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# Project HUMUS FOR THE BIOSPHERE Euki Project

Physical, chemical and biological  
parameters for the analysis of soil quality

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## Premise

The HUMUS PER LA BIOSPHERE project promotes the increase of soil organic matter, with particular reference to the formation of stable humus, to promote biodiversity, soil fertility and carbon sequestration in farms in the Etna river valleys, in Sicily, which represent an important ecological corridor between three natural parks.

In collaboration with the University of Catania (Department of Agriculture, Food and Environment and Department of Biological, Geological and Environmental Sciences) and through the participation of about 100 farmers, the aim of the project is to implement examples of good practices for a organic farming respectful of the climate and biodiversity and produce useful data and information for the drafting of the Regional Strategic Plan to be implemented as part of the new EU Agricultural Policy.

Furthermore, the project, in collaboration with 27 municipalities, intends to provide indications to local authorities for the creation of a regional composting system for organic waste.

These activities are complemented by environmental education measures, which involve young volunteers from all over Europe and around 3,000 students.

As part of the project, the research activities in the field provide for the assessment of the chemical, physical and biological parameters that influence the quality of the soil in order to develop a system for assessing and monitoring the increase in organic matter and stable humus. . This report describes the methods of analysis used for the detection of the aforementioned parameters and for the calculation of the relative indices.



## 1. The parameters for the analysis of soil quality

Soil is essential for food production and for the proper functioning of the global ecosystem. It originates through several dynamic processes. Soil quality is threatened by increasing human population, intensive management of available arable land, urbanization and soil degradation (Diamond, 2005).

The various types of soil are the substrate for primary production (plants, fungi, microorganisms) and for the survival of natural habitats. Furthermore, soil is the main carbon deposit on our planet, so much so that the 2030 Agenda underlines its importance and the need to preserve it with accurate soil management practices, since carbon sequestered in the soil can significantly reduce climate change. . Currently, the growing anthropogenic pressure on the environment leads, in most of the world, to a rapid change in land use and an intensification of agricultural activities (Menta, 2012).

Soil is increasingly recognized as a non-renewable resource on a human scale because, once degraded, its regeneration is an extremely slow process (Camarsa et al., 2014; Lal, 2015). Given the importance of soils for agricultural and livestock production and for the provision of wider ecosystem services for local and global societies, keeping soil in good condition is vital. To manage agricultural land use well, decision makers need science-based, easy-to-apply and inexpensive tools to assess changes in soil quality and function (Bai et al., 2018).

Soil quality is one of three components of environmental quality, in addition to water and air quality (Andrews et al., 2002). Water and air quality are mainly defined on the basis of the degree of pollution that has a direct impact on human and animal consumption and health or on natural ecosystems (Carter et al., 1997; Davidson, 2000). Soil quality, on the other hand, is not limited to the degree of soil pollution, but is commonly defined much more broadly as "the ability of a soil to function within the boundaries of the ecosystem and land use to supporting biological productivity, maintaining environmental quality and promoting plant and animal health "(Doran and Parkin, 1994, 1996).

This definition reflects the complexity and site specificity of the underlying part of terrestrial ecosystems, as well as the numerous links between soil functions and soil-based ecosystem services. Indeed, soil quality is more complex than air and water quality, not



only because soil is made up of solid, liquid and gas phases, but also because soil can be used for a greater variety of purposes ( Nortcliff, 2002).

Soil quality can be assessed both for agroecosystems in which the main, although not exclusive, ecosystem service is productivity, and for natural ecosystems in which the main objectives are the maintenance of environmental quality and the conservation of biodiversity (Bünemann et al., 2018).

Soil quality decisions and perceptions are strongly influenced by agricultural productivity and are usually based on soil quality indicators. Usually, farmers use physical and chemical indicators to assess soil quality (Bone et al., 2013; Moncada et al., 2013), such as bulk density, texture, organic matter, fertility (based on productivity of plants) and salinity (Tesfahunegn et al., 2013).

A chosen indicator must be related to a certain soil function, or an ideal indicator should integrate the physical, chemical and biological properties of the soil.

The United States Department of Agriculture has also prepared several indicators for assessing soil quality by the Natural Resources Conservation Service (NRCS). Soil health (soil health is used as a synonym for soil quality, but not all researchers agree) cannot be determined by measuring crop yields, water quality alone, or any other outcome. Soil health cannot be measured directly, so indicators are evaluated. There are three types of indicators: physical, chemical and biological.

However, the biological and biochemical properties of the soil can respond more quickly to management activities and perturbations (see, for example, Gianfreda & Ruggiero, 2006; Paz-Ferreiro et al., 2007). Furthermore, the biological component of the soil is responsible for many functions of the soil ecosystem, including the decomposition of organic debris, the nutrient cycle, the synthesis of humic substances, the degradation of xenobiotics and the fixation of nitrogen. Soil biological activity is influenced by land management and linked to soil erosion rates (García-Orenes et al., 2009, 2010, 2012).

For the description of the parameters, the official methods of analysis will be used, where present, according to the Ministerial Decree of 13 September 1999 "Approval of the official methods of soil analysis" of the Ministry of Agriculture and Forestry.

The report will describe the following parameters and indices:



### **Physical parameters:**

- granulometry
- soil moisture
- apparent density
- temperature
- water potential
- field capacity
- infiltration

### **Chemical parameters:**

- soil pH
- electrical conductivity of the soil
- total and reactive carbonate
- humic substances
- total organic carbon
- content of nitrogen and phosphorus
- content of potassium, calcium, magnesium and sodium

### **Biological parameters and indicators:**

- arthropods: QBS-ar
- earthworms: QBS-e
- soil breathing
- soil enzymes



## 2. Physical parameters

### 2.1. Granulometry

The mineral fraction of the soil constitutes on average 95-98% of the dry weight of the soil; in field conditions it represents about 40% of the soil volume, while the remainder is occupied by water, air and organic substances.

It can be said that it constitutes the support for all the physico-chemical and biological processes that take place in the soil; when we talk about earth or soil, in fact, the mass of inert material that constitutes it immediately comes to mind before the organic, biological, aqueous or atmospheric fractions which also contribute to determining its properties.

What differentiates soil and subsoil from other terrestrial environments and other natural resources is precisely the preponderance of this mineral fraction over the other components.

The deeper you go into the soil, the more the incidence of mineral materials increases; the composition of the deep layers is not very interesting for agricultural activity, but it is important to understand some phenomena such as the leaching of nutrients and substances that are distributed on the ground.

#### *2.1.1. The structure of the soil: Porosity*

Even before the size of the particles that form the soil, it is important, from an agronomic point of view, to know how they interact with each other to form more or less voluminous and compact aggregates.

The structure, that is the organization of these aggregates in the soil, conditions in particular the macro and microporosity, therefore the aeration (macropores) and the water retention capacity (micropores) of the soil, on which all biological activities and the degree of leaching of the profile.

Therefore, it influences both the physical environment in which plants develop, and the processes connected with the availability of nutritional elements, their transport or immobilization and the chemism of toxic elements.



Numerous systems of description and classification of aggregates have been developed as well as numerous methods to measure their structural stability, but since there are many factors that influence it, there is not, and probably cannot exist, a single and universal method.

In relation to what has been mentioned, the common laboratory analyzes do not involve research aimed at defining the structure of the soil and measuring its stability, but are limited to determining the particle size composition.

The analysis of the particle size composition aims only to know the content in primary particles of the soil, divided according to the size regardless of the way in which they are distributed in space.

Despite these limitations from its knowledge we can still draw useful information on the structural state of the soil as long as the results are interpreted together with the analytical data relating to the composition of the exchange complex, the reaction, the availability of organic material.

The primary solid component (i.e. not associated in structural aggregates) of the soil is characterized by particles of various sizes: from diameters of the order of a centimeter to millimeters and tenths of microns of the finer components.

These fractions can be classified according to the diameter and grouped into dimensional categories: many subdivisions have been proposed in this regard which fundamentally differ in the limits of the dimensional classes of the individual particles. The main ones are two and differ only in the limit that separates silt and sand: 0.02 mm for the International Society of Soil Sciences, taken up by Unichim, 0.05 mm for the USDA, taken up by the Italian Society of Sciences of the Soil; on the other hand, the upper limit for sand, 2 mm, and the silt-clay limit, 0.002 mm, coincide.

The method involves a first separation of the particles with a diameter of less than 2 mm, called "fine earth", which represents the fraction of the solid component of the soil most directly responsible for the fundamental characteristics of the soil and on which all subsequent chemical and physical determinations are carried out and related results reported.



The determination of the skeleton is performed in the laboratory using special sieves but, for a real evaluation, since the skeleton is distributed in the soil with great variability, it is always advisable to accompany the laboratory data with an evaluation in the field performed by observing, during an excavation, the profile and evaluating the extracted materials.

As the skeleton increases, the fine earth decreases and therefore the productive capacity of the soil, and the losses of nitrogen and potassium and the oxidation of organic matter can increase.

### ***2.1.2. Determination of the skeleton***

#### **Principle**

The method of preparation of the sample to be analyzed is aimed at allowing that:

- the smallest weight foreseen by the analysis methods is representative of the soil under examination;
- no changes in composition are made such as to significantly alter the various solubilities in the different extracting reactants;
- the quantity of particles with a diameter of less than 2 mm can be evaluated.

#### **Sample for the laboratory**

Soil quantity taken according to the defined sampling plan and delivered to the laboratory.

#### ***Raw sample for analysis***

Representative aliquot of the sample for the laboratory

#### ***Terrafine***

Aliquot of the raw sample for analysis, dried at a maximum temperature of 400C and passed through a sieve with 2 mm mesh.

#### ***Aggregate***

Set of "elementary" particles cemented by organic and / or inorganic constituents.

#### ***Skeleton***

Individual particles present in the soil that do not pass through the 2 mm mesh sieve,



## Equipment

- ventilation stove (optional);
- Rubber covered peg;
- apparatus for the crushing of
- brass or nylon sieve with 2 mm clear mesh opening;
- brass or nylon sieve with 0.5 mm clear mesh opening;
- brass or nylon sieve with 0.2 mm net opening;
- agate mortar.

## Method

### *Air dry sample, sieved to 2 mm (fine earth)*

Spread the entire sample in the laboratory on a flat surface, it is clean. After careful homogenization, separate from several points, randomly different, representative aliquots which, combined, constitute the raw sample for analysis.

Transfer the raw sample for analysis on a paper tray or plastic in a layer of 1-2 cm and dry it in the air, in a protected environment, at room temperature.

Exceptionally, the raw sample for analysis can be dried in a forced ventilation oven at a temperature not exceeding 400C. Weigh the sample at room temperature.

Crush the aggregates with a rubber-coated rolling pin and pass the sample through a 2 mm mesh sieve to separate the fine earth.

In the absence of a skeleton, a suitable device equipped with a separator can be used to crush the aggregates.

Store no less than 300 g of fine earth in clean, dry, closed and clearly identified containers.

Before weighing each analytical determination, carefully homogenize the fine earth sample.

### *Air dry sample, sieved to 2 mm (Skeleton)*

The material left on the sieve makes up the skeleton. Wash it with a jet of water to remove any adhering fine earth particles, dry and weigh it.



### ***Expression of results***

The quantity of skeleton, expressed in g / kg without decimal digits, is obtained from the relation

$$C = 1000 \cdot \frac{M_1}{M_2}$$

where is it

C = quantity of skeleton expressed in g / kg

M1 = mass of the skeleton, expressed in grams

M2 = mass of the crude sample for analysis, expressed in grams.

#### ***Air dry sample, sieved at 0.5 mm***

Crush a sieve aliquot with 0.5 mm mesh in an agate mortar.

Any material left on the sieve will need to be further pounded until it all passes through the sieve.

#### ***Air dry sample, sieved at 0.2 mm***

Crush a representative aliquot of the fine earth sample in an agate mortar and pass it through a 0.2 mm mesh sieve.

Any material left on the sieve will need to be further until it all passes through the sieve

### **Note**

The fine earth is used for those analytical determinations to be carried out on at least 5 g of sample. For particular determinations or when the method of analysis requires quantities of less than 5 g, it is more appropriate to use dry samples in the air, sieved at 0.5 0 to 0.2 mm.

### ***2.1.3. Dispersion of the soil sample for particle size analysis***

#### **Principle**

For the physical-mechanical characterization of the soil it is necessary to guarantee the optimal dispersion of the particles present in the sample used for analysis.



First, the partial or total elimination of the cementing substances is carried out and, subsequently, the physical dispersion.

## Reagents

- **Acetone ( $\text{CH}_3\text{COCH}_3$ )**

- **Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) [30% m / m in water (p = 1.122)]**

- **Solution (50 g \* L-1) of sodium hexametaphosphate**

Transfer to a 500 ml glass beaker, containing approximately 250 ml of  $\text{H}_2\text{O}$ , 40 g of sodium hexametaphosphate and 10 g of sodium carbonate. Shake on an electromagnetic stirrer until complete solubilization of the reagents and transfer the solution into a 1000 ml volumetric flask. Bring up to volume with  $\text{H}_2\text{O}$ . Determine the exact concentration by drying at 105°C after having evaporated a known volume of the solution in a water bath. The concentration (g \* L-1) will be given by the ratio between the weighted mass after drying (M in g) and the volume taken (V in ml), multiplied by 1000.

- **Sodium dithionite ( $\text{Na}_2\text{SO}_4$ )**

- **Diluted solution (100 ml \* L-1) of acetic acid**

Transfer to a 1000 ml volumetric flask containing about 600 ml of  $\text{H}_2\text{O}$ , 100 ml of acetic acid ( $\text{CH}_3\text{COOH}$ ) [(99.9% (p = 1.0499)]. Mix and make up to the mark with  $\text{H}_2\text{O}$ .

- **Solution (1 mole \* L-1) sodium acetate, buffered at pH 5**

Dissolve 136.08 g of acetate ( $\text{CH}_3\text{COONa}$ ) in  $\text{H}_2\text{O}$ , in a 500 ml glass. Bring the pH value to 5 by adding diluted acetic acid solution. Transfer to a 1000 ml volumetric flask. Bring up to volume with  $\text{H}_2\text{O}$ .

- **Solution (1 mole \* L-1) of barium chloride**

Dissolve 24.43 g of barium chloride ( $\text{BaCl}_2$ ) in  $\text{H}_2\text{O}$  in a 100 ml volumetric flask. Bring up to volume with  $\text{H}_2\text{O}$ .

- **Solution (1 mole \* L-1) of silver nitrate**

Dissolve 16.98 g of silver nitrate ( $\text{AgNO}_3$ ) in  $\text{H}_2\text{O}$ , in a 100 ml volumetric flask.

Bring up to volume with  $\text{H}_2\text{O}$ .

- **Solution (100 g \* L-1) of sodium chloride ( $\text{NaCl}$ )**

Dissolve 100 g of sodium chloride ( $\text{NaCl}$ ) in  $\text{H}_2\text{O}$  in a 1000 ml volumetric flask. Bring up to volume with  $\text{H}_2\text{O}$ .



- **Sodium chloride (NaCl) (saturated) solution**
- **Buffered solution of citrate-bicarbonate**

Prepare a 0.3 mol L<sup>-1</sup> sodium solution (88.4 g L<sup>-1</sup>). Add 125 ml of a 1 mole L<sup>-1</sup> solution of sodium bicarbonate (84 g L<sup>-1</sup>) to each liter of the sodium citrate solution.

### **Equipment**

- 120 + 140 cycles oscillating stirrer minute 'I with horizontal shaking;
- electromagnetic stirrer;
- bain-marie;
- centrifuge;
- soil blender.

### **Method**

#### ***Removal of soluble salts***

In soils with electrical conductivity of the aqueous extract 2: 1 greater than 1 dS / m, it is necessary to remove the soluble salts.

Transfer a weighed quantity of the fine soil sample to a 250-liter centrifuge tube.

Add 100 mL of H<sub>2</sub>O, shake and remove the clear supernatant by decanting. Add 50 mL of H<sub>2</sub>O and repeat the previous operations until the electrical conductivity of the centrifuge solution is less than 1 dS / m. If necessary, check that chloride and sulphate ions are absent using qualitative assays that involve the addition of a few drops of the solution (1 mole L<sup>-1</sup>) of silver nitrate and of the solution (1 mole / l) to different portions of centrifuged solution, respectively. ) of barium chloride.

#### ***Removal of carbonates***

They can be removed with the procedure already described (paragraph 4.1) by adding 10 ml of the solution (1 mole / l) of sodium acetate buffered at pH 5 to the water of the first wash. As a rule, the removal of carbonates is not performed and is completely not recommended in calcareous soils.

#### ***Removal of organic matter***



The soil sample, possibly pre-treated to remove soluble salts and carbonates, is transferred quantitatively with H<sub>2</sub>O (25 ml) into a 1000 ml beaker. 5 ml of hydrogen peroxide are added and stirred with a glass rod. If a large amount of foam forms, the glass is cooled from the outside by placing it in a tray containing water and ice. Continue adding H<sub>2</sub>O<sub>2</sub> until no more foam is noticed. Complete the destruction of the organic substance by heating to 90 ° C and subsequent additions of H<sub>2</sub>O<sub>2</sub>. Reheat for at least one hour after the last hydrogen peroxide addition. Transfer the sample into a 250 ml centrifuge tube, washing the sides of the beaker well with H<sub>2</sub>O.

Centrifuge and remove the clear liquid above by decanting.

### ***Removal of iron oxides***

Add approximately 150 mL of sodium citrate-bicarbonate solution to the centrifuge tube containing the pretreated sample as indicated above. After stirring and dispersing the sample, add 3 g of sodium dithionite gradually and avoid foaming. Transfer the centrifuge tube to a water bath heated to 80 ° C and shake continuously for 20 minutes. Next, add 10 ml of the saturated sodium chloride solution, centrifuge and decant the supernatant. If the sample is still colored, repeat the treatment. Wash the sample with 50 ml of sodium chloride solution (100 g L<sup>-1</sup>) and twice with H<sub>2</sub>O.

After each addition of saline solution or H<sub>2</sub>O it is necessary to shake, centrifuge and decant the supernatant. If this does not appear clear, centrifuge at a higher number of revolutions after adding, if necessary, 10 ml of acetone.

As a rule, the removal of iron oxides is not performed and is not recommended for soils characterized by a high content of these compounds.

### ***Physico-chemical dispersion***

Transfer the pretreated sample as indicated above into a 500 ml beaker. Dry in a bain-marie and, subsequently, in an oven at 105 ° C. After cooling, weigh and record the mass (M) with an accuracy of 0.001 g. To this material or to the possibly untreated fine earth, add 250 ml of H<sub>2</sub>O and a quantity of the sodium hexametaphosphate solution according to the separation method that will be used. Stir with a glass rod.

### ***Physical dispersion***

Follow one of the two following procedures:



- a) let the suspension rest in the glass for 24 hours. Transfer it quantitatively to the blender and keep stirring for 5 minutes;
- b) quantitatively transfer the suspension into a bottle and stir it overnight.

### Note

For the dispersion of the sample, the procedure that uses ultrasound is not suitable since at the moment the methodology is not sufficiently standardized and valid for all types of soil (Gee and Bauder, 1986).

In any case, indicate the treatment adopted for the preparation of the sample, taking into account the specific influence on the result of the analysis.

## 2.2. Soil moisture

### Principle

The residual moisture is calculated as the difference between the mass of fine earth sample and the mass of the same sample after drying at 105 ° C to constant mass.

### Equipment

- thermostated stove capable of maintaining a temperature of 1050°C ( $\pm 20$ );
- weighing filters ( $\varnothing$  8 cm).

### Method

Weigh 20 g of the fine earth sample in a weighing bottle, previously kept, for at least 2 hours, in an oven at 105 ° C and tared after cooling in a desiccator.

Keep the weighing bottle containing the fine earth sample for at least 16 hours in the preheated 105 ° C oven.

After cooling in the desiccator, weigh the weighing bottle with an accuracy of 1 mg.

### Expression of results



Moisture is expressed in g / kg, with one decimal place.

Perform the calculations using the following expression

$$C = 100 \times \frac{M_0 - M_1}{M_0}$$

Where is it

C = humidity, expressed in g / kg of fine earth

Mo = mass of the sample before drying, expressed in grams

M1 = mass of the sample after drying, expressed in grammi.

The multiplication factor f, to convert the analytical data obtained for dry soil in air into data referred to dry soil at 105 ° C, is calculated with the following expression

$$f = \frac{M_0}{M_1}$$

where the symbols have the meaning seen above.

### Note

Taking into account that the analytical results refer to the weight of the soil sample in the stove at 105 ° C, the residual moisture content must be ascertained before any other analytical determination.

For soils whose organic fraction is characterized by a high content of volatile or easily oxidizable compounds, it is advisable to determine the residual humidity by keeping the sample in an oven at 70 ° C for 48 hours. It must be pointed out, however, that there is no specific temperature that can allow the removal of moisture without causing loss of organic constituents.

## 2.3. Soil density

The density of the soil or bulk density refers to its apparent volume, including spaces, and is therefore given by the weight of the unit of volume of soil, including the pore spaces,



from which the water is removed by passage in the stove of the sample. It fluctuates, in most cases, between 1.0 and 1.6 g cm<sup>-3</sup>.

The density of the soil particles, or real density, is the average of the density of all individual particles, and is equal to the weight of the dry volume of soil, excluding empty spaces. The density of the soil particles varies less than that of the bulk density; in most cases it is between 2.6 and 2.7 g cm<sup>-3</sup>.

The organic substance causes a decrease in the apparent density of the soil, both because it is much lighter than a corresponding volume of mineral substance, and because it favors the stability of the aggregates. total porosity.

Excessively high bulk density values have negative effects on:

- Water penetration
- Root growth

The mechanical resistance of a soil, linked to its porosity and therefore to the apparent density, represents one of the most common limitations to root growth (sometimes accompanied by poor availability of oxygen and accumulation of carbon dioxide in compact layers).

Depending on the clay content, root growth and water penetration are hindered when the density is between 1.5 and 1.9 g cm<sup>-3</sup>.

The surface crust, which is formed following the beating action of the drops of water that fall on the bare ground, breaking the structure of the granules, can reach density values between 1.74 and 1.88 g cm<sup>-3</sup>.

True density is measured with an instrument called a pycnometer.

It is not influenced, neither by the size of the elementary particles, nor by the porosity.

## 2.4. Temperature

There temperature of a land is an element of great importance for its genesis and for the life of all organisms that make up the fauna. Temperatures below the point of freezing they inhibit almost any form of biological activity, as well as crystallizing the circulating solution and therefore preventing any type of translocation of elements inside the profile; the frozen ground is therefore in a sort of "suspended animation".



Positive but still low temperatures, below 5° C, allow the movement of water, but actually represent an obstacle to life in the ground: the germination of almost all seeds is made impossible, as is the development of roots; a layer with a temperature lower than 5 ° C constitutes a real physical impediment to plant growth, as much as one could be hardened layer or a layer with high concentration of saline.

The alternation between freezing and thawing periods also has important physical effects on the soil profile, producing the so-called *cryoturbations*.

This causes the temperature in a soil to be calculated essentially with reference to the first meter depth, which is the lower rooting limit for almost all plant species.

The temperature of a soil is essentially linked to the temperature of the mass of air immediately above; however, other factors may affect, such as:

- any coverages snowy, since the snow is a decent thermal insulator; in the presence of thick layers of snow, positive temperatures can also occur in the event of severe external frosts;
- quantity and distribution of rains: for example, rain in summer frequent prevents, in addition to desiccation, strong heating of the soil;
- the type of vegetation cover, which may or may not cause shading of the soil surface by limiting exposure to direct solar rays;
- the color, since dark soils absorb more heat than light ones;
- the agricultural processing;
- the presence and fluctuations of the aquifer.

The average annual temperature value of a soil is approximately equal to that of the average annual temperature of the atmosphere above: as you go deeper, however, the thermal excursions decrease. In a soil, the average annual temperature is approximately the same at any depth; this value is generally maintained unchanged even by proceeding in depth well below the lower limit of the soil.

The surface horizon of a soil is affected by both variations in seasonal temperature than the daily ones, albeit in a more attenuated way than the atmosphere; proceeding downwards the daily excursions gradually disappear, up to zero at a depth of a few meters.



ThereSoil Taxonomy, the soil classification developed by *United States Department of Agriculture* cites various examples of soils located in different climates:

- in a soil of a temperate environment, located in the surroundings of Belgrade in Serbia, seasonal temperature variations cancel out at a depth of 14 meters, while they are about 16 ° C at the surface level (the average annual variations in air temperature are, for that area, about 22 ° C)
- other studies have shown that, in the absence of an aquifer, seasonal temperature changes are zeroed at depths of around 20 meters in areas of high latitude (Alaska), 15 meters at mid-latitudes and 10 meters at triplets.

## 2.5. Water potential

It is a quantity that expresses the force with which the ground binds the circulating solution and, therefore, the work necessary to subtract the water to the ground.

By convention it is measured in bar. It almost always has negative values, so plants have to spend energy on root absorption.

The water potential is determined by humidity and by hydrological constants of the soil: reaches the highest values (virtually equal to 0 bar) when the humidity is at maximum water capacity and the lowest values (ranging from -100 bar to -1000 bar according to the relative humidity of the air) when the humidity drops to the hygroscopic coefficient.

The water potential is the algebraic resultant of several forces. As a rule, the following forces contribute:

- *Matrix voltage* or capillary tension: it is the force with which the solid matrix retains water by virtue of the phenomena of capillarity and of adsorption. The matrix tension increases with the content of fine particles and, in particular, those with colloidal properties. It is the most significant component of the water tension in agricultural soil under ordinary conditions.
- *Voltage osmotic*: is the strength with which the mineral salts of the circulating solution retain the water. In ordinary conditions it has a minimal contribution, but



it can become considerable in saline soils, arid soils and in those that have undergone a considerable contribution of chemical fertilizers.

- *Gravitational tension*: is the force with which the ground opposes the absorption of water by virtue of gravitational force. Since root absorption is normally able to exert suction pressures up to values of 15-25 bar, gravitational tension plays a negligible role in determining the water potential. Its contribution becomes significant only in the case of trees of considerable height: for example, a sequoia 100 meters high, it must overcome a gravitational potential of about 9.5 bar to allow the lymph to rise up to the upper parts of the canopy.

## 2.6. Field capacity

The field capacity or field water capacity or water holding capacity (CC or CIC) is a hydrological constant of the soil. Defines the content of water in the soil, in terms of humidity percentage, in optimal conditions as regards the relationship between water and air in the soil. These conditions occur when the soil moisture is at the field capacity all the micropores are saturated with water while in the macropores there is only air.

Field capacity is one of the most important hydrological constants from an agronomic point of view. In fact, it represents the limit to refer to when the irrigation is set to a soil water balance or on the measurement of water potential:

- is the level of humidity from which the progressive drying up by the action of evaporation and, above all, of radical absorption;
- it is the level of humidity that must be restored during an irrigation intervention.

Higher humidity levels represent an undesirable context because the excess humidity on the field capacity is represented by gravitational water and is therefore destined to percolate deeply and get lost in the flap. It is therefore an impromptu situation to which it is impossible to refer in a rational irrigation plan based on a water balance; moreover, irrigation water administered in excess constitutes a waste and, therefore, an unjustified cost from both an economic and environmental point of view. Finally, it should be



remembered that the persistence of humidity exceeds the field capacity due to insufficient drainage creates an unfavorable environment for a shortage in the supply of oxygen.

Lower humidity levels represent an ordinary condition: in the time interval between two irrigation interventions, the plants draw on the water reserved in the ground. During this interval, the duration of which in the season of maximum demand can vary, depending on the case, from a minimum of 2-3 days to a maximum of 10-15 days, the soil moisture level progressively lowers, the water potential is lowered and the plants exert an increasingly intense effort, until they show the first symptoms of water stress. Of fundamental importance is the choice of the optimal moment of irrigation intervention, which is based on criteria that contemplate overall economic, organizational, structural, agronomic aspects in the strict sense.

Regardless of the choices made, the level of humidity in the soil varies within an oscillation range whose minimum is represented by the critical intervention threshold and whose maximum, in correspondence with each irrigation intervention, is represented by the field capacity. A particular case is the adoption of irrigation systems with continuous delivery (for example, the drip irrigation) whose flow rate is calibrated on daily consumption. With these systems, optimal from the agronomic point of view, but not always adoptable for economic, organizational and structural reasons, it is possible to constantly maintain the humidity level on values close to the field capacity. This situation represents an ideal condition because the field capacity does not waste water resources and the plants do not show any state of suffering.

### ***2.6.1. Relationship between CC and Porosity***

The value of the field capacity is strictly dependent on the microporosity of the soil, i.e. the fraction of the total porosity made up of pores with a diameter of less than  $8\mu\text{m}$ . Unlike the other hydrological constants, the field capacity can be considered a real constant as it depends on intrinsic physical conditions of the soil and not influenced by processing or other exogenous factors.

The microporosity of the soil, regardless of the total porosity, is a property dependent on the content of fine particles, therefore in general it increases passing from sandy soil to



those silty and of medium mixture, until you reach the highest levels in those clayey. In land with colloid the state also affects the microporosity structural: soils compacted or subject to frequent and prolonged stagnant, processed in non-optimal conditions undergo an imbalance of the macroporosity / microporosity ratio in favor of micropores.

Ultimately, the field capacity settles on the highest values in clayey or rich soil humus and on the lower ones in stony or sandy soils, while it has intermediate values in soils with balanced texture and in loamy ones.

The following table shows the humidity values that the field capacity corresponds to on average in some types of soil.

Type of land	Field capacity (% of dry weight)
Sandy soil	10 - 15
Medium-textured soil	25 - 40
Clayey soil	45 - 50

### 2.6.2. Relationship between CC and Water Potential

When the soil is at field capacity, water is present in two states:

- *Hygroscopic water.* It is water adsorbed on the surface of colloids, wrapping the solid particles with a thin veil. It is withheld at tensions very high.
- *Capillary water.* It is water retained for phenomena of capillarity in the micropores of the soil, i.e. in the pores that have a diameter of less than  $8\mu\text{m}$ . It is retained at lower tensions than hygroscopic water.

Unlike the land at the maximum water capacity, therefore, the humidity corresponding to the field capacity does not include gravitational water, i.e. the water removed from retention because the force of gravity prevails over matrix voltage. The effect of the matrix



tension is such that the water present in the ground at the field capacity can only be removed from the radical absorption or forevaporation, therefore the field capacity represents the maximum limit within which water reserves can be built up in the ground, usable by plants.

The water potential with soil at the field capacity it assumes values that are not well defined. The differences are mostly due to the author, as there is no agreement in the literature on the value of potential to capacity. Many authors consider a water potential of -0.1 bar, other different values depending on the type of soil, for example, from -0.06 bar in a sandy soil to -0.3 bar in a clayey one, others -0.33 bar. In general, however, the literature mentions values between -0.1 bar and -0.3 bar. By adopting the scale of the  $\psi$  the field capacitance stands at values between  $\psi 2$  and  $\psi 2.5$ .

Regardless of the value adopted, it should be pointed out that the water potential, with the soil at the field capacity, always has a negative value. This implies that the roots must still exercise one pressure negative (voltage) and therefore make an energy expenditure. However, the effort that the plants must exert is modest in relation to the benefit they derive from it: humidity values higher than the field capacity would in fact be unfavorable, due to poor aeration conditions, and many species would actually suffer from excess water. prolonged.

### 2.6.3. Determination of the CC

The determination of the field capacity is carried out during the soil analysis. Being a humidity value, it is measured in percentage terms referring to the dry weight of the soil. The direct method for detecting soil moisture consists in subjecting the soil sample, after weighing, to drying in a stove to a temperature greater than 100° C (105-110 ° C). During the drying process the weight of the sample is periodically determined; when two consecutive weighings give the same value, the sample can be considered dried. Humidity is given by the following formula:

$$U = \frac{\text{peso umido} - \text{peso secco}}{\text{peso umido}} \times 100$$

The wet weight is that measured on the sample extracted before drying, the dry weight that measured in the last two weighings.



This method has the disadvantage of requiring execution in the laboratory and in a relatively long time, as on average it takes at least 48 hours for complete drying. For less accurate measurements, appropriately calibrated estimation methods can be used, which make it possible to identify the humidity value in more or less short times. In any case, since the soil moisture is not homogeneous, it is important to proceed with a correct onesampling and refer to a specific depth (35–40cm).

To obtain the value of the field capacitance humidity must be detected when specific conditions occur. This is possible using two criteria:

- empirically identify the condition in which the field capacity occurs;
- identify the condition based on the water potential.

In the first case, a significant surface (a few square meters) is irrigated and free of vegetation until saturation and the entire surface is covered with a plastic film. In this way both evaporation and transpiration are stopped (following radical absorption) and the only loss factor is represented by deep percolation. The latter stops when the matrix tension balances the force of gravity; at this point the soil moisture remains constant, as there are no other losses. To identify the field capacity, sampling is carried out at intervals and the humidity is determined for each sample. When the humidity of the last extracted samples remains unchanged, it can be assumed that the soil is at field capacity.

The previously described method, although conceptually simple, is long and laborious, therefore it is preferred to resort to methods that identify the field capacity from the corresponding value of the water potential. In this case it will be necessary to have adequate tools designed for such purposes, such as the tensiometer, L'Bouyoucos apparatus, L'Richards appliance.

## 2.7. Infiltration

The water that seeps into the ground after crossing the unsaturated zone, in turn partly feeds the aquifers underlying and is partly retained by the ground and thus remains available to any plant and animal organisms present in the soil. On an annual scale, the infiltration can be expressed as the volume of water that passes through a certain portion



of soil in the course of a year ( $m^3 / year$ ) or as the infiltration height, i.e. as the height in mm of the water column ( $mm / year$ ) that infiltrates the soil net of losses for runoff and evapotranspiration. The amount of water that infiltrates the soil, and therefore the permeability of the same, is measured with special instruments called infiltrometers.

The infiltration rate is the percentage ratio between the infiltration height and the annual precipitation height. Among the many factors that determine the infiltration rate, some are related to the type of soil considered, such as the presence of a crust or a compact surface layer, the weaving and the structure of the particles that compose it and its content of organic substance.

Other factors that influence the infiltration rate are the presence of vegetation, the water content of the soil and its steepness, as greater slopes of the land favor the runoff at the expense of infiltration.

### 2.7.1. Calculation of Infiltration

Experimentally found in the 30s of the twentieth century from Robert Elmer Horton for the infiltration of water into the soil, the formulas are valid when the rain ( $mm / h$ ) is greater than the infiltration capacity  $f(t)$  and the soil is dry. Dry means that at the time  $T_0 = 0$  in which it begins to rain the value  $f(T_0)$  is equal to  $f_0$ .

Instantaneous potential infiltration rate (Infiltration Capacity) at time  $t$  ( $mm / h$ )

$$f_t = f_c + (f_0 - f_c)e^{-kt}$$

Where is it:

- $f_t$  is the infiltration rate at generic time  $t$  (infiltration rate  $mm / h$  at time  $t$ );
- $f_0$  is the maximum infiltration rate for dry soil (maximum infiltration rate  $mm / h$  at time  $T_0$ );
- $f_c$  is the minimum infiltration value (constant) (minimum infiltration rate  $mm / h$  at time  $t = infinite$ );
- $k$  is the soil specific reduction constant or decay constant ( $1 / h$ )



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Volume of water infiltrated into the soil at time t (mm)

$$F_T = f_c T + \frac{(f_0 - f_c)}{k} (1 - e^{-kT}) A = \pi r^2$$



## 3. Chemical parameters

### 3.1. Soil pH

In general, pH is a measure of the concentration of hydrogen ions ( $H^+$ ) in an aqueous solution; the importance of this feature is linked to the influence it has on all reactions that occur in nature.

The pH of the soil measures the concentration of hydrogen ions in the circulating solution, i.e. the liquid phase found in the spaces left free by the solid parts. Since this solution interacts continuously with the solid surfaces of the soil, commonly called exchange surfaces due to the presence of these intense phenomena of interaction, the pH of the soil depends above all on the chemical nature of these surfaces, and in particular on the number of exchange sites. (positive or negative surface charges) and their degree of saturation and therefore the number and type of exchange bases (K, Mg, Ca, Na) present in the soil.

The possibility that the pH of a soil can change depends on the ability of the surfaces to release hydrogen ions or exchange bases to counteract these variations in the pH of the circulating solution (buffering power); it depends on the quantity and type of clay and organic substances present in the soil.

So the pH of the soil depends on:

- the presence of hydrogen ions in the circulating solution;
- the type and degree of saturation of the clayey and organic colloids;
- the nature of the exchange bases.

#### **Importance of soil pH**

The pH of the soil greatly influences the microbiological activity, the availability of mineral elements and the adaptability of the various plant species.

#### **Microbiological activity**



Most of the bacteria, on which nitrogen fixing, nitrification, some decomposition processes of the organic substance depend, prefer a sub-acid or slightly alkaline environment (pH 6.8 ÷ 7.2); the deviation from these conditions affects both the availability of nutrients and the humification process.

Fungi are favored by the acidic environment and in these conditions ensure the demolition of organic compounds. In slightly alkaline soils (pH 7 ÷ 7.5) rather dry, loose and therefore rich in oxygen, actinomycetes mainly develop which are able to compensate for the low activity of fungi and bacteria in periods of water scarcity.

### **Availability of mineral elements**

The pH of the soil conditions the solubility of the various mineral elements, determining their accumulation in forms more or less available to the plants or their leaching towards the deeper layers.

The knowledge of the pH therefore provides indications on the availability of mineral elements in the soil solution both from the decomposition of the original minerals and from the fertilizers distributed.

The best known and most important case for soil fertility is that relating to phosphorus; in the soil it is found in the form of poorly soluble phosphates. Their solubility depends on the pH: if the reaction is acidic there are iron and aluminum phosphates whose solubility increases with the pH, if it is basic there are calcium phosphates whose solubility decreases as the pH increases; the result is a greater solubility of phosphates and therefore of phosphorus at pH around neutrality.

#### ***3.1.1. Soil pH determination***

The pH of the soil is determined by potentiometry, after calibration of the measurement system on suspensions of:

- Soil-water: the values obtained are indicative of the degree of reaction of the system;
- Soil-solution of neutral salts (KCl or CaCl<sub>2</sub>); the values obtained are more correlated to the degree of saturation and to the nature of the exchange complex;



## Reagents

- Ready-to-use commercial buffer solutions (pH = 4, 7,10)
- Solution (1 mole  $\times L^{-1}$ ) of potassium chloride: dissolve 74.6 g of potassium chloride (KCl) in  $H_2O$  in a 1000 mL volumetric flask. Bring up to volume with  $H_2O$ .
- Solution (0.01 mole  $\times L^{-1}$ ) of calcium chloride; dissolve 1.11 g of calcium chloride ( $CaCl_2$ ) in  $H_2O$  in a 1000 mL volumetric flask. Bring up to volume with  $H_2O$ . The electrical conductivity value must be between 2.24 and 2.40  $dS \times m^{-1}$  at 25 ° C.

## Commonly used laboratory equipment

### In particular:

- pH meter with temperature compensation, glass electrode with reference electrode or combined electrodes;
- magnetic stirrer with adjustable speed;
- glasses in plastic material;
- stirring rods in PVC.

## Method

### Calibration of the measuring system

Calibrate the measuring system using a reference buffer solution with a pH close to that of the sample. Check the linearity of the system using at least one other reference buffer solution with a different pH.

### PH measurement (in $H_2O$ and in KCl or $CaCl_2$ solution)

Transfer 10 g of the fine earth sample to a 50 mL beaker. Add 25 mL of  $H_2O$  to each of the saline solutions. Shake for at least two hours. Let the suspension settle for a few minutes. Introduce the electrode system into the supernatant and measure the pH value.

### Expression of results

The degree of reaction is expressed as a pH unit, with one decimal place.



### 3.2. Electrical conductivity of the soil

High salt concentrations can, depending on the ionic species present, cause nutritional imbalances, toxic effects for plants, damage to the soil structure and, in some cases, changes in pH. Apart from these extreme situations, an increase in salinity generally determines an increase in the voltage of the circulating solution which in turn causes greater difficulty in absorbing water and mineral elements by plants: this phenomenon depends not so much on the content of salts soluble, as well as by the osmotic pressure exerted by them.

The electrical conductivity of the saturated soil extract, or alternatively of soil / water suspensions in different ratios, being strictly proportional to the osmotic pressure, is an effective and easy-to-use index for the diagnosis of salinity. It is not enough to consider the concentration of soluble salts to know the negative effect induced on plants by the increase in osmotic pressure as it is necessary to take into account, with the same saline content, also the different water retention capacity of the soil, an aspect capable of regulate the salt concentration and osmotic pressure of the soil solution.

The conductivity of the soil solution is measured with a conductivity meter on saturated extracts (ECe), or on soil suspensions in water in a ratio (weight / weight) 1: 2.5 (EC 1: 2.5) or 1: 5 (EC 1: 5) and is expressed in mS / cm. The values obtained by measuring the saturated extract are however the most correlated with the field conditions.

Specific electrical conductivity is one of the parameters considered for the classification of salty or sodium-rich soils; the crops have different sensitivity to salinity.

When a condition of excessive salinity is detected, it is extremely important to trace the causes that determine it to try to remove them. It may be due to the presence of aquifers or irrigation waters rich in salts, to a natural endowment of the soil or to the abuse of fertilizers especially in protected crops where the washing action of the rains is lacking.

To characterize the effect induced by fertilizers on the increase in salinity, a salinity index is used, obtained from the percentage ratio between the increase in osmotic pressure produced by the fertilizer in question and that produced by an equal quantity of sodium nitrate, which is considerably different between the various types of fertilizer.



However, it is always interesting to know the components of the salinity of the soil as there may be ionic species which, if in excess, can be simply eliminated by leaching without causing problems other than environmental ones (eg nitric nitrogen); others, on the other hand, such as sodium, chlorine, boron and aluminum can cause metabolic imbalances and / or toxic effects in plants and deteriorate the structure of the soil.

### **3.2.1 Determination of electrical conductivity**

The method consists in the direct (instrumental) determination of the electrical conductivity in aqueous soil extracts. Extracts can be used:

to saturation (saturated paste)

at a water / soil ratio 5: 1 (aqueous extract 5: 1)

at a water / soil ratio 2: 1 (aqueous extract 2: 1)

#### **Reagents**

##### ***Solution (0.1%) of sodium hexametaphosphate***

Dissolve 0.1 g of sodium hexametaphosphate  $[(\text{NaPO}_3)_6]$  in  $\text{H}_2\text{O}$  in a 100 mL volumetric flask. Bring up to volume with  $\text{H}_2\text{O}$ .

##### ***Standard solution (0.1 mol x) of potassium chloride $\text{L}^{-1}$***

Dissolve 7.455 g of potassium chloride (KCl) in  $\text{H}_2\text{O}$  in a 1000 mL volumetric flask. Bring up to volume in  $\text{H}_2\text{O}$

##### ***Standard solution (0.02 mol x) of potassium chloride $\text{L}^{-1}$***

Take 200 mL of the 0.1 mol KCl solution in a 1000 mL volumetric flask. Bring up to volume with  $\text{H}_2\text{O} \cdot \text{L}^{-1}$

##### ***Standard solution (0.002 moles x) of potassium chloride $\text{L}^{-1}$***

Take 20 mL of the 0.1 mol x KCl solution in a 1000 mL volumetric flask. Bring up to volume with  $\text{H}_2\text{O} \cdot \text{L}^{-1}$



## Equipment

- Oscillating stirrer at 120-140 cycles per minute;
- Vacuum pump;
- Buchner funnels (diameter 10 cm)
- Conductivity meter with measuring cell
- Round bottom porcelain capsules (diameter 13 cm)
- Horn or wooden spatula
- 

## Method

### *Preparation of the extract with a saturated paste*

Place 100-150 g of the fine earth sample in a round-bottomed porcelain dish in a cone shape. Slowly add, by sliding it along the inner wall of the capsule, a quantity of H<sub>2</sub>O sufficient to soak the sample. Cover with a glass and leave to rest overnight. Subsequently, stir the paste with the spatula and, with small additions of fine earth or H<sub>2</sub>O, try to obtain a fluid, shiny paste that flows well when taken with the spatula.

The saturation point is exceeded when the presence of free water is observed in a groove made with the spatula, it is not considered yet reached when the groove has difficulty closing. The saturated paste is immediately vacuum filtered on a Buchner funnel.

Add 1 drop of sodium hexametaphosphate [(NaPO<sub>3</sub>)<sub>6</sub>] solution (0.1%) to each 25 mL of extract.

### *Preparation of the aqueous extract 5: 1*

Transfer 30 g of the fine earth sample to a 250 ml conical Erlenmeyer flask. Add 150 ml of H<sub>2</sub>O. Shake mechanically for two hours and leave to rest for another two hours (if it is believed that calcium sulphate may be present in the soil, leave to rest overnight). Subsequently, filter on a Whatman® n ° 42 filter paper. If the filtrate is cloudy, pass it over the same filter. Add 1 drop of sodium hexametaphosphate [NaPO<sub>3</sub>)<sub>6</sub>] solution (0.1%) to each 25 ml of extract.

### *Preparation of the aqueous extract 2: 1*



Transfer 100g of the fine earth sample to a 500ml conical Erlenmeyer flask. Add 200 ml of H<sub>2</sub>O. Shake mechanically for two hours and leave to rest overnight. Next, filter on a paper filter (for example, Whatman® # 42). If the filtrate is cloudy, pass it over the same filter.

### *Measurement of electrical conductivity*

Using equipment that directly supplies the conductivity values reported at 25 ° C, it is sufficient to detect the ascertained value expressing it in dS / m.

### *Conductance measurement*

Using equipment that provides conductance values it is necessary, with the use of standard solutions of KCl, to calculate the cell factor (K). Subsequently, by introducing the appropriate correction factor (F) in the calculation, to bring the values back to 25 ° C, it is possible to trace the conductivity value.

### *Calculation of the cell factor*

The expression is used to calculate the cell factor

$$K = \frac{Ls}{G}$$

Where is it

K = cell factor

G = conductance of one of the standard solutions of KCl

Ls = conductivity of the same standard solution of KCl.

**Tabella 1 - Conduttività (L<sub>s</sub>) delle soluzioni standard di KCl a temperature diverse**

Temperatura °C	Conduttività specifica (dS · m <sup>-1</sup> )		
	0,100	0,020 (moli · L <sup>-1</sup> )	0,002
15	10,480	2,243	0,239
20	11,670	2,501	0,266
15	12,880	2,765	0,293
30	14,120	3,936	0,321

### *Calculation of electrical conductivity*



For the calculation of the electrical conductivity the expression is used

$$L_s = G \times F \times K$$

Where is it:

$L_s$  = electrical conductivity value at 25 ° C (dS / m) of the soil sample

$G$  = conductance value (dS / m) measured

$K$  = cell factor

$F$  = correction factor to bring the conductivity value back to 25 ° C

**Tabella 2 - Fattore di correzione per riportare il valore della conduttività elettrica a 25°C**

°C	F	°C	F	°C	F	°C	F
18,0	1,163	22,0	1,064	26,0	0,979	30,0	0,907
18,2	1,157	22,2	1,060	26,2	0,975	30,2	0,904
18,4	1,152	22,4	1,055	26,4	0,971	30,4	0,901
18,6	1,147	22,6	1,051	26,6	0,967	30,6	0,897
18,8	1,142	22,8	1,047	26,8	0,964	30,8	0,894
19,0	1,136	23,0	1,043	27,0	0,960	31,0	0,890
19,2	1,131	23,2	1,038	27,2	0,956	31,2	0,887
19,4	1,127	23,4	1,034	27,4	0,953	31,4	0,884
19,6	1,122	23,6	1,029	27,6	0,950	31,6	0,880
19,8	1,117	23,8	1,025	27,8	0,947	31,8	0,877
20,0	1,112	24,0	1,020	28,0	0,943	32,0	0,873
20,2	1,107	24,2	1,016	28,2	0,940	32,2	0,870
20,4	1,102	24,4	1,015	28,4	0,936	32,4	0,867
20,6	1,097	24,6	1,008	28,6	0,932	32,6	0,864
20,8	1,092	24,8	1,004	28,8	0,929	32,8	0,861
21,0	1,087	25,0	1,000	29,0	0,925	33,0	0,858
21,2	1,082	25,2	0,996	29,2	0,921	34,0	0,843
21,4	1,078	25,4	0,992	29,4	0,918	35,0	0,829
21,6	1,073	25,6	0,988	29,6	0,914	36,0	0,815
21,8	1,068	25,8	0,983	29,8	0,911	37,0	0,801

### Interpretation of the results

The conductivity value at 25 ° C is proportional to the concentration of salts in the soil solution.

If the conductivity measurement was carried out using the saturated paste extract, you will have:

Soluble salts (meq x) in the saturated paste extract =  $12.5 L_s L^{-1}$



Although the effects of salinity on plants are more closely correlated with the number of thousand-equivalent salts per liter of solution, however, in particular for alkaline soils it is also possible to take into account the quantity by weight of salts in the soil. Considering for the different salts present an average value of the equivalent mass equal to 51, it will be:

Soluble salts (mg x in the saturated paste extract = 640 x Ls, i.e.  $L^{-1}$ )

$$\text{Soluble salts (mg x in soil} = 0.064 \text{ Ls} \times kg^{-1}) \frac{W}{1000}$$

Where is it

W = water content in the saturated paste soil sample, expressed in  $g.kg^{-1}$

If the conductivity measurement was carried out using 2: 1 or 5: 1 aqueous extracts, the values must be multiplied by 2 and 5 respectively.

### 3.3. Active and Total Carbonate

Total limestone refers to the mineral component of the soil consisting mainly of calcium, magnesium and sodium carbonates.

Since the first is predominant over the others and the analytical method does not allow the distinction between the various forms, conventionally the limestone of the soil is expressed as calcium carbonate ( $CaCO_3$ ).

In some alkaline soils, it can constitute more than half of the solid fraction of the soil, contributing in a decisive way to defining its properties; in acid soils, on the other hand, it is rarely present and in any case in very low quantities, so much so that when the pH is lower than 6.5 the determination of limestone can be neglected.

The presence of limestone in the soil, within certain limits, is to be considered positive for the nutritional function of calcium in relation to plants and for its favorable effects on the structure and mineralization of organic substances. However, when it is present in excessive quantities and above all in very active mineralogical forms, the typical drawbacks of "constitutionally alkaline" soils can occur.



However, knowledge of the total limestone content does not give precise indications regarding its real ability to induce unwanted effects; in the soil, in fact, the possibility that the various components are involved in chemical processes depends above all on the degree of fineness of their particles. To overcome this limit, active limestone is determined which represents limestone present in more finely divided forms and therefore more hydrolysable and soluble.

The total limestone content affects, as much as the clay, the rate of degradation of the organic substance of the soil; the greater the quantity of limestone present, the greater the inertia of the soil towards the transformation processes of organic compounds.

The speed of this process is described by the mineralization coefficient which can be obtained empirically with the following formula proposed by Remy and Marin-Lafleche:

$$CM = 1200 / [(A + 20) * (CT + 20)]$$

where is it:

CM = is the mineralization coefficient,

A = is the clay content in%,

CT = is the total carbonate content in%.

The value assumed by this coefficient represents the percentage of mineralized organic substance in the course of a year; on the basis of the relationship described by the formula, the rate of degradation of organic materials in the soil is inversely proportional to the total limestone content.

This speed also affects the availability of nitrogen in the soil because it determines a minor or greater transformation of organic nitrogen (which is 95-98% of the total nitrogen of the soil) into mineral nitrogen, more easily absorbed by plants; the same applies to other nutrients, in particular phosphorus, which can be part of organic substances.

Active limestone represents the fraction that reacts more readily with the other components of the soil; it influences the availability of phosphorus and iron, forming with them highly insoluble compounds that cannot be assimilated by the plant.

Regarding phosphorus, the presence of high quantities of active limestone involves the formation of insoluble calcium phosphates which subtract the phosphorus from the



circulating solution of the soil from which the roots of the plants draw; this insolubilization can occur, according to Tombesi et al. (1985), to block up to 30-40% of the phosphorus present in the soil.

However, there are no precise indications on the type of relationship existing between the level of active limestone and the availability of phosphorus in the soil; the ARPAV Agrelan interpretation system considers an insolubilization of 4% for each percentage point of active limestone present in the soil.

What has been described for phosphorus also applies to iron; many authors have pointed out that the presence of active limestone is the cause of the occurrence of phenomena of iron chlorosis due to the insolubilization of the assimilable iron present in the soil with consequent difficulty of absorption by the plant. However, it is difficult to quantify this relationship between the level of active limestone and insolubilized iron, and therefore to interpret the analytical data of assimilable iron in the presence of discrete quantities of active limestone.

### ***3.3.1. Determination of "Total limestone"***

Gas-volumetry determination of CO<sub>2</sub> which takes place by treating a fine earth sample with hydrochloric acid.

#### **Reagents**

##### ***Diluted solution (1: 1 v / v) of hydrochloric acid (HCL)***

Add 500 ml of hydrochloric acid (HCl) [37% (p = 1.186)] to a 1000 ml volumetric flask containing approximately 450 ml of H<sub>2</sub>O. Mix and, after cooling, make up to volume with H<sub>2</sub>O.

#### **Commonly used laboratory equipment, in particular:**

- Dietrich-Fruhling calcimeter, containing slightly colored CO<sub>2</sub> saturated water for reading convenience, or equivalent equipment;
- Thermometer for measuring the ambient temperature
- Barometer



## Method

Transfer the following quantities of the fine earth sample, dry in the air and sieved at 0.5 mm to container "A" of the calcimeter:

- 5 g, for soils with carbonate content below 50 g / kg
- 1 g, for soils with carbonate content between 50 and 80 g / kg;
- 0.5 g for soils with carbonate content higher than 80 g / kg;

Insert a plastic test tube containing 10 ml of the diluted HCl solution into the container "A" of the calcimeter, together with the required quantity of sample. Connect the container A to the calcimeter using the appropriate closing cap.

Reset the equipment by equalizing the internal pressure to the external pressure using the tap C. Close the tap C and allow the CO<sub>2</sub> to develop, tilting the container A to the calcimeter using the appropriate closing cap.

The CO<sub>2</sub> that develops will lower the water level in the graduated tube "B". Provide a slight depression by lowering the level tube "D". Continue to shake container "A" until CO<sub>2</sub> is completely developed (approximately 1-3 minutes). Equalize the internal pressure to the external one, bringing the water contained in "D" to the same level as that contained in "B". Wait a few minutes until the level menisci stabilize and read the volume of CO<sub>2</sub> that has developed.

## Standardization of the gas volume carried out

Taking into account the temperature and atmospheric pressure at which the analysis was performed, standardize the volume of CO<sub>2</sub> developed at the temperature of 0 ° C and at the pressure of 760 mm of Hg (101, 325 kPa), by means of the expression:

$$\frac{V_t (P_t - \varphi) 273}{760 (273 + t)}$$

Where is it:

$V_0$  =volume of CO<sub>2</sub> carried out, corrected at 0 ° C and at an atmospheric pressure of 101.325 kPa, expressed in ml

$V_t$  =volume of CO<sub>2</sub> carried out at the temperature and atmospheric pressure at which the analysis was performed, expressed in ml



$P_t$  =atmospheric pressure, expressed in mm of Hg, at which the analysis was performed

T = temperature, expressed in ° C at which the analysis was performed

$\varphi$  = vapor pressure of water at temperature t, expressed in mm of Hg.

The water vapor pressure values (at different temperatures are shown in Table 1. $\varphi$ )

**Tabella 1 - Valori della tensione di vapore dell'acqua a temperature diverse**

Temperatura °C	Tensione di vapore mm di Hg	Temperatura °C	Tensione di vapore mm di Hg
10	9,2	24	22,4
11	9,8	25	23,7
12	10,5	26	25,2
13	11,2	27	26,7
14	12,0	28	28,4
15	12,8	29	30,0
16	13,6	30	31,8
17	14,5	31	33,7
18	15,5	32	35,7
19	16,5	33	37,7
20	17,5	34	39,9
21	18,6	35	42,1
22	19,8		
23	21,1		

The total limestone content is expressed in g / kg without decimal places. The expression is used for the calculation

$$C = \frac{V_0 \times 0,0044655 \times 1000}{M}$$

Where is it:

C = "total limestone" content, expressed in g / kg

$V_0$  = volume of CO<sub>2</sub> developed, corrected at 0 ° C and at an atmospheric pressure of 101,325 kPa expressed in ml

0.0044655 = gas-volumetric equivalent

M = mass of the fine earth sample used for the analysis, expressed in grams



### ***3.3.2. Determination of "active limestone"***

The name of active limestone indicates the calcium carbonate present in the soil which, due to its chemical nature, crystallinity and degree of subdivision, is characterized by high reactivity. By convention, the active limestone content is determined by cold reacting a fine earth sample with an excess of ammonium oxalate solution. The unreacted amount of ammonium oxalate is evaluated by titration with potassium permanganate solution.

#### **Reagents**

##### ***Solution (0.1 mol x) of ammonium oxalate $L^{-1}$***

Dissolve 14.212 g of ammonium oxalate in  $H_2O$  in a 1000 ml volumetric flask  $[(NH_4)_2C_2O_4 \cdot H_2O]$ . Bring up to volume with  $H_2O$ .

##### ***Solution (0.02 mol x) of potassium permanganate $L^{-1}$***

Dissolve 3.161 g of potassium permanganate  $KMnO_4$  in  $H_2O$  in a 1000 ml volumetric flask. Bring up to volume with  $H_2O$ .

##### ***Diluted solution (1:10 v / v) of sulfuric acid***

Carefully add, in a 1000 ml volumetric flask containing about 800 ml of  $H_2O$ , 100 ml of sulfuric acid  $H_2SO_4$  [96% (p = 1.835)]. Mix and after cooling make up to volume with  $H_2O$ .

##### ***Saturated solution (100 gx) of ammonium chloride ( $L^{-1}NH_4Cl$ )***

##### ***Solution (100 gx $L^{-1}$ ) of aluminum sulphate [ $Al_2(SO_4)_3 \cdot 18H_2O$ ]***

##### ***Ammonium hydroxide $NH_4OH$ solution [30% (p = 0.892)].***

#### **Equipment**

Rotating stirrer at 40 rpm or oscillating stirrer at 120-140 cycles / minute

Mechanical stirrer equipped with heating plate (70-80 ° C)



Spin at 3000 rpm

## Method

Transfer 10 g of the fine earth sample to a 250 ml volumetric flask. Make up to volume with the ammonium oxalate solution. Keep stirring for 2 hours. Filter the suspension. The filtrate can be colorless, or in the case of soils with a high humus content, brown due to the presence of organic substance. Collect 10 ml of the colorless filtrate in a 250 ml conical Erlenmeyer flask. Add 10-15 ml of the sulfuric acid solution and 60-70 ml of H<sub>2</sub>O. Titrate under heat (70-80 ° C) with the KMnO<sub>4</sub> solution until persistent pink color. Prepare the blank test following the same operating procedures. If the filtrate is colored brown due to the presence of organic substance, take 10 ml into a centrifuge tube, add 2 ml of the ammonium chloride solution,

Centrifuge for 5 minutes at 3000 rpm. Collect the supernatant and wash the residue again 3 times with the ammonium hydroxide solution previously diluted 1: 100. Heat the decanted assembled in a 250 ml conical Erlenmeyer flask and titrate as described above.

## Expression of results

The content of active limestone CaCO<sub>3</sub> is expressed in g / kg, without decimal places. The expression is used for the calculation

$$C = (A - B) \times M \times 0.25 \times (V1 / V2) \times (1000 / M)$$

Where is it

C = active limestone content, expressed in g / kg

A = volume of the KMnO<sub>4</sub> solution used for the titration of the blank, expressed in ml

B = volume of the KMnO<sub>4</sub> solution used for the titration of the test with the soil, expressed in ml

M = molarity of the KMnO<sub>4</sub> solution

0.25 = g of CaCO<sub>3</sub> corresponding to 1 ml of the KMnO<sub>4</sub> solution (0.02 moles x L-1)

V1 = initial volume of the ammonium oxalate solution



$V_2$  = volume of the sample solution used for titration with  $\text{KMnO}_4$

$M$  = mass of the fine earth sample used for the analysis, expressed in grams.

Taking into account that the oxalate solution also reacts with the  $\text{Ca}^{2+}$  ions in solution or present in the exchange position, the method should be used, more precisely, for the determination of the active calcium content. In any case, the reproducibility of the results depends on the pretreatment of the sample and on the method and time of extraction. If it is ascertained that active  $\text{CaCO}_3$  content is greater than 150 g / kg, the analysis must be repeated using a smaller amount of sample or a larger volume of ammonium oxalate solution.

### 3.4. Humic substance

The soil consists of a solid phase, a liquid phase and a gas phase; the solid phase in turn can be divided into mineral fraction and organic fraction.

The organic fraction in agricultural soils generally represents 1-3% of the solid phase by weight, while it is 12-15% by volume; this means that it constitutes a large part of the active surfaces of the soil and therefore has a fundamental role both for the nutrition of the plants and for the maintenance of the soil structure.

In natural soils the concentration of organic substance found in the soils is also significantly higher and often ranges between 5 and 10%; the soil is one of the great carbon sinks of the globe and all the soil management techniques that reduce the oxidation and mineralization of the organic substance contribute to reducing the emission of carbon dioxide into the atmosphere and therefore the negative consequences related to the effect greenhouse. This role of the soil as well as of plant biomass is recognized by the Kyoto Protocol for which the conservation and increase of soil organic carbon reserves are one of the priorities to be pursued.

However, this fraction is not homogeneous but includes groups of compounds that differ from each other in nature and chemical properties.



4 main components can be identified: plant and animal residues, living organisms, easily degradable substances and stable substances.

By residues we mean those substances that reach the ground such as leaves, woody parts, root exudates, animal waste, etc. and that even though they are already in the process of degradation, they maintain their original physical structure. In natural environments they form a surface layer called litter; they have a short lifespan because they are usually rapidly decomposed.

Living organisms include very different forms, each important for the particular role it plays in the soil, from the largest such as insects and earthworms that perform a cementing action on the soil structure, to the most microscopic ones such as fungi and bacteria that transform all of them. The substances present in the soil. This component has been significantly re-evaluated in recent years since the attention towards biodiversity has increased, precisely because the variety and diversity of organisms in the soil is very high.

The degradable organic substance is the set of products of the rapid transformation of residues carried out by soil organisms; it is still subject to further alterations and modifications and therefore is destined to evolve in a short time.

The stable organic substance is the one that has undergone processes such as to resist degradation by all organisms and therefore is characterized by long life times; this is what is called humified substance or humus.

Despite these differences there is no clear distinction in nature between the various groups of organic substances and also from the analytical point of view the separation of the humified material from other organic residues is anything but easy.

The processes that regulate the evolution of organic matter are quite complex but can be traced back to reactions of the "constructive" type (humification), which lead to the formation of humus, and of the "destructive" type (mineralization) which result in the disintegration of the organic matter and the release of mineral elements. In the soil the two processes tend towards equilibrium thus ensuring the maintenance of the organic component at a level that is a function of the climate (temperature, rainfall), of the pedological characteristics (structure, permeability, texture) and of any agronomic interventions (processing, fertilization, etc.).



In natural or slightly disturbed soils, the level of organic matter is generally higher than that of cultivated soils as in the latter the removal of organic material is greater and the destructive phenomena are more intense due to greater oxygenation of the soil due to processing.

For each cultivation system this level tends to assume a typical value for that land and those environmental conditions; it is possible to predict the direction taken by the evolutionary processes of the organic component following a change made to the system itself.

### **Importance of organic matter**

The importance of the organic substance of the soil is linked to the high number of nutritional and structural functions that it carries out in the soil-plant system.

### **Nutritional functions**

- a) The mineralization of the organic substance causes the release of the elements contained in it such as nitrogen, phosphorus, potassium, magnesium, calcium, etc. ; these can be absorbed and used by the plant;
- b) some classes of microorganisms important for soil fertility require organic substances for survival;
- c) organic compounds carry some microelements such as iron, boron, manganese, zinc, copper and phosphorus, and ensure that these are available for the roots of plants;
- d) some organic substances are themselves absorbed by the plants in which they perform hormonal functions favoring the development of some plant tissues;
- e) it constitutes a large part of the exchange complex, that is, of those surfaces of the soil capable of retaining the nutritional elements and preventing them from being washed away.

### **Structural functions**



- a) With the clays it forms stable aggregates called humo-clayey complexes which are able to give greater structure to the soil;
- b) in sandy soils increases the water holding capacity, preventing nutrient washout;
- c) in loamy soils it avoids the formation of superficial crusts or working soles and other impermeable layers;
- d) in clayey soils it contrasts the phenomena of compaction, summer cracking, erosion in sloping soils.

### 3.5. Organic Carbon

Organic carbon is oxidized to carbon dioxide, under standardized conditions, with potassium dichromate solution in the presence of sulfuric acid. The speed of the reaction is favored by the rise in temperature resulting from the abrupt dilution of the acid. After a set time, the reaction is stopped by adding a suitable quantity of H<sub>2</sub>O and the quantity of potassium dichromate which has not reacted is determined by titration with a solution of iron (II) sulphate heptahydrate. The final point of the titration is ascertained by adding a suitable redox indicator or by potentiometric method using a platinum electrode.

#### Reagents

- **Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [96% (p 1.835)]**
- **Phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) [85% (p - 1.695)]**
- **Solution (0.1667 moles • Cl) of potassium dichromate**  
Dissolve 49.032 g of potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) dried in an oven at 105 ° C in H<sub>2</sub>O in a 1000 mL volumetric flask. Bring up to volume with H<sub>2</sub>O.
- **Solution (0.5 mol • L<sup>-1</sup>) of iron (II) sulphate heptahydrate**  
Dissolve 139 g of iron (II) sulphate heptahydrate (FeSO<sub>4</sub> • 7H<sub>2</sub>O) in H<sub>2</sub>O, in a 1000 mL volumetric flask. Slowly add 20 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [96% (p = 1.835)] by sliding along the inner walls of the flask. Mix and, after cooling, make up to volume with H<sub>2</sub>O. The titer of the solution is not stable and must be checked for each run.
- **Oxred indicator**



Dissolve 0.2 g of sodium 4-diphenylaminosulfonate ( $C_{12}H_{10}NNaO_3S$ ) in 50 mL of sulfuric acid ( $H_2SO_4$ ) [96% ( $p = 1.835$ )], in a 100 mL volumetric flask. Make up to volume with  $H_2SO_4$  [96% ( $p = 1.835$ )].

- **Sulphate silver ( $Ag_2SO_4$ ), crystals**

### Equipment

- potentiometer or pH meter with the possibility of reading mV. Alternatively, automatic titrator equipped with a combined electrode and 5 mL automatic burette;
- magnetic stirrer;
- automatic precision burette;
- 20 mL dispenser.
- 10 mL dispenser.

### Method

#### *Sample preparation*

Transfer the following quantities of the soil sample, dried in the air and sieved to 0.5 mm, into a 250 mL conical Erlenmeyer pellet:

- 2 g, for soils with organic carbon content below 6 g / kg
- 1 g, for soils with organic carbon content between 6 and 18 g / kg
- 0.5 g, for soils with organic carbon content higher than 18 g • kg-i

In the case of peaty soils, sample quantities containing more than 21 mg of organic carbon must not be used. If necessary, add some crystals of  $Ag_2SO_4$ .

#### *Oxidation of organic carbon to carbon dioxide*

Take with a precision burette and transfer 10 mL of the solution (0.1667 mol / L-1) of potassium dichromate  $K_2Cr_2O_7$  into the conical Erlenmeyer flask. Then add 20 ml of sulfuric acid ( $H_2SO_4$ ) [96% ( $p = 1.835$ )], making them percolate slowly along the inner walls of the conical flask to avoid overheating the mixture. Shake carefully, avoiding that soil particles adhere to the flask walls. Cover with watch glass and leave to rest for 30 minutes.

Stop the reaction by adding 200 mL of  $H_2O$  previously cooled in the refrigerator.

#### *Volumetric titration*



Add 10 mL of phosphoric acid to the conical Erlenmeyer flask ( $\text{H}_3\text{PO}_4$ ) [85% ( $p = 1.695$ )] and 0.5 mL of the oxred indicator. Place the conical Erlenmeyer flask on the magnetic stirrer and titrate with the solution ( $0.5 \text{ mol L}^{-1}$ ) of iron (II) sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) until turning blue to green.

If less than 6 mL of the iron (II) sulfate heptahydrate solution was used, repeat the determination with a smaller amount of sample.

### **Potentiometric titration**

In the case of potentiometric titration, the potential varies from about 900-1000 mV to 650-700 mV beyond the equivalence point. If the automatic titrator is used, unitary additions not exceeding  $3 \mu\text{L}$  should be made.

### **Determination of the effective titer of the iron (II) sulphate solution**

Solutions of ferrous salts are not stable due to the oxidation of iron by oxygen. This oxidation process occurs, albeit slowly, also on the salt in the solid state. Therefore, for each series of analyzes, it is necessary to check the exact titer of the solution ( $0.5 \text{ mol L}^{-1}$ ) of iron (II) sulphate.

The method provides for treating a known quantity of the dichromate solution in the same way as the sample in order to simultaneously carry out the correction relating to the possible partial decomposition of the hot dichromate and check that this decomposition has not been excessive.

Take with a precision burette and transfer 10 mL of the solution ( $0.1667 \text{ mol L}^{-1}$ ) of potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) into a 250 mL conical Erlenmeyer flask. Then add 20 mL of sulfuric acid ( $\text{H}_2\text{SO}_4$ ) [96% ( $p = 1.835$ )], making them percolate slowly along the inner walls of the conical flask to avoid overheating the mixture. Cover with watch glass and leave to rest for 30 minutes.

Stop the reaction by adding 200 mL of previously cooled  $\text{H}_2\text{O}$  in the refrigerator and, successively, 10 mL of phosphoric acid ( $\text{H}_3\text{PO}_4$ ) [85% ( $p = 1.695$ )] and 0.5 mL of the oxred indicator. Place the conical Erlenmeyer flask on the magnetic stirrer and titrate with the solution ( $0.5 \text{ mol L}^{-1}$ ) of iron (II) sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) until turning blue to green.



## Calculation of the effective titer of the iron (II) solutionsulphate

Considering that

$$M_{Fe(II)} V_{Fe(II)} = V_{Cr_2O_7^{2-}} \cdot M_{Cr_2O_7^{2-}} \cdot 6$$

We have:

$$M_{Fe(II)} = 10 / V_{Fe(II)}$$

Where is it

$M_{Fe(II)}$  = effective molarity of the iron (II) sulphate solution

$V_{Fe(II)}$  = Volume of the iron (II) sulphate solution used for the titration of  $V_{Cr_2O_7^{2-}}$  expressed in ml

$V_{Cr_2O_7^{2-}}$  = volume of the potassium dichromate solution used for titration, expressed in ml (10 ml).

$M_{Cr_2O_7^{2-}}$  = molarity of the solution (0.1667 mol L<sup>-1</sup>) of potassium dichromate.



## Expression of results

The organic carbon content is expressed in g kg<sup>-1</sup>. The expression is used for the calculation

$$C = \frac{3}{2} \cdot 12 \cdot 1.30 \frac{(B-A)}{1000} \cdot \frac{MFe(II)}{6} \frac{1000}{M}$$

where is it

C = organic carbon content, expressed in g kg<sup>-1</sup>

3/2 = molar ratio of the redox reaction (2 moles of potassium dichromate react with 3 moles of C)

B = volume of the iron (II) sulphate solution used in the titration of the blank, expressed in mL

A = volume of the iron (II) sulphate solution used in the titration of the sample solution, expressed in mL

MFe (II) = effective molarity of the iron (II) sulphate solution

12 = atomic mass of carbon, expressed in g • mole

1.30 = empirical correction factor which takes into account the partial oxidation (70%) of the organic carbon

M = mass of the soil sample, expressed in grams.

from which

$$C = 3.9 \frac{(B-A)}{M} \cdot MFe (II)$$

## *Correction in the presence of chloride ions*

In the presence of a quantity of chloride ions greater than 2 g / kg, the actual organic carbon content is given by

$$C_e = C - (Cl^- / 12)$$

Where is it



Ce = actual organic carbon content, expressed in g / kg

C = organic carbon content, expressed in g / kg

Cl<sup>-</sup> = content of chloride ions present in the sample, expressed in g / kg

1/12 = conversion factor of the chloride ions consumed in the formation of chromyl chloride in the corresponding quantity of C.

### ***Evaluation of the organic substance content***

Considering the average carbon content in soil organic matter to be 58%, the factor 1.724 can be used to transform ig / kg of ascertaining organic carbon into the corresponding organic matter content:

$$\text{Organic matter} = C \times 1.724$$

### **Note**

As an alternative to the solution (0.05 mol L<sup>-1</sup>) iron (II) sulphate heptahydrate (FeSO<sub>4</sub> · 7 H<sub>2</sub>O) it is possible to use a solution (0.5 mol L<sup>-1</sup>) of iron (II) ammonium sulphate [Fe (NH<sub>4</sub>) (SO<sub>4</sub>)<sub>2</sub> · 6H<sub>2</sub>O]. In this case, dissolve 196.06 g of iron (II) ammonium sulphate in a 1000 ml volumetric flask, slowly add 20 ml of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [96% (ρ = 1.835)], mix and, after cooling, make up to volume with H<sub>2</sub>O.

Ferroin [solution of o-phenanthroline-iron (II) sulfate (C<sub>36</sub>H<sub>24</sub>FeN<sub>6</sub>O<sub>4</sub>S) in sulfuric acid] can also be used as an indicator. In this case phosphoric acid should not be added.

Under the conditions envisaged by the method, the oxidation reaction of organic carbon is not quantitative. Therefore, an appropriate correction factor (1.30) must be used which takes into account, with a good approximation, the average oxidation efficiency of the dichromate with respect to the organic substance of the soil. This is not the same for all organic compounds, being very low, for example for polycondensing aromatic compounds. Therefore, as a consequence of the different composition of the organic substance, specific correction factors should be used for different soils and horizons.

The presence of higher manganese oxides, ferrous compounds and chlorides can affect the accuracy of the test results.



Due to the negligible quantity of higher manganese oxides and the easy oxidation of ferrous compounds during air drying of soil samples, significant interference can derive exclusively from the presence of chlorides. The chloride ions react, in fact, with the dichromate, with the formation of chromyl chloride ( $\text{CrO}_2\text{Cl}_2$ ). If present in quantities not exceeding 2 g / kg, they must be eliminated beforehand by precipitating them as silver chloride. If the chlorides are present in higher quantities, the results obtained must be corrected on the basis of their content, ascertained with specific analytical determination. The organic substance content is positively correlated with the cation exchange capacity, with the formation and stability of the soil structure, with the pH value, with the quantity of macro- (nitrogen, phosphorus, sulfur) and micronutrients.

### ***3.5.1. Extraction, fractionation and determination of organic carbon***

The humic substances are solubilized by an alkaline solution of sodium pyrophosphate and sodium hydroxide which causes the dissociation of the acid functional groups and the replacement of divalent and trivalent cations with sodium ions. The replacement of the polyvalent ions with the dispersing cation is also favored by the chelating capacity of the pyrophosphate. However, the extractant can also solubilize non-humic substances and in particular carbohydrates and amino acids from vegetable and animal residues that are only partially decomposed. Therefore, it is necessary to separate the non-humic (NH) fraction from the humic (HA + FA) by means of solid phase adsorption chromatography (SPE) on polyvinylpyrrolidone resin. After having separated the humic acids by precipitation, the fulvic acid fraction, which is made up of polyphenolic substances, it is retained by the resin in an acid environment, due to the formation of hydrogen bonds with the phenolic groups, while the non-phenolic substances remain in solution and can be removed. Subsequently, the adsorbed fulvic acids are eluted with a sodium hydroxide solution which causes the destruction of the hydrogen bonds by ionization at high pH of the phenolic groups.

#### **Reagents**

- **Solution (0.1 mol L<sup>-1</sup>) of sodium pyrophosphate and sodium hydroxide**



Dissolve in a 1000 mL volumetric flask containing approximately 900 mL of H<sub>2</sub>O, 44.61 g of sodium pyrophosphate (Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> + 10H<sub>2</sub>O) and 4 g of sodium hydroxide (NaOH). After solubilization of the reagents, make up to volume with H<sub>2</sub>O.

- **Technical nitrogen gas.**
- **Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [96% (p = 1.835)]**
- **Phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) [85% (p = 1.695)]**
- **Diluted solution (50%) of sulfuric acid**

Carefully add 520 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [96% (p = 1.835)] in a 1000 mL volumetric flask containing approximately 400 mL of H<sub>2</sub>O. Mix and, after cooling, make up to volume with H<sub>2</sub>O.

- **Solution (0.005 moles • L<sup>-1</sup>) of sulfuric acid**

Take 10 mL of sulfuric acid solution (0.5 mol • L<sup>-1</sup>) of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) using a precision burette and a 1000 mL calibrated maraccio containing about 900 mL of H<sub>2</sub>O. Mix and, after cooling, make up to volume with H<sub>2</sub>O.

- **Solution (0.3334 mols • L<sup>-1</sup>) of potassium dichromate**

Dissolve 98.08 g of potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) previously dried in an oven for at least one hour at 130 ° C in H<sub>2</sub>O in a 1000 mL volumetric flask. Bring up to volume with H<sub>2</sub>O.

- **0.2 moles of iron (II) sulphate solution**

Transfer to a 1000 mL volumetric flask containing approximately 100 mL of H<sub>2</sub>O, 55.6 g of iron (II) sulfate heptahydrate (FeSO<sub>4</sub>•7 H<sub>2</sub>O). Slowly add 20 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [96% (p = 1.835)] by sliding along the inner walls of the flask.

Mix and, after cooling, make up volume with H<sub>2</sub>O. The solution is not stable and must be re-prepared frequently (2-3 days). In any case, the title of the solution must be checked for each run of analyzes.

- **Oxred indicator**

Dissolve 0.2 g of sodium 4-diphenylaminosulfonate (C<sub>12</sub>H<sub>10</sub>NaNO<sub>3</sub>S) in 50 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [96% (p = 1.835)], in a 100 mL volumetric flask. Make up to volume with H<sub>2</sub>SO<sub>4</sub> [96% (p = 1.835)]. The solution can be kept in the dark for a few months.

- **Insoluble polyvinylpyrrolidone resin (of the type PVP Code 85648/7, Aldrich, Germany)**
- **Solution (0.5 mol L<sup>-1</sup>) of sodium hydroxide**



Dissolve in a 1000 mL volumetric flask containing approximately 400 mL of H<sub>2</sub>O, 20 g of sodium hydroxide (NaOH). Mix and, after cooling, make up to volume with H<sub>2</sub>O.

- **Silver sulfate (Ag<sub>2</sub>SO<sub>4</sub>) crystals**
- **Fiberglass**

### **Equipment**

- agitator with thermostatic bath adjustable to 650°C;
- centrifuge equipped with rotor or adapters for 50 or 150 mL tubes;
- filtration system with membrane filters, equipped with 0.45 μm HA filters;
- 10 ml plastic hypodermic syringes;
- 200 flask for connection equipped with thermometer with scale up to 2000 ° C and graduations of 1 ° C
- Bunsen stove equipped with glass ceramic protection;
- Potentiometer or pH meter with the possibility of reading mV, Alternatively, automatic titrator equipped with combined platinum electrode and automatic 5 ml burette
- magnetic stirrer.

### **Procedure**

#### ***Extraction of organic carbon***

Transfer 10 g of the air-dried, 0.5 mm sieved fine soil sample to a 250 mL airtight container. Add 100 mL of the solution (0.1 mol L<sup>-1</sup>) of sodium pyrophosphate and sodium hydroxide and bubble nitrogen through the suspension for approximately one minute. Hermetically cap the container and keep it for 24 hours in a Dubnoff bath set at 80 shakes per minute and at 65 ° C.

Subsequently, cool the container in cold water and transfer the suspension into a 150 ml centrifuge tube. Centrifuge for 20 minutes at 2500 + 2700 rpm. Filter the supernatant by 0.45 μm membrane. Transfer the filtrate into a clean and dry container. Let nitrogen bubble for another minute and seal tightly. Samples not analyzed immediately can be stored for a few days at 4 ° C. Samples not analyzed immediately can be stored for a few days at a temperature of 40C.

#### ***Fractionation of the extract***

#### ***Preparation of the resin***



Transfer 50 g of polyvinylpyrrolidone to a transparent 1 + 1.5 L container. Add tap water and shake very carefully. Leave to decant for 10-15 minutes and discard the fractions still in suspension. Repeat the operation, in succession, twice with tap water and twice with H<sub>2</sub>O. Add enough of the solution (0.005 mol • L<sup>-1</sup>) of sulfuric acid to completely cover the resin. Shake and check that the pH value of the suspension is between 1 and 2. The resin thus prepared can be stored at room temperature, in a closed container and covered with solution.

### ***Preparation of the chromatographic columns***

Columns to be used for solid phase adsorption chromatography (SPE) can be prepared using 10 mL plastic syringes, replacing the hypodermic needle with a rubber tube closed with a small forceps.

Glass wool is introduced into the syringe and pressed on the bottom to form a layer of about 0.5 cm thick.

Pour into the syringe, fixed in a vertical position to a suitable support, a quantity of acidified resin sufficient to form, after sedimentation, a column of 4 + 6 cm<sup>3</sup>. Open the tweezers to let the liquid flow out, taking care that the resin does not dry out.

### ***Fractionation of the extracted organic carbon***

Collect and transfer 25 mL of the extract in alkaline solution of sodium pyrophosphate and sodium hydroxide into a 50 ml centrifuge tube. By adding the solution (50%) of sulfuric acid, bring the pH to a value below 2.

Centrifuge for 20 minutes at 2500 + 2700 rpm until satisfactory sedimentation is obtained. leave to rest for a few minutes. Using a Pasteur pipette, carefully transfer the supernatant to the column.

Let the liquid flow out, taking care that the resin does not dry out.

Wash the column with 25 mL of the solution (0.005 mol L<sup>-1</sup>) of sulfuric acid. Allowing the acid solution to flow down to a level just above the resin state.

Then elute the fraction adsorbed on the PVP with the solution (0.5 mol L<sup>-1</sup>) of sodium hydroxide.



Discard all the eluate derived from the previous operations until the first yellow drop appears at the exit of the tube or yellow-brown. Collect the fraction of fulvic acids (colored) directly in the centrifuge tube containing the precipitated humic acids.

Continue the elution with 20-25 mL of the solution (0.5 mol L<sup>-1</sup>) of sodium hydroxide or in any case until the liquid at the outlet appears completely colorless. Quantitatively transfer the fraction (FA + HA) into a 25 or 50 ml graduated flask and make up to the mark with the eluent sodium hydroxide solution. Even the fraction (FA + HA) can be kept for a few days at a temperature of 4 ° C, adopting the precautions foreseen to preserve the total extract.

### ***Oxidation of organic carbon to carbon dioxide.***

Take and transfer an aliquot (not exceeding 10 ml) of the total extract or fraction (FA + HA) containing an amount of organic carbon including 25 mg into the attachment flask. For volumes less than 10 ml, add a volume of the extraction solution or the solution (0.5 mol • L<sup>-1</sup>) of sodium hydroxide up to a total of 10 ml.

Take with a precision burette and transfer 20 ml of the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution into the flask for the attachment.

Place the flask for attachment in an ice-water bath and, being careful not to overheat the mixture, slowly add 26 ml of H<sub>2</sub>SO<sub>4</sub> [96% (p = 1.835)) and a few crystals of silver sulphate.

After inserting the thermometer, taking care that the bulb does not touch the bottom of the flask, heat on a Bunsen burner as quickly as possible to reach a temperature of 160 ± 2 ° C. Keep the temperature constant for exactly 10 minutes, shaking the mixture lightly. Cool rapidly to room temperature and quantitatively transfer the contents into a 200 ml volumetric flask. Bring up to volume with H<sub>2</sub>O. Mix and allow the solid mineral residue to settle.

### ***Volumetric titration***

Pick up with a precision pipette and transfer 20 mL of the clear solution into a 250 mL wide-necked conical Erlenmeyer flask. Add 100 mL of H<sub>2</sub>O and, in succession, 8 mL of H<sub>3</sub>PO<sub>4</sub> [85% (p = 1.695)) and 0.5 mL of the oxred indicator.



Place the Erlenmeyer conical flask on the magnetic stirrer and titrate with the dark violet to green iron (II) sulfate solution.

### ***Potentiometric titration***

In the case of potentiometric titration, the potential varies from about 900-1000 mV to 650-700 mV beyond the equivalence point. If an automatic titrator is used, unitary additions not exceeding 3  $\mu\text{L}$  should be made.

### ***Blank determinations***

Take and transfer an aliquot (not exceeding 10 mL) solution (0.21 mol L<sup>-1</sup>) of sodium pyrophosphate and sodium hydroxide or of the solution (0.5 mol L<sup>-1</sup>) of sodium hydroxide into the attachment flask .

### ***Determination of the effective titer of the iron (II) sulphate solution***

Solutions of ferrous salts are not stable due to oxidation of iron II by oxygen. The oxidation process occurs, albeit slowly, also on the salt in the solid state. Therefore, it is necessary to check the exact titer of the ferrous sulphate solution for each series of analyzes.

The method provides for treating a known quantity of the dichromate solution in the same way as the sample in order to simultaneously carry out the correction relating to the possible partial decomposition of the hot dichromate and check that this decomposition has not been excessive.

Transfer 5 ml of the sodium pyrophosphate and sodium hydroxide solution (0.1 mol L<sup>-1</sup>) or sodium hydroxide solution (0.5 mol L L) into a 200 ml volumetric flask placed in an ice-water bath. hydroxide. Take with a precision burette and transfer 20 mL of the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution into the same calibrated one. Slowly add 26 ml of H<sub>2</sub>SO<sub>4</sub> making them slowly percolate along the inner walls of the flask. Mix and, after cooling, make up to volume with H<sub>2</sub>O.

Pick up with a precision burette and transfer 20 mL of the solution into a 250 mL conical wide-necked Erlenmeyer flask. Add 100 mL of H<sub>2</sub>O and, in succession, 8 mL of H<sub>3</sub>PO<sub>4</sub> and 0.5 mL of the oxred indicator.

Place the conical Erlenmeyer flask on the magnetic stirrer and titrate with the dark violet to green iron (II) sulfate solution.



Carry out, under the same experimental conditions, - a hot white test ( $160 + 2 \text{ }^\circ \text{C}$ ) to ascertain the error possibly caused by the possible partial decomposition of the bichromate due to heating.

A difference greater than 0.4 ml between the titration of the potassium dichromate solution at room temperature or that as the sample indicates that there is an error either in the determination of iron molarity or in the heating procedure.

Calculation of the effective titer of the iron (II) sulphate solution

Considering that

$$M_{\text{Fe (II)}} V_{\text{Fe (II)}} = V_{\text{Cr}_2\text{O}_7^{2-}} M_{\text{CrO}_7^{2-}} \times 6$$

Yes it has

$$M_{\text{Fe (II)}} = 4 / V_{\text{Fe (II)}}$$

Where is it

$M_{\text{Fe (II)}}$  = effective molarity of the iron (II) sulphate solution

$V_{\text{Fe (II)}}$  = volume of the iron (II) sulphate solution used for the titration of  $V_{\text{Cr}_2\text{O}_7^{2-}}$  expressed in ml

$V_{\text{Cr}_2\text{O}_7^{2-}}$  = volume of the potassium dichromate solution used for the titration, expressed in ml

$M_{\text{CrO}_7^{2-}}$  = molarity of the solution ( $0.3334 \text{ mol} \times \text{L}^{-1}$ ) of potassium dichromate

### Expression of results

The extractable or humified organic carbon content is expressed in g / kg. The expression is used for the calculation

$$C = 12 \frac{3}{2} \frac{(B-A)}{1000} \frac{M_{\text{Fe(II)}}}{6} \frac{200}{20} \frac{1000}{M}$$

where is it

C = content of extractable or humified organic carbon, expressed in g / kg

3/2 = molar ratio of the redox reaction (2 moles of potassium dichromate react with 3 moles of C)



B = volume of the iron (II) sulphate solution used in the titration of the blank, expressed in ml

A = volume of the iron (II) sulphate solution used in the titration of the sample solution, expressed in ml

200 ml / 20 ml = volume ratio

MFe (II) = effective molarity of the iron (II) sulphate solution 12 = atomic mass of carbon, expressed in g mole<sup>-1</sup>

M = mass of the soil, expressed in grams

from which

$$C = 30 \frac{(B-A)}{M} MFe (II)$$

### Calculation of humification parameters

#### *Humification index (H.THE)*

It is given by the ratio between the content of non-humic organic carbon (CNH) and the content of humic carbon (CH = CHA + FA) in the alkaline pyrophosphate extract. Taking into account that the non-humic organic carbon content (CNH) is given by the extractable content (TEC) minus the humified one (CH), we have

$$HI = \frac{TEC - CH}{CH}$$

#### *Degree of humification (DH%)*

It results from the percentage ratio between the humic carbon content (CH = CHA + FA) and the total extractable carbon content (TEC)

$$DH\% = 100 \frac{CH}{TEC}$$

#### *Humification rate (HR%)*

It is given by the percentage ratio between the humic carbon content (CH = CHA + FA) and that of total organic carbon (TOC) determined according to the Springer-Klee method:

$$HR\% = 100 \cdot \frac{CH}{TOC}$$



## Note

Significant interference can arise from the frompresence of chlorides. The chloride ions react, in fact, with the dichromate, with the formation of chromyl chloride ( $\text{CrO}_2\text{Cl}_2$ ). If present in quantities not exceeding 2 g  $\text{kg}^{-1}$ , they must be eliminated in advance by adding some crystals of silver sulphate and causing them to precipitate as  $\text{AgCl}$ .

## 3.6. Content of Nitrogen and Phosphorus

### 3.6.1. Nitrogen

Among the elements that the plant absorbs with its roots, nitrogen is the most common in all soils, both acid and alkaline; the simplest chemical forms in which it is present are ammonia and nitric ions.

Above all, the former is able to form strong bonds with organic compounds and participates in the synthesis of complex substances, such as proteins and nucleic acids, which are also present in considerable quantities in the soil.

In the soil 97-99% of the total nitrogen is made up of organic nitrogen, while the remainder is present in ammonia and nitric form.

Plants mainly use inorganic nitrogen, especially nitric nitrogen; once absorbed this is reorganized to form new plant tissues.

The nitrogen cycle, which involves all living forms, has its key environment in the soil, as it is in this ecosystem that the two main processes that regulate the transformation of nitrogen into more or less available forms take place: the mineralization, ie the destruction of complex structures down to the simplest compounds, and immobilization, ie the use of simple forms for the synthesis of complex substances.

The main architects and agents of these processes are microorganisms and in particular bacteria; the presence of available nitrogen in the soil depends on their activity and on the prevalence of the species that operate one or the other process.

In the agricultural world it is commonly believed that nitrogen distributed with chemical fertilizers, such as urea, sulfate or ammonium nitrate, remains for a certain time readily absorbable by plants and available to aid their development.



More recent researches agree in indicating that 10-40% of the nitrogen given with fertilizers is organized in the soil, 5-10% is lost by leaching, 10-30% is lost in gaseous form and only 30-70% is assimilated by the plant.

It is therefore clear that only a part of the nitrogen distributed with chemical fertilizers is directly used by plants; a good part of it is incorporated in the organic substances of the soil, that is, it is immobilized to be released at a later time.

In particular, this nitrogen is made up of amino acids and proteins (25-50%), hexosamines (5-10%), nucleic acids (1%) and a fraction whose chemical structure is unknown (30-40%) (Nannipieri and Ciardi, 1982).

Nitrogen in modern agronomy is considered the main factor of soil fertility especially in determining the success of a crop; this is largely due to the evident positive effect caused by the nitrogen administration to the crops in the various phases of their cycle.

This has led to an excessive confidence in nitrogen fertilization which has induced and still induces excesses in the doses distributed to crops with the aim of maximizing yields.

As a consequence, in some cases there has been a flattening of the doses distributed to the different crops (from 200 to 300 kg / ha) without taking into account the specificity of the individual crops and the effects, in some cases deleterious, on the quality of the productions.

The nitrogen requirements vary considerably between the various crops; some, such as legumes, are self-sufficient thanks to the symbiosis with nitrogen-fixing bacteria that live in the roots and transform the nitrogen of the air into ammoniacal nitrogen; other crops have variable needs: the less demanding tree crops, the needs increase for horticultural crops, from crucifers, to cucurbits, to solanaceae, and finally the most demanding are grasses, and in particular corn, which, especially with the selection of hybrids more sensitive to nitrogen fertilization, it needs quantities often higher than 250 kg / ha.

However, the problem of nitrogen fertilization involves complex problems such as that of the supply of nitrogen available to plants with organic materials such as sewage, manure and other organic fertilizers, of the release of nutrients in the subsoil, of the quality of production.



### 3.6.1.1 Determination of total or organic carbon and total nitrogen with an elemental analyzer

#### **Principle**

The original analytical method is based on the complete and instant oxidation of the sample by "flash combustion" with consequent conversion of all organic and inorganic substances into gaseous products. The combustion gases are passed, in a helium stream, to the state of a suitable catalyst, to complete the oxidation process, and, therefore, to the copper layer, to remove oxygen and to reduce nitrogen oxides to molecular nitrogen (N). Subsequently, the gaseous mixture is separated by gas chromatography and CO<sub>2</sub>, N<sub>2</sub>, H<sub>2</sub>O and SO<sub>2</sub> are detected by a thermal conductivity detector.

Calcium carbonate, possibly present in the sample, can be removed before analysis by actuation with HCl.

#### **Reagents**

**Acetanilide (N-fennacetamide) (CH<sub>3</sub>CONHC<sub>6</sub>H<sub>5</sub>). Minimum title 99.5%**

**Magnesium perchlorate anhydrous [Mg (ClO<sub>4</sub>)<sub>2</sub>]**

***Diluted solution (10%) of hydrochloric acid***

Carefully add in a 500 ml volumetric flask containing approximately 300 ml of H<sub>2</sub>O and 135 ml of hydrochloric acid (HCl) [37% (p = 1.186)]. Mix and then bring to volume cooling with H<sub>2</sub>O.

#### **Equipment**

- elemental analyzer
- microbalance
- tin capsules (Ø 8 mm h 5 mm)
- silver capsules (Ø 12.5 mm h 5 mm)
- aluminum support with housing for the capsules
- 40 µl micropipette
- Heating plate



- steel tweezers
- fiberglass

## Method

### *Calibration of the elemental analyzer*

Weigh (in tin capsules for non-calcareous soil samples and silver for calcareous soil samples) at least four aliquots of acetanilide ranging from 0 to 3 mg ( $\pm 1 \mu\text{g}$ ). Close the capsules and place them in the sampler of the elemental analyzer

### *Sampling for the determination of organic and inorganic carbon and nitrogen*

Weigh, in a tin capsule, a quantity of the soil sample, dry in the air and sieved at 0.5 mm, between 15 and 20 mg ( $\pm 1 \mu\text{g}$ ).

Sampling for the determination of organic carbon and nitrogen in calcareous soil samples

Weigh, in a silver dish, a quantity of the soil sample, dry and sieved at 0.5 mm, between 15 and 20 mg. Place the capsule on the aluminum holder. Add 40  $\mu\text{l}$  of the diluted solution (10%) of HCl. Leave to rest overnight. Subsequently, add another 40  $\mu\text{l}$  of the diluted HCl solution. Leave to rest for 4 hours and, having placed the aluminum support on the heating plate, bring the temperature to 650 ° C. Allow the sample to dry for about 3 hours. After cooling, close the capsule and place it in the sampler of the elemental analyzer.

### *Determination of the quantities of carbon and nitrogen*

To determine the quantities of carbon and nitrogen present in the sample, follow the instructions and use the analysis conditions provided for the equipment used.

## Expression of results

To determine the elemental composition of the various samples, it is necessary to define the value of a calibration factor (factor K) by analyzing suitable quantities of a standard organic substance whose percentage content of carbon and nitrogen is known.

The value of the K factor is obtained using the expression

$$K = \frac{\% t \cdot Ms}{I}$$

where is it



% t = theoretical percentage content of the element in the standard substance

$M_s$  = mass of the standard substance used for the calibration of the analyzer, expressed in milligrams

$I$  = integrated area relating to each gaseous compound.

For the calculation of the content of each element, the expression is used:

$$C = \frac{K \times I}{M}$$

where is it

$C$  = content of each element

$K$  = mean value of the calibration factor

$I$  = integrated area relating to each gaseous constituent derived from the sample

$M$  = mass of the sample used, expressed in milligrams.

To evaluate the correct functionality of the analyzer, it is necessary to perform a blank test using an empty tin or silver capsule. The integrated areas obtained must be subtracted in the calculations for determining the value of the  $K$  factor or the elemental composition of the samples.

To calculate the percentage of carbon and nitrogen content, it is possible to use the software available for the computerized management of the system. The total or organic carbon and nitrogen content is expressed in g / kg.

### Note

40  $\mu$ l of diluted hydrochloric acid solution are sufficient to neutralize  $\pm$  6 mg of  $\text{CaCO}_3$ . For soil samples containing more than 500 g / kg of  $\text{CaCO}_3$  it is necessary to treat the sample several times with the same quantity of HCl.

The carbonate content can be calculated from the percentage of organic carbon from that of total carbon.



The H<sub>2</sub>O formed during the "flash combustion" process can be eliminated using a column of Mg (ClO<sub>4</sub>)<sub>2</sub>.

### 3.6.2. Phosphorus

Phosphorus is traditionally included among the macroelements, although it is contained in plants in much more modest quantities than nitrogen, potassium and calcium; the removals of phosphorus in a year of production for an arboreal crop are of the order of ten kg / ha, for the others they vary from 20 to 80 kg / ha.

However, since the practice of mineral nutrition has spread in agriculture, it has always been considered a fundamental element for maintaining a good level of fertility. This is a consequence of its scarce mobility in the soil and the insolubilization to which it is easily subjected in non-neutral soils; these conditions can make it a limiting factor for optimal plant development.

The phosphatic forms present in the soil are very stable; the speed with which the phosphorus is immobilized in insoluble forms depends on different factors, such as the pH of the soil, the content of calcium, iron and aluminum, the quantity and type of clay and organic substance.

In fact, phosphorus is found in the soil as mineral phosphates, in particular iron, aluminum and calcium whose relative presence depends on a balance regulated by the pH of the soil, or in the form of organic phosphorus present in animal and vegetable residues and which is gradually mineralized. .

The influence of pH is a function of the phenomena of insolubilization to which the phosphorus is subject: at pH lower than 6 the formation of insoluble and stable iron and aluminum phosphates prevail, while at pH higher than 7 calcium phosphates prevail for stability as well. insoluble.

The soluble form of phosphorus, and therefore assimilable by plants, is that of the orthophosphate ion, which has a negative charge; the reactivity of this ion with the mineral matrix of the soil is rather complex as it depends on the nature and extent of the surfaces, the quantity and nature of the other dissolved ions, the temperature, the pH and the water content.



The main reactions consist in the formation of numerous compounds between orthophosphoric ions and cations present on the exchange surfaces; they occur in several successive stages with the rapid formation of "adsorption phosphate complexes" and the slow release of the phosphoric ions present on the adsorbing surface.

In the balance between insolubilized phosphorus and soluble phosphorus, the role of organic substances and microorganisms is important; in particular the organic acids, secreted by the root systems of crops or formed by the degradation of the organic substance of the soil, can, after interposition of positive ions, act as carriers of phosphates from the adsorption sites to the root organs of the plant.

For their part, microorganisms regulate the phosphorus cycle by acting in particular on the demolition of organic phosphorus compounds and the organization of mineral phosphorus. It has also been ascertained that the presence of particular fungal organisms capable of forming symbioses with the roots of plants which take the name of mycorrhizae.

In ascertaining the reason for the greater development and vigor of mycorrhized plants, it has been seen that the assimilation of phosphorus is much greater in these plants; most likely mycorrhizae function as an "extension" of the roots, and are highly efficient for the absorption of phosphorus even in areas where the element is scarcely present in the assimilable forms.

### *3.6.2.1. Determination of assimilable phosphorus*

#### **Principle**

The method is applicable both to acid soils and to those characterized by the presence of calcium carbonate.

The presence in the sodium bicarbonate solution of carbonate and hydroxyl ions lowers the activity of  $\text{Ca}^{2+}$  and  $\text{Al}^{3+}$  with a consequent increase in the solubility of phosphorus (P). In calcareous soils, the increased solubility of calcium phosphate derives from the decrease in calcium concentration due to the high presence of carbonate ions and the consequent precipitation of  $\text{CaCO}_3$ .



In acidic or neutral soils, the solubility of aluminum and iron phosphates is increased by the increase in the concentration of hydroxyl ions which induces a decrease in the concentration of  $Al^{3+}$ , with the formation of aluminate ions, and of  $Fe^{3+}$ , with precipitation of oxides.

It must also be kept in mind that, at high pH, the increase in negative charges and / or the decrease in the adsorption sites on the surfaces of the aluminum and iron oxides can lead to the desorption of the fixed phosphorus.

The phosphorus content is determined by the ascorbic acid method.

## Reagents

### *Solution (2.5 mol · L<sup>-1</sup>) of sulfuric acid*

Carefully add 140 ml of sulfuric acid [96% ( $\rho = 1.835$ )] to a 1000 ml volumetric flask containing approximately 500 ml of  $H_2O$ . Mix and, after cooling, make up to volume with  $H_2O$ .

### *Solution (1 mol · L<sup>-1</sup>) of sodium hydroxide*

Dissolve 40 g of sodium hydroxide (NaOH) in  $H_2O$  in a 1000 ml volumetric flask. Mix and, after cooling, make up to volume with  $H_2O$ .

### *Solution (0.5 mol · L<sup>-1</sup>) of sodium bicarbonate*

Dissolve 42 g of sodium bicarbonate ( $NaHCO_3$ ) in a glass containing about 900 ml of  $H_2O$ .

By adding the solution (1 mole · L<sup>-1</sup>) of sodium hydroxide drop by drop, bring the pH to 8.5.

Transfer to a 1000 ml volumetric flask and make up to the mark with  $H_2O$ .

To avoid direct contact of the solution with atmospheric air, add an oil squeeze mineral.

## Activated carbon

It is advisable to check the purity of this reagent by carrying out the extraction with the solution (0.5 moles L<sup>-1</sup>) of sodium bicarbonate. In the presence of phosphorus, wash



several times with the same solution up to levels of P not detectable by spectrophotometry.

***Solution (0.25%) of p-nitrophenol***

Dissolve 0.25 g of p-nitrophenol ( $\text{NO}_2\text{C}_6\text{H}_4\text{OH}$ ) in  $\text{H}_2\text{O}$ , in a 100 ml volumetric flask.

***Solution (40 g · L<sup>-1</sup>) of ammonium molybdate***

Dissolve 40 g of ammonium molybdate [ $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ] in  $\text{H}_2\text{O}$ , in a 1000 ml volumetric flask. Bring up to volume with  $\text{H}_2\text{O}$ . Store the solution in a dark glass container.

***Solution (1 mg of Sb · ml<sup>-1</sup>) of antimony potassium tartrate***

Dissolve 0.2728 g of antimony potassium tartrate [ $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$ ] in  $\text{H}_2\text{O}$ , in a 100 ml volumetric flask. Bring up to volume with  $\text{H}_2\text{O}$ .

***Solution (0.1 moles L<sup>-1</sup>) of ascorbic acid***

Dissolve 1.76 g of ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ) in  $\text{H}_2\text{O}$  in a 100 ml volumetric flask. Bring up to volume with  $\text{H}_2\text{O}$ . Prepare the solution at the time of use.

***Sulfomolybdic reagent***

Mix, just before use, 50 ml of the solution (2.5 moles L<sup>-1</sup>) of sulfuric acid, 15 ml of the solution (40 g VI) of ammonium molybdate, 30 ml of the solution (0.1 moles L<sup>-1</sup>) of ascorbic acid and 5 ml of the solution (1 mg of Sb · ml<sup>-1</sup>) of antimony potassium tartrate.

***Standard solution (1000 mg · L<sup>-1</sup>) of phosphorus (P)***

Transfer to a 1000 ml volumetric flask containing about 500 ml of  $\text{H}_2\text{O}$ , 4.3938 g of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) dried in an oven at 400°C.

After solubilization of the salt, make up to volume with  $\text{H}_2\text{O}$ .

***Diluted standard solution of phosphorus (P)***

Take with a precision burette and transfer 10 ml of the solution (1000 mg · L<sup>-1</sup>) of phosphorus into a 1000 ml volumetric flask. Bring up to volume with  $\text{H}_2\text{O}$ . In this solution the phosphorus concentration is 10 mg · L<sup>-1</sup>.

**Equipment**



- pH meter with temperature compensator, glass electrode with reference electrode or electrodes
- combined;
- oscillating stirrer at 120 + 140 cycles / minute
- 0.45  $\mu\text{m}$  membrane filters
- spectrophotometer.

## Method

### *Extraction*

Transfer 2 g of the fine theme sample to Erlenmeyer conical flask or 125 ml plastic container. Add 0.5 g of activated carbon and 40 ml (VI) of the solution ( $0.5 \text{ mol} \cdot \text{L}^{-1}$ ) of sodium bicarbonate at pH 8.5. Stir for 30 minutes and pass several times through Whatman® n ° 42 paper collecting the filtrate in a plastic container with a cap. If necessary, pass through a 0.45  $\mu\text{m}$  membrane filter.

Prepare the blank test following the same operating procedures, omitting the soil sample.

### *Colorimetric determination*

Take with precision burette and in 50 ml volumetric flask, one aliquot of the clear solution (V2) containing from 2 to 40 days of P. Add 5 drops of the p-nitrophenol solution and, drop by drop, a quantity of the solution ( $2.5 \text{ moles L}^{-1}$ ) of sulfuric acid sufficient to change the color of the indicator to yellow.

The drops of sulfuric acid must be made to flow along the internal walls of the volumetric flask to avoid rapid development of  $\text{CO}_2$  and consequent losses of the solution.

Dilute to approximately 25 ml with  $\text{H}_2\text{O}$  and add 8 ml of the sulfomolybdic reagent. Bring up to volume with  $\text{H}_2\text{O}$ .

After 10 minutes, read the extinction value 882 nm on the spectrophotometer against a blank containing all the reagents except the phosphorus solution.

### *Calibration curve*

Take with a precision burette and transfer 0, 5, 10, 15, 20 and 25 ml of the diluted standard solution ( $10 \text{ mg} \cdot \text{L}^{-1}$ ) of phosphorus into six 50 ml volumetric flasks.

Dilute to approximately 25 ml with  $\text{H}_2\text{O}$  and add 8 ml of the sulfomolybdic reagent. Bring up to volume with  $\text{H}_2\text{O}$ .



In each of the six solutions, the phosphorus concentration is, respectively, 0, 1, 2, 3, 4 and 5 mg. the reagents excluding the phosphorus solution.

### Expression of results

The phosphorus content extracted with sodium bicarbonate solution from the soil sample is expressed in mg / kg without decimal places.

The expression is used for the calculation

$$C = (A - B) \cdot \frac{V_1}{V_2} \cdot \frac{50}{M}$$

where is it

C = content of assimilable phosphorus present in the soil, expressed in mg / kg

A = concentration of phosphorus in the sample solution, expressed in mg L-1

B = concentration of phosphorus in the solution of the blank test, expressed in mg · L-1

V1 = volume of the extract (40 mL)

V2 = volume of the sample solution used for the colorimetric determination

M = mass of the soil sample, expressed in grams.

### Note

All the products used must be silicon-free, taking into account the reactivity of this element with the sulfomolybdic reagent. For the same reason, it is preferable to use distilled water since deionized water can contain silica.

## 3.7. The Exchangeable Elements: Potassium, Calcium, Sodium and Magnesium

By exchangeable elements of the soil we mean those chemical elements which interact in considerable quantities, with an ionic bond, with the surfaces of the organic and mineral particles of the soil; since the charges present on these surfaces are negative for the most common pH values of the soil, between values of 5 and 8.5, these elements are cations,



ie positively charged ions. The most present is calcium, followed by magnesium and potassium in similar quantities, while sodium is almost always found in low concentrations; the presence of the latter in high quantities can cause loss of fertility (saline-alkaline soils).

Other elements with a positive charge and therefore exchangeable (iron, manganese, zinc, copper and other metals) are present in much lower quantities and therefore considered microelements.

These elements in the soil are found, as mentioned, linked to negatively charged surfaces; therefore, organic and clayey colloids; they exchange among themselves, in relationships that depend on the prevalence of one or the other cation, in a dynamic form, giving rise to phenomena of continuous release in the soil solution.

The presence of these substances which have a negatively charged external surface therefore generates exchange phenomena with the soil solution whose intensity is measured by the Cation Exchange Capacity (CSC); the greater this capacity, the greater the quantity of exchangeable potassium, magnesium and calcium present in the soil.

Since potassium, magnesium and calcium, together with the less present sodium, constitute the great majority of cations present in neutral and alkaline soils, the sum of their exchangeable forms corresponds to the CSC of the soil.

In acid soils the prevalence of hydrogen ions covers the negative charges of the surfaces which are no longer able to retain the exchange bases which are therefore washed away.

Depending on their availability to be absorbed by crops, the forms of potassium present in the soil can be divided into:

- not available: if constitutive of the primary minerals for which it must undergo long-term processes to transform into available forms;
- little available: if fixed in the interlayers of clayey minerals, it constitutes a potassium reserve in the soil because it is directly related to exchangeable potassium; its availability depends on the type of plants present and the extent of the exchangeable and soluble forms;
- available: if in exchangeable or soluble form; the first, readily available, is adsorbed to the exchange surfaces, the second is the one in solution that the plant absorbs directly.



In fact, this schematic subdivision into different forms can be of little importance for the plant which simply takes the potassium from the soil solution; however, this is continuously supplemented by the other more or less available forms.

Knowing the relationships between these fractions is more important than knowing each of them individually.

Each soil has a "buffering power" with respect to potassium, consisting in the ability to reconstitute the soluble form starting from the exchangeable one, and at the same time in the ability to transform the soluble form into exchangeable following the contribution of potassium fertilizer. In general, in a clayey soil this buffering power is greater than in a sandy one since it is correlated to the CSC of the soil and to the exchangeable potassium / CSC ratio (Villemain, 1988).

Due to the existence of this buffering power in a soil well endowed with potassium, the crops do not give a significant response, in terms of greater yield, to the increase in the doses of potassium distributed with fertilization; On the other hand, this response exists in medium-gifted and above all poor soils (Loué, 1985).

In the same way for magnesium the exchangeable magnesium / CSC ratio explains better than the exchangeable magnesium data alone the possibility that the plant obtains this element from the soil; for this reason the interpretation of the analytical result is more correct if the CSC value is taken into consideration. In any case, while for potassium we can distinguish a fixed or linked form, also called intermediate potassium, consisting of the ions placed between the layers of phyllosilicates, for magnesium and calcium this does not exist or is very small since the double valence and the dimensions of the hydrate ions prevent this retrogradation process from occurring.

Calcium in calcareous soils is present in substantial quantities in inactive carbonates, in coarse physical form with low chemical activity, and in more finely divided active ones that easily interact with the circulating soil solution.

Unlike nitrogen and phosphorus, the potassium, magnesium and calcium fractions contained in the organic substance are not very important as a reserve of the elements when compared with the mineral reserve constituted by the forms adsorbed or fixed on clay minerals.



### ***3.7.1. Determination of the potassium fixation power***

#### **Principle**

The method is based on the evaluation of the percentage of potassium, with respect to a known quantity added, that the soil is capable of fixing, preventing or limiting its solubility and, consequently, its availability for plants.

Potassium is extracted with ammonium acetate solution (1 mole \* L<sup>-1</sup>) at pH 7 from a soil sample as it is (reference test that allows to define the quantity of exchangeable potassium naturally present in the soil) and from a sample of the same soil to which a known amount of the nutrient has been added.

The amount of potassium added and not extracted is considered fixed.

The potassium content is determined by flame atomization atomic absorption spectrophotometry (FAAS).

This determination is of considerable importance in defining the degree of availability for plants of the K added to cultivated soils.

#### **Reagents**

##### ***Diluted solution (1: 10 v / v) of ammonium hydroxide***

Transfer to a 1000 ml volumetric flask containing about 600 ml of H<sub>2</sub>O, 100 ml of ammonium hydroxide solution (NH<sub>4</sub>OH) [30 0/0 (p = 0.892)]. Bring up to volume with H<sub>2</sub>O.

##### ***Diluted solution (1:10 v / v) of acetic acid***

Transfer to a 1000 ml volumetric flask containing about 600 ml of H<sub>2</sub>O, 100 ml of acetic acid (CH<sub>3</sub>COOH) (p = 1.0499). Bring up to volume with H<sub>2</sub>O.

##### ***Solution (1 mole \* L<sup>-1</sup>) at pH 7 of ammonium acetate***

Transfer to a 1000 ml beaker, containing approximately 900 ml of H<sub>2</sub>O, 77.08 g of ammonium acetate (CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>). After solubilization of the salt, with the addition of small quantities of the dilute solutions of ammonium hydroxide or acetic acid, bring the



pH value to 7. Transfer the solution to a 1000 mL calibrated solution and make up to the mark with H<sub>2</sub>O.

***Solution (2 moles \* L<sup>-1</sup>) at pH 7 of ammonium acetate***

Transfer to a 500 ml beaker, containing approximately 400 ml of H<sub>2</sub>O, 77.08 g of ammonium acetate (CH<sub>3</sub>CO<sub>2</sub>NH<sub>4</sub>). After solubilization of the salt, with the addition of small quantities of the dilute solutions of ammonium hydroxide or acetic acid, bring the pH value to 7. Transfer the solution to a 500 mL volumetric flask and make up to the mark with H<sub>2</sub>O.

***Solution (10 g \* L<sup>-1</sup>) of lanthanum***

Transfer 11.73 g of lanthanum oxide (La<sub>2</sub>O<sub>3</sub>) to a 1000 ml volumetric flask. Wet with distilled H<sub>2</sub>O and carefully add 100 ml of hydrochloric acid [37% (p = 1.186)]. After solubilization of the lanthanum oxide, mix and make up to volume with H<sub>2</sub>O.

**Standard commercial solution with guaranteed titre (1000 mg \* L<sup>-1</sup>) of potassium (K)**

***Potassium (K) solution (400 mg \* L<sup>-1</sup>)***

Take with a precision burette and transfer 40 ml of the standard commercial solution with guaranteed titre (1000 mg \* L<sup>-1</sup>) of potassium into a 100 ml volumetric flask. Bring up to volume with H<sub>2</sub>O.

***Diluted standard solution of potassium (K)***

Take with a precision burette and transfer 5 mL of the standard commercial solution with guaranteed titre (1000 mg Lei) of potassium into a 100 mL calibrated sample. Bring up to volume with H<sub>2</sub>O. In this solution the potassium concentration is 50 mg \* L<sup>-1</sup>

***Standard working solution of potassium (K)***

Take with a precision burette and transfer 0, 10, 20 and 30 ml of the diluted standard solution (50 mg \* L<sup>-1</sup>) of potassium into four volumetric flasks of 100 ml. Add to each flask 10 ml of the solution (10 g \* L<sup>-1</sup>) of lanthanum and 50 ml of the solution (2 moles \* L<sup>-1</sup>) of ammonium acetate. Bring up to volume with H<sub>2</sub>O.

In each of the four solutions the potassium concentration is, respectively, 0, 5, 10, 15 mg \* L<sup>-1</sup>).



## Equipment

- 120 + 140 cycles / minute oscillating stirrer;
- atomic absorption spectrophotometer equipped with flame atomization system (FAAS).

## Method

### *Preparation of the extracts*

Transfer 5 g of the fine earth sample to Erlenmeyer conical flask. Add 2.5 ml of the diluted solution (400 mg \* L<sup>-1</sup>) of potassium, taking care that the sample is homogeneously wetted.

Leave to stand for 16 hours at + 20 ° C closing the mouth of the conical flask with sealing film. Prepare the solution for the reference test following the same operating procedures, replacing the addition of 2.5 ml of the diluted solution (400 mg \* L<sup>-1</sup>) of potassium with the addition of 2.5 ml of H<sub>2</sub>O.

Prepare the blank tests following the same operating procedures, omitting the soil sample.

Subsequently, uniformly wet the sample with about 15 ml of the ammonium acetate solution (1 mole \* l<sup>-1</sup>) at pH 7, mix well and leave to stand overnight. Filter by Whatman® paper n ° 42 in a 100 ml volumetric flask. Make up to volume with the same ammonium acetate solution.

### *Preparation of the calibration curve*

Prepare the calibration curve on the atomic absorption spectrophotometer, using an air-acetylene flame (FAAS). Define conditions for the supplied instrument to respect the proportionality between absorbance and concentration of the standard working solutions. Use the specific lamp, selecting the wavelength: 766.5 nm

### *Dosage*

Read the absorbance values for the sample solutions.

If necessary, carry out an appropriate dilution respecting the ratios with the lanthanum solution and making up to the final volume with the ammonium acetate solution (1 mole \* L<sup>-1</sup>).



## Expression of results

The potassium fixing power is expressed as a percentage value, with one decimal place. The potassium content expressed with ammonium acetate solution (1 mole \* L-1) at pH 7 from a soil sample as it is (blank test that allows to define the quantity of exchangeable potassium naturally present in the soil) and from a sample of the same soil to which a known quantity of the nutrient has been added is expressed in mg / kg.

The expression is used for the calculation

$$C_k, C_w = \frac{(A - B) \cdot D \cdot V}{M}$$

where is it

CK, Cw = potassium content extracted from differently treated soil samples, expressed in mg / kg of K.

A = content of K in the sample solution, expressed in mg \* L-1

B = concentration of potassium in the blank tests, expressed in mg LD = dilution factor (D = 1 if the test solution has not been diluted)

V = volume of the extracting solution used, expressed in milliliters

M = mass of the soil sample used, expressed in grams.

For the calculation of the percentage of potassium fixed in the soil at 16 hours the expression is used

$$F_k = 100 \frac{200 - (C_k - C_w)}{200}$$

where is it

Fk = percentage value of the quantity of K fixed by the soil after 16 hours.

Cx = content of K extracted from the soil treated with KCl solution, expressed in mg / kg

Cw = content of K extracted from the soil in the reference test, expressed in mg / kg

Expressions are used to estimate the percentage of potassium fixed by different soils at 60 days



$$1) F_k = 9.0704 + 1.0601 F_k, 16h$$

$$2) F_k = 30 + 0.77 F_k, 6h$$

In particular, the first expression was tested for the non-acidic soils of the Po Valley (Perelli et al., 1994), while the second expression was tested for some soils of southern Lazio (Indiati et al., 1992).

### Note

The determination of the potassium content can be done by plasma emission spectroscopy (CP).



### ***3.7.2. Determination of the total content of alkaline and alkaline earth metals (sodium, calcium and magnesium)***

#### **Principle**

The sample is calcined to eliminate the organic constituents, which could incorporate some of the elements to be determined, and, subsequently, treated with hydrofluoric acid to break up the silicates. The salts are solubilized in an acid environment and the metals determined by atomic absorption spectrophotometry (FAAS) (Izza et al., 1994)

#### **Reagents**

**Hydrofluoric acid (HF) [500/0 (p = 1.155)]**

**Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [96% (p, 1835)]**

**Hydrochloric acid (HCl) [370/0 (p = 1.186)]**

***Diluted solution (1: 4 v / v) of sulfuric acid***

Carefully add 250 ml of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [96% (p = 1.835)] into a 1000 ml graduated flask containing approximately 600 ml of H<sub>2</sub>O. Mix and, after cooling, make up to volume with H<sub>2</sub>O.

***Diluted solution (1: 1 v / v) of hydrochloric acid***

Carefully add 500 ml of hydrochloric acid [37% (p = 1.186)] into a 1000 ml graduated flask containing about 400 ml of H<sub>2</sub>O. Mix and, after cooling, make up to volume with H<sub>2</sub>O.

***Solution (10 g \* L-1) of lanthanum***

Transfer 11.73 g of lanthanum oxide (1.2203) to a 1000 ml volumetric flask. Moisten with H<sub>2</sub>O and carefully add 100 ml of hydrochloric acid [37% (p = 1.186)]. Mix and make up to volume with H<sub>2</sub>O.

***Solution (15 g \* L-1) of cesium***

Dissolve 20, 42 g of cesium sulphate (Cs<sub>2</sub>SO<sub>4</sub>) in H<sub>2</sub>O, in a 1000 ml volumetric flask. Bring up to volume with H<sub>2</sub>O.

Store the solution in a plastic container.

**Standard commercial solution with guaranteed title (1000 mg \* L-1) of sodium (Na)**



**Standard commercial solution with guaranteed title (1000 mg \* L<sup>-1</sup>) of potassium (K)**

**Standard commercial solution with guaranteed title (1000 mg \* L<sup>-1</sup>) of magnesium (Mg)**

**Standard commercial solution with guaranteed title (1000 mg \* L<sup>-1</sup>) of calcium (Ca)**

***Diluted standard solutions of sodium (Na), potassium (K), magnesium (Mg)***

Take with a precision burette and transfer 5 ml of the standard commercial solution with guaranteed title (1000 mg L<sup>-1</sup>) of each of the four elements into volumetric flasks of 100 ml. Make up to volume with H<sub>2</sub>O.

In these solutions the concentration of lithium, sodium, potassium and magnesium is, respectively, 50 mg L<sup>-1</sup>.

***Diluted standard solutions of calcium (Ca)***

Take with a precision burette and transfer into two 100 ml volumetric flasks 10 ml of the standard commercial solution with guaranteed titre (1000 mg L<sup>-1</sup>) of the element. Bring up to volume with H<sub>2</sub>O.

In these solutions the calcium concentration is (100 mg \* L<sup>-1</sup>).

***Standard working solution of sodium***

With a precision burette, take 0, 5, 10 and 15 ml of the diluted standard solution (50 mg \* L<sup>-1</sup>) of sodium (Na) into four 500 ml volumetric flasks.

Add 50 ml of the cesium solution (15 g \* L<sup>-1</sup>) to each flask. Bring up to volume with H<sub>2</sub>O.

In each of the four solutions the sodium concentration is respectively 0, 5, 1 and 1.5 ml of the diluted standard solution (50 mg \* L<sup>-1</sup>)

***Standard working solution of potassium***

Take with a precision burette and transfer 10, 20 and 30 ml of the diluted standard solution (50 mg \* L<sup>-1</sup>) of potassium (K) into 500 ml volumetric flasks.

Add 50 ml of the solution (10 g \* L<sup>-1</sup>) of lanthanum to each flask. Bring up to volume with H<sub>2</sub>O.

In each of the four solutions the potassium concentration is 0, 1, 2, 3 mg \* L<sup>-1</sup>. Standard working solution of magnesium



Take with a precision burette and transfer 0, 5, 10 and 15 ml of the diluted standard solution ( $50 \text{ mg} \cdot \text{L}^{-1}$ ) of magnesium (Mg) into 500 ml volumetric flasks.

Add 50 ml of the solution ( $10 \text{ g} \cdot \text{L}^{-1}$ ) of lanthanum to each flask. Bring up to volume with  $\text{H}_2\text{O}$ .

In each of the four solutions the concentration of magnesium is, respectively, 0, 0.5, 1 and  $1.5 \text{ mg} \cdot \text{L}^{-1}$

### ***Standard solution of soccer work***

Take with a precision burette and transfer 0, 10, 20, 30 ml of the diluted standard solution ( $100 \text{ mg} \cdot \text{L}^{-1}$ ) of calcium (Ca) into four 500 ml volumetric flasks.

Add 50 mL of the lanthanum solution ( $10 \text{ g} \cdot \text{L}^{-1}$ ) to each flask. Bring up to volume with  $\text{H}_2\text{O}$ .

In each of the four solutions the calcium concentration is respectively 0, 2, 4 and  $6 \text{ mg} \cdot \text{L}^{-1}$ .

### **Equipment**

- platinum capsules
- muffle furnace;
- bain-marie;
- atomic absorption spectrophotometer equipped with flame atomization system (FAAS).

### **Method**

#### ***Preparation of the solution***

Transfer 1.5 g of the fine earth sample, dry in the air and sieved at 0.2 mm, into a platinum dish.

Keep the capsule for 4 hours in a muffle oven at  $450^\circ \text{C}$ .

After calcination, allow the platinum capsule to cool. Wet the residue with a few drops of  $\text{H}_2\text{O}$  and add, in succession, 15 ml of hydrofluoric acid (HF) and 15 ml of diluted solution (1: 4 v: v) of sulfuric acid ( $\text{H}_2\text{SO}_4$ ).



Keep the capsule on a boiling water bath, under the hood, until a syrupy residue is obtained.

After cooling, resume with a quantity of dilute solution (1: 1 v: v) of hydrochloric acid sufficient to solubilize the residue.

If necessary, heat slightly.

Quantitatively transfer the solution into a 500 ml volumetric flask. Bring up to volume with H<sub>2</sub>O.

Filter by Whatman paper n ° 42 in a dry plastic container.

Take two aliquots of 200 ml of this solution into two 500 ml volumetric flasks accurately.

To determine the sodium, take with a precision burette and transfer 50 ml of the cesium solution (15 g \* L<sup>-1</sup>) into one of the two 500 ml volumetric flasks. Bring up to volume with H<sub>2</sub>O.

For the determination of potassium, magnesium and calcium, take 50 ml of the lanthanum solution (10 g \* L<sup>-1</sup>) with a precision burette and transfer to the other 500 ml volumetric flask.

Bring up to volume with H<sub>2</sub>O.

Store the solutions in plastic containers

The blank tests are carried out with the same operating procedures, omitting the soil sample.

### ***Preparation of calibration curves***

Prepare the calibration curve for each metal on the spectrophotometer in atomic absorption, using an oxidizing air-acetylene flame. Define for the supplied instrument conditions such as to respect the proportionality between the absorbance and the concentration of the standard working solutions

Use specific lamp for each element, selecting the following wavelengths:

Sodium 589.6 nm

Potassium 766.5 nm

Magnesium 285.2 nm



Calcium 422.7 nr

### Dosage

Read the absorbance values for the sample solutions. If necessary, make an appropriate dilution. Using the calibration curves prepared, go back from the absorbance values to the concentration values.

### Expression of results

The content of each alkaline and alkaline-earth metal is expressed in g / kg, without decimal places.

The expression is used for the calculation

$$C = \frac{(A - B) 2,5 D L}{M}$$

where is it

C = metal content in the soil, expressed in mg kg

A = concentration of the metal in the sample solution, expressed in mg \* L-1

B = concentration of the metal in the solution of the blank test, expressed in mg \* L-1

2.5 = dilution factor (500ml / 200ml = 2.5)

D = dilution factor (D = 1 if the test solution has not been further diluted) final volume, expressed in milliliters

M = mass of the soil sample, expressed in grams.

### Note

To avoid possible losses of potassium, it is advisable not to exceed 4500C during calcination.



## 4. Biological parameters and indices

### 4.1. Arthropods: QBS - ar

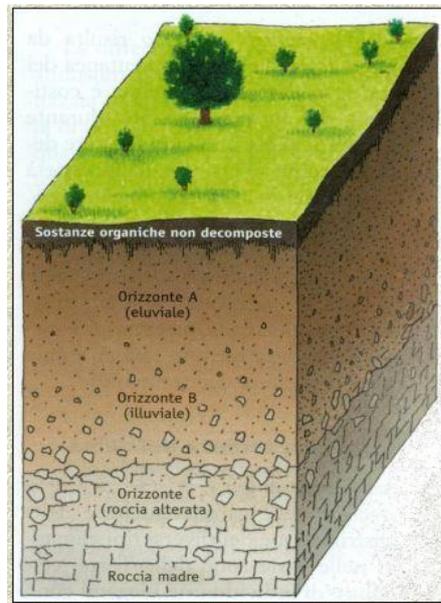
The Biological Quality Index of the Soil using Arthropods (QBS-ar) has proved to be a method of reading the soil environment that can be used in Didactics, with characteristics similar to IBE (Extended Biotic Index) and IBL (Lichen Biodiversity Index) .

The method is scientifically verified, not excessively specialized, capable of producing synthesis values of the investigation carried out and of providing a valid interpretation of the investigated environment. The QBS-ar was created and developed by prof. Vittorio Parisi of the Department of Evolutionary and Functional Biology of the University of Parma.

#### **The soil**

The soil is the result of a very long process of formation, called pedogenesis (from the Greek pédon = earth, soil and ghénesis = origin, process of formation), which gives it its own structure and a characteristic stratification, is an environment very rich in life, allows the closure of biological cycles by means of bio-reducers (organisms that transform organic substance into mineral salts, necessary for plants), is a thin and vulnerable layer, a limited and increasingly rare natural resource where the anthropic pressure is greater.

If we observe a vertical section of a natural soil, i.e. its profile, we note that it has stratifications or horizons which, starting from the surface, are defined as follows:



- **Organic horizon**(litter) consisting of the remains of more or less decomposed plants and animals, which lie on the ground;
- **Horizon A**(eluvial) usually dark, containing most of the organic substance, with plant and animal remains no longer morphologically recognizable (humus), in which the washing action of water removes the soluble materials from the surface downwards;
- **Horizon B**(illuvial) layer in which the organic substance is scarce and in which the materials transported by the percolation water, such as clay, aluminum and iron, accumulate;
- **Horizon C**(mother rock) layer not yet altered, which at the interface with layer B is more or less altered and coarse (this transition phase is called pedogenetic substrate). The material can be indigenous or allochthonous, coming from other areas, such as in the Po Valley.

Factors contributing to soil formation are:

- climatic factors (precipitation, temperature, erosive processes, irradiation) together with the interaction of living beings, starting from the pioneer forms such as lichens and mosses, which involve pedogenetic processes in the various regions and at different latitudes (to a greater quantity of water there is a greater possibility that the solids react



with the solvent and the longer the contact period between the solvent and the solid, the more intense the alteration is; moreover, the reactions are faster at higher temperatures). As a result, the alteration advances like a front slowly moving downwards.

- time, the formation of a soil is in fact an extremely long process: it takes hundreds or thousands of years for a horizon A to evolve to present a carpet of decaying organic matter mixed with altered minerals and clays and even longer times, from tens to hundreds of thousands of years, for the formation of horizon B.

Soil is made up of three components:  $\theta$

- solid phase (mineral component and organic component),
- liquid phase,
- gas phase.

Good soil is 50% full and 50% empty.

SOLID PHASE: includes mineral compounds and organic compounds. Mineral compounds are very numerous, however the most frequent are quartz ( $\text{SiO}_2$ ), calcite ( $\text{CaCO}_3$ ), sulphates such as gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ), phosphates and silicates. Clay minerals, such as kaolin, should be remembered for their ability to swell when moistened. The organic fraction is extremely complex, including compounds derived from the progressive demolition of plant and animal structures that reach the ground. In addition to sugars, fats, proteins, there are enzymes (released by microorganisms to break down organic compounds), phytotoxins (vegetable toxins, released from the leaves during the first stages of their demolition), antibiotics (of microbial origin, to control their density and distribution).

WATER PHASE: water, with its time-varying solutes, is present in the soil in various forms:

♣ gravitational water fills large or medium cavities, moving towards the bottom due to its own weight;



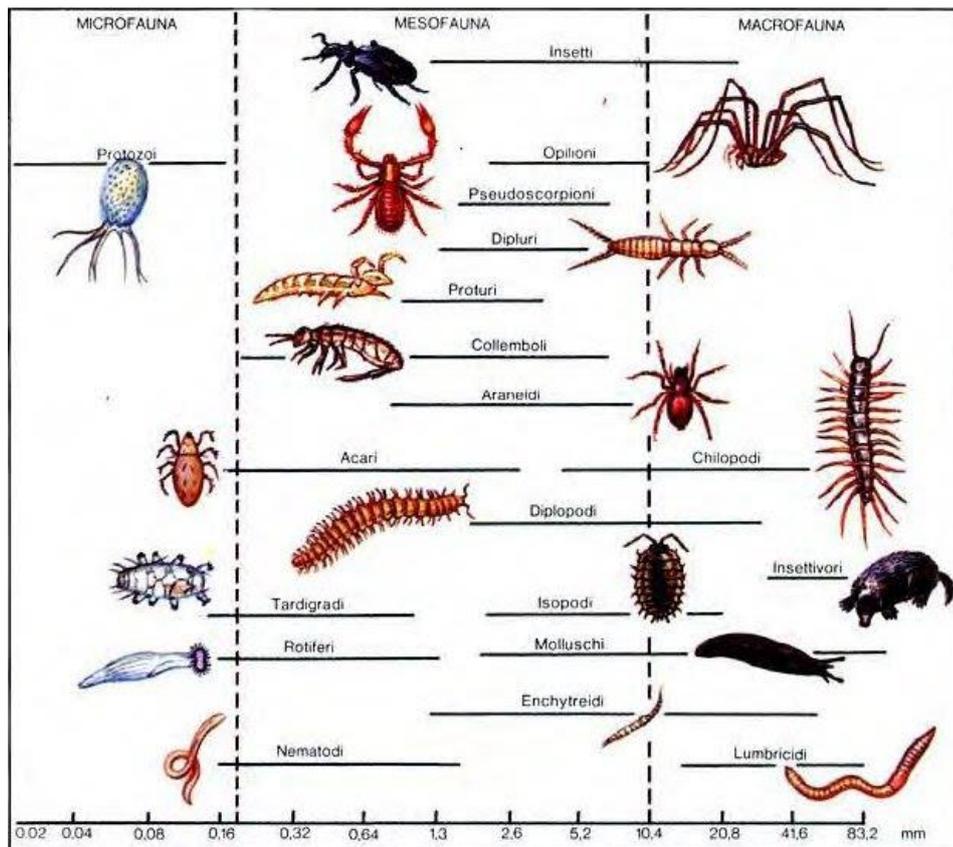
- ♣ capillary water fills smaller cavities;
- ♣ hygroscopic water is strongly linked to various substances present in the soil, varying in percentage according to its characteristics (in a sandy soil it is less than 1%, in an average one it is about 3%, in a peaty one about 18% and in a clayey even 23%);
- ♣ the water of crystallization or constitution, intimately linked to the substances that make up the soil, not available for the organisms that live in it.

GASEOUS PHASE: the hypogeal atmosphere has a composition similar to the epigeal one, differing for a higher concentration of CO<sub>2</sub> (from 0.3% to 1%, that is at least ten times as much) and for being saturated with water vapor. Due to the characteristics of this basal portion of terrestrial ecosystems, its biological population has at least a double origin:

- a - one part, called hydrobios (consisting of Bacteria, Algae, Protozoa, Nematodes, Rotifers, Tardigrades), colonizes the interstices with gravitational and capillary water;
- b - another part, called atmobios (made up of Mollusks, Arthropods and Vertebrates, Fungi), colonizes the cavities containing air.

The trophic webs of the soil in terrestrial ecosystems generally consist of:

- ♣ microflora (fungi and bacteria);
- ♣ microfauna (size between 20 µm and 200 µm; eg Protozoa and Acari);
- ♣ mesofauna (dimensions between 200 µm and 2 mm; eg Mites, Collembolae, Symphiles, Diplopods, Isopods, Tardigrades, Rotifers, Nematodes, Holometabolous insect larvae, etc);
- ♣ macrofauna (dimensions between 2 mm and 20 mm; eg. Annelids, Gastropods, Isopods, Diplopods, Chilopods, Araneids, Insects)
- ♣ megafauna (dimensions greater than 20 mm; eg. Annelids, Gastropods, Chilopods, Vertebrates)



The micro-arthropods that are dimensionally included in the mesofauna also play a fundamental role in the functioning of the soil through the following actions:

- shredding and shredding of plant residues;
- demolition of the organic substance;
- translocation of organic matter;
- control and dispersion of microflora and microfauna;
- predation of micro and mesofauna.

The pedofauna occupies the first 20-30 cm of soil plus the overlying litter; over 30 cm deep it becomes extremely rare and disappears

### The QBS-ar method

The QBS-ar method evaluates the biological quality of a soil through the biodiversity of the micro-arthropods used as bioindicators. These organisms present a complex series of adaptations and consequences to life in the edaphic environment and are sensitive to the state of suffering of a soil that can derive from agricultural work and compaction due to the passage of men and vehicles.



All organisms, regardless of their embryological origin, converge towards a Biological Form (FB) which allows the best adaptation to the environment.

Soil animals exhibit adaptations and consequences to life in this environment to varying degrees. These adaptations mainly concern:

- ✓ miniaturization;
- ✓ elongation and flattening of the body;
- ✓ the shortening of the sensory and locomotor appendages (possibly strengthened);
- ✓ the reduction or disappearance of appendages such as furca in Collembola or metathoracic wings in Coleoptera;
- ✓ the presence of sensory organs to perceive the degree of humidity, such as the post-antennial organ of Collemboli (PAO);
- ✓ depigmentation or possible cryptic pigmentation to be confused with earth particles as in mites;
- ✓ the reduction or disappearance of the sensory organs that receive light radiation.

The FBs (biological forms) therefore represent groups characterized by having the same series of converging characters. The convergence of adaptations and consequences leads to the confinement of these organisms to the edaphic environment, outside which they can no longer survive. The presence / absence of the most adapted organisms therefore becomes a good indicator of the level of soil disturbance. The FB criterion was adopted both to assess the level of adaptation to the edaphic environment and to overcome the classification difficulties of these groups of animals. Furthermore, for the determination of this quality index it is not necessary to calculate the density of the individuals of the various groups, considerably simplifying the method.



GRUPPI		EMI
Pseudoscorpioni		20
Scorpioni		10
Palpigradi		20
Opilioni		10
Aranei	Forme superiori a 5 mm	1
	Forme piccole e poco pigmentate	5
Acari		20
Isopodi		10
Diplopodi	Forme superiori ai 5 mm	10
	Forme inferiori ai 5 mm	20
Paupodi		20
Sinfili		20
Chilopodi	Forme superiori ai 5mm, ma con zampe ben sviluppate	10
	Altre forme (geoflomorfi)	20
Proturi		20
Dipluri		20
Collemboli	Forme epigee: appendici allungate, ben sviluppate. Apparato visivo (macchia ocellare e occhi) ben sviluppato. Dimensioni medio/grandi, presenza di livrea complessa	1
	Forme epigee non legate alla vegetazione arborea, con buon sviluppo delle appendici, con forte sviluppo di setole e squame. Apparato visivo ben sviluppato	2
	Forme di piccola dimensione con medio sviluppo delle appendici, apparato visivo ben sviluppato, livrea modesta, forme limitate alla lettera	4
	Forme emiedafiche con apparato visivo ben sviluppato, appendici non allungate, livrea con colore	6
	Forme emiedafiche con riduzione del numero di occhi, appendici poco sviluppate, con furca ridotta e assente, presenza di pigmentazione	8
	Forme eudafiche con pigmentazione assente, riduzione e assenza di occhi, furca presente ma ridotta	10
	Forme eudafiche depigmentate, prive di furca, appendici tozze, organo postantennale sviluppato (ma non necessariamente presente)	20
Microcorifi o Archeognati		10
Zigotomi		10
Dermatteri		1
Ortotteri	In generale	1
	Famiglia Gryllidae	20
Embiotteri		10
Isotteri		10
Blattari		5
Psocotteri		1
Tisanotteri		1
Emitteri	Forme epigee	1
	Larva cicalea	10
Coleotteri	Forme epigee	1
	Dimensioni <2 mm	+4
	Tegumenti sottili, con colori testacei	+5
	Micreatterismo e atterismo	+5
	Macrofalma o anofalnia	+5
	Nel caso di forme edafobie	20
Imenotteri	In generale	1
	Formicidi	5
Ditteri	Adulti	1
Coleotteri (larve)		10
Ditteri (larve)		10
Imenotteri (larve)		10
Lepidotteri (larve)		10
Altri olometaboli	Adulti	1

In the QBS-ar reference is not so much to taxonomy, but rather to the rate of adaptation to the soil, to evolutionary convergence. Each character showing the adaptation to the ground is assigned a score, up to a maximum total of 20; each form found is then assigned a score from a minimum of 1 to a maximum of 20.

When in a sample of pedofauna taken from the ground, there are several FBs belonging to the same group, for example Collembola or Coleoptera, only the highest EMI value found is taken into account (NOT the sum).



The studies and experiences carried out so far have identified a gradient of values that goes from the lowest ones for the soils subjected to plowing to the highest ones shown by the undisturbed woods. An absolute scale of values has not yet been defined.

An index has so far been developed for soils in Northern Italy and some of the values obtained fall on average and indicatively in the intervals of QBS-ar shown in the following table:

TIPOLOGIE DI SUOLO IN BASE ALL'AMBIENTE O ALLA DESTINAZIONE D'USO	QBS-ar	NOTE
Suolo arato	40-50	La diminuzione di biodiversità si ha dopo un certo periodo di tempo dall'aratura
Barbabietola	40-60	Generalmente è la coltura che mostra i valori più bassi
Mais	40-100	Certi campi molto inerbiti possono dare valori anche superiori a 100
Frumento	60-100	Mediamente tra i seminativi è la coltura che mostra i valori più alti
Erba medica	60-180	I valori più alti si hanno al terzo anno di coltura perché diminuiscono gli effetti della preparazione del letto di semina
Prati stabili	90-180	Sono i prati permanenti che durano oltre i 10 anni
Boschi	150-250	Generalmente le aree boschive hanno valori maggiori di 130

## 4.2. Earthworms: QBS - e

New index of Biological Quality of the Soil applied to earthworms, evaluates the quality and health of the soil based on the monitoring of the community of earthworms housed in it. As they are not very mobile and closely tied to the ground and easily sampled in a standard way with the hand sorting technique.

However, this method cannot be used in all environments (earthworms are not found in environments with a too dry climate, or in sandy or too stony soils).



It is based on the identification of the ecological category, on the age and number of individuals.

#### **4.2.1. Sampling-Monitoring Hand-sorting technique**

Taking a clod of soil (30 cm X 30 cm X 20 cm deep).

Crushing the clod on a white cloth in search of earthworms.

These are picked up and cleaned from the earth using a container with water.

Repeat the sampling 5 to 10 times at a minimum distance of 6-10 m.

Sampling period: Spring - Autumn (sample after rainy periods).

#### **4.2.1. Ecological categories**

##### **EPIGEI**(*Lumbricus rubellus* - *Lumbricus castaneus*)

Dorsally pigmented, they are found in the litter (L) and the soil horizon A01 (up to 2.5 cm deep). High reproductive capacity (65-106 cocoons per year)

##### **ENDOGEI**(*Allolobophora chlorotica* - *Allolobophora caliginosa*)

Sometimes less pigmented are found from the A02 horizon to the A1 horizon. They dig mainly horizontal tunnels (within the first 50 cm of depth). Poor reproductive capacity (8-27 cocoons per year)

##### **ANECICI**(*Lumbricus terrestris* - *Eophila tellinii*)

Even large, they can reach the A2 and B horizon. They dig vertical tunnels that can even reach a few meters in depth. They often rise to the surface at night to feed on litter. Low reproductive capacity (1-8 cocoons per year)

##### **COPROPHAGES**(*Eisenia foetida* - *Dendrobaena veneta*)

They live in manure or compost and are closely associated with them and therefore rarely collect in soils where they do not survive for long. High reproductive capacity (107 cocoons per year)

##### **HYDROPHILS**(*Eiseniella tetraedra*)



Lovers of soils with a superficial water table, even near rivers. Average reproductive capacity (41 cocoons per year)

#### 4.2.2. EMI

YOUNG: with indistinguishable clit. They indicate the rate of generational change

ADULTS: with clearly visible clit. They indicate that the environmental conditions are favorable for growth and development and are potential reproducers

Categoria ecologica	Stadio	EMI
Idrofilo (IDR)	Giovane (G)	1
Idrofilo (IDR)	Adulto (AD)	1
Coprofago (COP)	Giovane (G)	2
Coprofago (COP)	Adulto (AD)	2
Epigeo (EPI)	Giovane (G)	2,5
Epigeo (EPI)	Adulto (AD)	3
Endogeo (END)	Giovane (G)	2,5
Endogeo (END)	Adulto (AD)	3,2
Anecico (ANE)	Giovane (G)	10
Anecico (ANE)	Adulto (AD)	14,4

### 4.3. Soil Respiration

According to Xu and Shang (2016), Soil Respiration (SR) commonly refers to the efflux of CO<sub>2</sub> from the soil surface to the atmosphere. The annual CO<sub>2</sub> emission through the SR on a global scale is about ten times that of current fossil fuel emissions (Bond-Lamberty & Thomson, 2010).

Apparently, soil respiration is critical to the global carbon cycle, as global soils store twice as much carbon as the atmosphere (Post & al., 1982; IPCC, 2013; Scharlemann et al.,



2014) and ecosystem feedback through the SR is important for the projection of future atmospheric CO<sub>2</sub> concentrations (Cox et al., 2000; Heimann & Reichstein, 2008).

SR measured at the soil surface can come from multiple sources, such as root respiration (RR), heterotrophic respiration (HR), soil fauna respiration (FR) and non-biological CO<sub>2</sub> production (NR).

RR includes root maintenance respiration, in which breathed energy is used to keep roots alive, root growth respiration for the growth of new roots or new root tissues, and rhizospheric respiration (RZ), in which microbes breathe CO<sub>2</sub> into the rhizosphere by feeding on radical exudates. HR is due to the decomposition of dead organic matter by microbes in the soil in bulk. FR includes the respiration of soil fauna, such as earthworms, ants, beetles, earwigs and scale insects. NR arises from chemical and physical processes in the soil, such as CO<sub>2</sub> released from soil solutions when water evaporates or the solubility of CO<sub>2</sub> changes with fluctuations in soil temperature.

Soil CO<sub>2</sub> efflux measured at the soil surface is often considered an approximation of "true soil respiration", although this hypothesis was recently challenged by the discovery that breathed CO<sub>2</sub> can be dissolved in soil solutions (Emmerich, 2003; Hastings et al., 2005; Stevenson & Verburg, 2006; Schlesinger et al., 2009; Tamir et al., 2011; Ma et al., 2013; Roland et al., 2013; Angert et al., 2015) or in the water of the root xylem and carried upwards in the transpiration stream (Aubrey & Teskey, 2009; Bloemen et al., 2013).

#### ***4.3.1. Components of soil respiration***

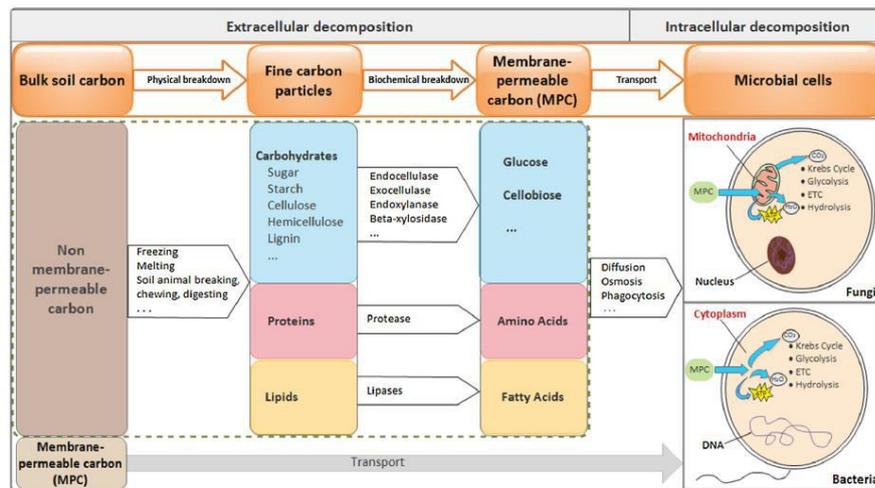
##### ***4.3.1.1. Heterotrophic soil respiration (HR)***

HR refers to the decomposition of soil dead organic matter (SOM) by soil microbes. The two main groups of these decomposers are bacteria and fungi, involved in the aerobic and anaerobic degradation of organic matter. These microbes produce extracellular enzymes to convert the polymeric compounds, such as cellulose, hemicellulose and lignin, present in the SOM into smaller molecules that can be further assimilated within the microbial cells (Romaní et al., 2006). Fungi are more efficient than bacteria in degrading highly recalcitrant organic matter because they can produce a wider range of extracellular enzymes than bacteria (Szafranek-Nakoneczna & Stępniewska, 2014).



Although actinomycetes have been found capable of degrading lignin (Benneret al., 1984), it is generally believed that fungi decompose most large molecule compounds and that bacteria mainly decompose polysaccharides and polymeric compounds upon decomposition. by mushrooms (Romaní et al., 2006). Some polymeric carbohydrates can be degraded by both aerobic and anaerobic microbes, but complete degradation of lignin requires aerobic microbes. The decomposition of soil organic matter begins with physical and biophysical processes that break down large pieces of organic matter into smaller pieces, followed by the biochemical transformation of complex organic molecules into simpler organic and inorganic molecules (Fig. 6).

Soil organic matter is known to exist in the soil in the form of bulk complexes. A very small part of the SOM, such as amino acids, glucose and fatty acids, is soluble and permeable to membranes. These SOM molecules can directly penetrate cell walls and membranes and be oxidized in the mitochondria or cytosol. Most of the SOM present in soil is made up of larger molecules, such as starch, cellulose, lignin, proteins and lipids, which cannot penetrate cell walls and microbial membranes. Therefore, these large molecules must be biochemically broken down into membrane-penetrable carbon molecules (MPCs) before they can be used by soil microbes. First, soil animals, such as mites and earthworms, they break down the complicated mass of SOM into small pieces through various activities such as movement, chewing or digestion. Freezing and melting of water in loose soils can also facilitate the breakdown of large pieces of SOM. Thereafter, the biochemical breakdown of SOM begins with fine particles, including carbohydrates, proteins and lipids. Biochemical breakdown involves various extracellular enzymes to break specific chemical bonds (Vandermeer, 2011). For example, proteases are used to break down proteins into amino acids, while lipases can break down lipids into fatty acids (Whiteley & Lee, 2006). Large carbohydrate molecules are usually broken down into random chains and then broken down into MPC units by the non-reducing end of the molecular chain by cutting specific bonds, such as the  $\beta$ -1,4 bond in cellulose (Berg & Laskowski, 2005; Wilson, 2011). The resulting small units (MPCs), such as monosaccharides and disaccharides, can be transported into microbial cells by diffusion, osmosis or phagocytosis (Sauer et al., 1994).



**Figure 6. Microbial decomposition of soil organic carbon (Xu & Shang, 2016)**

Fungi are able to spread their hyphae through the empty spaces between or within organic matter over a long distance, which allows them to access organic matter and secrete extracellular enzymes to break down fine particles more easily. carbon. Unlike fungi, bacteria usually work in groups to break down fine particles, due to the limited enzymes produced by each bacterial species.

Finally, MPCs are further broken down within microbial cells by intracellular enzymes in the mitochondria for fungi or in the cytosol for bacteria and archaea. Through cellular respiration, microbes break down substrate carbon molecules and capture energy for biosynthesis and nutrient transport. Many biochemical processes, such as the Krebs cycle, glycolysis, electron transport chain and hydrolysis, are involved in the metabolism of carbon, with the release of CO<sub>2</sub> as the final product of the processes.

#### 4.3.1.2. Radical breathing (RR)

Root respiration is one of the main factors contributing to the production of CO<sub>2</sub> in the soil and therefore one of the main factors influencing soil respiration (Raich & Tufekciogul, 2000). Radical respiration includes all the respiratory processes that occur in the rhizosphere according to the definition of Wiant (1967), according to which "root respiration includes all respiration derived from organic compounds from plants, including respiration of live root tissues, respiration of mycorrhizal symbiont fungi and associated microorganisms, and the decomposing organisms that operate on root exudates and recent dead root tissues in the rhizosphere".



Root Contribution (RC), which is the relationship between root respiration and soil respiration, varies considerably between different ecosystems. Hanson et al. (2000) examined methods of dividing SR into different biomes and ecosystems and found that the mean RC with respect to total respiration was 45.8% and 60.4%, respectively, in forest and non-forest ecosystems, but the CR ranged from 10% to 90% in different studies. Subke et al. (2006) updated the Hanson et al. Database. (2000) and found that the RC was 2-97% in coniferous forests, 4-73% in hardwood forests, 10-75% in grasslands, 14-73% in cultivated land and 53- 75% in shrubs. CR has shown a great variability even within the same ecosystem, due to the

#### **4.3.1.3. Soil fauna respiration (FR) and non-biological CO<sub>2</sub> production (NR)**

Soil fauna respiration (FR) refers to the CO<sub>2</sub> produced by soil animals through metabolic processes. In general, RF represents less than 5% of total soil respiration (Petersen & Luxton, 1982). However, in some ecosystems, RF can represent up to 10% of the SR (Sorensen et al., 2006). Non-biological CO<sub>2</sub> production (NR) is the emission of CO<sub>2</sub> at the soil surface resulting from physical and / or chemical processes in the soil. Physical CO<sub>2</sub> production is rare under normal conditions and generally occurs when soil porosity is altered by rain storms (Tang et al., 2003), groundwater fluctuation, soil compression or wind (Xu et al., 2004a). The chemical production or absorption of CO<sub>2</sub> in the soil refers to pedochemical and geological processes, such as geothermal and volcanic degassing of CO<sub>2</sub> (Werner & Brantley, 2003), photochemical degradation of litter (Austin & Vivanco, 2006) and absorption of CO<sub>2</sub> by alkaline soils (Xie et al., 2009). Under normal conditions, the non-organic production of CO<sub>2</sub> is negligible, but it could be significant in some particular ecosystems (Rey, 2015).

#### **4.3.1.4. Factors affecting soil respiration**

SR is determined by the quantity, quality and availability of SOC, which are the main substrates of HR. In the same ecosystem, HR is positively correlated with soil organic carbon content, because more C provides more sources and more reaction sites for microbial decomposition (Wang et al., 2013). In addition to the quantity of C, the quality of soil C is also important for determining the HR both physically and chemically.



Larger C particles have a smaller surface to volume (or mass) ratio and therefore have relatively smaller surface areas for contact with microbes and enzymes. Therefore, in theory, the smallest soil organic particles favor HR. In temperate or boreal climates, freezing and thawing of the soil in winter and spring help break down large soil organic particles into smaller pieces. Soil animals, such as earthworms, also facilitate the breakdown of organic soil particles (Bocock, 1964; Lee & Foster, 1991; Brussaard, 1998; Jennings & Watmough, 2015). The chemical composition of soil organic C, known as substrate quality, is also a determinant of HR (Rustad et al., 2000; Hartley & Ineson, 2008).

Small C molecules, such as glucose and cellobiose, can easily penetrate cell walls and microbial membranes. Large C molecules, such as starch, cellulose and lignin, are not penetrable by cell membranes and must be broken down into small molecules before they can be used by microbes. Therefore, microbes prefer labile C sources (small C molecules from fresh litters) over recalcitrant C sources, which are more resistant to decomposition and are usually composed of large C molecules. Furthermore, HR is also influenced by the availability of organic C in the soil (Ryan & Law, 2005). If organic C is absorbed or protected by soil particles, it is not available for microbes, making decomposition very difficult even if the C molecules are small. Therefore, the recalcitrant C of the soil is not necessarily composed of large C molecules.

#### *4.3.1.5. Oxygen content in the soil*

Under aerobic conditions, molecular oxygen is an ideal terminal electron acceptor in root respiration and soil microbial decomposition. The supply of oxygen is essential for the synthesis of ATP and the production of CO<sub>2</sub> in the breathing processes. Calhoun et al. (1993) found that oxygen supply can modify the efficiency of the electron transport chain through the use of different types of ubiquinol oxidase in prokaryotes. Under highly aerobic conditions, cells used an oxidase with a low affinity for oxygen, capable of carrying two protons for each electron. When the oxygen level drops, they switch to an oxidase that transfers only one proton per electron, but with a high affinity for oxygen. When the oxygen content in the soil is reduced to low levels, the metabolic pathway shifts from aerobic respiration to anaerobic fermentation and the products shift from CO<sub>2</sub> to



CH<sub>4</sub> (Bridgham & Richardson, 1992; Kane et al., 2013; Fan et al., 2014). Therefore, CO<sub>2</sub> efflux into the soil drastically decreases when soils are flooded, such as in wetlands (Krauss et al., 2012; Liu & Xu, 2015).

#### 4.3.1.6. Soil enzymes and microbes

All biochemical processes involved in SOC degradation require enzymes to catalyze chemical reactions. Major soil enzymes include amylase,  $\beta$ -glucosidase, cellulase, chitinase, dehydrogenase, phosphatase, protease and urease and are produced by various microbes (Dick & Tabatabai, 1984; Gupta et al., 1993; Makoi & Ndakidemi, 2008).

Although new DNA sequencing technologies have made it possible to investigate the composition of microbial communities (Torsvik & Øvreås, 2002; Cong et al., 2015; Hartmann et al., 2015; Pitombo et al., 2015), we have limited knowledge of the details of soil enzymes, such as quantities, types, distribution and efficiency, and little is known about individual microbial species and the corresponding enzymes produced. The importance of soil enzymes for ecosystem functions, including soil respiration, was examined by Makoi and Ndakidemi (2008). Soil communities and microbial activities are regulated by climate, oxygen supply, nutrient levels, pH and substrate availability (Zogg et al., 1997; Yao et al., 2000; Barnard et al., 2014; Zhalnina et al., 2015).

#### 4.3.1.7. The climate

Like many other biochemical reactions, soil respiration is temperature dependent. When the soil temperature is below 0 °C, the SR is very low due to the weak metabolic rate of the roots and microbes. Soil microbial activity has been reported at temperatures below -10 °C (Schroeter et al., 1994; Vorobyova et al., 1997; Rivkina et al., 2000). Soil respiration usually increases non-linearly with increasing soil temperature.

Soil moisture can affect soil respiration both directly and indirectly. Soil respiration is low in drought conditions and increases until it reaches its maximum at an intermediate humidity level, until it begins to decrease when the moisture content excludes oxygen. Soil moisture affects the diffusion of low water soluble substrates and high water oxygen diffusion, both of which can limit soil microbial respiration (Davidson et al., 2006).



Microbial activity depends on soil moisture and reaches maximum levels at water contents where the limiting effects of substrate diffusion and O<sub>2</sub> supply are equal (Skopp et al., 1990). Furthermore,

In short, soil moisture affects SR

1. reducing the mobility and availability of carbon; And
2. reducing microbial activities and therefore decreasing the production and mobility of enzymes (Barnard et al., 2014). Furthermore, the hydrolytic process to break the glycosidic bond in polysaccharides requires water molecules as reactants in both extracellular and intracellular decomposition.

#### 4.3.1.8. Vegetation and other factors

Vegetation affects SR primarily through its impact on root respiration (RR), litter production and root exudation. Vegetation can also influence SR through photosynthesis of the canopy above ground, which is the source of root exudates (Högberg et al., 2001; Tang et al., 2005a; Mencuccini & Hölttä, 2010).

The respiration of the rhizosphere (ZR) strongly depends on the contribution of carbohydrates from photosynthesis, which also depends on physiological and morphological factors of the plant, as well as on the climate. Therefore, ZR is probably the most dynamic component of SR due to rapid changes in climate and vegetation growth.

Vegetation can also affect SR through litter fall (both above ground and root), which is the main source of SOC.

Human activities, such as land use change, crops, fertilization and irrigation, significantly affect soil respiration. When soils are disturbed by cultivation, their organic matter content decreases (Schlesinger & Andrews, 2000). This is due to the fact that cultivation improves soil aeration and moisture, which leads to higher soil respiration rates. Furthermore, the supply of fresh plant debris to the soil decreases when native vegetation is converted to agriculture. Cultivation also disrupts soil aggregates, exposing stable, adsorbed organic matter to decomposition (Elliott, 1986; Six et al., 1998).



Other factors, such as soil properties, fires (Wüthrich et al., 2002) and pollution (Ilangovan & Vivekanandan, 1992; Liang et al., 2013) can also influence SR through their impact on carbon availability in the soil, nutrient supply, microclimate and microbial activities (Boshoff et al., 2014).

#### 4.3.1.9. Soil respiration measurements

The chamber method has been widely used for SR measurement. During the measurement, a chamber is placed on the soil surface and the CO<sub>2</sub> molecules emitted from the soil are trapped inside the chamber. The first measurements of SR used the chemical absorption (CA) method, in which the respired CO<sub>2</sub> molecules were absorbed by alkaline solutions and determined by titration (Norman et al., 1992). With the advent of the infrared gas analyzer (IRGA) in the 1970s, the accuracy of SR measurements improved significantly (Norman et al., 1992; Pongracic et al., 1997; Davidson et al., 2002a).

Collars are often made of PVC pipes and have a height of approximately 4-10cm. The collar is inserted into the ground vertically (parallel to the gravitational field) approximately 1-2 cm from the ground surface. When measuring SR on slopes, higher collars are required to ensure that the underside of the collar can be inserted deep enough into the ground. The vertical installation avoids having to correct the measured SR flow and minimizes the effects of the collar wall on the alteration of water movement in the ground during rain events. However, tall collars have other problems.

Measuring SR on steep slopes requires higher ground collars, with most of the collar embedded in the ground, especially on the upper side of the slope. According to the molecular diffusion theory, the diffusion direction of the CO<sub>2</sub> molecules in the soil should be perpendicular to the slope surface to minimize the path length and thus the resistance to diffusion. When the collar is inserted vertically into the soil, the top side of the collar wall will block the CO<sub>2</sub> molecules moving upward from deeper soils.



## 4.4. Soil enzymes

### Glucosidase ( $\alpha$ , $\beta$ ) Galactosidase ( $\alpha$ , $\beta$ )

Glucosidases and galactosidases are widely present in soil and represent key enzymes in the carbon cycle. They hydrolyze the organic substance by releasing glycosidic residues (glucose or galactose) used by soil microorganisms as an energy source. The activity, which in many soils was significantly correlated with agronomic practices, is to be considered an excellent index for evaluating production performance and the degree of evolution of the soils.

### Urease

This enzyme catalyzes the hydrolysis reaction of urea into carbon dioxide and ammonia. Urease is ubiquitous in nature and has been found in microorganisms, plants and animals. The enzymatic dosage of urease, which is important for evaluating the effects produced on the nitrogen cycle in the soil following the burial of vegetable biomass or organic, animal or vegetable amendments, may be altered following fertilization interventions with ammonia products.

### Amidase and Protease

Amidases and proteases are important enzymes of the nitrogen cycle in soil. They promote the degradation of proteins by hydrolysis of the CN bond releasing nitrogen in the ammonia form. They are widely distributed in soil, plants, yeasts and fungi. They represent a very useful index of the evolution of organic matter in the soil. The amidasic activity is correlated with that of  $\beta$ -glucosidase.

### Phosphatase

Phosphatase activities participate in the phosphorus cycle as they catalyze the release of phosphate (phosphomonoesterase) or pyrophosphate (phosphodiesterase) from organic matrices, making the element available for the mineral nutrition of plants. Phosphomonoesterases are classified into acidic and basic based on their optimum activity in response to soil pH. Phosphatase activity is considered a good index to evaluate the mineralization potential of organic phosphorus. The addition of phosphatic mineral fertilizers significantly depresses the phosphatase activity.



## **Dehydrogenase**

Dehydrogenases are a class of enzymes common to most microorganisms, with a predominantly intracellular localization. They are studied as they participate in the evolution of the organic substance of the soil, in the degradation of lignin, in the synthesis of humus, in the degradation of xenobiotics. However, the dehydrogenase activity is not correlated with other important biological activities of the soil such as: oxygen consumption, evolution of carbon dioxide and microbial biomass. Furthermore, the presence of extracellular phenol oxidase and the possibility of dehydrogenase-like abiotic reactions catalyzed by metal surfaces present in the soil, can easily produce overestimated readings of this enzymatic activity.

## **Arylsulfatase**

Sulfatases mobilize the sulphate contained in 40-70% of the total in the soil organic pool in the form of esters. Sulfatases are considered potential indicators of the effects produced by agronomic practices.

## **FDA-hydrolase**

The hydrolysis of fluorescein diacetate (FDA) summarizes the hydrolytic activity of numerous enzymes such as: protease, lipase, esterase. It is related to the hydrolytic demolition activities of fungi and bacteria. In this sense, the activity of the FDA-hydrolase represents an overall index of the potential for the release of inorganic nutrients from organic matrices.





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