

Once a soil, even if primary (*protosoil*), has been established, the further development will depend not only in the kind of protosoil, but also on climate, surrounding vegetation and time. This *secondary succession* will lead to the establishment of *climax* vegetation. The formation of a soil includes chemical processes, such as weathering of the bedrock and alluvial deposits, oxidations and reductions, hydrolysis, chelation or solution of ions, hydration, interactions with organic substances (rotting, humus formation), and physical processes (erosion), like freezing, thawing, leaching, wetting, drying, and different kinds of transportation and depositions. The biota will also help: the organisms mix soil materials and create pores that allow the lower layers to be affected by the other factors; some of them (*e.g.* plant roots, bacteria and fungi) produce substances that are freed to interact with the other soil components. The net of roots and hyphae keep the stability of soils, and create an irregular system that also stores organic matter.

Over all these factors, the main ruler is *time*. None of these processes will take place if not enough time is allowed for them to act, and for all the factors to interact. Interrupted soil forming processes will start again on the new basis created by the disturbance. These processes may be very fast or take millions of years, until a mature soil is formed.

The soil is important because of this interaction between its abiotic and biotic components, because of its action as a substratum of wild plants and crops, conveying the nutrients for the upper trophic levels in the life pyramid, and because its biota are a fundamental part of the unknown biodiversity.

The science of studying soils is known as Soil Science, and has two main branches: Edaphology (the influence of soil in living things, including man's uses - agriculture and related disciplines) and Pedology (study of soils in natural environments), although this distinction is denied by some schools.

1.2. Structure of soils

Soil is composed of layers (*horizons*). Every layer has its own peculiarities in the proportions and characteristics of the three phases. From bottom to top, the soil becomes less and less similar to the original parent rock, and the signs of interaction with the atmosphere, the hydrosphere, and most of all, the biosphere, become more and more evident.

Typically, a mature soil must have four horizons, which are separated according to colour, structure (form and aggregation of grains, porosity), texture (proportion of clay, silt and sand), consistency, rhizosphere, pH and some other characters (Fig. 1). These are called by using letters, from top to bottom, O, A, B, C. Sometimes the bedrock or parent rock in the bottom is called horizon R. Usually they are easily separable by sight and by texture.

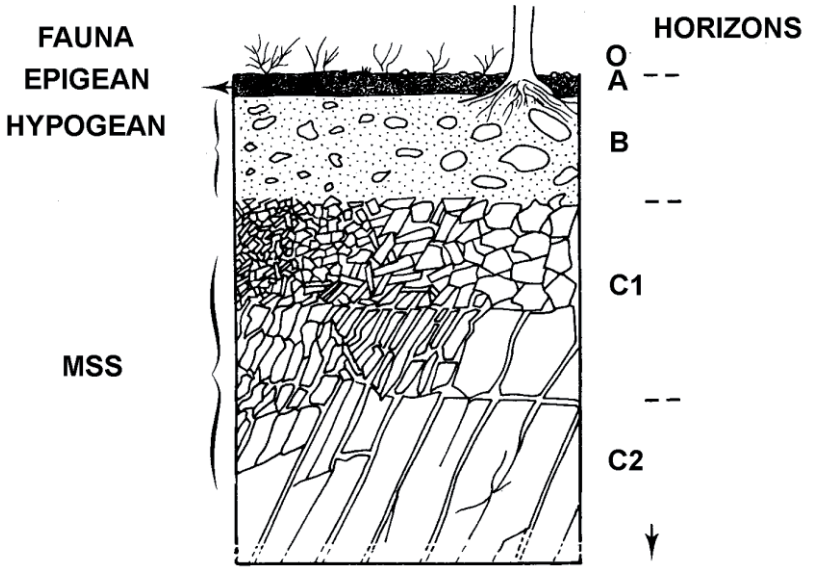

Fig. 1. Soil horizons and associated fauna (redrawn from Juberthie *et al.,* 1980b).

- **Horizon O.** This is a horizon composed of organic matter which is not yet decomposed (raw humus). It corresponds to litter in the usual meaning of this word in biology. Mineral matter is almost absent. For some authors, this layer is not a part of soil in fact, since it does not show clearly any of the processes leading to soil formation. It is usually divided into two subhorizons (from top to bottom): O_1 and O_2. The first has vegetal remains that can be recognized by sight (pieces of leaves, etc.); in the second, recognition is not immediately possible.
- **Horizon A.** This is the top layer of the "true" soil. It is usually darker in colour than lower horizons, because of the accumulation of humus (the stable colloidal, uniform, dark substance resulting from chemical transformation of the raw humus, the general organic matter of soil). This humus helps to buffer soil pH, retains water, increases the soil capability of storing nutrients, and sticks mineral grains together, thus improving the texture and structure of soil, among other valuable properties. Horizon A is also poor in clay and sesquioxides, and is where most of the biological activity takes place, so most of the organisms are concentrated here.
- **Horizon B.** This is the intermediate layer of the soil, usually containing concentrations of clay and minerals of elements like iron or aluminium, or a little organic material which arrives from above by leaching. Consequently, it is usually reddish or brownish. It is also called the "illuviated" horizon because it receives materials from above by filtering (illuviation) through horizon A.
- **Horizon C.** This is a horizon which is little affected by processes occurring in the soil, showing a poor development from the parent rock that lies below, being sometimes just a layer of (sometimes boulder-like) fragmented rock on top of it.

In some classifications, horizons D, E and P are also recognized.

The horizons B and C are also united, from a biological point of view, by the MSS ("milieu souterrain superficiel" (Juberthie *et al.,* 1980a, b) or "mesocavernous shallow stratum" (Ashmole *et al.,* 1990), also called less often "superficial underground compartment" (Juberthie & Delay, 1981) or "shallow subterranean compartment" (Howarth, 1983)) with caves and void subterranean spaces below (lava tubes, etc.). This MSS is a network of cracks and crevices, mostly in C_1, acting as corridors between the upper and the lower horizons, and into the caves, a kind of living highway for exchange of biota both horizontally and vertically, subject to seasonal temperature changes.

Another particular structure in the soil is the *rhizosphere*. This is defined as the region of soil that is immediately adjacent to and affected by plant roots, forming a boundary layer between roots and the surrounding soil (Cardon & Whitbeck, 2007). It is the interface where roots and their secretions (usually hormone-like, called *exudates*, *e.g*. strigolactones, or *allelochemicals*, which prevent other plants' roots from growing), microorganisms, soil, nutrients and water interact. The soil not affected by the rhizosphere is called *bulk soil* and is poorer in organic matter and biota. Larger organisms tend to concentrate in the

rhizosphere, where they can find food easily, since the exudate attracts microorganisms and smaller fauna, and favours the growing of fungal mycelia. Plant root growth (and thus the increase of the rhizosphere) is facilitated by the burrowings of earthworms.

The rhizosphere and MSS are intimately linked, since the crevice system tends to be occupied by roots even at very great depths, and root feeders may follow them downwards.

1.3. Soil types

Soil being a complex entity, it is not surprising to learn that there are many types. However, two major factors (climate and parent rock) around the globe produce four main *pedogenic regimes*, giving rise to four major types of *zonal soils*:

- **Podzolization.** This occurs where temperatures are cool and precipitation is abundant. Even with substantial plant growth, microbial activity is inhibited causing *humus* to accumulate in the upper horizons and its soluble components to be leached to lower horizons. Illuviation also reduces cations in the soil, and acidifies it. The typical forests on these soils are coniferous forests, or sometimes deciduous temperate forests.
- **Laterization.** This happens where temperatures are warm and precipitation is heavy. In these circumstances, microbes quickly break down all organic matter, and there is no time for *humus* to accumulate. Oxides of iron and aluminium precipitate to form a hard bricklike red layer (laterite). Cations are leached with heavy rainfall, leaving behind a hard, poor and infertile soil if the tropical cover forest is cut.
- **Calcification.** This process develops in arid grasslands and shrublands with a cool to hot climate, but with a very scanty precipitation. Cations are not leached out, but they precipitate in the lower levels as a calcium carbonate rich layer (this, if uncovered, forms a rocklike layer named *caliche*). If there is enough rain, cations and other nutrients are mobilized upwards and distributed in the soil, which is highly prized for agriculture.
- **Gleization.** This is the typical process occurring in waterlogged areas, *e.g*. in cold and wet polar regions. The water table is very high, preventing decomposition, and accumulating acidic organic matter. Below this layer, usually a layer of bluish-grey clay (*gley*) appears, containing partially reduced iron (FeO). Nutrients are scarcely available, so the vegetation is grassy and sparse.

However, certain rock types (*e.g*. gypsum, serpentine or limestone) or peculiar soil conditions (like extreme acidity, or salt) may form *azonal soils*, which can appear interspersed anywhere between the main kinds and its varieties.

1.4. Classifications

There is no single classification of soils. Since the original classification of the father of edaphology, Vasilij V. Dokučaev around 1880, many systems have

been proposed. Of those still in use, some put special emphasis on the pedogenetic processes and some on the recognizable features of the soils, so these classifications are not equivalent. The most used are:

- **The French Soil Reference System.** The "Référentiel pédologique français" is based on pedogenesis (Baize & Girard, 2009) and widely used in French territories and former colonies.
- **USDA Soil Taxonomy.** This is a descriptive system based on soil morphology (Soil Survey Staff, 1999), which allows the use of identification keys (in English or Spanish) to name a soil (Soil Survey Staff, 2006). It is mostly used in the USA and surrounding areas, but it has also been adapted to other countries.
- **The FAO system.** Originally envisaged as a legend to its famous *Soil Map of the World*, it is a worldwide system, which underwent an important improvement (FAO, 1988) and includes no climatic criteria. This system was replaced in 1998 with the *World Reference Base for Soil Resources* (WRB), which is now the only international standard system adopted by the International Union of Soil Sciences (IUSS Working Group WRB, 2007).

However, many countries have developed their own classification systems, suitable to their own pedological units.

2. The soil fauna

2.1. Groups, size and distribution

Soil fauna is abundant, rich and diverse. High numbers of individuals and species belonging to all terrestrial phyla can be found here (Rotifera, Annelida, Mollusca, Tardigrada, and most of all, Nematoda and Arthropoda).

Usually Arthropoda show the highest diversity, although there are accounts showing that they are probably equalled, if not surpassed, by the Nematoda (far less studied and understood). Representatives of all the arthropodan subphyla and of all of their terrestrian classes can be found in the soil: Cheliceromorpha (scorpions, pseudoscorpions, spiders, harvestmen and mites, and other rarer groups), Crustacea (amphipods and woodlice), Myriapoda (centipedes, millipedes, and rarer groups), and Hexapoda (insects and close allies). The latter are very well represented in the soil with the orders of entognathous hexapods (considered by some to be three classes different from insects: Collembola, Protura and Diplura), and 20 out of the 26 orders of ectognathous hexapoda (true insects) (Greenslade, 1985, with the addition of Mantophasmatodea).

Nevertheless, apart from taxonomic classifications, some other kind of classifications based upon horizontal distribution or body size can be more useful for soil fauna. This classification on body size has widespread repercussions on sampling and study of the different groups. Most authors (*e.g.* Wallwork, 1970) (Fig. 2) differentiate three size classes: micro-, meso- and macrofauna.

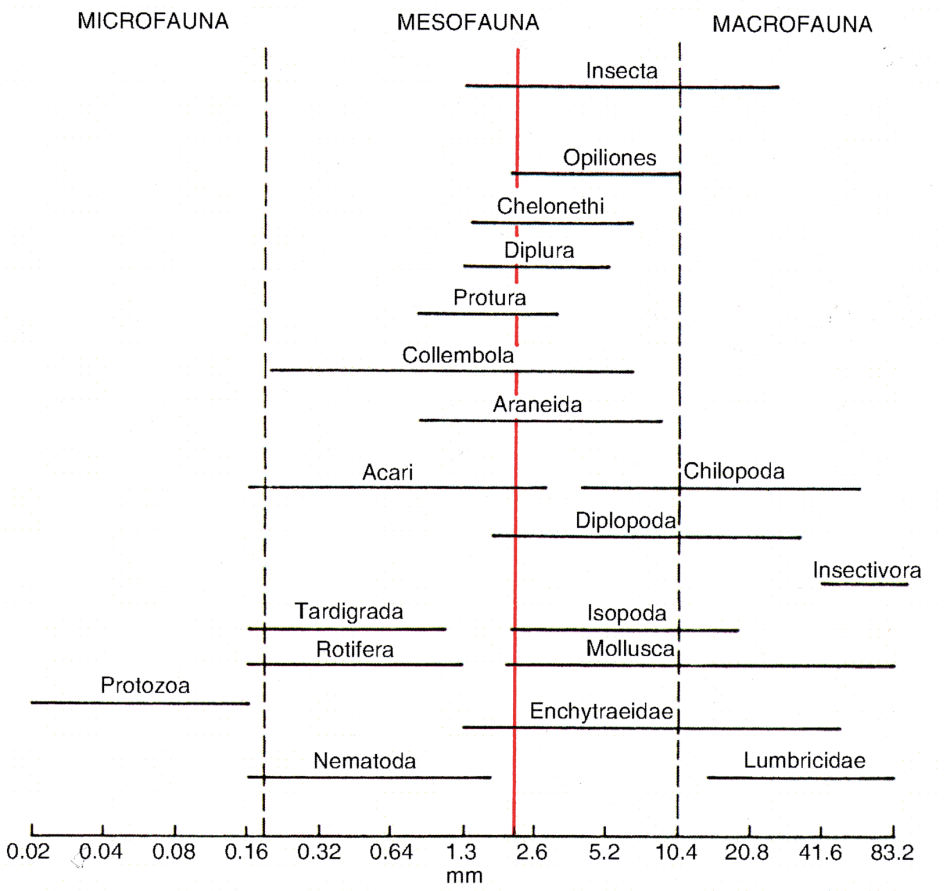

Fig. 2. Size classes in soil fauna groups, according to Wallwork (1970), modified to show the 2 mm boundary (red line).

However, there is a fundamental division in the sampling methods for microfauna and the other two groups (which will be referred to as macrofauna in the following): separating specimens under 2 mm and substratum components in the field is very difficult, when not impossible. So usually the methods intended to collect microfauna under 2 mm (Nematoda, Tardigrada, Collembola, Acari, etc.) the size of which is equal or less than the soil grain, are '*blind*'. This small size does not allow the collector to make a separation of the sample into its faunal and non-faunal components *in situ*, so it must be carried "as it is" to the laboratory and processed there (see *Laboratory extraction methods*). The collector is then compelled to extract an *in toto* sample of the soil under research (containing litter, rhizosphere parts, mycelia, sifted soil, etc.) and to carry it to the laboratory.

If, however, the target is the macrofauna (earthworms, macroarthropoda, etc.), the researcher can usually separate faunal and non-faunal elements *in situ*, and carry to the laboratory only the desired specimens.

Other factors affecting the sampling are:

- **Size of the animals.** Usually, the larger the body size of the target animals, the wider the area and the bigger the sample that must be taken;
- **Dispersion of populations.** The more disperse the populations of the target animals, the wider the area to be sampled;
- **Horizontal distribution.** Usually there is a general tendency to think of two separate behavioural guilds: *epigean* (*epiedaphic*) vs. *hypogean* (*hypoedaphic*, *endogean, subterranean*) faunal components (the first as walkers or crawlers on ground surface, the last either as burrowers or diggers under the surface, or as crevice or cave dwellers). But this difference is blurred by the existence of both daily and seasonal vertical migrations. They are very important in places where the diurnal and nocturnal temperatures are extremely different, or where wet and dry seasons alternate. Depending upon the sampling targets, precautions must be taken to avoid, estimate or measure these impinging factors. It must not be forgotten that soil also acts as a *refugium* for animals that feed or perch above ground (mostly at night). *Hypogean* elements can be divided into those living buried (usually either burrowing or moving along crevices), those which are true soil dwellers (*edaphobionts, edaphobites*), and those living in underground spaces much larger than their own size, like tunnels, caves, lava tubes, etc. (*troglobionts, troglobites*). In some groups, like Arthropoda, the adaptations shown by either of these two *hypogean* guilds are quite different; it is also relatively common to find *edaphobionts* invading (and being collected in) the habitats of the *troglobionts*, but not the reverse. Other authors (*e.g*. Jennings, 1985) distinguish between *endogean* and *hypogean* elements, being the first those "pertaining to the biological domain immediately beneath the ground surface *i.e*. in the soil or plant litter" and the second, those "pertaining to the domain below the endogean, including the dark zone of caves". As mentioned above, these differences are not essential.

2.2. Importance and conservation

Edaphic flora and fauna are very important for life on Earth. The living component of soil is the one carrying out the numerous functions taking place in it, among them, the recycling of nutrients. For a good functionality of the soil, its fauna must be appropriately conserved.

Moreover, from an anthropocentric point of view, the edaphic fauna performs some of the commonly called "nature services". For example, predators (spiders, ground beetles, etc.) are fundamental to keep possible pests under control in agrosystems (Goehring *et al.,* 2002, Duan *et al.,* 2004). They have even been used in developing integrated control strategies against pests (Juen & Traugott, 2004), or in measuring the success in restoring prairie ecosystems (Peters, 1997), tropical forests (Jansen, 1997), riverside forests (Williams, 1993) or coastal vegetation (Longcore, 2003), among others.

At the same time, some species play a key role in ecosystems. For example, dung beetles (Scarabaeinae) are the primary decomposers par excellence. If for

any reason their abundance decreases in a prolonged way, the decomposition rate of the organic matter will decrease as well (Klein, 1989). As a consequence, nutrient recycling in the soil will become slower, soils will become impoverished and plant communities will become fragmented; with some other unpredictable imbalances appearing as well (Goehring *et al*., 2002).

Unfortunately, negative factors affecting the soil fauna are numerous: fires, desertification, erosion, abusive agricultural management, urbanization, contamination with pesticides and heavy metals, etc. These disruptions may cause serious imbalances in soils and provoke an irreversible loss of biota. Because of this, we must pay much attention to the high species- and community-richness inhabiting soil, if we want to conserve the terrestrial ecosystems.

Nevertheless, there is great ignorance of the taxonomy and the biology of many soil groups of taxa. For example, there is information about only 10% of the microarthropodan populations in soil and only a 10% of its species have been formally described (André *et al*., 2002). To overcome this impediment, we must increase the effort to study the edaphic fauna, among other things.

3. Sampling methods

When a biological inventory is to be done, the first assumption that must be made is that it is not possible to collect all the species that are present in the target area (for example, Gotelli & Colwell, 2001), even more when the study focus on hyperdiverse and poorly known taxonomic groups (Colwell & Coddington, 1994).

This impediment may oblige evaluation of the collected samples and relativization of the observed richness to be able to make meaningful comparisons. Keeping this in mind, it will be very useful to undertake sampling in a methodical way and to quantify the invested effort.

Soil zoology has tried for a long time to find a sampling method that allows collection of the greatest fraction of fauna as possible. However, there is now a growing general agreement that a method allowing a good sampling of one community of species may fail for other communities (Southwood & Henderson, 2000). Thus sampling protocols combining different methods must be established if maximal efficacy of sampling is to be achieved.

The selection of the methods most suitable to the objectives must be exhaustive when trying to delimit the inventory, both taxonomically and in relation to soil horizons. Thus, the order of decision should be first the horizons and then the taxonomical or functional groups to be studied and after this, a second decision on the most suitable sampling methods is to be reached, taking into consideration the other factors affecting sampling, like body size of the target group and its distribution (see above).

With all this in mind, the next sections will treat in order the most general methods first (allowing the capture of a wider set of taxa), following with those

specifically fitted to sample particular horizons (MSS samplings), and ending with the most usual extraction techniques to be done in laboratories.

3.1. Field techniques

This section includes the methods used to collect in the field the target fauna or to collect the 'blind' soil samples to be carried to the laboratory for extraction.

3.1.1. General field techniques

These general field techniques allow the capture of a wide set of taxa. Even if the research sampling is focused in a very specific group of animals, it is desirable and highly recommended to use some of these as a tool for 'completing' the inventory.

Direct sampling

This is the basic sampling method. The researcher will locate and capture the target fauna searching for the specimens in their habitat by eyeing the ground, turning stones, searching among litter, digging around plant bases, etc. This method usually allows the capture of macrofauna only. As an exception, smaller individuals may be collected with magnifying glasses and brushes.

This method allows the sampling of the upper horizons of the ground. However, it can also be used when sampling underground inside caves, etc. In this case, it is convenient to introduce some plant matter at the first visit, and check it for specimens in subsequent visits.

In any case, direct sampling is essential if a reliable inventory, containing at least 80% of the species present in the target area, is to be realised. It allows the inclusion of the species that cannot be collected under other protocols in the inventory, which is thus completed with the rarest species, the most difficult to collect just by chance. The collecting success using this method is heavily dependent on the collector's experience and training (pers. obs.).

When using this kind of sampling, special attention must be paid to the collecting habitats. This valuable information must be included in data labels, together with other data, such as locality, date, altitude, etc. This will increase the knowledge about the biology of the target taxonomic group and will raise the probability of collecting the rare species.

Even in this kind of sampling, it is convenient to use sampling units in a systematic way, measuring allotted time, sampled area, energetical effort and other factors that may influence the results. In many studies, the unit of effort measurement is a search of 15 min. However, trained collectors should estimate whether more units are needed to give a satisfactory result of the biodiversity of the target area. If there is a suspicion that a single unit is not enough, sampling with a different number of units should be previously carried out to ascertain the most profitable set. A previous estimation of the aggregation of the populations may be also important for design (Zhou & Griffiths, 2007).

Sifting methods

Usually, sifting methods are used for the study of epigean and litter macrofauna. A sample of litter and the first centimetres of soil is sifted using sieves of different mesh, so that two (or more) fractions are obtained *in situ*; a finer one, smaller than the mesh used and a larger one. Either of both fractions can be discarded if they are of no interest for the research; the grosser fraction can be checked *in situ* to notably reduce the volume of material to be carried to the laboratory, as the finer one usually is. Sifting thus allows the separation of the macro- and the microfauna.

Fig. 3. Winkler-Wagner eclector ready to be used in the field (© MNCN; photographer: Manuel Sánchez-Ruiz).

One of the usual devices used for this purpose in entomological research is the Winkler-Wagner eclector (Fig. 3), described for the first time by Holdhaus (1910). In the case of extraction of microfauna, it is usually coupled with the Berlese-

Tullgren method or with the Moczarski eclector (see below the section *Laboratory extraction methods*). In summary, a Winkler-Wagner eclector is a funnel in strong cloth like sailcloth or similar, ca. 80 cm in length, 30 cm in diameter in the widest opening and 10 cm in diameter in the opposite opening. This narrow opening must have some kind of tight closing device, like a cord or rope to be tied around. The wider opening is circled with a metal ring and a handle at right angles (more or less like a frying pan) fit to be held with the left hand. Some 25 cm below the wider opening, a second ring with another handle will be placed, but this will have inside a flat steel sieve with a mesh as required (usually 2 mm). The handle should be prepared to be held with the right hand (beware left-handed people of swapping handle orientation in both rings!). Handles should make an angle of ca. 80º. The procedure entails placing a sample of soil and litter in the upper part of the Winkler-Wagner eclector and, while keeping the upper ring still, the second ring will be vigorously shaken. After a given time for this treatment, the upper gross fraction that did not pass through the sieve will be placed on a light colour (white, pale yellow, cream) cloth under the sun and extended with the hands to create a thin layer. Specimens fleeing from heat and drying will be directly detected and caught by using fine brushes, forceps, entomological aspirators, or the hands. The fine fraction can be treated in the same way, placing it on the opposite side of the cloth under the sun. Otherwise, the finer fraction can be placed in a dark plastic bag to be transported to the laboratory, where an adequate extraction method will be selected and applied to it. Sample sizes are dependent of the above mentioned factors: Longino *et al.* (2002), in a survey of ants in a tropical forest, extracted samples of 6 l each and sifted them using the Winkler-Wagner eclector, while they collected 1.7 l samples to be directly placed in Berlese-Tullgren funnels in the laboratory. Anderson & Ashe (2000) recommend for obtaining leaf litter beetles the sifting of litter until 4.5 l of fine fraction is obtained and transported to the laboratory, where it could be divided into 3 equal portions of 1.5 l each, and placed in separate Berlese-Tullgren funnels or Moczarski eclectors. For other types and methods of use, Besuchet *et al.* (1987) can be consulted.

Pitfall traps

Purpose and design

Pitfall traps are containers buried with their rims level with the ground surface (Fig. 4). They are gravity collectors and used in general for sampling the epigean fauna, walking or crawling on the ground surface. However, they have also been used to sample hypogean fauna, placing them inside caves or excavations in slopes (Fig. 5). Although they are purpose-built for macroarthropodan collecting, they allow collection of a wider set of taxa belonging to different trophic levels and habitats. They have been used with success in monitoring the small mammal, amphibians and reptile diversity in temperate and tropical forests (*e.g*. Santos-Filho *et al.,* 2008; Lehmkuhl *et al.,* 2008; Lima & Junca, 2008), with appropriate modifications.

Pros and cons

Pitfall traps have been used in a wide variety of studies because of their obvious, numerous advantages. They are cheap, simple to construct, use and maintain, and provide an efficient relation between number of captures and invested field collecting effort. Even so, they show several constraints, based on the fact that the obtained data do not have to reflect the actual structure of the sampled communities (Fabricius *et al*. 2003). Although this inconvenience is also shown by many other sampling methods, the generalized use of pitfall traps makes that their disadvantages take on special significance. According to Topping & Sunderland (1992), almost 40 % of the studies that used pitfall traps did not take into account this constraint in their interpretation of results, and therefore they obtained erroneous conclusions based on the absolute values of the captures.

It has often been mentioned that pitfall traps measure "surface activity", a complex parameter in which size, activity and abundance interact, so that these traps, in fact, do not sample the faunal composition of a site (M. Morris, pers. comm.).

Some authors (*e.g.*: Luff, 1975; Topping & Sunderland, 1992) have looked at the factors causing biases and at the measure in which these distort the collecting. Thus, for example, Mommertz *et al*. (1996) point out that the factors affecting efficiency of pitfall traps can be divided in:

- **Those related to the trap characteristics.** These are: diameter, material, preservatives and baits, disturbance.
- **Those related to the sampled habitat.** These are: composition, structure and properties of the soil.
- **Those related to the specific characters of the target species.** These are: body size, activity, "capturability".

The next section will study how trap design (container depth, rim diameter, preserving liquids and baits used, distance among trap units, etc.) can affect the efficiency of collecting, since these are the only factors (i.e., first type) the researcher can modify according to his/her needs.

Variety in design

As mentioned above, the efficacy of this method depends of many factors, among these the design and the disposition in the field of the pitfall trap units (Weeks & McIntyre, 1997). Consequently, there are as many design as studies. When very particular objectives are pursued, these designs may even become very specific: *e.g*., time-sorting traps, directional traps or ramp traps.

Fig. 4. Pitfall trap from outside, ready to work, showing its rim flush with the gound surface and ethylene glycol solution inside (© MNCN; photographer: Antonio Sánchez-Ruiz).

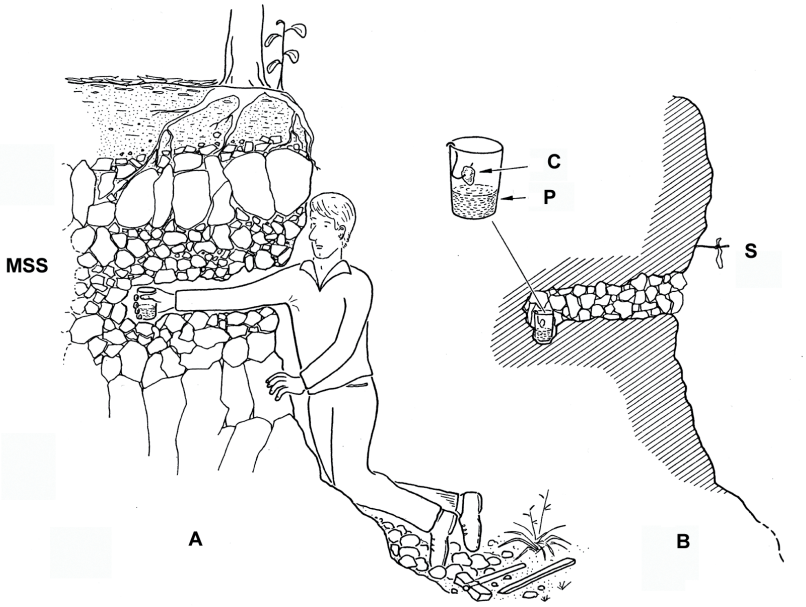

Fig. 5. Trapping by slope boring (redrawn from Machado Carrillo, 1992). A. Placement of the trap in the MSS after making a hole in the slope. MSS = mesocavernous shallow stratum. B. Final position of trap after blocking the hole. C = cheese for bait. P = preservative. S = signal for retrieving the trap.

Trap units can be disposed in different kinds of arrays depending upon the hypothesis to be tested. A usual one is difference in attractant or repellent efficiency, or interferences. They can be arranged in rows along a transect, or in square plots, or in other ways, changing trap diameter, number, spacing and layout as variables. Descriptions of complex arrays can be found elsewhere (*e.g*. Collett, 2003).

However, pitfall traps have also been modified for its use in answering more complicated questions:

- **Directional traps.** An array separated with drift fences to sample either a larger area or the direction of animal movement (upon design) (Hossain *et al*., 2002; Juen & Traugott, 2004).
- **Time-sorting traps.** A complex array inside a box where a timing device exposes to the collecting funnel one container every so often. It is used in ecological studies of soil fauna activity (Blumberg & Crossley, 1988) (Fig. 6).

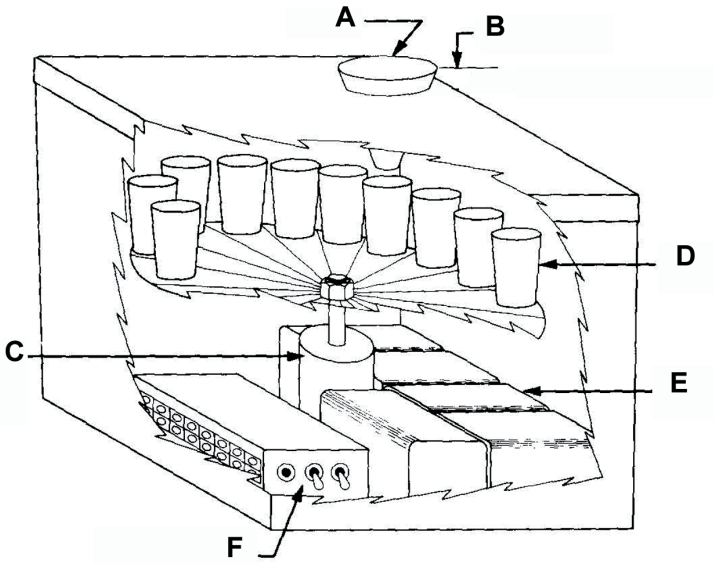

Fig. 6. Diagram of a time-sorting trap, redrawn from Blumberg & Crossley (1988). A = funnel. B = ground surface. C = rotary stepping solenoid. D = containers. E = batteries. F = timing circuit.

- **Barber trap.** Originally, this was a stone-covered and grill-baited pitfall. Barber tested different preservatives and discarded those containing acetic acid and ethanol, because of their deterring effects. He used Galt's solution mixed with ethylene glycol or glycerine, or ethylene glycol alone (Barber, 1931). In subsequent years, this term has been used for any kind of pitfall trap, but particularly for uncovered pitfall traps with three elements: an outer large receptacle, an upper wide funnel and an inner container with preservative (Fig. 7).

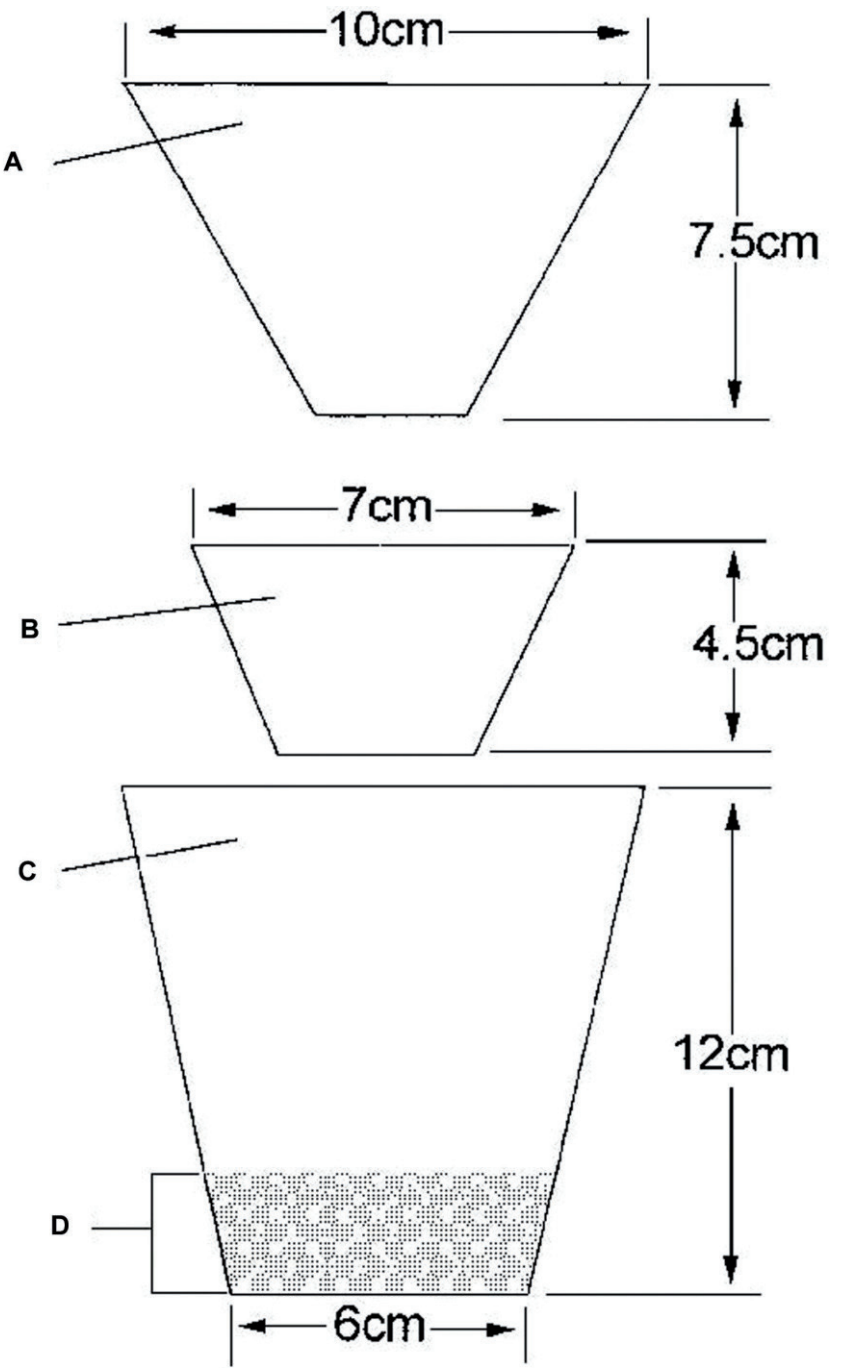

Fig. 7. Uncovered Barber style trap, according to Weeks & McIntyre (1997). A = funnel. B = inner container. C = outer container. D = preservative.

- **Ramp pitfall trap.** Used for sandy, stony or hard substrata where no digging is advisable. The trap is placed on the substratum and has ramps climbing to the rim (Bouchard & Wheeler, 2000).
- **Special-purpose traps.** There are several types. Perhaps the most well known is the Nordlander trap, originally designed for capturing weevils (Nordlander, 1987) and later used for capturing ants (Higgins & Lindgren, 2006).

Materials, shapes and collecting perimeter

Collecting containers are usually made of plastic nowadays, this being a very resistant material, although glass is also used when it is desirable to avoid climbing species from escaping from the trap, plastic being usually less polished than glass. Other authors use aluminium (Bellocq *et al.,* 2001). When the design uses a receptacle where the collecting container is fitted in, other materials can be selected for the former, like metal (Bess *et al*., 2002) or PVC (Collett, 2003), but in the case of inverted truncated cone containers (beaker cups), it is best to use another of the same kind (Witmer *et al.,* 2003). Usually the containers are deeper than wide at rim, but the contrary is also found in the literature (Bellocq *et al.*, 2001; Bellocq & Smith, 2003).

Almost all the studies use containers with a circular section, but sometimes they can have other kind of section (square, for example, in Bellocq *et al*., 2001). However, even if trap diameter is used as a token of their overall size (even if it is not the same along non-cylindrical containers, it is to be understood as rim diameter), the most influential dimension for capture efficiency is rim perimeter.

In the examined literature, the diameter of the container ranged between 18 and 210 mm (Collett, 2003; Verdú *et al*., 2000, respectively). Although most of the works do not justify the use of any specific diameter, most of the studies usually used traps with a diameter between 70 and 115 mm. However, Collett (2003) expressly recommends traps of 18 mm in diameter to avoid flooding during storms while still capturing the largest arthropods. Majer (1978) recommends this diameter as well, for the same reasons, and uses a digging method minimizing the so-called *'digging-in effect'* (see below). However, this rim diameter, which can be enough for large arthropods in cold or temperate areas, may be grossly inadequate, *e.g*., for the largest walking beetles in warm and tropical areas.

Installation, layout and distance between trap units

Traps are placed by digging or drilling a hole of the appropriate depth and width in the selected ground. The container is placed inside the hole and the ground around is fitted to the container rim. It is convenient to interfere as little as possible in the soil qualities (physical, chemical, structural) around the trap unit, not only during the installation, but also when collecting the sampling results, avoiding excessive trampling. Traps must be handled with care (Ruano *et al.,* 2004), avoiding preservative spillage and soil and litter destructuring by trampling

Fig. 8. Placement of inner beaker cup container with killing-preserving liquid inside an outer similar container (© MNCN; photographer: M.A. Alonso-Zarazaga).

and repeated digging (Goehring *et al*., 2002). Most of these problems are avoided by using an outer receptacle (Fig. 8) for the container (Witmer *et al.,* 2003, Thomas & Marshall, 1999, Weeks & McIntyre, 1997). The receptacle will serve to keep the soil in place when the container is being extracted, saving time during container replacement and rim levelling. In places where trap flooding by rain is frequent, this receptacle can be prepared to act as a water drainage system (Collett, 2003).The 'digging-in effect' is the disturbance in the collecting efficiency of a trap after the installation. To avoid this undesirable effect, some time must be allowed to go by, before reliable samples may be collected. This period fluctuates between one (Gibb & Hochuli, 2002) and two weeks (Collett, 2003). Data obtained from samples collected during this period must be carefully considered.

In most studies, a lid is placed some 3-5 centimetres above the rim of each trap unit. This will prevent the evaporation of preservative and the entrance of water and debris (Bess *et al*., 2002), but it also acts as an attracting shelter for specimens (pers. obs.). The lid may consist of a white plastic plate (Borges & Brown, 2003) or a small tin roof (Mommertz *et al*., 1996) or a ceramic tile (Bess *et al*., 2002) separated from the ground by small pieces of wire, nails, etc., or of a handier one, just a stone enough large to cover the rim with the underside rather flat, placed on three smaller ones (Domingo-Quero *et al.,* 2003). It is always convenient to use topped traps, unless the lid may hinder the capture of the target group somehow (for example, Orthoptera).

Traps may be placed single or in arrays, functioning as a single complex trap. There are many criteria about the layout of the traps, either as units or as arrays. Some authors plead for a random placement (Goehring *et al*., 2002; Mathews *et al*., 2004; Witmer *et al*., 2003), or in linear transects (Borges *et al.,* 2005), in the corners of predetermined plots (Bellocq *et al.,* 2001) or in specific layouts (Juen & Traugott, 2004; Perner & Schueler, 2004).

Another parameter influencing as well the abundance, the richness and the composition of the collected fauna is the distance between traps or arrays. Thus the election of a particular distance must avoid interferences and maximize the efficiency of each trap unit. Many authors do not pay much attention to this point; however, some use or recommend a minimum separation of 7.5 (Bellocq *et al.,* 2001), 10 (Samu & Lövei, 1995; Bess *et al*., 2002), 20 (Longcore, 2003), 25 or even 30 m (Albajes *et al.,* 2003). Although these distances may be adequate for the sampling of many macroarthropodans, distances can be reduced or widened according to the presumed size of the feeding or foraging area of the target fauna.

Sample preservatives

Traps can be set dry (*live traps*) without preservative or bait, making at least a bottom hole for drainage. They are suitable for trapping living animals, but they must be tended frequently (every 24 h or less), since animals may attack each other, or may escape, or in some cases, trapped females may attract big numbers of males overflowing the trap. They are also used in arrays as a control trap unit.

Usually, traps are provided with a killing-preserving agent (usually a liquid) and called *wet traps* or *kill traps*. There are many killing-preserving agents: water, salt water, vinegar, ethanol, propylene glycol, ethylene glycol, Turquin's liquid, etc., in different purity degrees (see Appendix). All of them present pros and cons, since any single compound may result attractive for some taxonomic groups and repellent for some others. However, in general it is advisable to use some kind of killing-preserving agent, since in its absence the animals may escape or attack each other, taking into consideration the hazardous effect of most preserving agents (Weeks & McIntyre, 1997) (see Appendix). Apart from the preservative selected for the sampling, some drops of liquid detergent should be added. This additive acts as a wetting agent by reducing the surface tension, favouring the sinking of the captured specimens and avoiding thus their escape. On the other hand, several killing-preserving agents, among those considered to be more suitable, can be used at the same time. Borges (1992) recommends the simultaneous use of three of these (5% formalin, vinegar and Turquin's liquid) to capture a wider diversity of epigean arthropods in the Azores. In arrays or pilot tests, a dry trap may serve as a test control unit.

Baits

Depending upon the kind of study and the target group, the use of some particular type of bait or attractant may be suitable. For example, for the sampling

of coprophagous or necrophagous animals, respectively a bait of excrement or of some kind of carrion (meat, squid, etc.) should be used. Other matters may also be used as attractants, like cantharidine (for some beetles), rotting fruit (for flies) or heavily scented cheese (for pitfalls placed inside caves or lava tubes) (García *et al*., 2001), or specific feromones, among others. Attractants may be solid (and then usually placed in the middle of a wide mesh grill on top (Fig. 9) or hanging from this point, or liquid and mixed with the preservative. Some preservatives may act either as attractants or repellents for different groups of animals, and thus bias the results.

Fig. 9. Dung-baited uncovered pitfall trap for collecting coprophagous beetles (© MNCN; photographer: Jorge M. Lobo).

Sampling period and frequency

Sampling periods and frequencies should be established after analysing the results of a pilot study. To increase the collecting probability, the most favourable periods for the target fauna should be selected.

The sampling frequency will be determined by the objectives and by the project budget (*e.g.,* Marshall *et al*., 1994). For studies in hot and dry places, collecting frequencies above once per week will allow the use of killing-preserving agents with a high evaporation degree (like ethanol or water). However, selecting frequencies below once per week will oblige to use mixtures containing liquids with a low evaporation rate (for example, ethyleneglycol or propyleneglycol) (Bess *et al*., 2002). In the consulted literature, sampling frequency fluctuates between daily and monthly, being the most usual a collecting frequency of once every 1-2 weeks (*e.g*., Albajes *et al.,* 2003). Weekly collectings are most versatile, materials will not decompose and enough time is allowed for mending any kind of wear in the traps, usually meteorological, animal- or human-made, etc.

Recommendations

Several experimental works (Weeks & McIntyre, 1997; Borges, 1992; Borges *et al.,* 2005) have not yet got to a single solution regarding the selection of trap size, distance and killing-preserving agent to use with pitfall traps. Even so, if the target is a complete inventory of the arthropodan fauna of an area, it is very convenient to use them combined with others. The most advisable point is to do

a pilot study before starting the project sampling. This study may give important information leading us to replace a single trap with an array (or the contrary) or a simple trap design with a more complex one, or may allow for a test of different preserving agents and collecting frequencies. However, from our experience, we recommend the following:

1) Use killing-preserving liquid if the sampling is not a 'capture-mark-recapture' design.
2) Different killing-preserving agents can be used in different trap units within the same trap array, provided the distance is enough to avoid interferences, in the same locality. Samples will be evaluated separately and will give useful information on the efficiency of the different agents used.
3) Standard containers with screw tops are most useful. They can be prepared in the adequate number and with the needed amount of preservative in the lab and carried to the field, where the lids will be used to cover the replaced containers and the new containers will be immediately placed instead, fitting the rims and letting them working with minimal disturbance.
4) In the laboratory, samples will be carefully filtered with a sieve of small mesh, avoiding the loss or deterioration of the specimens. Distilled water will be used to drag the preservative agent, and after that samples will be rinsed, placed in clean containers, with a definitive preservative liquid (usually 70º ethanol), and properly labelled.

Pitfall traps are also very useful in combination with other kinds of traps (*e.g.* Malaise traps, yellow pan traps, etc.) to give a most complete inventory of the fauna of a given area, as requested in ATBIs. They can be placed in different layouts (*e.g.*, Basset *et al.,* 2004).

3.1.2. Specific sampling methods

These methods are aimed at obtaining specimens of precise horizons, communities or taxa. In this chapter, we will deal only with the active and passive sampling of the MSS, i.e., the fauna of the lower part of horizon B and of horizon C. It can be done in an active (collecting by hand samples out of these deep horizons) or passive manner (using MSS traps).

Active sampling

Active sampling can be done by quick digging to the sampling depth or by turning big stones or rocks using levers, at a depth of 20-30 cm or more. Both activities are very hard and the latter is risky of injuries as well, and must be undertaken by several people. The samples should be taken from the bottom of the turned stone (by brushing) as well as from the hole. This method yields mostly hypogean microfauna. A large amount of substrate must be collected to make sure that there is enough sampled material for the study. The fauna can be separated *in situ* by flotation (Marshall *et al.,* 1994), using water, a (better light-coloured) plastic bucket, a mug-like jar and a fine meshed sieving system or a paper filter. The procedure is as follows: fill ¾ of the bucket with water; drop the

sample into the water and stir carefully; as the specimens tend to float, the supernatant will be recovered with the mug and filtered. Once fixed by washing with 70% ethanol (and perhaps re-filtering), the material will be carried to the laboratory to be studied under the binocular.

Passive sampling

Passive sampling of the MSS include the use of vertical traps (here called *MSS traps*) and slope boring. They are aimed at obtaining edaphobionts from the MSS. In the first case, the method adopted here is a slight modification of that devised by García *et al.* (1997).

Construction of the MSS trap

The trap is made of several pieces (Fig. 10):

Fig. 10. Assembled main components of a MSS trap (© MNCN; photographer: M.A. Alonso-Zarazaga).

- **Component A.** A PVC tube 150 mm in inner diameter and 600 mm in length (of the grey kind used for pipes); its widest part is to be considered the upper rim.
- **Component B.** A resistant plastic container ca. 150 mm in diameter and ca. 1 l of capacity.
- **Component C.** Strong nylon thread or wire (not too thin).

- **Component D.** A PVC plug, of those prepared to plug pipes of 150 mm in diameter (to fit into component A).
- **Component E.** An eyebolt with eye as wide as to put a forefinger through.

Step 1: Take component A and delimit a zone between 250 and 450 mm below top rim.

Step 2: Drill holes 15 mm in diameter following a regular pattern (6-8 vertical rows around) (Fig. 11). Make sure that no burrs or other irregularities project inwards. If so, erase them with emery board.

Fig. 11. Detail of holes drilled along middle part of component A of a MSS trap (© MNCN; photographer: M.A. Alonso-Zarazaga).

Step 3: Cut transversally component B at a distance of 110 mm from bottom (better use an electric saw). Do not leave burrs or other irregularities on the rim. Erase them with a file or emery board and give the rim a slant inwards.

Step 4: Make two small opposite holes 20 mm below rim of component B, adjusted to diameter of component C.

Step 5. Make a handle in component B by using 450 mm of component C, passing the ends of the thread or wire through the holes from the outside to the inside. Place stopping knots in the inner part of component B. This handle will allow extraction of component B from within component A by pulling the handle out.

Step 6: Test that component B fits along component A and glides smoothly from bottom to top and back. Detect any irregularity in component A or B and erase them (Fig. 12).

Step 7: Screw component E in the center of the outer side of component D.

Fig. 12. Top view of component B placed in the bottom of component A of a MSS trap with handle up in pulling position (© MNCN; photographer: Miguel A. Alonso-Zarazaga).

Installation of the MSS trap

Be sure to carry all the components, plus some strong plastic bags or similar, a container with 200 ml of a hypersaturated salt water solution and some drops of washing-up liquid (killing-preserving agents, see Appendix), some strong nylon thread or elastic bands, and a soil drill 150 mm in diameter. Protecting gloves, a tape measure and a lever may also be useful.

Step 1: Find a suitable place in the area to be sampled. Drill a hole 150 mm in diameter and 550 mm deep into the ground. Try to avoid extreme disturbance of soil, digging with a spade is to be discarded. Use hand or power (electric, motor) drills. In extremely loose soil, like volcanic ones, a lever may suffice. Work may be hard and extracting stones in the drill path by hand every so often is commonplace.

Step 2: Once the hole is finished, introduce component A in it. It must stick out some 50 mm, so that the holes drilled in this component will be located between 20 and 40 cm in depth. This will be the sampled horizon. Make sure that the ground around the trap fits closely its neck.

Step 3: Pour the killing-preserving agent into component B.

Step 4: Descend component B to the bottom of component A, taking care of not spilling, its handle up (Fig. 12). Make sure that the rim of component B lies below the level of the lower holes around.

Step 5: Plug component A with component D to avoid contamination with surface fauna. Cover with a strong plastic bag or similar and tie it with nylon thread or elastic bands around the projecting end of component A, to waterproof it.

Step 6: Cover the top of the trap with vegetal matter, stones, litter or sand and gravel, depending upon the surroundings. Conceal it as well as possible.

Step 7: Make a precise note or sketch of the position of the trap (to be sure to find it later) and write down the date of installation.

Collecting the results

The MSS traps are functional over long periods of time. No collecting should be done before one month, even better three months. These traps have a "*maturation time*" after their setting, while the soil around the trap recovers its normality. During this maturation time, number and diversity of the captures will increase to a normal level. This time will be shorter or longer depending upon how "traumatic" for the soil the installation of the trap has been. These traps should be exploited during a long period of years to have a real inventory of the edaphobiont fauna moving through the MSS in a certain area.

After accessing the trap, the top will be carefully cleaned, and the bag and the plug removed, avoiding the drop of debris inside the trap. Putting a hand inside, the component B will be hold by its handle, and carefully extracted. Some meters away from the trap, the killing-preserving agent will be filtered with a gauze (adding more fresh water if needed) and the captures placed in 70º ethanol. Component B will be cleaned and new killing-preserving agent will be placed. The trap will be reset as mentioned above for a new collecting period.

These traps can be flooded by heavy rain making the water table to raise close to surface, in which case most of the captures will get lost. The captured specimens may need a long wash with distilled water to get rid of salt encrusting.

Other measures can be used in constructing this kind of traps upon availability of the components, but inner diameter of component A must allow for an arm to go in. Rows of holes can be made at different depths depending upon the upper and lower depths of the MSS in a particular area for a proper sampling; however, care must be taken that the rim of component B does not lie higher than the lower holes. Anyway, deeper traps (more than 600 mm) are not advisable because of the difficulties in grasping the handle of component B and extracting it or in placing it well in the bottom.

Slope boring

This method lies in making a hole of an adequate size in a bank or slope, preferably in fresh cut ones because of public works. A suitable depth must be selected (usually 60-80 cm), always above the parent rock. A hole where an arm can be introduced has to be horizontally drilled. Natural cavities at the appropriate depths can also be used. Normal pitfall traps can be placed inside the hole (Fig. 5A).

Some attractant pieces (strongly scented cheese is very appropriate) can be dispersed inside the small tunnel or hung inside the pitfall trap, and the opening will be carefully closed and concealed (Fig. 5B). Some days later, the area will be carefully brushed out or specimens picked up by hand or aspirator first, around the trap, and this will be extracted later. A passive approach may use also baited

ramp pitfall traps, instead of normal ones. Replace the bait and the preservative, and conceal the opening again.

3.2. Laboratory extraction methods

Sampling very small animals (*microfauna*) has the disadvantage that they cannot be separated in the field. In this case, carrying samples to undergo a laboratory extraction is obligatory. According to the nature of the methods, two kinds of extractions are to be distinguished: mechanical or passive methods, and dynamical or active methods. On the other hand, samples can be manually separated under the binocular. This is a quite unusual method since it takes up too much time, however, it may help us to evaluate the efficiency of other methods, since this is very variable, and target taxon and target horizon dependent (Southwood & Henderson, 2000).

3.2.1. Mechanical or passive methods

They are based on physical principles and sample organisms do not move. The commonest are filtering, flotation, decantation, elutriation and flotation-centrifugation. Separation protocols are very variable, since every research team tends to modify them in order to adjust them to their particular needs.

Filtering

This technique may be used in combination with those mentioned below. It is used separately when the difference between the body size of the specimens and the soil grains is very wide. In the laboratory, the sample is usually suspended in water to help the filtering process. Successive filtering can be done through a series of sieves descending in mesh size and ending in a paper filter, separating thus size fractions. Motorized sieve shakers can be found in the commerce and piled in descending mesh size.

Flotation

Is a widely used technique when the specific gravity of the fauna and of the soil grains is very different. Different liquids can be used as suspension media: solutions of 25% magnesium sulphate, of sodium chloride, of 75% of zinc chloride, of sucrose or directly heptane (Southwood & Henderson, 2000). Sometimes it is needed to do a pretreatment of the soil sample if it is too clayey, by gently shaking in solutions of sodium citrate (200 g/L) or sodium oxalate (saturated solution), or if heavily clayey, with a solution of sodium hexametaphosphate (50 g) and sodium carbonate (20 g) in 1 l of water, and placed in a vacuum desiccator under reduced atmospheric pressure until desiccation, before resuspending in the flotation medium. The basic heptane protocol is as follows: Put the sample in a cylinder with flat stopper and add 1 l of 50% ethyl alcohol and 10 ml of heptane. Replace stopper and invert cylinder without shaking. Allow the heptane to rise. Repeat inversion twice. Allow the cylinder to stand for 4 h. The sediment will settle. Decant the heptane

supernatant layer into a sieve. Rinse the sieve with 95% ethanol to remove the heptane and wash the sample into a sorting dish.

Decantation

This technique lies in washing the sample several times filtering the supernatant with a 63 µm meshed sieve. It is mostly used for specimens able to go through a sieve of 1 mm mesh, mostly soil nematodes. There are several variants of this technique (Southwood & Henderson, 2000). They are considered to be less efficient than other methods, such as elutriation.

Elutriation

This technique lies in separating the organisms by washing the sample in a constant current of water. Thus the specimens, floating more or less, are swept and later filtered, while the sediment, being heavier, is kept in the bottom of the device. This method is able to process a large amount of sample in a short time. It has been used to separate pauropods and springtails but is often used to separate soil nematodes in slightly modified devices (Southwood & Henderson, 2000). The **soil washing** technique uses a washing apparatus (Fig. 13) made of a stack of two sieves (a coarse one on top of a medium one) placed over a settling can. This can has a pivoted lateral drainage that allows floating animals to pass into the Ladell can, which has a 0.2 mm fine phosphor-bronze sieve in the bottom. Its lower opening is inmersed in the drainage tank, so that the water level in the tank is always slightly above the sieve of the Ladell can. When water is poured over the sample placed in the upper sieve, specimens are filtered: large animals are caught in the coarse sive, medium sized animals in the medium sieve and small animals (depending upon mesh size) are washed to the Ladell can sieve, where they can be recovered.

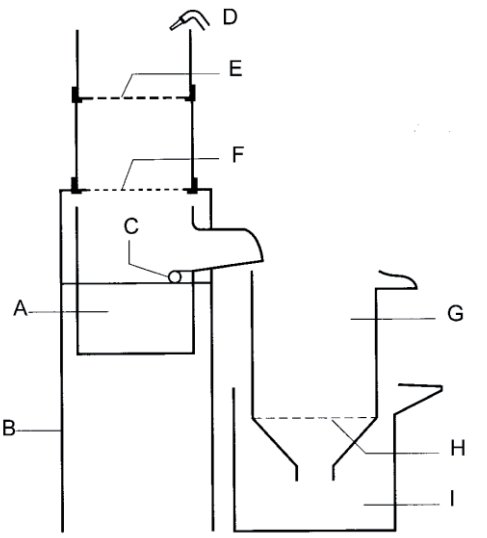

Fig. 13. Soil-washing apparatus, redrawn from Southwood & Henderson (2000). A = Settling can. B = Stand. C = Pivot. D = Nozzle of hose. E = Coarse sieve. F = Medium sieve. G = Ladell can. H = Fine phosphor-bronze gauze. I = Drainage tank.

Specifically for Nematodes, two different and widely used elutriators have been designed: the Oostenbrink and the Seinhorst elutriators.

Flotation-centrifugation

As a previous technique to this, decantation and elutriation should be used to obtain an extract of the sample. The technique in itself lies in centrifuging this extract in a saturated salt or sucrose gradient, allowing thus a final purification. This technique is not much used because it can process only a few samples at a time, but it is the election technique to extract soil nematoda and water bears in inventory studies (Coleman *et al*., 2004).

3.2.2. Dynamic or active methods

They are based on the migration of the sample organisms as a response to the alteration of the physicochemical conditions of their environment. The most common methods are the Berlese-Tullgren funnel, the Moczarski eclector and the Baermann funnel.

Berlese-Tullgren funnel

This technique was devised for dry samples. It was originally designed by Berlese (1905) with a hot water jacket to heat the sample and posteriorly modified by Tullgren (1918) by eliminating the jacket and adding a bulb on top. After this basic design, other authors have developed more sophisticated devices, like the horizontal extractor, the high gradient extractor and the Kempson extractor (Southwood & Henderson, 2004). All of them are based in the negatively phototropic and positively geotropic behaviour of the soil fauna, which migrates downwards to fall in a collector container. The Berlese collector (Fig. 14) consists of a funnel with smooth inner surface, a lab tripod to keep it upright, a sieve fitting inside the funnel (mesh size 2 mm), a container with the appropriate killing-preserving liquid (usually 70% ethanol, added or not of up to 25% ethylene glycol; hypersaturated salt water can also be used) and a top. The sample is carefully placed in the sieve on a piece of paper and extended, then the sieve is placed inside the funnel and the debris on the paper added, the collecting container is placed below and the top covering the sieve. If the organisms sampled tend to die quickly because of drying, the environmental gradient must be soft, and the top may consist of a square gauze (square side longer than sieve or funnel diameter) with four lead weights, each sewn in one corner, and placed flat on the sieve rim; the sample will dry under the environmental conditions (Berlese model). If they are more resistant, the environmental gradient can be made harder by using a conical metal top with a light bulb inside (Tullgren model). This bulb will be on during the whole extraction process and its intensity will be determinant of the desiccation speed. These funnels can be placed in arrays and bulb tops can be powered with a single

battery or socket. It is particularly good at collecting mites, small myriapods and insects (mostly springtails and microcoleoptera) and minute spiders. According to the target fauna, the researcher can introduce particular modifications. Reca & Rapoport (1975) commented on the efficiency related to mesh size in temperate areas, observing that a 2.3 mm mesh collects only 70% of the total soil fauna, recommending instead a 4 mm mesh to be near the optimum size for collecting most of the fauna.

Fig. 14. Berlese collector formed by a sieve, a funnel, a holder and a plastic container with preservative. Leaded gauze is not shown. (© MNCN; photographer: Teresa Domingo-Quero).

Moczarski eclector

This eclector is constructed in a similar way to the Winkler-Wagner one and is widely used in temporary labs in the field (Fig. 15) or in closets in hotel rooms while travelling. The main difference with Berlese/Tullgren funnels is that the extraction is by desiccation of the sample through the surrounding cloth, and not with an external energy source drying the sample from top to bottom. It is made of a strong cloth (sailcloth or similar), two equal square frames in wood or aluminium (ca. 38 cm), one strong hook and a (usually square) sieve of 2-3 mm mesh fitting the size of the frames. Both frames are sewn with a band of fine mosquito netting cloth. The upper frame is provided in each angle with one string, all four tied at their free end to the base of the hook. One funnel-like piece

is sewn by uniting four pieces of sailcloth cut like isosceles triangles, whose base must fit once sewn 38 cm, the longer side of the triangles being ca. 1 m.

Fig. 15. Array of Moczarski eclectors in place at a field station in Mont Nimba (Guinea). (© and photographer: Didier VandenSpiegel).

The four apices are cut and sewn round to allow its placement around the mouth of a collecting jar with preservative, placing previously a metal ring around the outside to force the opening of the cloth funnel to be smaller than that of the jar. The jar can be fastened under the funnel by placing an elastic band around the funnel apex and the jar. Four similar triangles will be sewn by their bases to the upper frame, being kept free on their sides, and can be united with a string under the hook, forming a hood, or conversely, they can be sewn by their sides and attached under the hook by a string, so it can be pulled up and down and fitted externally to the upper frame. The sieve will be placed on the lower frame and hold with small twisted plastic-coated wires. The sample will be placed on the sieve, and the eclector hung in a closet bar or similar. For a more sophisticated device and other details, Wheeler & McHugh (1987) can be consulted.

Baermann funnel

This technique is devised for wet samples. The original model consisted of a glass funnel full of water, with a sieve at midlength, where the sample, wrapped in a gauze, is deposited (Fig. 16). A later modification is the addition of a lamp heating the water, which accelerates the separation process.

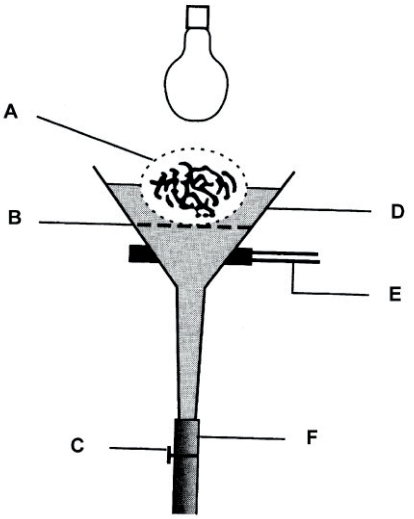

Fig. 16. Baermann funnel, redrawn from Southwood & Henderson (2000). A = muslin wrapped over sample. B = sieve. C = pinchcock. D = water. E = funnel stand. F = rubber tubing.

This technique is recommended for the extraction of animals extremely sensible to desiccation. It works well for the separation of nematodes and rotifers, but it is less advisable for that of water bears.

4. Recommendations

Sample conservation and transport

Care must be taken with the particular needs of each group for preservation and/or conservation for their later study. Most of the samples will be adequately kept in 70º ethanol at room temperature. However, with animals that must arrive alive to the laboratory, some special precautions must be taken, like trying to keep the samples in a fresh place or in a cool box and process them at once, when the laboratory is reached. If the processing should have to wait, the samples ought to be kept in a refrigerator (ca. 5ºC) until this moment. If in the field the temperature and humidity conditions are unbearable for the fauna being collected, the quick use of a cool box is absolutely necessary.

Anderson & Ashe (2000) recommend the use of cotton cloth bags for the transport of samples to the lab, and processing them before 24 h of their collection, not exposing them to extreme variations of temperature and humidity. In general, processing in laboratory after field collection of the samples must be carried as soon as possible (Marshall *et al.,* 1994).

Another general recommendation the authors of these lines have made above is repeated here: the importance of pilot studies to help finely tune the parameters of the collection.

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7. Appendix: Preservatives

Do not forget in every case to add a few drops of liquid detergent (washing-up liquid) as a wetting agent. Uncommon preservatives have not been listed.

Ethanol. Also called ethyl alcohol or simply alcohol, it is abbreviated sometimes as EtOH. Usually used as a 70% ABV water solution, that can be obtained from the commercial absolute alcohol (95,6% ABV, azeotropic mixture) by adding to one liter of the latter 391 ml of distilled water (beware, mixing is exothermic!). It is

volatile, flammable and a psychoactive drug, and it is irritant for skin and eyes. Never use denatured ethanol for conservation purposes. Percentage of alcohol by volume (ABV) is also called *degree Gay-Lussac* (º).

Ethylene glycol. It presents differential attractiveness. A very widely used killing-preserving liquid because of its slow evaporation. The cheapest way to obtain a suitable solution is to use car coolant, reduced to 50% with distilled or soft water. However, it is an eye irritant, and toxic by oral consumption, affecting the central nervous system, the heart and the kidneys. Antidotes are ethanol (strong spirits may be used until a hospital is reached) and fomepizole.

Formalin. Pure formalin is a hypersaturated solution (ca. 40% by volume) of formaldehyde in water. Commercial formalin has 10-12% methanol as a stabilizer. Its use should be discarded because of health hazard: allergenic, carcinogenic, eye and mucous membranes irritant, intoxication by aspiration provokes headaches, burning throat and difficult breathing.

Galt's solution. A mixture of 5% common salt (sodium chloride), 1% potassium nitrate, 1% chloral hydrate to be completed with water up to 100%. To be mixed for use with ethylene glycol or glycerine. Not recommended, since the captured specimens deteriorate too soon, potassium nitrate is moderately toxic, irritant for skin and eyes, and chloral hydrate is a sedative and hypnotic drug with a strong potential for health hazard.

Isopropanol. Also known as IPA or isopropyl alcohol, it is a cheap dissolvent with many uses. It is moderately toxic to humans, being a central nervous system depressant. It is also highly flammable, and should be used only in well-ventilated areas.

Picric acid. Also known as TNP, it is 2,4,6-trinitrophenol. Its use should be discarded because of health hazard, being corrosive, explosive, toxic by inhalation, oral consumption or skin contact, damaging lungs, liver and kidneys.

Propylene glycol. Proposed as an alternative to ethylene glycol by some authors because of its lesser toxicity, it presents similar properties, but may be more difficult to obtain. Even so, it is an eye and skin irritant, may harm the respiratory tract, and it is also allergenic and mutagenic.

Turquin's liquid. Original Turquin's (1973) formula modified after Ashmole & Ashmole (1987): 10 g chloral hydrate, 5 ml formalin, 5 ml glacial acetic acid, 1 ml detergent and dark beer added to make one liter. It is hazardous because of the presence of chloral hydrate and formalin (see above), and of glacial acetic acid, which in pure state is a strong corrosive burning skin and mucous membranes, and is flammable in contact with air over 39ºC.

Vinegar. It is usually a 4-8% acetic acid solution in water (typically 5%). Natural vinegars contain other acids in addition. A good preservative, the commercial brands from white wine ought to be selected.

Water. It may repel certain species, and as such is not a good preservative liquid. When hypersaturated with salt, it can be used for long stay traps. A saturated sodium chloride brine depends upon temperature, hot water admits more salt than cold. Solubility at standard state (25ºC, 100 kPA) is 35.9 g / 100

ml, so if this amount of salt is placed in 100 mL of distilled or soft water, it will be probably soon hypersaturated in cold conditions because of the lowering of the temperature or in hot conditions because of the evaporation. Probably the most innocuous preservative agent, both for users and environment. In addition, it is inexpensive.