



UNIVERSITÀ
DEGLI STUDI
DI PADOVA

UNIVERSITY OF PADOVA

**Department of Agronomy Animal Foods Natural Resources
and Environment (DAFNAE)**

**PHD SCHOOL IN ANIMAL AND FOOD SCIENCE
Course : Agro Food Productions
Cycle : XXVI**

Composting in viticulture: effects on microbial activity and soil fertility

**Head of Doctorate School: Professor Martino Cassandro
Coordinator: Professor Viviana Corich
Supervisor: Professor Giuseppe Concheri**

PhD student : Fabio Stellin

To my Dad ...

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SUMMARY

The soil carbon improves the physical and chemical properties of soil and it widely accepted that the carbon content in the soil is an important factor in the overall health of the soil and crops. In Italy and especially in Veneto region the loss of soil organic matter is a major problem for all sectors of agriculture and certainly also for the wine business. In order to restore soil organic matter has been proposed in this work a model of sustainable management for the re-use of plants and animals biomass (cattle manure, vine branches and grape marc) derived from agricultural practices. In particular, the research has focused on the study of the effects caused by the distribution of composted soil on the major aspects of production and quality of two vineyards, both located in the Conegliano-Valdobbiadene D.O.C.G. zone. The characterization of composted soil improver has been obtained by the study of the evolution of some microbial groups active in fermentation processes and monitoring the changes in temperature, moisture, pH, carbon content, nitrogen, sulfur and heavy metals. The introduction of some innovative indices such as the capacity of the biodegradable organic substance evaluated by the degradation of cotton and silk wires inserted in the soil has allowed the diagnosis of the nutritional status of vine. The variables studied were subjected to one-way analysis of variance (ANOVA) using the statistical software "STATISTICA 12" identifying in some cases large and significant differences ($p < 0.05$).

The treated vines showed an improvement in productivity and quality aspects of the grapes due to the contribution both of compost manure both of prunings residues and grape marc, with some variability related to the amount distributed and the method of distribution in the field. This improvement is certainly consequent on an increased biological fertility

soil shown by a greater degradation of cotton and silk wires relating to the treated thesis compared to untreated control. ARISA analysis performed on samples of mature compost also showed unexpected and important processes of selection of useful microflora (bacteria and fungi).

In conclusion, the compost has proved an excellent soil improver capable of restoring the soil fertility of the vineyards and improve the nutritional status of the vines.

Key words: soil, organic matter, compost, vineyard, grape quality

RIASSUNTO

Il carbonio del suolo migliora le proprietà fisiche e chimiche del terreno ed è ampiamente accettato che il contenuto di carbonio è un fattore importante nella salute generale del terreno e delle colture. In Italia e soprattutto in Veneto la perdita di materia organica del suolo è un problema importante per tutti i settori dell'agricoltura e sicuramente anche in viticoltura. Al fine di ripristinare la materia organica del suolo è stato proposto in questo lavoro un modello di gestione sostenibile per il riutilizzo di biomasse di origine animale e vegetale (letame bovino, tralci di vite e vinacce) derivate dalle pratiche agricole. In particolare, l'attività di ricerca si è concentrata sullo studio degli effetti determinati dalla distribuzione di ammendante compostato sui principali aspetti della produzione e della qualità di due vigneti, entrambi situati nella zona D.O.C.G. di Conegliano-Valdobbiadene. La caratterizzazione del materiale compostato è stata ottenuta mediante lo studio dell'evoluzione di alcuni gruppi microbici attivi nei processi di fermentazione e il monitoraggio delle variazioni di temperatura, umidità, pH, contenuto di carbonio, azoto, zolfo e metalli pesanti. L'introduzione di alcuni indici innovativi come la capacità biodegradativa della sostanza organica valutata tramite la degradazione di fili di cotone e seta inseriti nel terreno ha permesso la diagnosi dello stato nutrizionale della vite. Le variabili studiate sono state sottoposte ad analisi della varianza ad una via (one-way ANOVA) utilizzando il software statistico "STATISTICA 12" ottenendo in alcuni casi ampie e significative differenze ($p < 0.05$).

Le viti trattate hanno mostrato un miglioramento della produttività e degli aspetti qualitativi delle uve grazie al contributo sia di compost da letame sia di compost da sarmenti e vinaccia, con una certa variabilità

correlata alla quantità distribuita e al metodo di distribuzione in campo. Questo miglioramento è certamente conseguente ad un aumento della fertilità biologica del terreno mostrato da una maggiore degradazione dei fili di cotone e seta relativi alle tesi trattate rispetto al controllo non trattato. L'Analisi ARISA effettuata su campioni di compost maturo ha anche mostrato inaspettati e importanti processi di selezione della microflora utile (batteri e funghi).

In conclusione, il compost si è rivelato un eccellente ammendante in grado di ripristinare la fertilità dei suoli vitati e di migliorare lo stato nutrizionale della vite.

Parole chiave : suolo, materia organica, compost, vigneto, qualità dell'uva

Chapter 1

GENERAL INTRODUCTION

1.1 Background

The global economic crisis originated in the financial sector, is affecting many production activities, including the wine sector. The Italian wine industry is characterized by a sharp decline in individual consumption while increasing competition from new foreign countries. In addition, the consumer has been directed toward the use of higher quality wines by limiting the amount consumed. Therefore, the objectives to be pursued are to reduce the production costs, to focus on the quality and uniqueness of the productions offering wines at competitive prices. These goals can be achieved by improving the understanding of the complex interactions between soil and plant.

This will make it possible to identify the tools that can be better used to maximize the quality of grapes and wine, and the most appropriate strategies for disseminating knowledge about Italian wines in the rest of the world, enhancing the territory of origin and the landscape.

1.2 Introduction and spread of the vine (*Vitis vinifera* L.) in Italy

1.2.1 The plant of grapevine

The grape plants belong to the genus *Vitis* L. of the family Vitaceae. Almost all of the vine varieties belonging to *Vitis vinifera* L., native of the Mediterranean and the Near East.

Originally the *Vitis vinifera*, spread from Europe to Asia during the Pleistocene glaciations, took refuge in the territories of the Mediterranean basin and in the Asian territories that today correspond

to Armenia, Georgia and Iran. Growing up in very different environmental conditions, the vine has diversified itself, giving rise to two subspecies: *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi, Europe, and *Vitis vinifera* L. subsp. *sativa* Hegi (*V. vinifera* Caucasian Vavilov) in the East.

1.2.2 Origins and spread of the vine cultivation

According to many scholars, the cultivation of *Vitis vinifera sativa* for winemaking dates back at least 4000 years before Christ. It can be assumed that the first cultivation was the center of the area located around Mount Ararat in the Caucasus, the mountain where the Bible says that Noah's Ark ran aground.

The cultivation of the vine would spread along three routes. The oldest way is the one that goes from Mount Ararat through Mesopotamia and Egypt to Greece, under the influence of various nations, while according to some authors, the vine would instead arrived in Greece through Anatolia. The second route starts from Greece and arrive to the Magna Grecia (Sicily, southern Italy), France (Marseille) and in Spain, under the influence of the Greeks and Phoenicians. The third route goes from France to northern Europe, especially through the Rhone, the Rhine and the Danube, under the Roman influence.

1.2.3 The vine in the ancient Greece

The first documents on grape growing and wine production can be dated to the late third millennium (2300 B.C.). The findings of the surface in the basement of Godin Tepe in western Iran show that the wine was produced in the Near East since the middle of third millennium. From this time the words grapes, dried grapes and wine are

always more numerous in Mesopotamian cuneiform texts (Mc Govern & Michel, 1995).

A few centuries later (1800 B.C.) similar citations appear in the texts found at Alalah and Mari (now Syria). Between the fourteenth and thirteenth centuries there are numerous literary quotations from the Canaanite city-states (now Palestine) ... *“drink a glass of wine, in the golden cup the blood of the vine.* The rich Mesopotamian and Egyptian iconography illustrates the different aspects of the grape harvest, winemaking and wine consumption, highlighting the elitist character of this ritual and consumption.

Another ancient culture dedicated to the cultivation of wine grapes was the Mycenaean (II millennium B.C.), to which was attributed the first major commercial movement to the West. The iconographic documentation (the crater of Enkomi) and literary (the number of claims in the Odyssey), as well as that archaeological (the finds of Mycenaean pottery), show the attendance of Mycenaean and fast ships for the transport of wine (Od. IX 151 -171, Od. IX, 194-213) in the last quarter of the twelfth century along the Italian coast of the Adriatic, the Tyrrhenian Sea and Sicily.

The second millennium ends in the East with a serious collapse of the city-state, called by historians *“the crisis of 1200”*. The wine trade resumes with the Phoenicians and coincides with the resumption of the mercantile city of Tyre (IX-VIII century B.C.). The Phoenician amphorae, very similar to those Canaanite, are always present in archaeological finds of the western seas.

During the ninth century B.C., the Greeks began to slowly enter in the wine trade as a result of frequent contacts who settled in that period with the coasts of Asia Minor, Syria and the Caucasus region. The activity started in the Aegean Sea, was then developed in the Ionian

Sea and the Tyrrhenian sea, often along the routes followed by the Phoenicians who traded wine for religious or elite use.

The myth of the wine spreads so even in the West, thanks to the Etruscans, as evidenced by the numerous finds of “*kylix*”, cups for consumption of wine, especially in symposia devoted to Dionysus .

The production and the use of wine in southern peninsular Italy and Sicily were documented during the late Minoan and Mycenaean age. The period of Greek colonization in the West, where there was the spread of the myth of the wine can be dated from the eighth to the sixth century B.C., precisely between 750 B.C. and 540 B.C., the alleged date of the founding of Cuma and Elea.

1.2.4 The wines of Greece and their introduction in Italy

The vineyards of Magna Grecia have developed in a very long time (about 500 years), during which they spread some varieties, such as *Aminee* or *Biblino*, and have established the winemaking techniques of Greek origin. So the spread of precious vines of oriental origin, forms of cultivation with low strain and the pruning of the South of Italy can be attributed to Ancient Greece.

The wines of the Greeks, traded throughout the Mediterranean basin, distinguished mainly by the area of origin, the islands of the Aegean, in particular that stood out for the high specialization of culture (Chios, Lesbos, Thasos) and the Crete island or coastal areas, such as the Chalkidiki peninsula . The best greek wine was sweet for the withering of the grapes harvested late, and then very alcoholic. It was then diluted with water consumed, including sea and embellished with various herbs and spices. The most famous Greek wines were produced with grapes Byblinos, whose name literally means "vine that twists or vine that clings" came from the Aegean Sea and eastern Europe. and

rappresenatnti. Byblinos the vine was one of the first to be introduced in ancient Greece and with its grapes the wine of the same name was produced in various places in Sicily (Syracuse, Gela) and Campania (Vandermesch, 1994).

Other famous wines were the *Lagaritanos* (sweet and delicate, recommended medicine) product on Lagaria hills of Capo Spulico, not far from the Grumentum city, the *Thourinos*, and the *Murgentinum* that was produced in Morgantina (ancient Greek and Sicilian city, archaeological site in the Aidone territory, Italian town in the Enna province, in Sicily) then circulated in Campania. A special mention deserves the *Capnios* vine known since the IV - III century B.C. and introduced by Greece to Sybaris, from which was obtained the wine of the same name.

1.2.5 The Etruscans and the native Italian vine

The vine has been cultivated in Italy before the Greek colonization, especially in places of Etruscan expansion (Forni, 1996), and was the result of the domestication of wild vines in lowland forests.

In the period before the Greek colonization, the vine was known and appreciated by indigenous peoples Italian, paleo-Ligurian and of the Po Valley. The Etruscans cultivated *V. vinifera sylvestris* since the eighth century B.C., before the Greeks and then the Romans spread it the Italian *V. vinifera sativa* with its many varieties. The Etruscan wines have become the subject of export from the coastal regions of Tuscany, Lazio and Campania to the southern Gaul and Catalonia, as demonstrated by the findings of the characteristics Etruscan amphorae typical of the period from the seventh century until the beginning of the fifth century B.C. (Ridgway , 1992). During the period in which they had

to co-exist in Italy the Greek and Etruscan civilizations, between the two there was almost a “hidden frontier”.

Among the diversity there was also the choice of grape varieties and the cultivation methods of the vine. Significant in this regard is the coincidence between the area of dissemination and cultivation of the vine to support live with the area of maximum Etruscan expansion, not only in the region of northern Italy, but also in Campania (Scienza, 2000). Probably the vines are descendants of the wild vines domesticated by the Etruscans, starting from *V. vinifera sylvestris*.

1.2.6 The vine in ancient Rome

In the first half of the second century B.C., according to Cato (*De Agriculture*), the vineyard was already on the top of the crops list. The vineyard, as well as the olive grove, were present in small family gardens, although a part of the wine-growing was widespread in mixed farming areas, where the vines were grown in intercropping with cereals, figs, olive trees.

The system of vine cultivation were different, but the tree and support died have continued to prevail in most of the South Italy and in Liguria, while others system of vine cultivation as live support prevailed in the Po Valley and in the Capua plains. The greatest merit of the Roman scholars is represented by their descriptions of vine varieties grown and of organoleptic characteristics of the produced wines. The authors of this period (Polybius, Virgil, Columella), which continued until the fall of the Roman Empire (400 A.D.), have helped to define the cultivation techniques of grapes, that have been used practically until 1700. The simplest classification was that which divided the varieties into two major categories: table grapes (*ad mensam, ad edendum, cibaria, suburbanae*) and wine grapes (*ad Bibendum, ad vindemias*).

In Roman times the concentration of the elite vineyards in Campania is unmatched in the rest of the peninsula. The cultivated varieties in this area are described in the *Historia naturalis* (Book IV) of Plinio and in *De re rustica* of Columella and classified into the following three classes:

- varieties of highest quality, the noble grape varieties that give the wines of the most famous vineyards (*Amineae* and *Nomentanae*); Plinio within this group identifies the indigenous grape varieties and imported varieties
- varieties that combine good productivity to a decent quality (*Murgentina minor*, *Argitis*, *Graecula*)
- very productive variety, but of poor quality (*Scirpula*, *Horconia*).

Some of the varieties grown today are largely considered to be direct descendants of varieties of Ancient Rome (Scienza, 2000) (Table 1.1).

Vines cultivated in Roman times	Existing varieties
Aminee	Greci di Posillipo, Chasselas
Aminea lanata	Pinot meunier, Riesling renano
Aminea gemella	Greco di Tufo, Riesling renano
Apianae	Moscato
Albuelis	Gaglioppa, Elbling
Basilica	Cocolubis, Picardaut
Pergulana	Uva Rota di Napoli
Biturica	Cabernet, Genouillet, Gamay
Helvolae	Pinot grigio, Ribolla
Pretia	Chasselas laciniè
Allobrogicae	Mondeuse, Syrah, Nebbiolo
Alopecia	Coda di Volpe
Conseminea	Lanaiole
Helvenaciae	Pinots
Oleagina o Tiburtina	Olivetta o Olivella
Carbonica	Cabernet

Table 1.1 - Correspondences between varieties described by the Latins, and some varieties currently grown

1.2.7 The vine from the Middle Ages to the 19th century

Since ancient times, for many centuries in Italy were grown old and new cultivars of *V. vinifera sativa* and *V. vinifera sylvestris*, using techniques and tools now completely abandoned.

Between 1347 and 1353 the Black Death swept across Europe, killing at least one third of the continental population and depopulating the Italian countryside. In the absence of the labor force much of the wine-growing was abandoned and only the recovery, the vineyards planted before the epidemic were replaced by highly productive vines, but of poor quality. For this reason, among the wealthier classes spread the fame of the wines from Greece.

These wines were scattered on the tables of the nobility and clergy through the importation of Venetian merchants. As a result, began importing of some vines, or of the Greek wine-making technique, to the point that by the name of "greek" were also referred to the local varieties that were suitable to be bred like Greek vineyards.

Around the mid-nineteenth century, the European vineyards began to be increasingly attacked by fungal pathogens, including powdery mildew (*Uncinula necator* Burr., Powdery Mildew *tuckeri* Berk.) .

Therefore, in subsequent decades, in particular through the work of the French, some vines selected for their resistance to disease of fungal origin have been imported from direct grapes producers. The introduction and dissemination of new American varieties, however not very productive, unfortunately made the deployment in Europe of a new disease, phylloxera (*Phylloxera vastatrix* Planchon) caused by a tiny insect, an aphid approximately 1 mm in length, greenish yellow, which destroys the roots of European vines .

The presence of phylloxera has been reported in Italy for the first time in 1877, while two years later (1879) added another pathogen is widely

known among growers, downy mildew (*Plasmopara viticola* Berl. Et De Toni), also this of american origin (Goidanich , 1964). All this has caused the death of most of the Italian crops of *V. vinifera sylvestris* and *V. vinifera sativa*. A few years later, however, were identified other species of the genus *Vitis*, also of american origin that proved particularly resistant to pest attacks of phylloxera. Thus began importing from America and spread to Europe from other species , including *Vitis berlandieri* Planch , *Vitis riparia* Michx and *Vitis rupestris* Scheele, who had a root system able to withstand the parasite attacks (Traverso, 1926) , but at the same time were not very productive. For this reason, it was started a work of grafting of all Italian cultivars on American rootstock obtaining varieties characterized by resistance to phylloxera typical of the American species and the attitude to the production of European vines. From America was also imported *Vitis labrusca* L. (Saccardo, 1971), which, crossed with *Vitis vinifera*, has produced cultivars used for the production of fruit grapes and wine grapes. It is particularly resistant to pest attacks of phylloxera, powdery mildew and downy mildew and for these characteristics, it is not grafted. There are several varieties, the most common are called strawberry grape (white and black).

Currently Italy is the world's largest producer of wine, with an average annual production of 60 million hectoliters and 18 million hectoliters per year, mainly exported to Germany, to the United Kingdom and to the United States of America. Most Italian wine is produced in Tuscany, Piedmont, Veneto, Puglia, Sicily, Emilia Romagna and regions where there are intensive production. In recent years, mainly thanks to the activities of some important wineries, the attention is directed towards the expansion of areas for the cultivation of the vine of merit and accordingly increased the production of wines with denomination of origin, at the expense of rampant production of table wines. Since 1980,

the D.O.C. wines were up 19% and increased the amount of wine sold in bottles compared to the direct selling in barrels or carboys.

1.3 Conegliano - Valdobbiadene D.O.C.G. zone

1.3.1 History and Description

The viticulture in the Conegliano hills is present since the Iron Age (first millennium B.C.), thanks to the first domestication of the vine by Paleoveneti, but it was the arrival of the Romans to favor the appearance of the first vineyards ordered. Subsequently, the Benedictine monastic orders developed the progress of viticulture in the hillside with the first religious groupings around the fortified castles of Ceneda, Serravalle, Conegliano and Susegana. Among the finest examples of this settlement there is the famous Abbey of Santa Maria in Follina and the Camaldolese monastery of Rua Feletto.

The vocation to the production of white wines in the Conegliano hills is documented in particular by "*Statuti Coneglianesi*" (1282). The transition of this area under the control of the Republic of Venice enhances the reputation of the wines of Conegliano hills.

The grape varieties grown in the area, including the Marzemino, are cited by Giacomos Agostinetti in "Cento e dieci ricordi che formano il buon fattore di Villa" of 1679. After the freeze of 1709, to respond to the difficult situation in Conegliano was founded in 1769 the Academy of Agriculture to improve agricultural and vines techniques. In particular, the investigations have been carried out on the land vocation for the vines cultivation, such as Prosecco (today Glera) and Marzemino.

The tradition and interest in wine sphere, buyed new enthusiasm and vigor with the founding in 1869 of the Treviso Wine Company, but it is

only after the publication of the book "*La vite e il vino in Provincia di Treviso*" by Vianello and Carpenè that born a modern viticulture in the Conegliano. The culture of wine growing and wine making in this region takes on greater importance with the creation in 1876 in Conegliano of the first School of Viticulture and Enology of Italy, from which in 1923 it developed, the first Experimental Station of Viticulture and Enology, even today seat of reference for the research and experimentation in viticulture for the Italian Ministry of Agriculture.

The twentieth century is a period of great interest for the wine and respected scholars, originating of these hills, including G.B. Cerletti, A. Carpenè, A. Caccianiga, deepen the research on value varieties, such as Prosecco (Glera), Riesling, Marzemino, but also on the international varieties like Cabernet Franc, Cabernet Sauvignon and Merlot that in these places find the soil and climate suitable for winemaking that celebrates the uniqueness of the area. Immediately after the second world war Professor Luigi Manzoni, headmaster of the School of Viticulture and Enology, with the work of selection and crossing accomplished in more than two decades of studies was able to create a heritage cultural priceless. The clear testimony is contained in the historical sequence of artificial insemination that led to the selection of vines 6.0.13 (Riesling x Pinot Blanc) white grape and 2.15 (x Prosecco Cabernet Sauvignon) black berry, made up of numerous tests in the laboratory and in the field, to derive the variety that they could settle and acclimatise perfectly in this range.

The success in national and international field of wine of Conegliano hills, has led to the obtaining of the D.O.C. in 1993 (Ministerial Decree of August 3, 1993) and in 2011 the recognition D.O.C.G. (Ministerial Decree of September 14, 2011).

1.3.2 Geomorphological characteristics of the area

The territory of the D.O.C.G. is part of a geomorphological system consists of gentle hills in Eastern Veneto. From this area the Belluno Dolomites are just a few kilometers to the north, while Venice only a few tens of kilometers to the south. These ridges run through the area from east to west of high Treviso Province, enclosing an area with a large wine growing, for white grapes and also for black grapes.

The two chains, Alpine and pre-Alpine, form a natural barrier to entry of cold air currents, while the discrete proximity to the sea allows a mitigation of the climate that can be described as temperate, favoring the establishment of viticulture in ancient history and tradition . The hilly provision allows a vineyards ideal arrangement for maximum solar radiation and high temperature ranges. The soils are derived from the processes of formation of the Alpine chain which caused the lifting of that in the Cretaceous was a marine platform. The rocks have been eroded once emerged, from the atmospheric elements and also from a series of glacial sequences that have shaped drawing pads visible today and stratified soils that make up the hilly soils. These consist mainly of alluvial deposits, fluvial or lacustrine, but with a great skeleton, due in large part to the presence of glacial moraine profile. This structure allows the land drainage of rainwater falling abundant in this area and allow the vines to withstand the hot summers. The winds, especially in the northern part of the Conegliano hills favor the thermal inversion, on the one hand very good for the quality of the grapes, on the other hand to promote drying of the vines that defend themselves in a natural way by fungal diseases. Precisely for this particular climate, Refrontolo and Fregona are known as places where the currents of fresh and dry air allow to obtain the natural drying of the grapes from which are derived the two finest raisin wines.

1.3.3 Climate of D.O.C.G. Conegliano Valdobbiadene zone

The wine-growing area between the Conegliano hills and Valdobbiadene show climatic characters related to warm temperate values and belonging to the mesothermic climates. The D.O.C.G. covers an area of approximately 3.600 ha in the hills between Conegliano and Valdobbiadene, on the sunny slopes, located at an altitude between 50 and 100 m above sea level and annually produces an average of 400 thousand tons of grapes. The slopes are east-west direction and come up from the plains to the Pre-Alps. They distance themselves equally by the Dolomites, which provide them with protection and the Adriatic Sea.

Regarding temperatures, the annual average is 16,1 °C with values of more than 23 °C in summer and values lower than 2 °C in the winter months. The coldest month is January with average monthly temperatures of 4 °C and minimum values around an average of 0 °C.

Temperatures will reach the highest values in July and August with monthly mean values around 24-25 °C. In these two summer months, maximum temperatures are found with higher values that often exceed 30 °C, reaching, on hot days, the threshold of 37 °C. At the end of the summer, the area is characterized by large temperature variations between day (average maximum temperature: 29,9 °C) and night (average minimum temperature: 14,9 °C), allowing the development of aromatic substances in grapes during ripening.

The average annual rainfall is around 1250 mm: the wettest months are those autumn (October and November) during which rain falls on a third of the annual total. Even in the spring (March and April) the precipitation are good while they are scarce in the winter months. In percentage terms, the rainfall is broken down by 50% in the three autumn months,

25% during the spring, 15% in the months of July-August and the remaining 10% in the winter months.

The other elements that contribute to this climate are exposure to south of the most part of the hills territory and of the larger valleys, reddish-brown coloring of the land. All of these elements, in the well-exposed hillsides, promote during the winter a good absorption of solar radiation and the heat returned during the night mitigates the surrounding atmosphere to avoid cold damage to plants. From the point of view of water in the hills there are numerous sources that have a flow-sensitive even in seasons with low rainfall.

1.3.4 Vine cultivation in the D.O.C.G. Conegliano Valdobbiadene zone

The territory of the D.O.C.G. encloses an area that has a great attitude to the vine cultivation, for the production of white and red wines.

The wines D.O.C.G. of Conegliano hills are produced in a twenty municipalities in the eastern foothills of Treviso. The white and red wines are produced in the municipalities of Conegliano, Susegana, Pieve di Soligo, Farra di Soligo, Refrontolo, San Pietro di Feletto, Miane, Follina, Cison di Valmarino, Revine Lago, Tarzo, Vittorio Veneto, Fregona, Sarmede, Cappella Maggiore, Cordignano, Colle Umberto, San Fior, San Vendemiano e Vidor. The production of Refrontolo is limited to the municipalities of Refrontolo, Pieve di Soligo and San Pietro di Feletto and that of Torchiato and Fregona to the municipalities of Fregona, Sarmede e Cappella Maggiore.

The white wine is produced from grapes of the Manzoni Bianco, which must be present for at least 30%, to which are added Pinot Bianco and / or Chardonnay, not less than 30%, with possible use of grapes obtained with Sauvignon and Riesling varieties, up to a maximum of 10%. The red wine must be composed of Cabernet Franc, Cabernet Sauvignon,

Merlot and Marzemino - present for at least 10% for each grape variety, with Merlot which may not exceed 40% - to which can be added, to the extent maximum of 20%, the variety Manzoni 2:15 and / or Refosco. The Refrontolo wine is obtained for the 95% minimum Marzemino, while the Torchiato of Fregona is obtained from grapes Glera - at least 30% - Verdiso - at least 20% - and Boschera - a minimum of 25% - with eventual addition, not more than 15% of other non-aromatic white grape varieties suitable for cultivation and in the Treviso area.

One of the most important native grape varieties grown in the area D.O.C.G. Conegliano - Valdobbiadene is the Glera. According to some authors, the origin of this variety was already known in the days of the Roman Empire under the name of Pucino, from which was obtained a wine particularly appreciated by the Empress Livia Augusta.

The main ampelographic and phenological characteristics of the Glera vine are the following (Fregoni M., 2009) :

Bud: top bud expanded, white, cottony with slight shades of pink

Leaf: adult large, pentagonal, three-lobed

Bunch: medium-large, elongated pyramidal, winged (wings), sparse

Berry: medium, spheroidal with waxy skin, thin but fairly consistent, golden yellow, slightly speckled

Budding: early

Flowering: early

Veraison: mid-late

Ripening: mid-late

This vine prefers hilly soils, not too dry in areas protected against spring frosts and is grown mainly in espalier, in particular to "Capuccina" and "Guyot" pruning a long winter. Because of the vegetative vigor also needs pruning summer, but it is generally not very resistant to spring frosts. It has a certain sensitivity to the millerandage and dripping in vintage unfavorable. It resists acid rot, and has a higher sensitivity to

powdery mildew and downy mildew. Among the parasites are to be reported mites, leafhoppers, and moths. The wines it produces are pale yellow in color, fresh, fruity and floral, balanced acidity, smooth and velvety.

The Glera is the grape that provides the basic structure of the Prosecco wine of Conegliano-Valdobbiadene, but Verdiso, Perera and Bianchetta, considered minor varieties can be used for a maximum of 15% in the production of Prosecco D.O.C. may, in some years and in some areas, contribute their specificity to maintain the organoleptic balance of the wine. The Verdiso is grown in the Conegliano area since 1700 and in the nineteenth century was already widespread with greater production than that of any other variety in the area. Used in the vinification of Prosecco to increase the acidity and flavor and balance the acidity in hot years. The Perera, varieties mentioned as cultivated in the Treviso area already in the nineteenth century, it was used in small quantities in Prosecco wine, especially in the Valdobbiadene area, in order to increase the aroma and flavor. The name is perhaps due to the particular flavor (pear) of the pulp or, more likely, to the berry shaped like a pear upside-down. The Bianchetta, mentioned as early as 500 and by some authors considered original of Treviso area, is used to "soften" the Prosecco especially in cold years as mature before: because of this is often grown in highest and most difficult areas with the Verdiso.

1.4 Physical and chemical properties of the soil and vine nutrition

The term "pedogenesis" literally means *soil formation* and it represents a very complex evolutionary process that, starting from the parent rock, originates a soil used by the vegetation as a support and as source of nutrients. The soil consists of three phases:

- Solid phase: includes minerals, organic matter and soil organisms;
- Liquid phase: it represented by the water present in the macro and micropores in which they are dissolved minerals;
- Gas phase: it represented by the air that is located in the soil interstices not occupied by water, much richer in CO₂ than the atmosphere.

The main factors that contribute to the soil formation process are :

- The nature of parent rock
- Climate
- Topography
- Living organisms
- Time

The main soil physical properties are:

- Texture: it represents the dimensional distribution of the components of the fine earth (diameter less than 2 mm) and is one of the more stable characters since it does not change with time;
- Porosity: it expresses the volume of the soil empty spaces as the percentage ratio of the total volume. This directly affects the dynamic properties of the soil liquid and gaseous phase and, indirectly, fertility;

- Structure: it is defined by the spatial arrangement of the soil solid particles and by the consequences that result from all possible combinations of their state of aggregation;
- Toughness: it is the result of the force that tends to unite together the soil particles. Therefore expresses the resistance that opposes the penetration of the soil working tools;
- Adhesiveness: it is the property that the soil particles to adhere to the surfaces of the working parts;
- Cohesion: it is the force with which the soil particles are bound together and oppose the posting;

The main chemical characteristics, however, are the following:

- Soil reaction (pH): the reaction of the soil, acidic, neutral or alkaline, is expressed by the pH value, is understood in terms of the chemical activity of hydrogen ions. The determination of pH is very important because it affects the physical, chemical, and biological availability of nutrients needed by plants;
- Cation Exchange Capacity (CEC): it is the amount of exchangeable ions, expressed as meq/100g of the soil, which can hold a heat exchanger for ion exchange. The ion exchange is the main mechanism by which the soil provides and retains the elements. Ca^{2+} , Mg^{2+} , K^+ and Na^+ adsorbed to clay minerals are replaced by other cations to vary the composition of the soil circulating solution;
- Degree of base saturation: it expresses the percentage of soil exchangeable bases (Ca^{2+} , Mg^{2+} , K^+ and Na^+) on the CSC;
- Buffer capacity: is the soil's ability to resist the change in pH after the addition of acids or bases;
- Total Limestone : it is the amount of all carbonates in the soil even if the analytical result is expressed as a% of CaCO_3 . It

consists of coarse elements and if abundant inhibits the Fe and P absorption making them insoluble;

- Active Limestone: it is the fraction of carbonates potentially present in the soil solution. it is made from fine limestone particles and reactive purposes that interact with the physiology of the radical. The knowledge of this value is important to the choice of rootstocks tolerant;
- Organic carbon: the content of soil organic carbon is closely related with that of the organic substance, even if the composition of the latter has a high degree of variability (0-5%). It contributes to the structural stability of the soils, helping to bind the particles into aggregates, increases the cation exchange capacity (CEC) and the and water-holding capacity. The soil organic matter, of which carbon is an important part, contains a good amount of nutrient and trace elements that are of great importance for the plants growth. It prevents the leaching of nutrients and form stable aggregates with Cu, Mn, Zn and other multivalent cations by improving the availability of minerals for plants. It represents an important buffer because protects the soil from strong changes in pH.
- Total nitrogen: this element in the soil is found in different forms but the plants absorb nitrate form NO_3^- , very mobile in soil (110cm/year), and the ammonia form NH_4^+ which is more adsorbed colloids and exchanged by other cations such as potassium. Ivaasky (1965) found that the NH_4^+ favor more than NO_3^- the shoots growth, the shoots weight and roots growth. Lafon et al. (1966) found that the most NO_3^- stimulates greater the bunches growth, increases the green color of the leaves and the content of amino acids in the leaf blade;

- C/N ratio: it expresses the trend of organic matter mineralization. In soils rich of organic matter well humified the relationship is between 8-12 while in the soil biologically less active and with slow mineralization the value is greater than 15;
- Sulfur: in the soil it is presents in organic form (amino acids, S-lipids, vitamins), inorganic form (pyrite, gypsum, chalcopyrite, anhydrite), adsorbed to soil colloids and in solution. It is absorbed as sulphate anion SO_4^{2-} and the higher speed of absorption is pH 6-7. The sulfur, once absorbed, is activated, reduced and incorporated into amino acids (methionine and cysteine) to form proteins.
- Phosphorus: in soil it is present in the form of orthophosphate, and it is found in different forms: organic (P-lipids, sugars, nucleic acids), inorganic (variscite, strengite, hydroxyapatite, fluoarapatite), in solution (liberated by enzymes) and adsorbed to colloids. It is absorbed by plants as HPO_4^{2-} ion in acidic pH and form H_2PO_4^- at alkaline pH.
- Potassium: it is present in 90-98% in the crystal lattices of primary minerals (feldspars, micas) and for 1-10% in secondary minerals (vermiculite, montmorillonite). Potassium is an essential element for life because it affects the quality characteristics: elevates the flavor and fragrance, improves the flavor and keeping qualities, increases the title sugar, vitamins B1 and C, but decreases the acidity (Melnick and Kossavewa, 1964).
- Calcium : it is found in igneous rocks (feldspar and plagioclase) and sedimentary ones (gypsum, calcite, dolomite). With increasing of soil acidity increases its availability but is also quickly lost to runoff (200-300 kg/ha/year). The excess of this element involves, on the grapevine, classical anomaly: chlorosis.

- Magnesium: it is contained in the iron-magnesium minerals (biotite, olivine) in the clay (illite, chlorite, vermiculite, montmorillonite) and in the form of $MgSO_4$, $MgCO_3$, $CaCO_3 \cdot MgCO_3$ (dolomite). Calcium favors the transport and accumulation of the products of synthesis, it activates the phosphatase, the ATP-ase and carboxylase, it is also the central atom of the chlorophyll molecule (25% of total Mg of the plant) and contributes to the maintenance of cellular turgor. It is very important the Mg/K ratio which must be between 2 and 5.
- Iron: it is present in ferromagnesian minerals (olivine, biotite, serpentine, augite), in silicates, in oxides or carbonates (hematite, siderite, magnetite) in clay minerals (illite) and in the form of hydroxide $Fe(OH)_3$. The iron is adsorbed by organic compounds, chelates, which keep it in solution, making it available for absorption by the roots. Its availability depends on the pH of the soil: with increasing of pH decreases the availability by a thousand times per pH. So normally in the basic soils occur a deficiency symptoms (yellowing internervale with the ribs that remain green).
- Manganese: it is present in iron-magnesium minerals, but especially in the oxides (manganite, braussite) and it is absorbed as Mn^{2+} . The availability decreases of a hundred times with increasing of pH so, like iron, there will be no shortage in basic pH. It is essential for the synthesis of carbohydrates, some vitamins, for the reduction of nitrate to NH_3 and is involved in the reaction of Hill. Contributes to higher production (favoring the fertility of the buds and fruit set), resistance to cold (promotes lignification of shoots), the density of the wort (which increases

the fructose), anticipates the maturation and reduces the acidity (Vnukova and Ryza, 1957; Miniberg, 1959 Rija, 1959).

- Zinc: it is present in iron-magnesium minerals (augite, biotite) in zinchite, smithsonite and sphalerite. As Zn^{2+} cation is absorbed and binds strongly to carboxylic groups present in the root (in fact 90% of the total absorbed zinc is bound to the cell walls). This element is important for cell permeability, intervenes in breathing, in the metabolism of sugars and proteins, stimulates the synthesis of auxin through the production of tryptophan, catalyzes the alcohol dehydrogenase and carbonic anhydrase.
- Copper: it is contained in silicates, carbonates and sulphates. It is absorbed as Cu_2^+ cation and it is the most immobile nutritious in the soil (in fact 98% of the copper in solution is bound to organic compounds). It is 'involved in various enzymatic activities such as Cu-Zn superoxide dismutase, ascorbate oxidase, laccase and fenolasi, is a component of plastocyanin, and finally it is important for the metabolism of proteins and indole acetic acid.
- Boron: it is absorbed in the form of boric acid (H_3BO_3) and it represents a very important micro-nutrient because it is a structural element of the cells walls and membranes, increases the pollen germinability, is involved in the transport of carbohydrates, promotes the synthesis of amino acids and proteins , enters the synthesis of β -indole acetic acid (Mc-Illroth Skok, 1958).
- Molybdenum: it is absorbed by plants as molybdate anion (MoO_4^{2-}) and can be complexed to sugars or amino acids. It is strongly adsorbed to colloids in acidic pH that is more available

to alkaline pH. It is important for higher plants because it enters into the constitution of nitrogenase and nitrate reductase.

- Chlorine: it is absorbed as a chloride anion Cl^- and is a very mobile element in the plant. It is important as it accompanies the potassium to counterbalance the positive charges, is located in the reaction of Hill, stimulates the ATP-ase of the tonoplast, is a strong competitor of nitrate and acts to maintain the osmotic cell turgor.
- Cobalt: it is essential for the synthesis of chlorophyll, can increase the alcoholic sugar and reduce the acidity, balancing the vegetative growth and enters into the constitution of several enzyme systems.
- Cadmium: if it is available in small doses it promotes the synthesis of fructose and vitamin C also stimulates enzyme activity.
- Chromium: if it is available in small doses has a positive effect on the weight and quality of production, the sugary title bud differentiation and fertilization, and it is also part of several enzyme systems (catalase, peroxidase, invertase).
- Vanadium: it increases the sugary title and the berries weight, it reduces the acidity of the must and in small doses is involved in oxidation-reduction potential.

1.5 Soil microbial communities

The soil is considered to be the matrix with the major source of genetic diversity per unit volume present in nature. The solid phase of the soil is composed of mineral particles such as sand, silt and clay, which mainly belong to the inorganic component, representing about 96-97% of the

solid phase. The remaining 3-4% affects the organic component, of which a small fraction (about 5%) is made from biomass, or from living organisms. This fraction is characterized by the presence of bacteria, actinomycetes, fungi, micro-algae, yeasts, protozoa, worms and arthropods. It is estimated that one gram of soil may contain more than one billion cells of microorganisms and probably thousands of different species (Knietsch et al., 2003).

Microorganisms perform very important functions in the soil and therefore are considered to be indicators of the quality of the matrix that contains them. Some of these functions relate to the formation and maintenance of soil structure, regulation of water flow, the decomposition of organic material through biodegradation processes and control of biogeochemical cycles, all employees by microbial activity. In fact a key role is played by micro-organisms that govern the mobilization of mineral elements such as nitrifying bacteria that convert ammonium into nitrate, denitrifying bacteria, which reduce nitrate to nitrogen gas and the bacteria involved in the carbon cycle and in the transformation of phosphorus. The recycling of these elements is essential to make them directly available to the plant and therefore for the maintenance of soil fertility. The structure and composition of the microbial community are extremely variable in time and depend on many factors: the composition and physical-chemical nature of the soil in terms of moisture, pH, temperature and nutrients present, the presence of plants and their relative stage growth and health, climate change and human activities, and finally the interaction between the individual species that constitute the community itself (Garbeva et al., 2004). All these factors will select the most suitable microorganisms, thus promoting the development of some microbial species than others (Rovira, 1965). A particularly suitable habitat for growth and microbial activity is that portion of the soil in direct contact with the root system,

defined **rhizosphere**. This region has the highest rate of microbial growth mainly due to the abundance of nutrients derived from the roots. In fact, the roots release large amounts of organic compounds assimilated by microorganisms. One example is the lysates radicals who derived from senescent epidermal cells, exudates in the form of sugars and phenols that leak from intact root cells and the mucilage secreted by peripheral cells of the root cap (Bolton et al., 1993).

The bacteria that inhabit the rhizosphere, the rhizobacteria, depend on plants and nutrients that they release, but at the same time they are useful to the same plants for a variety of metabolic activities relating to the production of plant hormones such as auxin, cytokinins and ethylene that further promote the growth of the root system. Another essential component of soil microbial communities is represented by mycorrhizal fungi that form mutualistic symbiosis with the majority of plant species, which are essential for their nutrition, soil fertility and for the maintenance of stability and biodiversity of plant communities (Smith and Read, 1997).

1.6 Biological soil fertility

The soil microbial communities discussed in the previous section, are directly responsible for the soil fertility, understood as the soil ability to make productive crops. In fact, without a telluric microflora the agricultural soils would be mostly a mechanical passive support and inert towards the plants that should nourish. More precisely, it is important to consider a fertility concept which differs from the one with valence purely physical-chemical commonly understood: **the biological fertility**. This is the expression of soil microbial life in terms of metabolism and microbial turnover and is strictly dependent on the

organic matter present in the soil itself, the main source of nutrients for the microorganisms growth. The organic matter is formed from the remains of plants, animals and micro-organisms affected by a slow process of transformation, decomposition and accumulation in the soil. The decomposition is carried out by the micro and macrofauna of the soil and mostly affects plant residues mainly composed of cellulose and lignin. The reasons that make a productive soil are to be attributed to the ability of organic matter to promote the soil structural stability, the aeration and the water runoff. It is important to point out the negative effects that can lead to some agronomic practices, such as those based on intensive deep tillage, monocultures and the use of pesticides and herbicides, to the detriment of soil fertility. Among these effects can clearly detect an alteration in the amount of organic matter and biodiversity of the rhizosphere. The consequence of this is a decrease of the biological fertility of the soil and consequently in the crop productivity. This destabilization change the structural balance of the microbial community present and the composition of the various population who make up that community (Bolton et al., 1985). Miller et al., 1999, have shown as an agricultural soil contains much lower amounts of organic matter compared to a forest or swampy ground. A depletion which results in a substantial decrease in the total number of bacteria observable under the microscope.

1.7 Composting process in viticulture

1.7.1 Introduction

Towards the end of the nineties, under the pressure of a necessary renewal of the vineyard, you spreaded the need to address the

viticultural activities with the adoption of more dense vineyards and less vigorous plants, smaller yields, improved quality and greater attention to the use of agrochemicals. In the later years this trend has increased and today the viticulture is facing a new crucial change that will improve the use of natural resources and conservation of soil, air, and water and finally restore or maintain the biological soil fertility. In recent decades the soil in the vineyards has suffered an obvious loss of soil organic matter is therefore necessary to evaluate the possibility of his reinstatement with the use of organic matter also derived from viticultural and oenological by-products. In order to reduce air pollution you will have to make use of non-toxic active principles, of an integrated pest management, of facilities that allow a considerable saving of the product and able to center the target only. It will have to start taking into account that the vineyard is a consumer and kidnapper of atmospheric CO₂, head of climate change. To optimize the use of water will have to adopt new irrigation systems (ex. dripline underground) and strategies that allow water savings up to 40%. All this is now possible thanks to the studies and research of the last decade and the possibility of resorting to precision viticulture. This allows to improve the status and function of root systems backed by airy and not compacted soils. In this new context of production, is spreading the use of certain by-products of wine-growing and winemaking, for centuries regarded as waste and from a few years became a resource as they can be reintegrated into the production cycle. Among these the **vine branches** obtained from the winter pruning of the vines and the **grape marc** arising from the pressing of grapes, suitably processed into compost, are a viable solution for the maintenance of soil organic matter and the conservation of the soils physical properties mined by the increasingly frequent compaction of areas under vines (loss of structure, permeability and asphyxial phenomena).

Up until a few years ago it was common practice to burn vine branches on the edge of the vineyards, or proceed to a coarse chop. The need and the obligation to abandon the practice of field burning of the production cycle waste is reached with the European Directive 96/62 on "the assessment and management of the air quality and the environment". This provision was first introduced at the national level with the Legislative Decree no. 351/99 and later in the 2000s taken over by the provincial and regional structures (for example, in the Veneto region has been inserted in the resolution of the Regional Council no. 57/2004 "Regional plan of the atmospheric reorganization"). The aim is expected to fight air pollution and in particular due to fine particulate matter (PM₁₀), ensuring the protection of public health. Due to the ban on open burning of any residual plant pruning, the vine branches have assumed considerable importance as a by-product and as such reintegrated into the production cycle or used in corporate plants to produce heat, power or biogas.

Currently the vine grower therefore has three alternatives:

- 1) **cutting on the field**, leaving the material deposited on the sward; currently it is the most common practice and it is definitely the least expensive option, but also the one that does not allow an optimal reuse for poor mineralization of organic matter and which can promote the spread of escoriosis whether the vineyard is affected;
- 2) **pressing and packaging** of pruning in packages of different sizes and shapes and their subsequent **chipping and burning** at high temperatures in low-emission boilers for heat production (2,4 kg of dry wood chips produce the same energy as a liter of diesel or a m³ of methane) (Spinelli R. *et al.*, 2010);

- 3) **shredding and tearing** of the material, adding or less of grape marc, **transformation into compost** and its use as a soil conditioner and fertilizer.

1.7.2 Composting process : chemical and microbiological aspects

The term composting is defined as the process for the biological controlled decomposition and maturation of organic substrates in the solid phase, which takes place in an aerobic environment (rich of air). This process leads to the production of materials to simpler molecular chain, more stable, sanitized, rich in humic compounds, which are useful for the crops fertilization and for the restoration of soil organic matter. The process is carried out by different strains of microorganisms involved in an aerobic environment as bacteria, fungi, actinomycetes, algae, protozoa, naturally present in organic biomass or artificially made with any material inoculum.

Briefly, the process can be described by the following equation:



The composting process is very interesting under different points of view:

- from the point of view of ecological-environmental, because it transforms biomass of different nature and composition to materials useful for fertilization of agricultural soils, because do not more phytotoxic, which bring nutrients and enhancers of the soil structural characteristics

- from the point of view of sanitation, because the organic material is sanitized in the process, thanks to the high temperatures that they are caused in the initial phase
- from the energy point of view, given that the process is self-sustaining energy, with the energy deriving from the demolition of the links biochemical characterizing the complex molecules of the organic substance (Tuomela M. *et al.*, 2000).

1.7.3 Compostable organic matrices

The compostable organic matrices must have biochemical characteristics such as to ensure a smooth implementation of the process, in particular, must contain sufficient compound easy to degrade, to ensure the nutrition of microorganisms agents of the process (Confesor R.B. *et al.*, 2008).

The organic materials used in the production of quality compost are regulated by the Decree of February 5, 1998 and, in the Veneto region, by the D.G.R.V. no. 766.

The product obtained in order to be marketed and used in agriculture without contraindications as soil conditioner / fertilizer, must possess the specific characteristics and requirements set by the legislation on fertilizers, that is by law no. 748/1984.

1.7.4 Agents of the composting process

Among the different populations of microorganisms that alternate within the organic matrix at different stages of the composting process there is a synergistic interaction. In fact the metabolic products of a type of microorganism can be used as nourishment of others. A useful example

is that of microorganisms secreting amino acids and vitamins, which are essential to the lives of other microbial strains. Even insects and other invertebrates are useful to the physical disintegration of many organic structures making them, thus, available for bacteria.

On the distribution of microorganisms in the pile, the bacteria are found everywhere while fungi and actinomycetes are found predominantly in the surface layer (0,05 to 0,15 m from the surface).

In numerical terms the bacteria represent the dominant part of the agents of the process, making it about a hundred times higher than the other groups of microorganisms.

The different microorganisms operate at thermal regimes defined and their activity is closely influenced by the process temperature, so it can be classified in psychrophilic, mesophilic and thermophilic (Table 1.2). The psychrophilic microorganisms or cryophilic are extremophile organisms able to grow and reproduce at low temperatures between 0 and 20 °C and are certainly less important than the mesophilic and thermophilic for composting process. The mesophilic microorganisms grow well at temperatures ranging between 20 °C and 35 °C, while they tend to arrest the development (some species less resistant also to die) above 45 °C. The thermophilic microorganisms start to develop at temperatures close to 40 °C and have an optimum development at 50-60 °C. During composting process when changes the temperature, vary the active microbial populations: in the early stages, which involve a rapid metabolism of carbon compounds most simple (monosaccharides, lipids and peptides), operate initially psychrophilic and mesophilic microorganisms.

MICROBIAL GROUPS	TEMPERATURE (°C)
Psychrophilic microorganisms	0 < t < 30
Mesophilic microorganisms	0 < t < 45
Thermophilic microorganisms	45 < t < 90

Table 1.2 - Classification of micro-organisms in relation to the thermal regime

Subsequently, due to the temperature rise, resulting from an intense metabolic activity, there is a strong selection among bacterial populations for the benefit of thermophilic species. At lower temperatures they greatly reduce growth to a standstill, but without being killed. If it rises above 80-90 °C occurs the death of most of the microorganisms with the exception of some ultra-thermophilic bacterial species, which are found in the organic matrix in the form of spores (structures of resistance to heat). At such conditions corresponds, however, a progressive decrease of microbial activity, up to the stop of the same. The subsequent cooling leads to reactivation of organisms, from those thermophilic, then move on to those mesophilic and psychrophilic in chronological order.

A further classification of microorganisms that operate in the composting process can be done in order to their biochemical functions (Table 1.3).

CARBON CYCLE	
Function	Microorganisms
amidolytic	bacteria, actinomycetes, fungi
pectinolytic	bacteria, actinomycetes, fungi
emicellulosolytic	bacteria, actinomycetes, fungi
cellulosolytic	actinomycetes, fungi
ligninolytic	actinomycetes, fungi
chitinolytic	actinomycetes, fungi
NITROGEN CYCLE	
Function	Microorganisms
nitrogen fixation	bacteria
proteolytic	bacteria
ammonification	bacteria
nitrification	bacteria
denitrification	bacteria
SULFUR CYCLE	
Function	Microorganisms
sulfur mineralization	bacteria

Table 1.3 - Micro-organisms active in the various transformations of the cycles of carbon nitrogen and sulfur

Bacteria

The bacteria (Figure 1.1) are the smallest known living organisms: they are mostly unicellular (cocci, bacilli, vibrios, spirilla), but you also have associations multicellular (diplococci, streptococci, staphylococci, streptobacilli, sarcina). They are always present in the soil and in plants at concentrations of a few million per gram and largely dominant scale and type of activity. Are present in at least 1,000 different species and are susceptible to strong growth in high humidity environment, and especially in the presence of easily biodegradable material (Yamamoto N. *et al.*, 2010). They have a broad spectrum of activity and ability to operate in a wide pH range, although they not tolerate acidic pH. For

the purpose of composting are, however, especially important bacteria capable of degrading the cellulose compounds.

Fungi

The fungi (Figure 1.1) are multicellular organisms composed of filamentous structures (hyphae) very abundant in soil and present almost everywhere in nature (Gregory B. *et al.*, 2010). In particular, they are the agents of the decomposition of soil organic matter, especially in acidic conditions. Metabolically are comparable to heterotrophic bacteria : in fact, they use mostly the same substrates, being therefore often competing with these and can dissolve the solid substrate via secretion of extracellular hydrolytic enzymes . Unlike bacteria, fungi can live in areas with low humidity and can grow on dry substrates, using the atmospheric humidity. The fungi are favored compared to bacteria from the high C/N, live in a wide pH range, being able to operate between 2 <math>pH < 9</math> , and often they have lower demands for nitrogen than bacteria. For the purpose of composting are especially important that species able to degrade lignocellulosic materials (Shawn L.T., 2014). The fungi can be divided into :

- Molds have an aerobic metabolism and tend to form filamentous structures. They play an important role in the final stages of the process, with the oxidation of materials rich in lignin.
- Yeasts are mainly unicellular and have aerobic and anaerobic metabolism. They play an important role in the humification phenomena.

Actinomycetes

The actinomycetes (Figure 1.1) present in the composting process are aerobic and thermophilic. Generally they are similar to fungi (for the formation of hyphae) and bacteria. If the environmental conditions are unfavorable, as a survival strategy, they do not reproduce sexually but with the production of endospores. They are heterotrophic microorganisms therefore mainly use organic nitrogen (only some *Mycobacterium* are able to fix nitrogen gas); as carbon source use that content in organic matter and in particular in the cellulose and lignin. As a source of energy used that released by the breaking of biochemical bonds. The most part of them living in the soil, which gives the typical smell of earth. The actinomycetes attack the organic substance is not degraded by bacteria and fungi, such as chitin; they are neutrophils, but bear slightly basic pH. They operate in the final stage of the process in difficult working conditions for fungi and bacteria and become visible in the static piles in the form of powder or strands gray color from white to light green. If the compost is turned mechanically, these large colonies are not visible or extremely limited.

Microfauna of the compost

The organisms described in the preceding paragraphs belong to the category of so-called *primary consumers*, which feed on decaying organic matter. Also some species of nematodes (Steel H. *et al.*, 2010), earthworms and mites belong to this category: their role in the process of composting is to contribute to the physical disintegration of the organic matrix and to facilitate the action of microorganisms, creating tunnels - especially earthworms and nematodes - that increase the porosity and improve the aeration of the compost; they also produce

excrement rich in organic compounds useful in the composting process. The microfauna of the compost is made also by the *secondary* and *tertiary consumers* that perform essentially the role of predators: among them we can mention arachnids, myriapods (centipedes) and insects (springtails, staphilinidae).

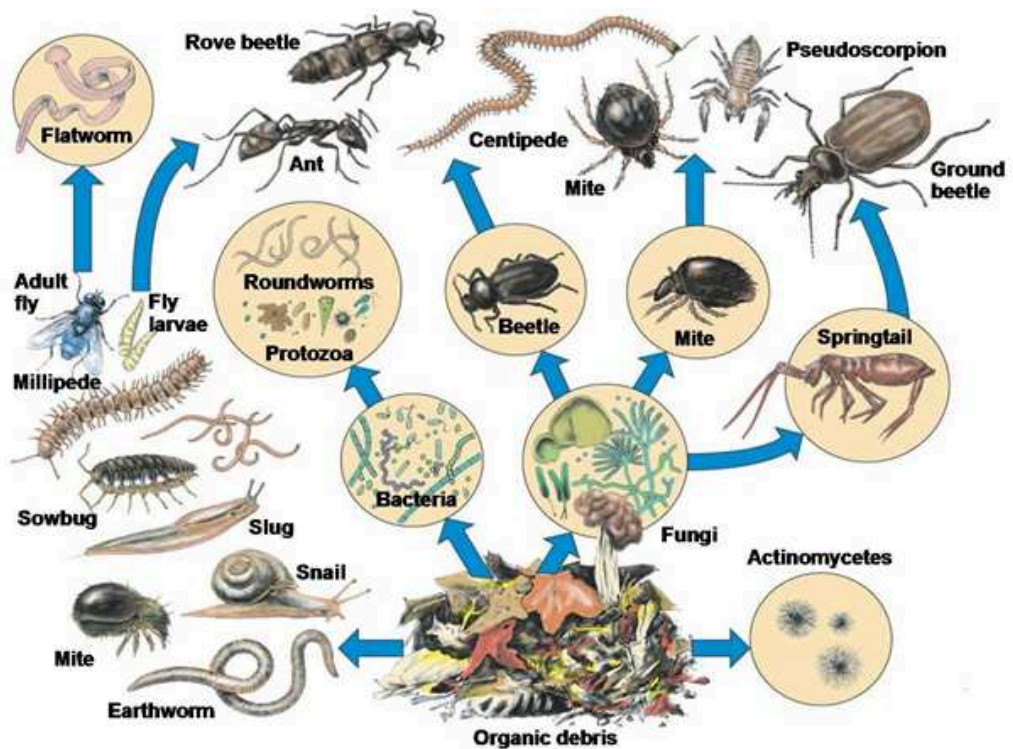


Figure 1.1 - The composting ecosystem : primary consumers (bacteria, fungi, actinomycetes), secondary consumers (protozoa, beetle, mite, springtail, nematode,) and higher-level consumers (earthworms, ground beetle, millipede, centipede, ant, spider).

1.8 Stages of composting process

The transformations that undergoes the organic matter in the composting process may be divided into two stages: **decomposition** and **maturation** (Adani F. *et al.*, 1999).

The composting process begins as soon as the organic substrate is correctly placed in piles with the phase of decomposition of the most easily degradable organic fraction (sugars, organic acids, amino acids) at the hands of aerobic microorganisms with oxygen consumption, production and release of CO₂ of energy, necessary to bring the temperature of the cumulation progressively up to the expected scheme thermophilic: this is obtained by breaking the chemical bonds of the different organic compounds. This phase, purely thermophilic, is also known as *high-rate phase* and can last for several weeks and even more than a month: the duration is affected by the characteristics of the substrate and by the technique of composting adopted. The increase in temperature is very marked in the 12-48 hours after the setting up of the pile and the trend of rapid growth up to 55-60 °C. If the heat is not adequately dissipated temperatures may increase leading to inactivation of most microorganisms. The thermophilic phase involves the devitalization of weed seeds that may be present in the starting materials. Therefore the overturning of the pile is indispensable to allow the cooling of the substrate and to maintain oxygenation of the biomass to above the critical values for the activity of the aerobic microbial population. With the advancement of the process, the soluble substances decrease and at the same time begin to form pseudo humic substances. The high temperatures, the conditions of pH and moisture that develop in this phase inside the mass carry the bacteria to be the most active organisms.

With the exhaustion of more easily biodegradable compounds metabolized in the first phase of composting, the metabolic processes of decomposition involving the more complex organic molecules and are implemented with slower processes, even after the death of a large proportion of the microbial population due to lack of nourishment. With the consequent progressive lowering of the temperature change that populations of active microorganisms, with a passage from the thermophilic ones to mesophilic before and psychrophilic then. In fact, in this phase, also called *curing phase*, the temperatures drop to values of 40-45 °C and then descend gradually, stabilizing slightly above the ambient temperature (Zmora-Nahuma S. *et al.*, 2008). During this phase (mesophilic phase) that can last several months, the actinomycetes actively degrade starch, cellulose and lignin compounds essential for the synthesis of humic substances. The work of the actinomycetes is fundamental to the humification which is mainly due to the oxidative polymerization of phenolic acids and phenols obtained from the catabolism of lignin, tannins and polyphenols, or for microbial neosynthesis. One of the most well-known effects of the actinomycetes activity concerns the production of aromatic compounds, such as geosmin, which give the final product the typical smell of the forest soil. It also has an intense colonization of the material by small animals (such as springtails, mites and centipedes) that contribute significantly to the shredding and mixing of the organic and minerals compounds. At the end of this phase, you get a stable mature compost that is similar in appearance to that of a good soil: the size of the product is reduced compared to the original one, but it may have glomerular aggregation.

1.9 Evolution of the composting process : assessment of main parameters

The evolution and the speed of the composting process are closely dependent on the factors that influence the optimal conditions for the life of the microorganisms involved in the different stages of the process. Parameters such as oxygen, humidity and temperature are normally inspected for correct performance of the process (Bernal M.P. *et al.*, 1998), but there are other parameters that affect the living conditions of microorganisms and in particular:

1. substrate porosity;
2. moisture content of the material;
3. presence of oxygen;
4. process temperature;
5. C/N ratio;
6. pH;
7. presence of substances that inhibit the transformation process.

It is evident, therefore, that the evolution of a composting process depends not only on a correct composition of organic biomass, but also from the maintenance of optimal process conditions. A proper monitoring of the piles, especially in the start-up phase, it is essential for the detection of abnormalities of the process (Witter E. *et al.*, 1987).

1.9.1 Substrate porosity

The *total porosity of the pile*, or *lacunar space*, is the ratio (expressed as percentage) between the volume occupied by the empty spaces within the biomass and that occupied by the biomass itself (Van Ginkel J.T. *et al.*, 1999). The empty spaces are filled partly by air and part by

water: the *free porosity*, which in the bibliography is also indicated with the term F.A.S. (*Free Air Space*), indicates the percentage of volume occupied by the air.

$$\text{TOTAL POROSITY : } (V_v) / V_t$$
$$\text{FREE POROSITY : } (V_v - V_a) / V_t$$

V_v = volume of voids (empty spaces)

V_a = volume occupied by water

V_t = total volume

This parameter is of great importance, as it influences the possibility, or not, to maintain in the composting mass in the amount of oxygen required for the process. In composting is, therefore, in the presence of a system of three variables: air, water and organic material. Under optimal conditions, the lacunar space amounted to values between 35 and 50%. The porosity of the mass is closely dependent on:

- Particle size of the material to be composted,
- Moisture content,
- Thickness of the material layer (height of the pile).

More the particles are thin and with a low dry matter content, more the piles tend to compact and lower will be its porosity; to other factors being equal, then, to a greater height of the pile corresponds a lower material porosity, especially in the layers close to the base. During the process, the porosity of the mass should theoretically decrease, because as a result of the decomposition of the product and of the pile settlement, the granulometry of the product is reduced. In fact, considering that the humidity of the product tends to decrease with the progress of the process, the porosity tends to remain constant, and in some cases to increase.

1.9.2 Moisture content of the material

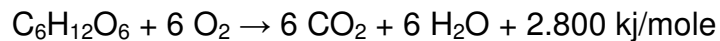
The water is an essential element in the life of most active microorganisms in the composting process, because:

- It is essential for the nutrients exchange across the cell membranes;
- It is the vehicle for extracellular enzymes;
- It is the vehicle for soluble substrates;
- Is the medium in which chemical reactions take place.

The values of optimal moisture of the material are between 40 and 65%: with values less than 40% is a considerable slowdown of the biological activity, which stops reached the limit of 25-30%. With a moisture higher than 65% the diffusion of oxygen in the mass is difficult, could also lead to the establishment of anoxic conditions. It is possible, however, to use for the composting some materials with moisture close to 70% (for example fresh grape marc or cattle manure): in this case, it will be necessary to mix the product with materials with a high content of dry matter, (for example, vine branches), so that the average humidity of the mass does not exceed 65%. The composting process leads to a decrease of natural moisture in time due to evaporation, facilitated by the exchange of air in the mass. Excessive reduction involves, however, the block of the process: the material is apparently stable but biological phenomena resume in case of rehydration. It is therefore essential to ensure that the values of this parameter are within the optimal range, this is achieved by appropriately mixing the compost matrices in the initial phase, and during the process using humidification of the product periodically wetting the pile.

1.9.3 Presence of oxygen

Under aerobic conditions the degradation of a substrate rich in carbon determines a strong oxygen consumption, with production of carbon dioxide, water and heat, as indicated below, for example for carbohydrates:



In the composting process there is a continuous decrease in oxygen available to microorganisms. The aerobic microorganisms use approximately 1,6 kg of oxygen to process 1,0 kg of organic matter. This oxygen demand must be met from the O₂ introduced in the pile. The higher oxygen consumption is observed at the beginning of the process, when the demolition of organic matter by microorganisms reaches its maximum intensity: in this phase the availability of oxygen in the lacunar spaces must be maintained in 5-15% in volume. In these conditions do not reach the anaerobic conditions but not even those of the complete demolition of the organic matter typical of combustion phenomena. In fact, the process should not lead to a total mineralization of organic matter (combustion), but to its stabilization and humification. The maturation-humification phase requires a lower level of oxygenation, with O₂ between 1% and 5%, so it is possible to minimize the operations of oxygenation of the pile. The oxygenation of the mass is obtained with techniques for introducing air in the pile (forced ventilation in compression and depression) or with techniques of overturning. With these interventions as well as to ensure the necessary supply of oxygen to the mass, it promotes the removal of excess heat, the removal of water vapor and gases formed in the lacunar spaces.

1.9.4 Process temperature

The temperature of the mass during composting is the parameter that best predicts the trend of the process and is also easier to monitor.

In addition to a correct transformation of organic matter by microorganisms in order to make it available for agronomic uses, it should be noted that the thermophilic phase is very important in the process, because it leads to:

- Sanitation of the product, with the destruction of pathogenic microorganisms, which occurs at temperatures not lower than 55 ° C for at least 1 day;
- Inactivation of weed seeds and plant pests, which occurs at temperatures not lower than 60 °C.

In the thermophilic phase, should be avoided raising the temperature above 70 °C, because this involves a high microbial mortality, since there are few bacterial strains able to survive in such conditions: that would undermine the efficiency of the process.

For this reason, not to exceed the optimal temperature (55-60 °C), it is necessary to intervene with the overturning of the mass. By monitoring the pile temperature is also possible to assess the level of oxygenation of the mass: the lowering of temperature should be placed in the first instance in relation to oxygen deficiency. The control of this parameter is implemented mainly through the overturning mechanic that determines an immediate cooling of the mass. The restoration of aerobic conditions determines, in turn, increase the activity exothermic: the temperature resumes, therefore, to increase.

The greater or lesser rapidity with which you can reach high temperatures at the beginning of the process depends on the characteristics of the starting material: a higher and more easily biodegradable, leading to faster thermophilic regimes.

The heat production varies with the degraded material: a low fermentability is typical of lignocellulosic materials, which also show a limited ability to degradation, while the grape marc tends to ferment more quickly because of the high water and sugars content. Temperature values of 35-45 °C are typical of the second phase of the process; levels of temperature slightly different from that of the environment indicate, as a rule, the end of the process.

1.9.5 C/N ratio

During the composting process is extremely important to have a balanced ratio between carbon and nitrogen (Bustamante M.A. *et al.*,2008) : the lack of one of these elements (but also of other nutrients) is a limiting factor for microbial activity, as well as for its development (Larsen K.L., McCartney D.M. 2000). Indeed, the heterotrophic microorganisms use the carbon as a source of energy and need nitrogen to synthesize proteins. The nitrogen is obtained by cleavage of proteins into peptides and free amino acids, and these may be subject to a direct assimilation or to further cleavage with production of ammonia. This can come back to be part of organic compounds when used by microorganisms and can be lost through volatilization or converted into nitrate. During the different phases of the process the microbial populations using about 30% of the carbon combining with the nitrogen to form the cellular protoplasm while the remaining part is oxidized to carbon dioxide. Considering that are typically used thirty carbon atoms for each nitrogen atom, it is evident that the optimal ratio C/N at the beginning of composting should be 30. Under conditions of excess carbon (C/N>30) there is a slowing of decomposition, with lengthening of the duration of the process. With a value of C/N<20, occurs the release of excess nitrogen in the form of NH₃, in very high

concentrations in the presence of a high nitrogen content and high values of temperature and pH.

The C/N ratio decreases over time because there is a loss of carbon (CO₂ emission), while the nitrogen component of the aromatic structures of humic substances, tends to remain in the mass. At the end of the composting process the C/N is lower than the initial value and in optimal conditions, is between 15 and 20. With too low value of C/N ratio, the compost can be toxic for plants and for the potential release of ammonia. With too high value, however, can lead to a competition between roots and soil micro-organisms for the available nitrogen.

1.9.6 pH

The composting process takes place with pH extremely variable, but the optimal values for the starting mixture are between 5,5 and 8,0, considering that the bacteria prefer a pH close to neutral and that the fungi prefer acidic pH. At the beginning of the process there is a natural shift of pH to acidic values following the formation of CO₂ and organic acids; subsequently the pH rises to 8-9 because of the elimination of CO₂ with aeration and further to the decomposition of proteins with production of ammonia. At the end of the process, the pH of the mature compost is neutral or slightly alkaline.

1.9.7 Presence of substances that inhibit the transformation process

The biological degradation of organic matter are negatively affected by the presence in the substrate of heavy metals in concentrations higher than those compatible with microbial activity. Too high values determine toxic effects that in the best case only induce the inhibition of the

biological activity, but may also lead to the death of all the bacterial strains that are not resistant.

A strong negative effect is explicate by chromium ions, particularly hexavalent chromium which penetrates the cell membrane more easily than the trivalent and interferes with cellular functions.

Chapter 2

COMPOST FROM VINE-WOOD PRUNING AND CATTLE MANURE IN THE VINEYARD : EFFECTS ON PRODUCTION AND GRAPE QUALITY

2.1 Introduction

The soil is a complex and dynamic biological ecosystem that interacts with the root system and whose physical, chemical and biological influence the fertility from which depends the crop productivity. The importance of soil organic matter has long been known, because it can determine beneficial effects on soil structure, water retention on the cation exchange capacity and mineral endowment influencing growth and productivity of the vineyard. Recent studies have also shown that the organic substance, and in particular humic acids contained in the soil organic matter are able to promote root elongation and the formation of new roots in corn plants (Cannellas et al. 2002).

The soil organic substance is a chemical tank being the main source of nitrogen for the plant and also containing 65% of the total P (Baur and Balck, 1994) thereby affecting the vigor and productivity of the plant. There are, in this regard, many works in the literature where it is reported as nitrogen stimulates the growth and yield of the plant, on the contrary, others refer only to a minimal effect. In an experiment conducted in sandy soils in South Africa (Conradie, 2001), were compared the effects on plants caused by an intake of 50 kg ha⁻¹ of mineral and organic N (cattle manure), at different times. The beneficial effect of manure (Rossi L. and Guercini S., 2001) has determined an increase in production and vigor of the plant compared to the unfertilized control, and the wines have achieved higher scores. In general, it should be returned annually 2-3 t ha⁻¹ of dry matter in order that the soils cultivated with vineyards maintain a balanced value of organic matter.

2.2 Aim of the study

To assess the effects on the plant determined by the distribution of organic matter to the soil were analyzed vegetative and productive responses of a vineyard in the north-east Italy. The first objective was to compare two types of compost, one obtained by pruning residues and another from cattle manure, with the second goal, we aim was to verify the correct amount of compost to be returned (2 or 4 t ha⁻¹) to maintain a balanced force of plants and adequate quality of the grapes. The third objective was to verify the importance of the compost location, comparing a distribution in the space between rows and a location under the row.

2.3 Materials and methods

The test was conducted on a Cabernet sauvignon vineyard (ISV-FV clone) grafted on rootstock 3309C located in the lower venetian plain within the “D.O.C. Piave” zone (45° 44' 30" N and 12° 30' 34" E) at 12 m above sea level and placed in a north – south direction. The vineyard has a planting pattern of 2,2 m x 0,9 m and is bred to counter-espalier with Guyot pruning. The soil is characterized by clayey texture throughout the profile with difficult drainage and groundwater present at one meter deep.

In Figure 2.1 is shown the location of the vineyard analyzed (Google Earth).



Figure 2.1 - Location of the Cabernet Sauvignon vineyard (Google Earth pictures).

The experimental design used was randomized blocks and each block were established nine treatments repeated three times:

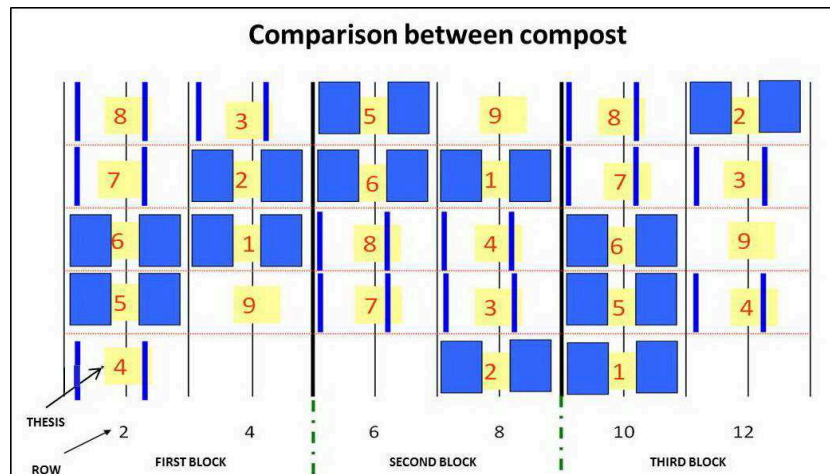


Figure 2.2 - Experimental scheme

The red numbers from 1 to 9 with a yellow background identify the thesis of the study. The blue areas and the blue lines indicate where the compost was distributed.

Below is a description of each thesis:

1=distribution along the inter-row space of 2 t ha⁻¹ with compost made from pruning residues (INSA2);

2=distribution along the inter-row space of 2 t ha⁻¹ with compost made from cattle manure (INLE2);

3=distribution under the row of 2 t ha⁻¹ with compost made from pruning residues (SOSA2);

4=distribution under the row of 2 t ha⁻¹ with compost made from cattle manure (SOLE2);

5=distribution along the inter-row space of 4 t ha⁻¹ with compost made from pruning residues (INSA4);

6=distribution along the inter-row space of 4 t ha⁻¹ with compost made from cattle manure (INLE4);

7=distribution under the row of 4 t ha⁻¹ with compost made from pruning residues (SOSA4);

8=distribution under the row of 4 t ha⁻¹ with compost made from cattle manure (SOLE4);

9=untreated control (TEST);

The surveys carried out involved the vegetative and productive parameters of the vineyard. In particular were monitored at the time of grape harvest, on 6 plants per replication (total 18 plants), production per plant, the average bunch weight and the number of bunches per vine. The following winter, in one day, on the same plants was harvested wood pruning. From the relationship between yield per plant and pruning wood was deducted the Ravaz index. During the grape harvest, were collected 60 berries for reply in order to calculate the average weight. Later was taken a sample of bunches and were analyzed the soluble solids content (° Brix), the acid profile and the pH. The content in malic acid and tartaric acid was done with a Thermo Finnigan HPLC chromatograph UV detector and reverse phase column C18 250 x 4 mm. It was used a mobile phase with H₃PO₄ to a concentration of 5 x 10⁻³ M, with a flow of 0,6 mL min⁻¹, at room temperature. For the extraction of polyphenols and total anthocyanins from the grape skins, have been used 75 mL of a solution at pH 3,2 containing 2 g L⁻¹ of SO₂ and 12% of ethanol (Di Stefano and Maggiorotto, 1995) for a time contact of 4 hours. The whole was homogenized and centrifuged, the clear extract diluted 50 times with hydrochloric ethanol (ethanol, water, and concentrated HCl, 70:30:1 v/v/v) was read the absorbance at 540 nm and 280 nm .

2.4 Results

2.4.1 Productive results

The productive results were analyzed by the study of the treatments effects in the three years of experimentation on some parameters considered particularly significant, such as:

- yield per plant;
- berry weight;
- pruning wood;
- Ravaz.index.

Yield per plant

In the first year the yield per plant was higher than the control thesis, especially for thesis, "INLE4", "SOSA4" and "SOLE4" where the compost was distributed to the higher doses (4 t ha⁻¹). These differences were maintained even in following years with increases, of about 50% in the thesis SOSA4 compared to the control. The thesis SOSA2 is the only one that was not affected by the contribution of compost. The increased yield per plant of almost all theses treated can be explained by a better fruit set that resulted in an increase in the bunch weight, which does not reaches the 90 g for the untreated control in the average on the three years, while in the thesis dealt with 4 t ha⁻¹ of compost is increased by about 25% and is equal to 112 g. This effect is due, probably, to the greater availability for the plant in terms of N that, especially during the flowering period may have affected the percentage of fruit set and then the greater number of berries per bunch (S. Bell, 1999 M. Keller , 2001 M. Keller, 2005).

Berry weight

There are no differences between the different thesis in comparison (with the exception of the thesis SOSA4 that has the highest value, equal to 1,59 g, compared to an average of 1,51 g). This result is in agreement with findings by other authors in similar experiments (Morlat and Symoneaux, 2008), and also in line with other works that have found as high inputs of nitrogen, minimally affect the final size of the berry (Wolf 1988). This is a good thing for the grapes quality, especially for black berry cultivars, because the quality of the wines is closely linked to the smaller berry size during the harvest (Holt *et al.* 2008 Nuzzo and Matthews, 2007).

Pruning wood

It is also evident the effect of compost on plant vigor. In particular for the three years of the test, the thesis "INLE4" is that with the increased production of pruned wood and significantly different from the control. On the contrary, the witness and the thesis "SOSA2" are those who have shown less vegetative growth.

Ravaz index

It is expressed as the ratio between the yield per plant and the pruning wood and is a fundamental parameter for good performance of production while maintaining adequate quality of the grapes (Howell, 2001). We can say that, basically, lower is the value of this index, better will be the vegetative-productive balance and the grapes quality. In the test conducted not seem to be large differences between treatments if not a slightly higher value for the control, although not statistically

significant, in agreement with other authors (Morlat R., 2008). The results suggest that the action of the compost was not limited only to stimulate production, but has favored the plant vigor without disturbing the vine balance. Among of all thesis, INLE4 during the test period was one that showed the lowest index and statistically different from the other thesis.

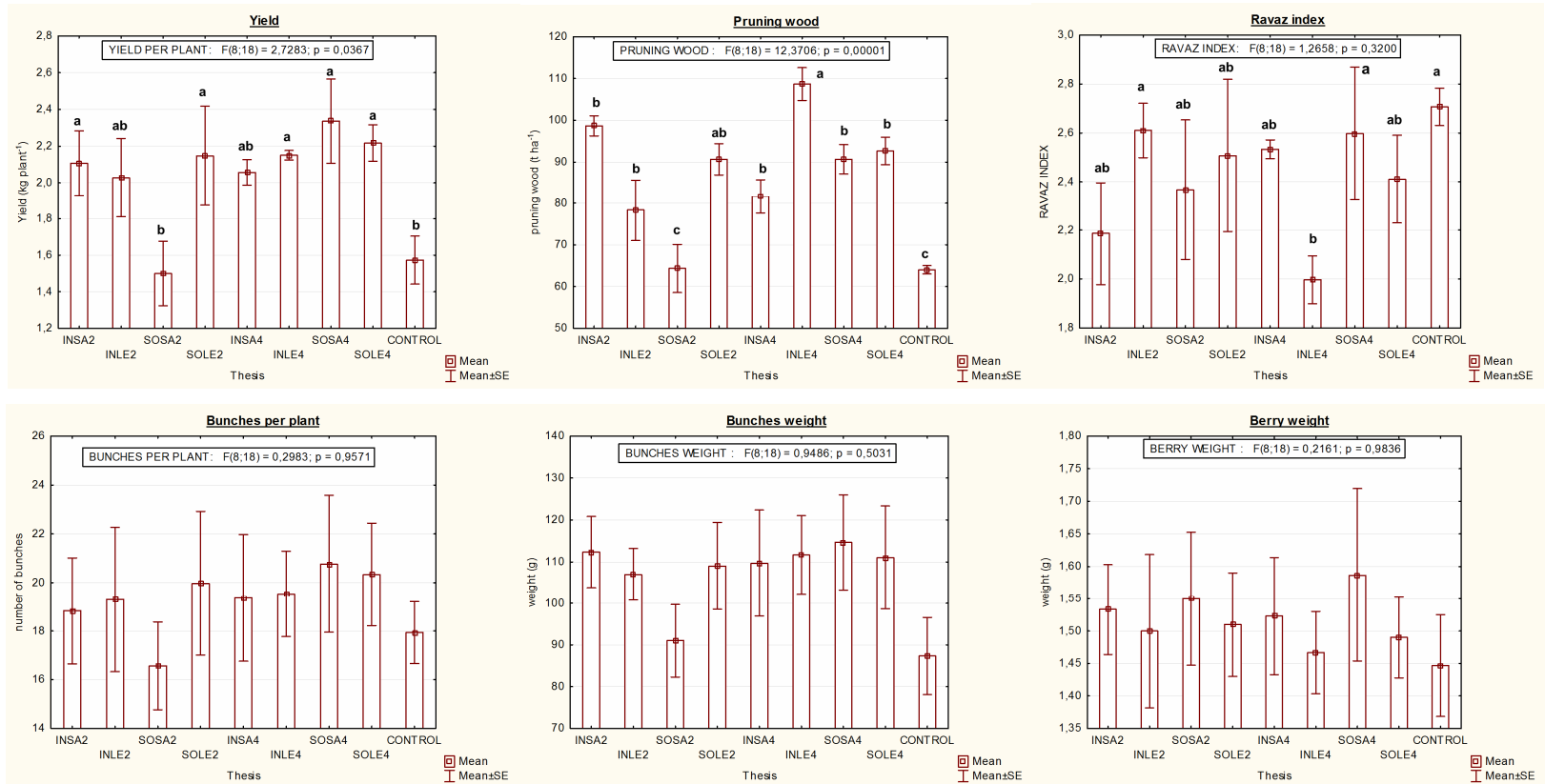


Figure 2.3 - Treatment effects on productive parameters (mean of three years). Means within treatment followed by a different letter are significantly different at the 0.05 probability level.

THEESIS	Yield	Bunches per plant	Bunches weight	Pruning wood	Ravaz index	Berry weight	Sugar	pH	Tritatable acidity	Tartaric acid	Malic acid	Total anthocyanins	Total flavonoids
	Kg plant ⁻¹	g	g	t ha ⁻¹		g	° Brix		g L ⁻¹	g L ⁻¹	g L ⁻¹	mg Kg ⁻¹ grape	mg Kg ⁻¹ grape
<u>YIELD PER PLANT</u>	1,000000	0,683978	0,380503	0,608216	0,847758	0,503464	0,037638	0,493248	-0,014954	-0,008469	0,310206	-0,097306	0,078623
<u>BUNCHES PER PLANT</u>	0,683978	1,000000	-0,409366	0,378110	0,314842	0,814411	-0,286939	0,192929	0,438534	0,234129	0,835708	-0,211262	0,550045
<u>BUNCHES WEIGHT</u>	0,380503	-0,409366	1,000000	0,269080	0,043441	-0,403507	0,435619	0,397796	-0,592730	-0,282278	-0,674155	0,178194	-0,577339
<u>PRUNING WOOD</u>	0,608216	0,378110	0,269080	1,000000	0,801827	0,137469	-0,380962	0,003683	0,357532	-0,117868	0,347488	-0,534360	-0,299989
<u>RAVAZ INDEX</u>	0,347758	0,314842	0,043441	-0,501827	1,000000	0,355045	0,455707	0,509116	-0,394057	0,105237	-0,046176	0,457977	0,407897
<u>BERRY WEIGHT</u>	0,503464	0,814411	-0,403507	0,137469	0,355045	1,000000	-0,080922	0,339521	0,216172	0,200476	0,749322	0,067384	0,723588
<u>SUGAR</u>	0,037638	-0,286939	0,435619	-0,380962	0,455707	-0,080922	1,000000	0,780571	0,937055	-0,052361	-0,541773	0,767770	0,119837
<u>pH</u>	0,493248	0,192929	0,397796	0,003683	0,509116	0,339521	0,780571	1,000000	-0,699098	0,002012	-0,102212	0,578214	0,293419
<u>TRITATABLE ACIDITY</u>	-0,014954	0,438534	-0,592730	0,357532	-0,394057	0,216172	0,937055	0,699098	1,000000	0,164295	0,681225	-0,751780	0,068083
<u>TARTARIC ACID</u>	-0,008469	0,234129	-0,282278	-0,117868	0,105237	0,200476	-0,052361	0,002012	0,164295	1,000000	0,311098	0,065106	0,384838
<u>MALIC ACID</u>	0,310206	0,835708	-0,674155	0,347488	-0,046176	0,749322	-0,541773	-0,102212	0,681225	0,311098	1,000000	-0,366854	0,566469
<u>TOTAL ANTHOCYANINS</u>	-0,097306	-0,211262	0,178194	-0,534360	0,457977	0,067384	0,767770	0,578214	-0,751780	0,065106	-0,366854	1,000000	0,469087
<u>TOTAL FLAVONOIDS</u>	0,078623	0,550045	-0,577339	-0,299989	0,407897	0,723588	0,119837	0,293419	0,068083	0,384838	0,566469	0,469087	1,000000

Table 2.1- Pearson correlations among the evaluated traits. Significant *P* values ($p < 0.01$) are in bold

2.4.2 Qualitative results

The qualitative results were evaluated by monitoring of certain parameters considered among the more important for to know the grapes quality produced. The parameters studied are the following:

- berry composition in the grape harvest;
- acid content : tartaric acid and the malic acid;
- anthocyanins content;
- flavonoids content.

Berry composition in the grape harvest

In all three years of the test there were no differences in the sugar content of the grapes among the different treatments. Important instead the differences between vintages, with values significantly higher in the first and third year of the test, characterized by a more favorable course climate. the type and quantity of compost instead did not affect the accumulation of soluble solids. As pointed out by other authors (Bell and Henschke, 2005), the contribution of organic matter and therefore of available nitrogen for the vine may alter the balance of source / sink in the plant favoring vegetative growth and limiting the accumulation of secondary organic compounds in the bunch. In the specific case of the test, the addition of organic matter increased vigor, but the value of the Ravaz index has remained the same between the thesis. The addition of organic matter did not cause an alteration in the vegetative and productive balance of vine allowing a greater leaf development and promoting the grapes maturation. Even the tritatable acidity of the grape must has not felt the effects of the treatment, as already shown in other field trials (Spayd *et al.*, 1994). The pH was minimally affected by

the effect of the compost, even if the thesis where the organic substance has been made to a higher concentration, irrespective of the nature of the compost, are those with the higher pH values. This effect is probably due to the greater availability of nitrogen to the plant, but especially potassium which has a direct action on the pH, in agreement with the results of similar experiments (Morlat and Symoneaux, 2008).

Acid content : tartaric acid and malic acid

In the three years of study, the tartaric acid content of the grapes was not affected by the input of organic matter and the differences between the thesis are minimal. Unlike the effect on the content of malic acid, which is a result very variable between the different treatments and especially between vintages. During the second year of the trial, there was greater differentiation between the treatments and the final content of malic acid which was higher in the thesis with supply of compost made from cattle manure, in quantity equal to 4 t ha⁻¹. This effect is probably due to the greater contribution of nitrogen to the vine that has favored the vegetative stage and slowed down the degradation of malic acid content (Keller *et al.*, 1999).

The addition of organic matter to the soil depresses the accumulation of anthocyanins and flavonoids in the berry not only for the competition with the vegetative development of the plant but also for the direct and negative effect of nitrogen on the anthocyanins biosynthesis, as evidenced by other authors on experiments conducted under controlled conditions with Merlot (Hilbert *et al.*, 2003).

Anthocyanins content

The average value of the anthocyanins (expressed in mg kg^{-1} of grapes) was about 16% lower than the witness for the thesis where the compost was added in quantity equal to 4 t ha^{-1} , and it was about 12% lower when the addition of compost was equal to 2 t ha^{-1} (average values of three vintages). Important differences are also evident if we compare the two types of compost. The thesis affected by the contribution of cattle manure showed a 17% reduction in the accumulation of anthocyanins compared to the untreated control, while the decrease was 11% for the thesis treated with compost obtained from vine branches, effect more evident to higher intakes. No differences found on the different methods of distribution.

Comparing the data expressed in $\text{mg}/100$ berries, we observed a decrease in the differences between the thesis with respect to the untreated control, especially when the compost is obtained from pruning residues (SOSA2, INSA4 and SOSA4). This suggests that the compost, particularly from vine branches, did not interfere with the kinetics of anthocyanins accumulation and when the data are expressed in mg kg^{-1} , the differences are mainly due to the different berry weight.

Flavonoids content

The flavonoids showed a similar behavior to that of the anthocyanins, with lower accumulations corresponding to the higher contribution of compost and when it is obtained from cattle manure. As observed for anthocyanins there is a direct effect due to the different plant vigor, which has affected the sink storage and modified the canopy architecture of the vineyard, thereby resulting in a different position of bunches to direct sunlight.

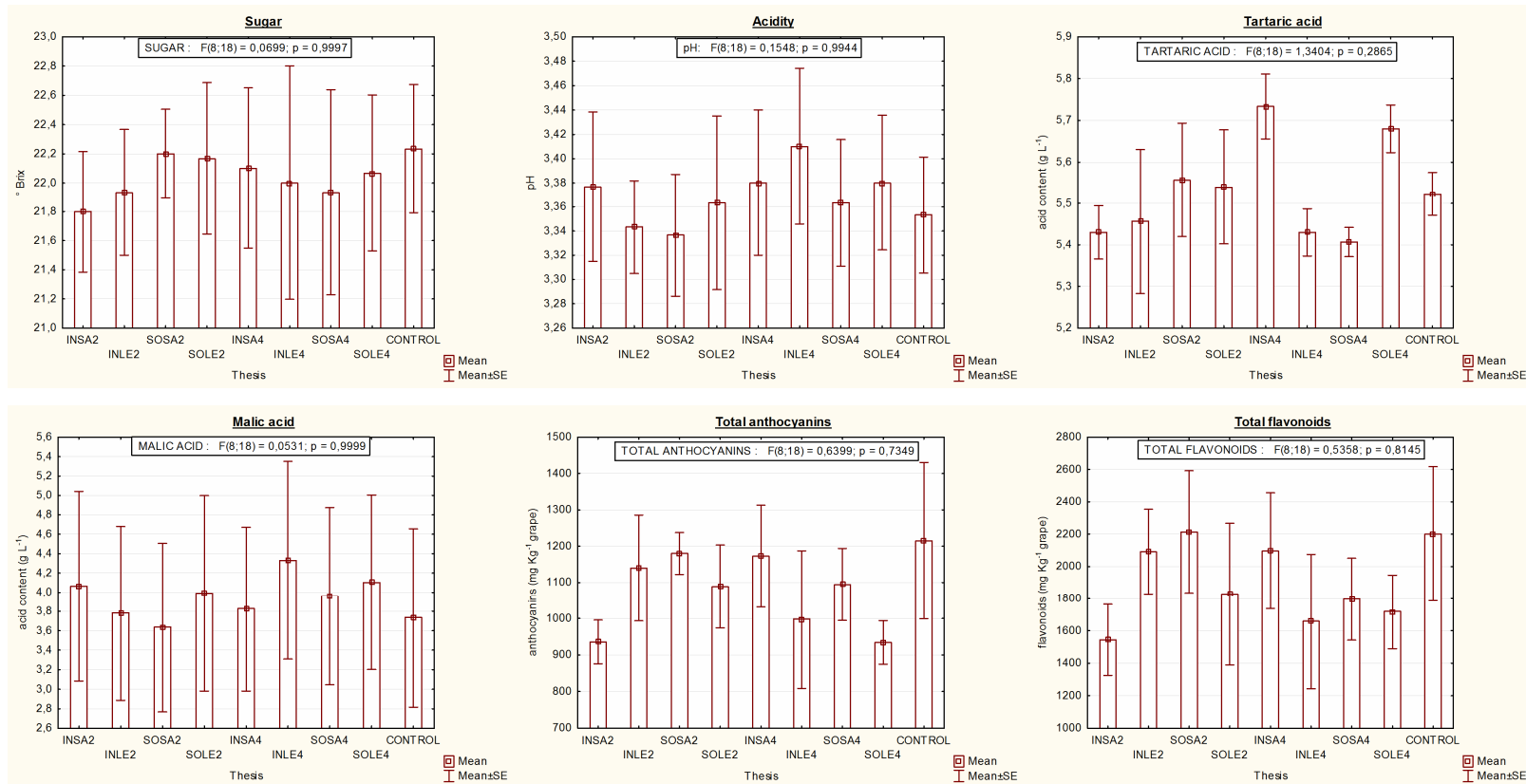


Figure 2.3 - Treatment effects on qualitative parameters (mean of three years).

2.5 Conclusions

The addition of both type of compost (from cattle manure and from pruning residues) directly affects the productivity, the plant vigor, the grapes maturation and the chemical / microbiological properties of the soil. The response of the vine occurred already in the first year of test with higher production, especially when the addition of compost was equal to 4 t ha^{-1} . This effect is due to the increased availability of nitrogen, which has probably contributed to the fruit set of the bunch. Even the plant vigor has been affected positively, but without affecting the value of Ravaz index which is nay lower for the thesis dealt. No significant difference was observed for the sugar content of the grapes, while the effects on the polyphenolic profile were more evident penalizing the thesis dealt with compost from cattle manure. When instead the compost from pruning residues was added in quantity equal to 4 t ha^{-1} , the differences of the flavonoids and anthocyanins content were minimal compared to the untreated control. This test has therefore allowed to demonstrate that the compost from pruning residues and cattle manure is a viable alternative to restore soil fertility by stimulating the productivity, the plant vigor and preserving the grapes quality.

Chapter 3

COMPOST FROM VINE BRANCHES AND GRAPE MARC IN THE VINEYARD : EFFECTS ON CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF SOIL

3.1 Introduction

The wine production in the countries of the European Union represents about 53% of the entire worldwide production, being Spain, with France and Italy, the main producers (International Organization of Vine and Wine - OIV, 2013). Such high levels of production involve the production of large amounts of by-products (vine branches from operations of winter pruning and grape marc from the pressing of the grapes after harvesting). The pruning residues does not currently represent for vineyards a source of income, but are in most cases a problem and at the same time a production cost (Esteban A. *et al.*, 2007).

Until today the disposal of such waste consisted of two main solutions:

- shredding in the field between the rows and their possible burial;
- collecting and burning the branches.

The shredding with landfill can be useful in the presence of healthy vineyards: in these cases the shoots do not constitute sources of infection or spread of diseases. This practice, however, may present a phytosanitary negative return in the case of vineyards affected by various diseases, including excoriose and grapevine esca, as well as encouraging the spread of root rot. In these circumstances, the burying of pruning is to be avoided, as the pathogen in the soil is a favorable environment for the winter and re-infect the following spring, the new shoots. In many cases, however, the shoots are collected and taken to the perimeter areas of the plots to be burned later. At present this solution is banned for its negative environmental returns for reasons of air quality-related emissions of fine particles and a precaution to prevent fires.

The grape marc are made from solid parts (skins, seeds and stalks) that remain after the pressing of the grapes. They represent a material rich

in phosphorous potassium and magnesium but have some disadvantages such as low pH (<4), high C/N ratio (25-40) and the presence of phytotoxic compounds (especially ethanol, acetic acid, lactic acid, polyphenols). In the distillery are processed to produce alcohol, brandy, grape seeds (from which we get then seed oil) and hulls (a supplement for animal feed). Alternatively, they can be used in the composting process to improve and accelerate the transformation of other materials less rich in nutrients.

Composting is becoming an ecological and economical alternative for reuse of plant biomass, such as pruning residues and grape marc obtained from the effects of agricultural practices in the vineyard (Fauci *et al.*,1999).

3.2 Aim of the study

The winter pruning of the vines and the pressing of the grapes after harvest produce large quantities of by-products whose management is not always easy, although regulated by a specific legislation. In recent years the need to identify a system of sustainable viticulture has become of great importance (Ramos M.C. *et al.*, 2007).

The purpose of the present work was to study and propose a model of sustainable management for the re-use of plant biomass (vine branches and grape marc) arising from agricultural practices in the vineyard. The research activity is mainly focused on the study of artificial humification process that could respond on the one hand to the needs of environmental sustainability (Sequi P., 1996) and on the other hand could be a safe resource to be included among the inputs of the vineyard ensuring recovery of vineyard fertility soils. In particular, this work has focused on the possibility of getting a quality compost from the combined fermentation of vine branches and grape marc obtained from the wine-making process; on the characterization of the microorganisms present in the plant mass during composting and on the study of the evolution of the microbial population during fermentation (Lei F., Vander Gheynst J.S., 1999); on the monitoring of physical/chemical and parameters to counter the development of grapevine pathogens and thus ensure the sanitization of biomass (Santos M. *et al.*, 2008) and on the study of the early effects of compost in the vineyard as a soil amendment to improve the nutritional status of grapevines and the soil biological activity.

3.3 Materials and methods

The trial was conducted in the three years period 2011-2013 at the “Dal Cin” farm. The farm complex is located a few kilometers from the center of Cordignano, in the venetian plain in the "D.O.C. Piave" zone (45° 57' 36" N and 12° 22' 21" E) at about 83 m above the sea level and covers an area of 16 ha, of which 15 involved in vineyard (Glera, Pinot, Merlot) and 1 dedicated to the cultivation of Actinidia.

In Figure 3.1 is shown the location of the farm (Google Earth).



Figure 3.1 - Location of the Dal Cin vineyard (Google Earth pictures).

In September 2011, were set up three static piles (Finstein M.S. *et al.*,1985) (Figure 3.2) using for each of them the amount of residues (vine branches and grape marc) obtained from 1 ha of vineyard: 3,5 t of vine branches and 3 t of grape marc. In February 2012 the same amount of pruning residues was added to the pile 1. The main idea was try to integrate cyclically the piles through the addition of the branches

in February and of the grape marc in September. Finally once a year, after checking the optimal course of composting, the mature material would be distributed in vineyard using at least 70-75% of the entire pile. The residue compost would have served as the inoculum for the newly added fresh material, accelerating the development of the whole process.



Figure 3.2 - Piles set up in September 2011 at the Dal Cin farm (Cordignano)

From september 2011 (T_0), the sampling was carried out every two months by taking three sub-samples from different parts of the plant mass, at different depths and exposure, from each of the two experimental piles. The collected samples were subjected to physical/chemical, microbiological (Chefetz B., 1996) and molecular analysis, in order to correlating the variations of each parameter with the evolution of the composting. In addition, during the same period were monitored some environmental parameters such as temperature and rainfall to better understand the influence of these factors on microbial activity and thus on the progress of fermentation process.

3.3.1 Physical/chemical analysis

The compost samples (in the case of samples chilled or frozen, after being equilibrated to room temperature) were thoroughly agitated and divided into two aliquots of 200 g each:

- the first (wet sample) was used for the determination of total moisture and pH. Because of the heterogeneity of the wet sample it was necessary to mix thoroughly the material to be analyzed.
- the second was used for the preparation of the dried sample in an oven, which was subsequently ground and sieved. The obtained sample was subjected to elemental analysis (CNS, ICP-OES).

Total moisture

The total moisture is determined on the wet sample by drying in an oven. This determination allows to know the total water content and at the same time to report the analytical data obtained for the wet sample, to the dry matter (Bozzolo A.,2010).

For each sample were weighed about 100 g (wet weight = ww), and then the sample was subjected to drying in a stove at 80 °C for a time of 24 hours. After the drying process is completed, the sample was weighed again (dry weight = dw). The total moisture was then calculated as follows :

$$\text{TOTAL MOISTURE \% : } (ww - dw) \times 100 / ww$$

ww = wet weight

dw = dry weight

pH

In chemistry, pH, notation that really means "power" (in the mathematical sense) of hydrogen, is used to indicate the magnitude that measures the acidity or alkalinity of an aqueous solution, expressed by the co-logarithm of the concentration (or, more properly, the activity) of hydrogen ions. Therefore: $\text{pH} = -\log [\text{H}^+]$. This analytical report is derived from the potentiometric technique (introduced by SPL Sorensen in 1909) which were made the first accurate measurements of the concentration of hydrogen ions, for which the measured value of the potential is a function of the logarithm of the concentrations.

The pH meter was calibrated using two buffer solutions of known pH: the first with a pH of 4,00 and the second at pH 7,00. Following the official methods of soils chemical analysis (September 1999), 10 g of each sample were weighed and placed in 25 ml of ultrapure water for 1 hour observing a sample/water ratio of 1:2,5 (v/v). The measurement was performed directly on the aqueous suspension keeping the bulb of the glass electrode immersed in the murky and the salt junction in the supernatant clear liquid.

CNS elemental analysis

A representative quantity of the dry sample was below ground to an extent that pass entirely through a sieve with a mesh diameter of 0,5 mm (500 μm). The grinding must be conducted with equipment that does not give rise to contamination, for example, due to the release of metals and must be terminated only when almost all of the sample has passed through the sieve.

The CNS analysis requires the preparation of homogenized and sieved samples in capsule: to this purpose have been used tin foil of 35 x 35

mm adequately shaped inside of which it is possible to pour the material to be analyzed (Figure 3.3). For each sample was weighed approximately 70 mg of material using an analytical balance (A&D, model GH-252) and then were added about 140 mg of tungsten trioxide (WO_3). This substance prevents the alkali and alkaline earth metals present in the sample react with the quartz combustion tube causing the destruction of tube itself, it also prevents the formation of alkaline sulphates that are difficult to burn. Once prepared the capsule is closed and placed in a chamber in which the air is expelled through manual pressure exerted on a small piston (Figure 3.4). In this way were obtained the capsules of all samples to be analyzed.



Figure 3.3 – on the left: plastic stencil for the creation of the vessels from the tin foil. On the right: capsules or vessels.

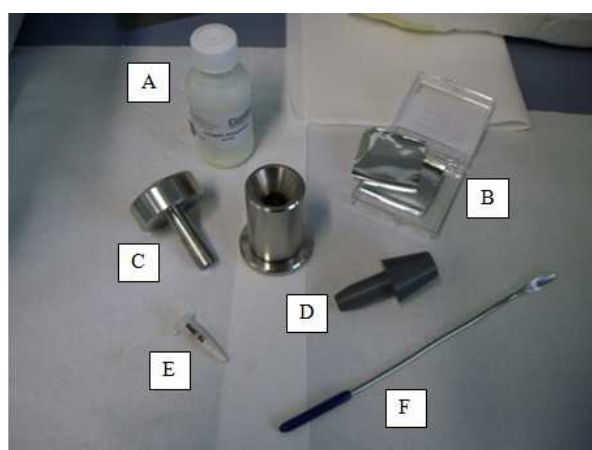


Figure 3.4 - Tools for sample preparation: A) tungsten trioxide, B) tin foil, C) piston, D) plastic stencil, E) sieved sample, F) spatula for weighing chemicals.

The analyses were performed using the "vario MACRO" elemental analyzer (Figure 3.5), a completely automatic tool which allows rapid quantitative analysis of C, H, N, S, starting from various kinds of materials (solids or liquids). The operating principle is based on the Dumas method (1831), which provides a complete and instantaneous oxidation (flash combustion) of the sample with conversion of all organic and inorganic substances in gaseous products. Inside the instrument take place the following reactions: in the combustion tube (first reaction tube) the high temperatures (1150 °C) and the O₂ presence determine the incineration of the sample. The products that arise during the combustion are CO, CO₂, H₂O, NO_x, SO₂ and SO₃ and they are transported by a He flow, used as a carrier gas, until the detector. In the reduction tube (second reaction tube containing Cu in the reduced state) NO_x and SO₃ are quantitatively reduced to N₂ and SO₂ and the excess oxygen is bound by silver wool (present in the upper side of the tube). The moisture present in the gas stream is removed with a first passage through a membrane and with subsequent transfer of Sicapent (highly hygroscopic compound). Inside the post-combustion tube (third reaction tube containing CuO and Pt) there is a complete oxidation to CO₂ of carbon compounds not completely oxidized (CO). The separation of N₂ from CO₂ and SO₂ is made blocking temporarily the CO₂ and SO₂ inside specific heated columns. The nitrogen (N₂) comes instead directly to the detector which detects the concentration. Following the CO₂ adsorption column is heated up to 230°C causing the liberation of the compound which can be transported up to the detector. Finally, to effect the heating of the SO₂ column up to 210°C the sulfur oxides are released and detected.

The quantification of the various elements is performed with creation of a calibration curve generated by the use of a standard (Sulfanilamide)

containing known concentrations of the interest elements (N=16,25%; C=41,81%; S=18,62%; H=4,65%).

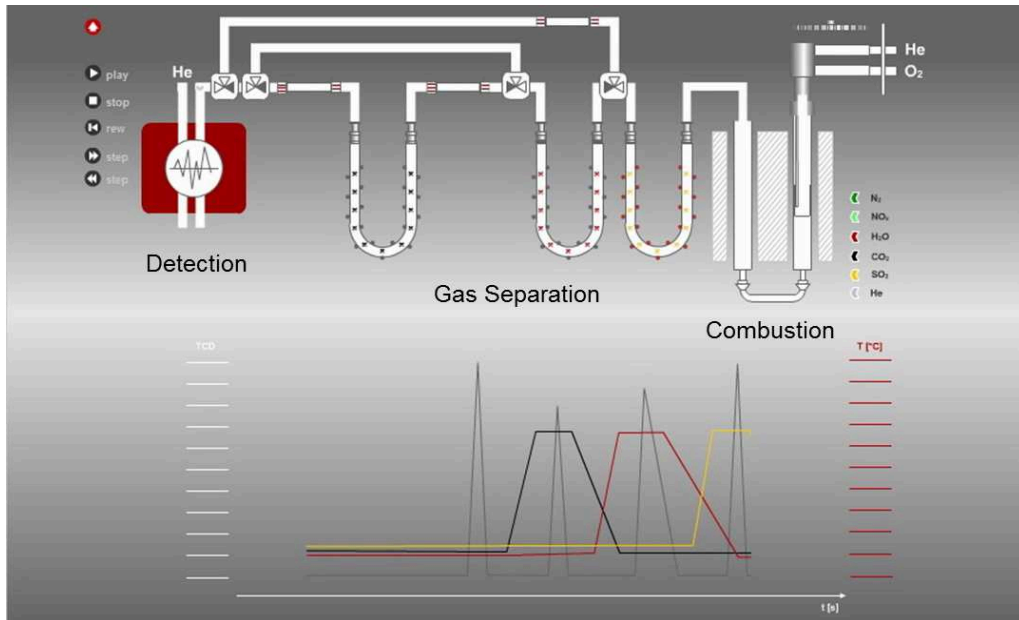


Figure 3.5 - Functional diagram of the elemental analyzer CNS "Vario Macro". Combustion: combustion chamber (furnace), where occurs the flash combustion; Gas Separation: reaction tubes, where the sample reacts with the gaseous state of substances that allow the reduction or oxidation of the elements measured by the spectrometer; Detection: detectors that allow the evaluation quantitative chemical elements (CNS). Source: www.elementar.de

ICP-OES elemental analysis

To assess the possible presence of heavy metals, a representative portion of the dry sample (5 g) was placed in a muffle furnace for 2 hours at 550 °C. The ashes obtained were acidified with 1 mL of HCl 37% ($\rho = 1.186 \text{ g mL}^{-1}$) and after 5 minutes were added 9 ml of ultrapure water (conductivity at 25 °C = $0,054 \mu\text{S cm}^{-1}$) to obtain a suspension with dilution ratio of 1:10. This operation was repeated to reach a final dilution ratio of 1:100.

The samples were analyzed with a spectrometer ICP-OES (Inductively Coupled Plasma - Optical Emission Spectroscopy). The tool allows to simultaneously detect with variable sensitivity and precision all the elements between lithium and uranium with the exception of oxygen, fluorine, noble gases. The characterizing part of the plasma optical emission spectrometer is the plasma itself which is constituted by argon gas with a high degree of ionization and at very high temperature (6000 to 8000 °C depending on the analysis), produced by the ionization of the gas being continuously flushed through the system.

The spectrometer consists of four parts:

1. Sample introduction system: the liquid sample is aspirated from the tubes through a peristaltic pump whose action is combined with a nebulizer. The liquid pours into the spray chamber. The purpose is to transform the sample solution and the Argon in an aerosol formed by droplets of diameter less than 10 μm .
2. Radiofrequency generator and torch: an electric current of 27,12 MHz creates a magnetic field that passes along the torch axis in which the energy produced by the generator is transferred by the electrons to the gas and so for collision the gas is heated. The plasma ignition is guaranteed by an injector and the plasma is confined inside the torch from a toroidal magnetic system. It has

thus a kind of plasma "nut" where is injected the aerosol containing the sample. The action of the magnetic field produced by the radiofrequency generator heats the plasma exciting the atoms of the elements to be analyzed. These atoms, returning to the lower energy state, emitting radiation. At the same time the torch is cooled by multiple streams of Argon to prevent breakage.

3. Optical bench and detectors: the radiations emitted go to collimate on a fixed system composed of 2 diffraction gratings that provide to separate them between 125 and 770 nm. The monochromatic beams are sent directly separated on 22 semiconductor detectors CCD arranged and secured in a semicircle (Rowland circle). The primary radiation is then analyzed simultaneously without loss of energy. In this way it is possible to simultaneously determine the elements on all of their emission lines. The core of the reading system is represented by CCD detectors through which it is possible to measure continuously all wavelengths between 125 and 770 nm. In the particular case of the ICP used, the detector is constituted by the union of two high resolution diffraction gratings Paschen-Runge which allow to overcome the drawbacks that are usually associated with the use of CCD technology. The signal arriving at the CCD is reworked on the CCD itself, sent to 3 electronic boards on ICP and then, once "clean", sent to the PC that controls the instrument with TCP/IP protocol, the same used in networks intra/internet .
4. Software: the system control is entrusted to a computer which, through a specific program, allows to make the instrument more easily usable and in many cases to reduce operator intervention only to the choice and optimization of the method. To proceed with the samples analysis is necessary to choose the length or

the most appropriate wavelengths (each element emits radiation at most frequencies). The samples inserted into Falcon tubes are taken through an autosampler that automates much the whole analytical procedure. Every ten samples was added to a control standard with a tolerance of +/- 10%. Before the reading of each sample, the instrument provides for the washing of the system through the aspiration and the flushing of demineralized water for 30 seconds through all parts of the machine in contact with the sample followed by the aspiration and the flushing of the sample for 60 seconds. The analysis consists of three readings, of variable duration depending on the determination, carried out consecutively. The result is obtained from the arithmetic mean of the three readings.

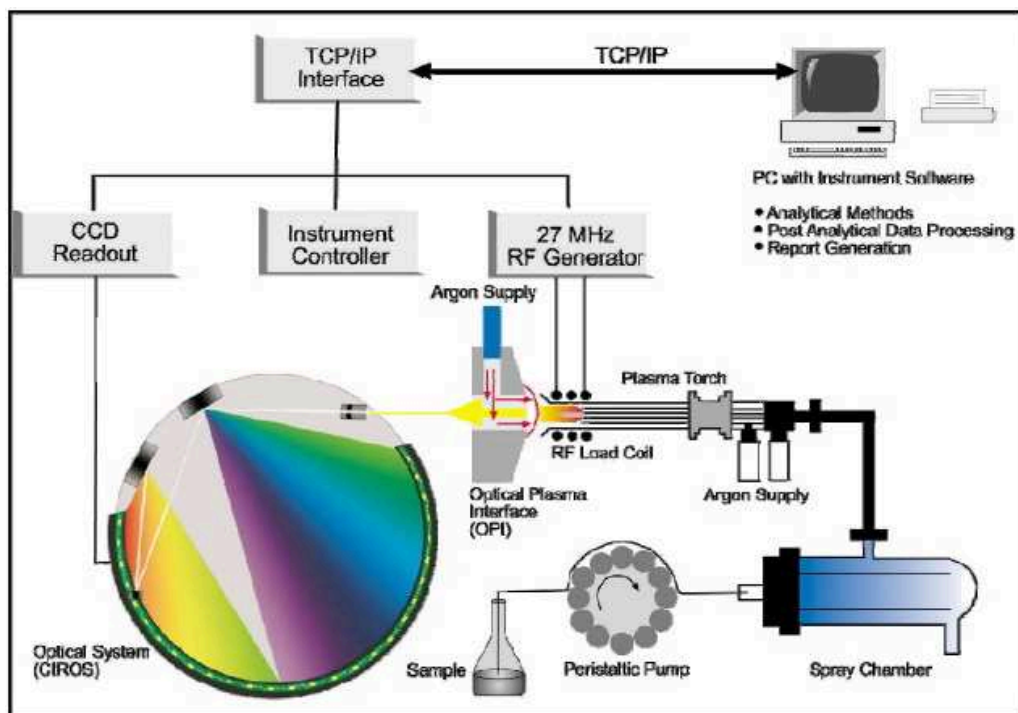


Figure 3.6 – The SPECTRO CIROS ICP-OES schematic diagram as provided by SPECTRO S.A. Source: www.spectro.com

3.3.2 Microbiological analysis

The composting process takes place thanks to the combined action of several microbial species naturally present in soil and plant materials (Ryckeboer J. *et al.*, 2003). The microorganisms grow by consuming the nutrients present in the mass and transforming complex polymeric materials (mainly cellulose and lignin) in compounds of low molecular weight. Microbial activity is high at the beginning of the composting process due to increased availability of nutrients can be easily used (fermentable sugars) and when occurs the addition of new plant material (vine branches, but especially grape marc that are more rich in simple sugars).

The present level of understanding microbial community dynamics in composting processes is largely based on studies carried out with traditional methods such as the cultivation on plate, the isolation and identification of bacteria, actinomycetes and fungi (Raso E. *et al.*, 2003). The determination of the number of microorganisms present in the material collected at each sampling was performed by standard counting of viable cells on the plate (Standard Plate Counts, SPC). This method is based on the principle that live cells present in a given sample rate (g or mL), once transferred (seeded, inoculated) in or on a suitable nutrient medium agar after incubation at room temperature and atmosphere appropriate, will multiply (in variable times depending on their growth rate in the conditions used for their cultivation), giving rise to visible colonies that can be counted (Francesco Villani *et al.*, 2006).

The cultivation methods of microorganisms require a series of steps that must be performed in the following order:

1. Preparation of culture media;
2. Sample preparation;
3. Preparation of serial decimal dilutions;

4. Sowing on nutrient agar;
5. Incubation and counting of colonies.

Preparation of culture media

During the two years of composting test has been studied the evolution of five differential microbial groups: total bacteria, cellulose-degrading bacteria, actinomycetes, molds, cellulolytic fungi. The cultivation of each group required the preparation of suitable media, for chemical composition, to the optimal microbial growth. The culture media used are:

TSA (Trypticase Soy Agar) is a culture medium for *total bacteria* composed of casein peptone (pancreatic) 15 g L⁻¹, soya peptone (papainic) 5 g L⁻¹, sodium chloride 5 g L⁻¹, agar 15 g L⁻¹ with final pH of 7,3 +/- 0,2 at 25°C. The solution was prepared by weighing 40 g L⁻¹ of powder and dividing the quantity to be weighed to the volume of interest (for the experiment were prepared 2 L of solution divided into five Erlenmeyer flasks of 400 mL, then for each flask the quantity to be weighed has been determined with the following equation - 40grams : 1 liter = x grams : 0,4 liters). The weighed amount was transferred into the flask , after adding 400 ml of deionized water, and then the flasks were sterilized in an autoclave for about 2 hours at 121 °C. After the sterilization, the antibiotic was added (TSA allows the total bacteria growth then is added the cycloheximide to prevent the fungi growth) in quantities (4 mL) calculated with the following formula :

$$C_i \times V_i = C_f \times V_f$$

C_i = stock concentration (0,2 g L⁻¹);

V_i = volume of flasks (400 mL);

Cf = final concentration (for chloramphenicol was selected a concentration of 100x or $20 \text{ g L}^{-1} = 0,2 \text{ g L}^{-1} \times 100$)

Vf = final volume (amount of antibiotic to be added into the flask).

BC is a selective culture medium for *cellulose-degrading bacteria* composed of ammonium sulphate 1 g L^{-1} , potassium hydrogen phosphate 1 g L^{-1} , magnesium sulfate heptahydrate $0,5 \text{ g L}^{-1}$ sodium chloride 1 mg L^{-1} , agar 15 g L^{-1} , carboxymethylcellulose 10 g L^{-1} . The solution was prepared by weighing the reagents $[(\text{NH}_4)_2\text{SO}_4, \text{K}_2\text{HPO}_4, \text{MgSO}_4 \times 7\text{H}_2\text{O}]$ in the amounts indicated in the recipe (see above) and dividing the quantity to be weighed to the volume of interest (2 L of solution divided into five Erlenmeyer flasks of 400 mL). The weighed amount has been transferred to a 2 L plastic glass containing about 1,5 L of deionized water placed on a mechanical shaker. During the stirring was added the stock solution of NaCl (the stock concentration is $0,001 \text{ g L}^{-1}$ and it was decided to use a concentration of 10000x, for which the quantity to be added is calculated with the following formula:

$$C_i \times V_i = C_f \times V_f$$

Ci = stock concentration ($0,001 \text{ g L}^{-1}$);

Vi = volume of plastic glass (2000 mL);

Cf = final concentration (for NaCl was chosen a concentration of 10000x or 10 g L^{-1} ($0,001 \text{ g L}^{-1} \times 10000$))

Vf = final volume (amount of NaCl to be added to the solution).

After autoclaving the antibiotic was added (BC allows the growth of cellulolytic bacteria, then is added the cycloheximide to prevent the fungi growth) in the same quantity of TSA (4 mL).

AIA (Actinomycete Isolation Agar) is used for isolation and propagation of *actinomycetes* and it is composed of sodium caseinate 2 g L⁻¹ , L-Asparagine 0,1 g L⁻¹ , sodium propionate 4 g L⁻¹ , dipotassium phosphate 0,5 g L⁻¹ , magnesium sulphate 0,1 g L⁻¹ , ferrous sulphate 1 mg L⁻¹ , agar 15 g L⁻¹ with final pH of 8,1±0,2 at 25°C. The solution was prepared by weighing 22 g L⁻¹ of powder and dividing the quantity to be weighed to the volume of interest (2 L of solution divided into five Erlenmeyer flasks of 400 mL). After the transfer of the culture medium in the flask were added 5 g L⁻¹ of glycerol (for flask - 5grams : 1 liter = x grams : 0,4 liters) and then 400 ml of deionized water. After sterilization and cooled the the culture medium to avoid the inactivation of antibiotic were added 4 mL of cycloheximide.

PDA (Potato Dextrose Agar) for *molds* : composed of potato extract 4 g L⁻¹ , dextrose 20 g L⁻¹ , agar 15 g L⁻¹ with final pH of 5,6±0,2 at 25 °C. The solution was obtained by weighing 39 g L⁻¹ of powder and dividing the quantity to be weighed to the volume of interest (2 L of solution). At the end of the two hours of autoclaving was added the antibiotic (in this case the chloramphenicol because the PDA enables the growth of molds and therefore it is necessary to prevent the development of bacteria). The amount (4 mL) was calculated using the known formula:

$$C_i \times V_i = C_f \times V_f$$

C_i = stock concentration (0,1 g L⁻¹);

V_i = volume of flasks (400 mL);

C_f = final concentration (for chloramphenicol was chosen a concentration of 100x or 10 g L⁻¹ (0,1 g L⁻¹ x 100)

V_f = final volume (amount of chloramphenicol to be added to the solution).

FC for *cellulolytic fungi*: a selective culture medium obtained by adding to a first solution (composed of carboxymethylcellulose 10 g L^{-1} and agar 15 g L^{-1}) with a second solution named YNB (yeast nitrogen base) characterized by a complex chemical composition (biotin $2 \text{ } \mu\text{g L}^{-1}$, calcium pantothenate $400 \text{ } \mu\text{g L}^{-1}$, folic acid $2 \text{ } \mu\text{g L}^{-1}$, inositol 2 mg L^{-1} , niacin $400 \text{ } \mu\text{g L}^{-1}$, p-aminobenzoic acid $200 \text{ } \mu\text{g L}^{-1}$, pyridoxine hydrochloride $400 \text{ } \mu\text{g L}^{-1}$, riboflavin $200 \text{ } \mu\text{g L}^{-1}$, thiamine hydrochloride $400 \text{ } \mu\text{g L}^{-1}$, boric acid $500 \text{ } \mu\text{g L}^{-1}$, copper sulfate $40 \text{ } \mu\text{g L}^{-1}$, potassium iodide $100 \text{ } \mu\text{g L}^{-1}$, ferric chloride $200 \text{ } \mu\text{g L}^{-1}$, manganese sulfate $400 \text{ } \mu\text{g L}^{-1}$, sodium molybdate $200 \text{ } \mu\text{g L}^{-1}$, zinc sulfate $400 \text{ } \mu\text{g L}^{-1}$, potassium phosphate monobasic 1 g L^{-1} , magnesium sulfate 500 mg L^{-1} , sodium chloride 100 mg L^{-1} , calcium chloride 100 mg L^{-1}).

The culture medium was obtained by preparing a first solution with carboxymethylcellulose (10 g L^{-1}) and agar (15 g L^{-1}). The amount weighed were transferred into each flask of 400 mL, in this case adding 360 mL of deionized water. During autoclaving was prepared the second solution (YNB - Yeast Nitrogen Base) starting from the stock concentration (6,7 g in 100 mL), in a quantity equal to 200 mL whereas the addition of 40 mL per flask (360 mL of solution 1 + 40 mL of YNB). The solution obtained cannot be sterilized in an autoclave, and then must be filtered under a laminar flow hood using millipore filters, within falcon tubes of 50 mL and then added to the culture medium with a pipette.

As for the PDA, also in this case was added to the culture medium 4 mL of chloramphenicol for avoid the bacterial growth.

Hereinafter, operating under laminar flow hood, the Petri dishes were prepared by pouring in each about 15-20 mL of liquid medium and allowed to dry for 15 minutes until complete solidification of the agar medium. The plates were then stored in a refrigerator at $4 \text{ } ^\circ\text{C}$ until the sampling day (about for a week).

Sample preparation for analysis

First of all, it was necessary to ensure conditions of absolute sterility in order to avoid external contamination that would otherwise have invalidated the analysis, for this reason all glassware used (Duran glass bottles, measuring cylinders, flasks) was sterilized autoclave. The standard for sterilization in a laboratory environment provides for the attainment of a temperature of 121 °C and a pressure of 2 bar maintained for 20 minutes. Before counting, the microbial cells contained in the matrices to be analyzed (mix of vine branches and grape marc) must be transferred into the appropriate nutrient medium. To this end, the three sub-samples were homogenized in a suitable diluent, in order to disperse the microorganisms in a liquid phase that could be easily manipulated for analysis. The rate of the sample intended to prepare the homogenate must be large enough so as to be representative of complex microbial composition of the sample. The homogenate was then prepared by diluting the sample in a ratio of 1:10 in a suitable diluent. The choice of diluent is a very important stage of the analysis, because not suitable diluents, such as water, can cause damage to the microbial cells, resulting in underestimation of the microorganisms number. The diluent used for this type of analysis is represented by a Ringer solution obtained by dissolving in 500 mL of deionized water a tablet containing the following salts: sodium chloride, potassium chloride, calcium chloride, sodium hydrogen carbonate. The suspension-final dilution, also called first dilution or dilution mother, was obtained for each of the three sub-samples by adding 200 mL of diluent (Ringer solution) to 20 g of sample, weighed into a Duran glass bottle. The three bottles were placed in agitation at 150 rpm for 2 hours at a controlled temperature of 22 °C.

Preparation of serial decimal dilutions

The quantitative microbiology aims to determine the microorganisms number or, more precisely, the number of Colony Forming Units (CFU) per g or ml in a given sample. The microorganisms number present in a sample (not only for compost sample), in general, is so high as to be counted only after being subjected to serial dilutions.

For the preparation of serial decimal dilutions, the diluent used was brought to a temperature close to that of the sample, to avoid thermal damage to the microorganisms. The first dilution was left at rest, to facilitate the settling of coarse particles and the dispersion of microorganisms in the diluent. In any case, the time interval between the preparation of the first and subsequent dilution should never exceed 15 minutes, while seeding dilutions to Petri dishes must be prepared no later than 20-30 minutes after preparation of the initial dilution.

The procedure involves withdraw with a sterile pipette 1 mL of the first dilution and transfer to 9 mL of sterile diluent (1:10) avoiding contact between the pipette and the diluent and in this way is obtained a 1:100 dilution (10^{-2}) of the sample. This dilution is homogenized carefully by an automatic stirrer for 5 minutes. The entire procedure was repeated a number of times equal to the dilutions number required for the CFU count (Figure 3.7). For the five microbial groups studied in this work, the serial decimal dilutions were defined on the basis of CFU counting results obtained from similar experiments, concerning the study of the microbial community dynamics during green waste composting (Gazi A.V. *et al.*,2006).

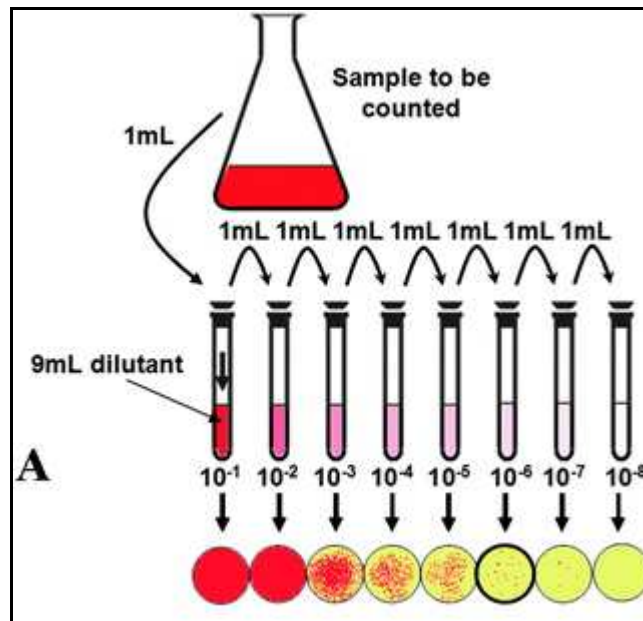


Figure 3.7 - Scheme of operations for the preparation of serial decimal dilutions

Sowing on nutrient agar

Once prepared the serial decimal dilutions of the sample (and therefore of the microorganisms present in it) it was possible to transfer with the use of a pipette, 1 mL of the dilutions on the nutrient medium. The goal was to inoculate the plate, with rates of dilutions, an ever smaller number of microorganisms so that at least 1 or 2 plates have after incubation under optimal conditions, between 30 and 300 well-isolated colonies, so as to be easily counted.

The used technique known as “spread plate” provides the sowing of the inoculum for surface distribution on the solid substrate. The inoculum was deposited with a pipette on the agar surface and distributed on it with a sterile glass rod in the shape of “L”. Once adsorbed the inoculum and dried the culture medium under laminar flow hood, the plates were transferred into the thermostat in upside down position.

Incubation and counting of colonies

Once inoculated, half of the plates (270 for each sampling) were incubated at 60 °C for 3 days to ensure the growth of thermophilic strains, and the other half was placed at 30 °C for 7 days to evaluate the development of mesophilic microorganisms.

After incubation of the plates, if the inoculum was homogeneously distributed in the substrate, theoretically every cell present in the initial sample has given rise, after multiplication, to a single colony visible to the naked eye. For the counting of colonies are selected only the plates that are accounting, then those that present a number of colonies between 30 and 300. This interval is chosen because it was considered statistically significant. When on a plate there are less than 30 colonies, even small errors in the dilution technique of the sample or the presence of a few cells of contaminated (arising, for example, from contamination during counting operations) may have significant effects on the final count. Instead, a plate with a number of colonies greater than 300 may be difficult to count. In fact, when they are deposited on the substrate with the inoculum many cells, these will tend to develop in a confluent manner, giving rise to colonies not enough isolated to be counted.

For counting, the number of colonies counted on a plate was multiplied by the number of times in which the initial sample has been diluted. In particular for each of the three sub-samples collected from each of the two experimental piles were provided three replicates for three different sample dilutions. Therefore it was calculated the average of the three values obtained for each dilution and the mean value was multiplied by the dilution ratio. Finally, the average value so calculated was multiplied by the dry weight of the corresponding sub-sample by obtaining the number of CFU g⁻¹ of dry weight.

3.3.3 Molecular analysis: ARISA technique

The ARISA technique (Automated Ribosomal Intergenic Spacer Analysis) is used to estimate the microbial diversity within an environmental sample and is based on the use of fluorescent-labeled primers for the amplification of intergenic region ITS of ribosomal operon (intergenic spacers). The forward primers used in the PCR ARISA are labeled at the 5' fluorophore with the phosphoramidite (6-FAM) suitable for detecting the automatic sequencer. In this thesis, the ARISA analysis was performed for bacterial and fungal communities using the primers ITSF and ITSReub (Table 3.1).

The concentration of DNA to be amplified is 10 ng, but can increase up to 300 ng depending on the sample analyzed. The reaction mixture comprises 1xPCR buffer, 200 μ M each dNTP, 1,5 mM $MgCl_2$, 0,25 mM each primer and 1,5 units of Taq DNA polymerase, for a total volume of 25 μ L (Cardinale *et al.*, 2004). The PCR thermal profile ARISA is the following:

- 94 °C for 3 minutes (*initial denaturation*),
- 30 cycles at 94 °C for 45 seconds (*denaturation*),
- 55 °C for 1 minute (*annealing*),
- 72 °C for 2 minutes (*elongation*),
- 72 °C for 7 minutes (*final extension*).

The resulting amplicons, once subjected to a DNA gel electrophoresis to check the success of the reaction, are denatured in the presence of formamide. The denaturation occurs within a thermal cycler for 3 minutes at 95 °C. The denaturation mixture comprises 0,6 μ L of ROX, a ladder labeled with a fluorescent probe, 18,9 μ L of formamide which acts as a denaturing agent and 1 μ L of DNA at a concentration of 1,2 ng L^{-1} for a total volume of 20,5 μ L. Once denatured, the samples were

immediately placed on ice and subsequently introduced into a sequencer that performs an automated capillary electrophoresis which guarantees a high reproducibility of the technique. The amplicons that migrate into the capillary electrophoresis system are detected by a laser system that recognizes employee fluorescence-labeled nucleic acids. Each peak detection is recorded in the form of an electropherogram that at the end of the analysis will be translated into a complex electrophoretic profile, a kind of fingerprinting of the microbial community structure. The sensitivity of the method is very high due to the resolution capabilities of the capillary electrophoresis system able to distinguish differences of a single nucleotide. The fingerprint ARISA generated were analyzed using GeneMapper software version 4 (Life Technologies, UK).

Primer	Sequence (5'→3')	Dye
ITSF	GTCGTAACAAGGGTAGCCGTA	6-FAM
ITS Reub	GCCAAGGCATCCACC	

Table 3.1 - Sequences of the primers used for the ARISA analysis

3.3.4 Environmental parameters

The process of artificial humification began in September 2011 with the piles preparation was closely followed by monitoring of some parameters such as temperature (of air and of the piles) and rainfall.

The process temperature was detected by the use of two probes (data logger) for each of the two experimental piles, inserted inside the mass to a depth of about 50 cm and adequately protected with plastic housings in order to avoid that the humidity inside the biomass could

damage the electronic components of the probes and thus lead to loss of recorded data. The data loggers were programmed to detect and store up to 24 temperature data per day: the measured values were drawn by using a special software (MicroLab Lite v3.6.5, Fourier). The main objective of the trial was to demonstrate the validity of composting in the field then it is considered appropriate to detect the temperature and rainfall environment: the data were obtained as the average of the daily values provided by three meteorological stations (Conegliano , Vittorio Veneto and Gaiarine) located a few km from the Dal Cin farm. The data processing has produced the two graphs below:

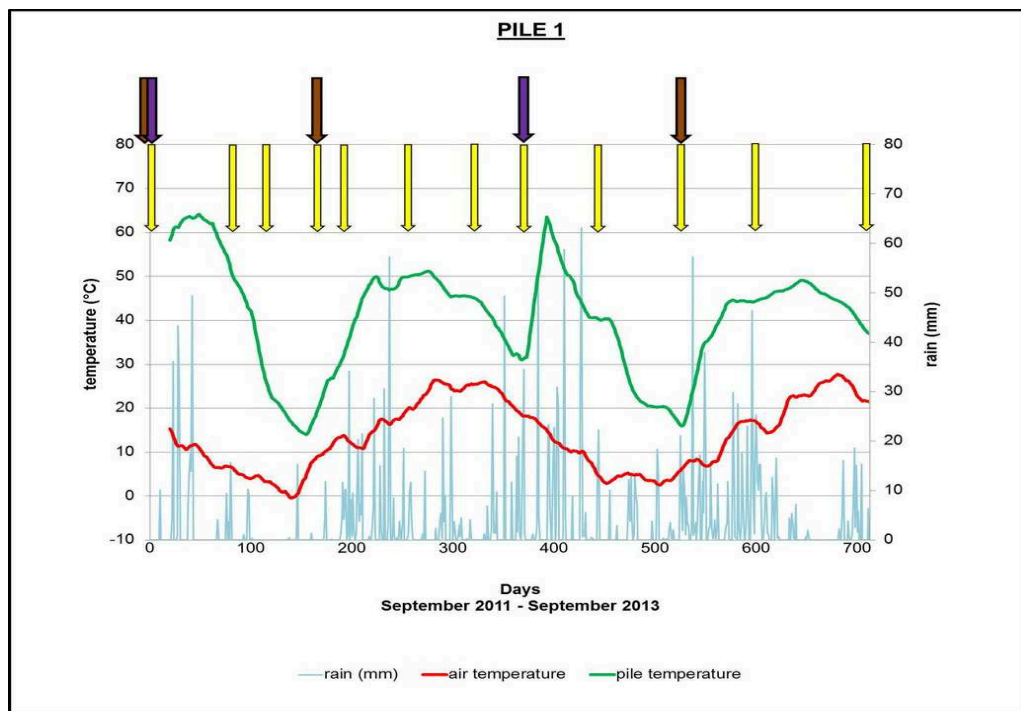


Figure 3.8 - Temperature trend of the pile 1 during the composting process. The red line represents the average air temperature and the blue histograms show the contributions of rainwater. The brown arrows indicate the time of the prunings addition (initially in September and then, always in February) while the purple arrows show the additions of grape marc (September). Are also visible the samplings time for microbiological analysis (yellow arrows).

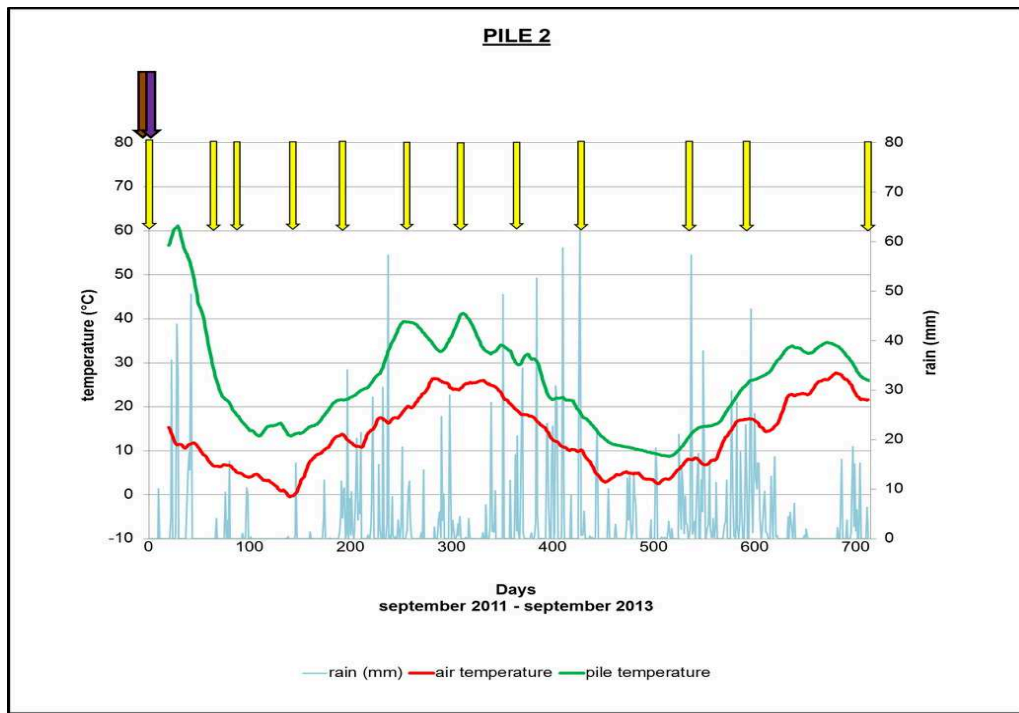


Figure 3.9 - Temperature trend of the pile 2 during the composting process (see caption of Figure 3.8).

The temperatures of the pile 1 show an initial active phase with temperature values above 60 °C for several days: during this stage the material undergoes a major sanitation which causes inactivation of weed seeds, and especially, pathogenic organisms as long as the temperature is maintained at values greater than 55 °C for at least a week (Bustamante M.A. *et al.*,2007). In this regard, it is clearly see that the addition of vine branches and grape marc lead to new active phases (sanitizing) subsequent to the contribution of fresh materials causing temperature rises that provide a more effective sanitizing. This is particularly evident after the intake of grape marc. On the contrary, the pile 2 manifests only an initial active phase, then the biomass temperatures follows without further elevations, the air temperatures determined by climatic conditions.

3.3.5 Laboratory composting test

In order to assess the ability of by-products of the vineyards to natural composting, a homogeneous sample of biomass, deriving from the pruning residues of vineyards mixed with grape stalks (size approximately 6 cm), was subjected to composting test in adiabatic laboratory fermenter of 20 L (Figure 3.8). Before the test, the sample has been optimized for the moisture content bringing it, as required by the Method UNI/TS 11184 - October 2006), to the 75% of maximum Water Holding Capacity (WHC).



Figure 3.8 – the adiabatic fermenter used for composting test

After about 9 days of active phase in the fermenter, during which the sample was mixed periodically monitoring the trend of the Potential Dynamic Respiration Index (PDRI), the biomass temperature, the

oxygen concentration at the reactor outlet and the specific air flow ($5,82 \text{ m}^3 \text{ h}^{-1} \text{ t}^{-1} \text{ dm}$), the material was placed in a container, wet and reshuffled weekly, in order to simulate the subsequent maturation phase, so as to reach a treatment time of 90 days.

The table 3.2 shows the main characteristics of the sample during the composting test.

Parameter	UM	t=0	t=7	t=15	t=90
pH		5,78	7,21	7,23	8,31
Moisture	%	39,47	43,12	46,95	29,31
Dry Matter	%	60,43	56,88	53,05	70,69
Volatile substances	% d.m.	89,52	91,56	91,43	88,84
PDRI	$\text{mgO}_2 \text{ kg SV}^{-1} \text{ h}^{-1}$	1024	//	//	329
Total nitrogen	% d.m.	1,22	1,3	1,13	1,27
Organic carbon	% d.m.	41,58	44,26	42,31	43,07
C/N ratio		34,08	34,05	37,44	33,91

Table 3.2 - Characterization of the matrices used in the composting test

The graphs below show, respectively, developments of PDRI and temperature during the composting test.

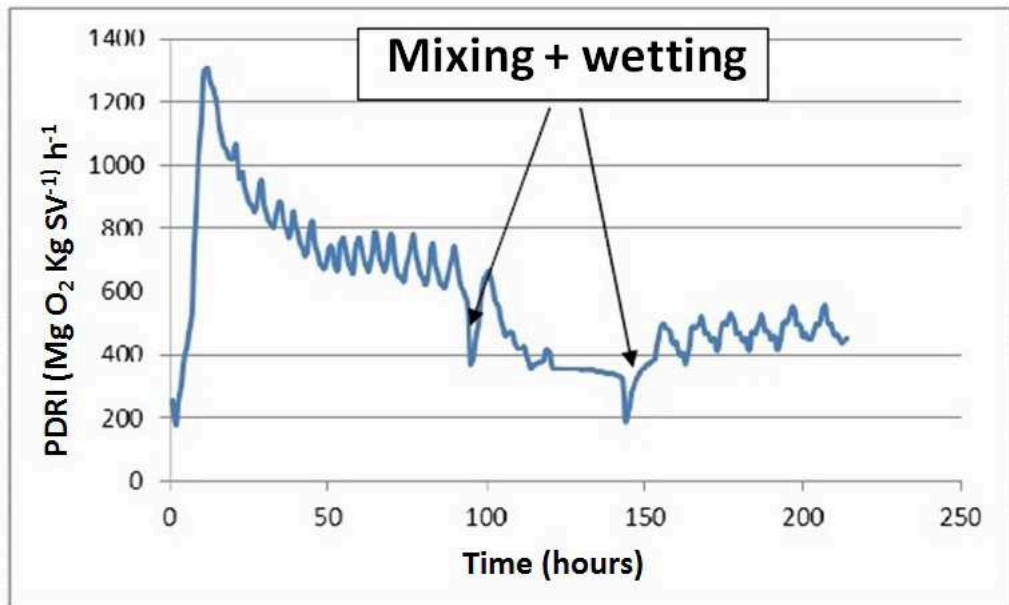


Figure 3.9 – PDRI trend during the composting test

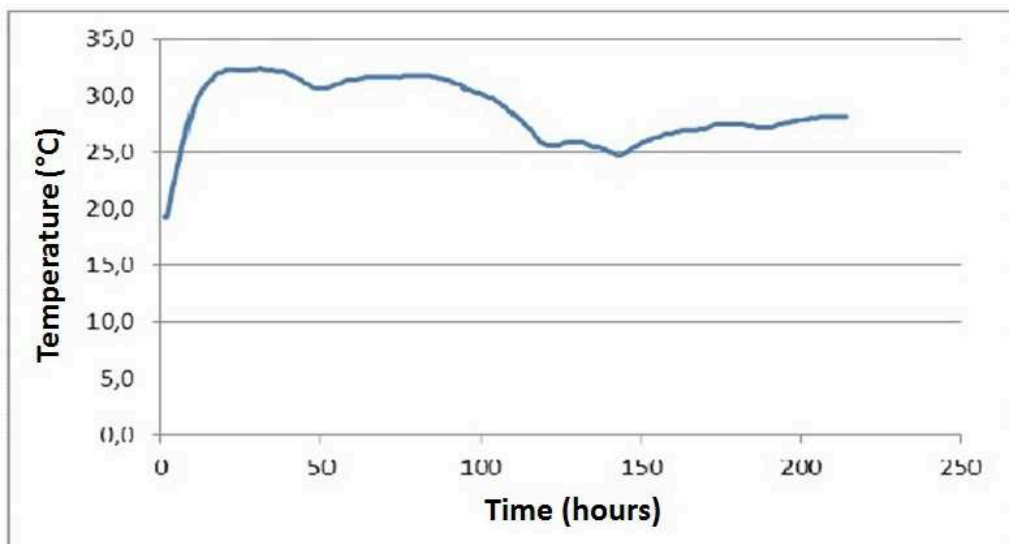


Figure 3.10 – Temperature trend during the composting test

As shown in Figure 3.9, the biomass during the active phase in the fermentor was stirred at the 4th and 7th day from the beginning of the

test, when it was detected a trend towards decreasing of the PRDI values (Scaglia B. *et al.*, 2000). Following the result obtained after the second mixing in the presence of low levels of respiration ($<500 \text{ kg mgO}_2 \text{ SV}^{-1} \text{ h}^{-1}$), it was deemed no longer necessary to submit the sample to forced ventilation and then proceeded to transfer in a container to continue the maturation phase by exploiting the mechanism of passive aeration of the material, also considered the high porosity of the mixture. There is also to be considered that the values of specific air flow rate, equal to $5,82 \text{ m}^3 \text{ h}^{-1} \text{ t dm}^{-1}$, required by the biomass (the test was set up with a feed-back controlled by the O_2 concentration in the biomass that is not should never be less than 14%, this being the concentration considered optimal for the activity of aerobic microorganisms) were below the air requirements normally expected for the biostabilisation mixtures with high organic load (eg. lignocellulosic waste in mixture with FORSU or generic agro-industrial waste). This is to mean that, probably, the tested mixture (pruning residues of vineyards mixed with grape stalks) can, obviously with longer times, be composted without forced air insufflation, with undoubted advantages from the economic point of view (Michel F., 1999). At the end of the composting test, the biomass has been analyzed to evaluate the main chemical and microbiological parameters (Table 3.3), which were compared with the parameters required by Legislative Decree no.75 of April 29, 2010 in relation to the qualitative characteristics of the composted soil improvers. It is evident that the measured values are within the expected limits. The only data that exceeds the law limits the C/N ratio, equal to 33,91 with respect to the maximum expected value of 25.

Parameter	UM	Measured Value	Legal Limit*
Moisture	%	29,31	<50
pH		8,31	6-8,5
Organic carbon	% d.m.	43,07	>20
UA+FA	% d.m.	11,15	>7
C/N ratio		33,91	<25
Total nitrogen	%	1,27	//
Organic nitrogen	% total N	84	>80% total N
Germination index (dil.30%)	%	112	>60%
Cd	mg kg d.m. ⁻¹	0,04	<1,5
Cr ^{VI}	mg kg d.m. ⁻¹	0,09 (total Cr)	<0,5
Cu	mg kg d.m. ⁻¹	45,05	<230
Hg	mg kg d.m. ⁻¹	0,05	<1,5
Ni	mg kg d.m. ⁻¹	0,93	<100
Pb	mg kg d.m. ⁻¹	0,85	<140
Zn	mg kg d.m. ⁻¹	11,07	<500
Plastic matter, glass, metals (dimension >2mm)	% d.m.	0,0041	<0,5
Inert lithoid (dimension >5mm)	% d.m.	absent	<5
Salmonella	MPN	absent	absent
Escherichia coli	CFU g ⁻¹	<10	<1000

Table 3.3 - Characterization of final composted soil improver (*Legislative Decree no.75 of April 29, 2010).

The final compost is characterized by values close to zero for inert materials (plastic and glass) as well as could be expected given the high quality of the initial matrix. Even the analysis of the possible presence of pathogens, in particular Salmonella and E. coli, shows typical values of high quality compost.

The most interesting aspect is certainly relative to the germination test with *Lepidium sativum* (cress), aimed to assessing the compatibility of biomass with the growth of plants. The test was performed following the procedures for evaluation of the phytotoxicity of composted soil improvers (Microbiological methods of analysis of the compost, 2003). The basic principle of the test involves the assessment of the effect of

an aqueous extract of the compost to be analyzed, on the germination of the plant to be tested. For this purpose the test sample (200 g) is brought to a humidity of 85% and left for two hours in contact with the water addition. The suspension is centrifuged at 6000 rpm for 15 minutes and the supernatant is then filtered under pressure to 3,5 atm. with sterilizing membrane. The aqueous extract is diluted to a concentration of 75% and 50%. Then five aliquots of 1 mL each for each of the two samples obtained (as many more controls with water) are placed in Petri dishes containing tissue paper. In each capsule were placed 10 seeds of *Lepidium sativum* made to swell for one hour in distilled water. The capsules are incubated at 27 °C for 24 hours. After this period, the germinated seeds were counted and measured the root length. The Germination Index (GI) is calculated as follows:

$$GI \% = (Gs \times Ls / Lc \times Gc) \times 100$$

Gs = average number of germinated seeds in the sample

Gc = average number of seeds germinated in the control

Ls = average root length in the sample

Lc = average root length in the control

The compost obtained showed a germination index equal to 112% compared to the minimum value required by law equal to 60% (Table 3.3 and Figure 3.11), indicating not only the total absence of phytotoxicity but that the product is able to stimulate the plant activities.

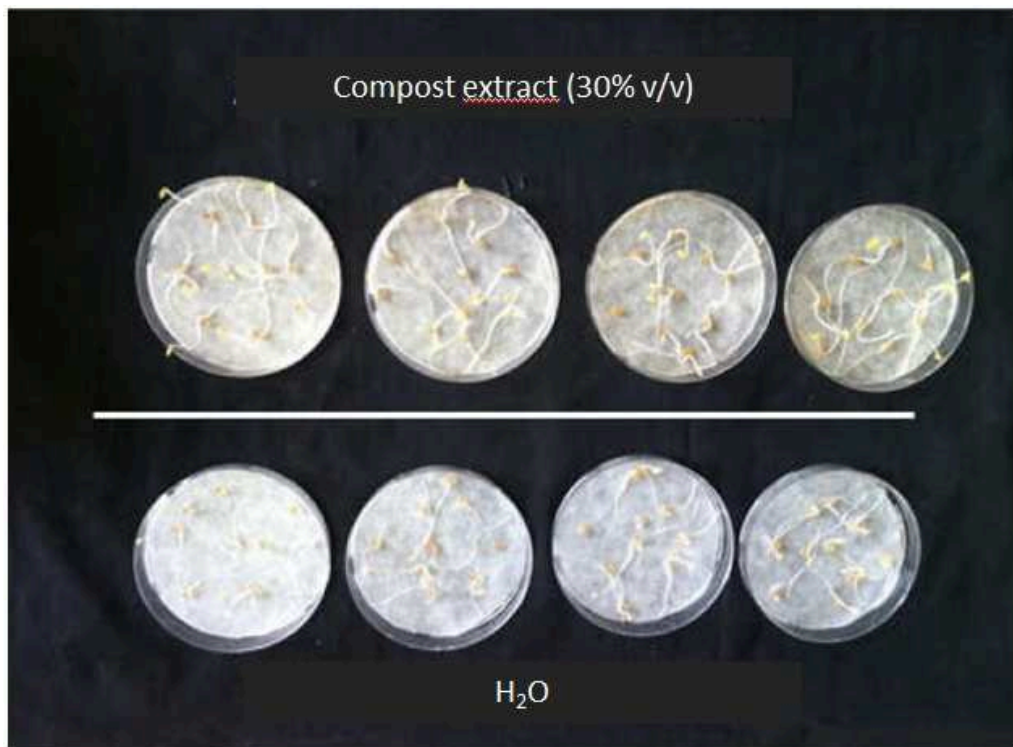


Figure 3.11 - Results of the germination test with cress (*Lepidium sativum*).

3.3.6 Compost distribution in the vineyard

After 18 months from the start of the experiment, the analysis of all monitored parameters showed the end of the composting process. Thus was identified a suitable site to proceed with the distribution of composted soil improver in order to assess the effects of the return to the vineyard of native organic matter. The compost of prunings residues and grape marc was distributed on March 4, 2013 on 4 rows (approximately for 1.000 linear meters under the row space) of Livieri vineyard located in the "D.O.C. Piave" zone (45° 57' 12" N and 12° 19' 98" E), about 4 km from the Vittorio Veneto centre (adjacent to the cooperative winery), at about 99 m above the sea level and covers an area of 5 ha planted with Glera (Block C, Figure 3.12).



Figure 3.12 - The rows affected by the distribution are highlighted by yellow segments.

Before the spill, the composted soil improver was evaluated on the basis of the main chemical and microbiological parameters (Table 3.4), which show the high quality of the material. The compost obtained proved to be a high-quality fertilizer that meets the criteria required by the Italian Composting Association for the award of the Quality Mark, as well as, all the parameters of the reference standard (Legislative Decree no.75 of April 29, 2010).

Parameter	UM	Measured Value	Legal Limit*
Moisture	%	59,2	<50
pH		8,24	6-8,5
Organic carbon	% d.m.	28,7	>20
UA+FA	% d.m.	13,5	>7
C/N ratio		13,5	<25
Total nitrogen	%	2,2	//
Organic nitrogen	% total N	88	>80% total N
Germination index (dil.30%)	%	109	>60%
Cd	mg kg d.m. ⁻¹	0,04	<1,5
Cr ^{VI}	mg kg d.m. ⁻¹	0,32 (total Cr)	<0,5
Cu	mg kg d.m. ⁻¹	26,55	<230
Hg	mg kg d.m. ⁻¹	0,04	<1,5
Ni	mg kg d.m. ⁻¹	1,07	<100
Pb	mg kg d.m. ⁻¹	0,87	<140
Zn	mg kg d.m. ⁻¹	13,42	<500
Plastic matter, glass, metals (dimension >2mm)	% d.m.	0,023	<0,5
Inert lithoid (dimension >5mm)	% d.m.	0,018	<5
Salmonella	MPN	absent	absent
Escherichia coli	CFU g ⁻¹	<10	<1000

Table 3.4 - Characterization of final composted soil improver after about 520 days of composting (*Legislative Decree no.75 of April 29, 2010).

The study in field of the effects caused by the contribution of organic matter in the vineyard involved some nutritional parameters (the leaf chlorophyll content and length of bud) measured during the fruit set stage (late spring) and the veraison stage (mid / late summer) and a new indicator of fertility.

Chlorophyll plays a key role in plant photosynthesis by light absorption and synthesis of sugars and molecular complexes with high content of chemical energy (as ATP during photophosphorylation) (Gang Lv and Haiqing Yang, 2011). It was confirmed that the chlorophyll content is closely related to the nitrogen status in leaves. Traditional extraction

methods of measuring chlorophyll content are expensive, time-consuming and skill-needed. Nondestructive methods have therefore been developed and inexpensive optical chlorophyll meters, such as the SPAD-502 (Konica Minolta, Osaka, Japan), are today frequently used (Coste S.*et al.*, 2009).

The leaf chlorophyll content was measured for four rows treated with compost (row no. 2, 6, 10, 14) and for four untreated rows (row no 4, 8, 12, 16). For each row monitored were counted from twenty to thirty-six spaces between poles (distance between each concrete pole and the next one). To eliminate the variability due to the different lengths of the rows monitored, it was decided to take the length of the shorter row. So for each row were identified ten spaces between poles alternating with ten other non-monitored. Between two consecutive poles, the central plant (of 5 plants present) was chosen for the measurement of chlorophyll content performed on the 7th leaf starting from the bud apex. For each leaf were carried out four readings at different points of the leaf blade by using a SPAD (*Soil Plant Analysis Development*).

The distribution of compost increases the availability of nitrogen in the soil and hence improves the vegetative growth of the vines. The magnitude of this effect was assessed by measuring the length of the same bud before identified for the readings with the SPAD.

In order to better evaluate the effect of the compost soil improver on fertility and on some enzymatic activities of the soil (Bolton Jr.H. *et al.*, 1985), were employed textile threads of varying composition (cotton and silk) called "**fertimetri**" (Patent Cooperation Treaty PCT/IB2012/001157, June 13, 2012, Squartini, Concheri, Tiozzo). The method developed and the corresponding probe (Figure 3.13) allow to determine the microbial activity of mineralization, in soil or any other substrate in which microorganisms are present. The method provides that the soil fertility, understood as attitude to support plant production

depends not only on the availability of nutrients (fertilizers containing nitrogen, phosphorus and potassium and / or organic fertilizers such as manure or compost), but also by the activity of telluric microorganisms that promote radical assimilation of nutrients through mineralization and mobilization processes (Bolton Jr. H. *et al.*,1993).

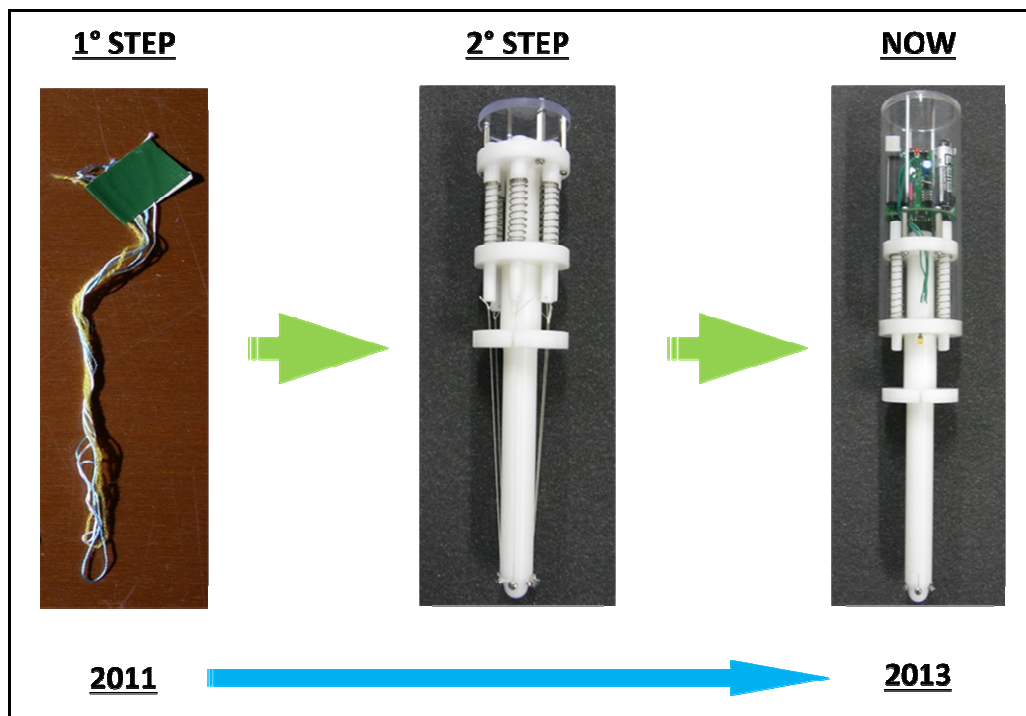


Figure 3.13 - Development of the probe in the period 2011-2013.

This fertility indicator is a very simple tool which measures the degradation of silk and cotton filaments kept in contact with the surface horizon of soil. In particular have been used 3 sets of wires: the first made with untreated silk and cotton (control), the second compound from the treated with a solution of nitrogen (3 g L^{-1} of NH_4NO_3) and the third compound with the wires treated with a solution containing phosphorus and potassium (6 g L^{-1} of $\text{Na}_2\text{HPO}_4 + 3 \text{ g L}^{-1}$ of KH_2PO_4). The treatment is done by immersing the fibers in the respective

solutions for 15 minutes and subsequently letting them air dry for one day. After treatment, the filaments were installed on the probes (the prototype contains six filaments: one of untreated cotton, one of untreated silk, one of cotton added with nitrogen, one of silk added with nitrogen, one of cotton added with potassium phosphate and one of silk added with potassium phosphate). During the fruit set stage and the veraison stage, the probes were placed in four rows treated (2, 6, 10, 14) and four rows untreated (4, 8, 12, 16), two for each row, equally spaced between them and with respect to the beginning and the end of the row. The wires degradation in the field was monitored daily for a week, after which, the probes were extracted from the ground to check the status of the wires and then to evaluate the cellulolytic (Khalil A.I. *et al.*, 2001) and proteolytic activity. The cellulolytic activity (for cotton, cellulose fiber) and proteolytic activity (for silk, protein fiber of the silkworm) is common to most of the telluric microflora and can therefore be studied by using these probes. In addition, the residual strength of the wires treated with nitrogen or phosphorus (and potassium), when were lower than the untreated wires, allowed to obtain useful information regarding the availability of these important macro-nutrients for the crop. This allows the evaluation of soil microbial fertility without costly laboratory analysis (Stevanato P., 2013). Furthermore, the difference in the degradation times of the different wires used to schedule the fertilization with a high degree of accuracy, allowing a saving of fertilizer, a yield optimum crop (the excess of nutrients often depresses the yield and / or compromise the harvest quality, especially in the vineyard) and environmental protection (leaching of nutrients from the roots not intercepted in groundwater and eutrophication; see Nitrates Directive 91/676/EEC).

3.4 Results and Discussion

3.4.1 CNS elemental analysis

The results of CNS analysis related to the collected samples are reported in the graphs below (Figure 3.14) that presents the evolution of carbon, nitrogen and of respective ratio and the percentage of sulfur during composting. The first graph show the classic trend of the process that leads to increased nitrogen (% d.m.) , the decrease of organic carbon (% d.m.) and then the C/N ratio. This parameter is certainly the most significant because it defines the proper performance of the composting process. If there is shortage of nitrogen, the decomposition of the materials will proceed more slowly, resulting slowed the microorganisms action, by contrast with an excess of nitrogenous substances, eventuality very rare, occurs a release of nitrogen in the form of ammonia. Generally a C/N ratio equal to 12 is the limit accepted for mature compost (Bernal *et al.*, 1998). The sulfur instead presents a trend almost constant for the whole duration of the process but is maintained at higher values, even if no significant differences with respect to sulfur values detected for the second pile. This effect can be explained considering adding periodic pruning (February) that concerns only the first of the two experimental piles. In fact in viticulture about 38% of plant protection treatments are carried out against fungal diseases using fungicides based on sulfur, that remain on the woody parts of the plant even after a long time and that have been observed during the analysis of compost samples. This effect can be explained considering the periodic adding of pruning residues (February) that concerns only the first of the two experimental piles. In fact in the vineyard about 38% of plant protection treatments are those carried out

against fungal diseases using fungicides based on sulfur, that remain on the woody parts of the plant even after a long time and that have been observed during the analysis of compost samples.

In the pile 1 have been identified higher final percentages even for nitrogen and carbon, although the second pile present substantially the same tendency. The differences observed between the two piles relating to the content of the three studied elements is designed in accordance with the different management applied to the first pile.

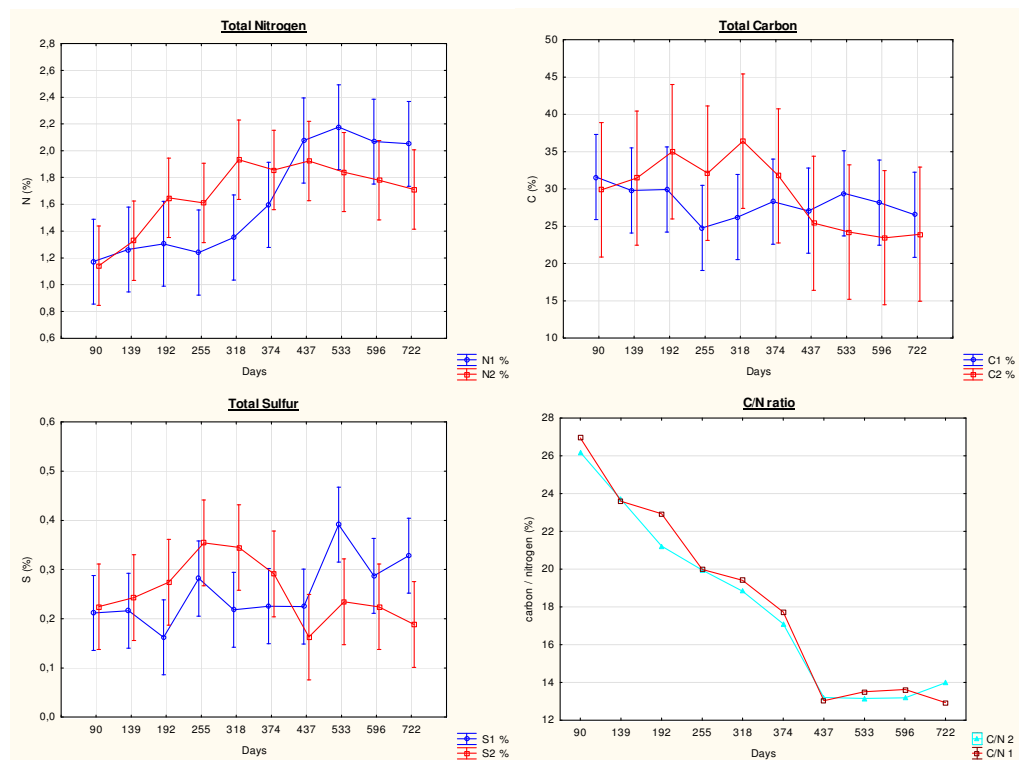


Figure 3.14 - Percentages of carbon, nitrogen, sulfur, and C/N ratio in the two experimental piles during the humification process.

3.4.2 Microbiological analysis

The seeding on Petri dishes of small quantities of compost samples and the subsequent counting CFU allowed to obtain the results shown in the graphs below (Figure 3.15).

At the beginning of composting is evident a lower microbial concentration, explained by the necessity of microorganisms to adapt to the new environment before starting to multiply. The mesophilic microorganisms seem to remain throughout the period on constant values with small fluctuations not statistically significant. The thermophilic instead show a steady slight increase during the entire period. This could be explained by considering that the plant biomass protects the microbes from damage related to extreme temperature changes, especially on the low values.

The pile 1 shows a decrease of the microbial concentration in the autumn 2012, that is readily recovered after the addition of grape marc, thanks to the large intake of sugar contained in the grape marc itself. The last graph shows the sum of all categories of microorganisms monitored separately for the two piles. It clearly shows that in both piles, the values of total microbial population remain quite stable and high (between 1 and 10 billion g^{-1} of compost) for 2 years of the experiment duration. This is a very positive fact, because it highlights an adequate microbial concentration throughout the process, regardless of the composting method used, which ensures proper and efficient performance of the biological transformation (Steenwerth K.L. *et al.*, 2008). The sum of the two graphs show that in the course of the experiment the total population remains fairly stable, but it enriches quality of thermophilic component.

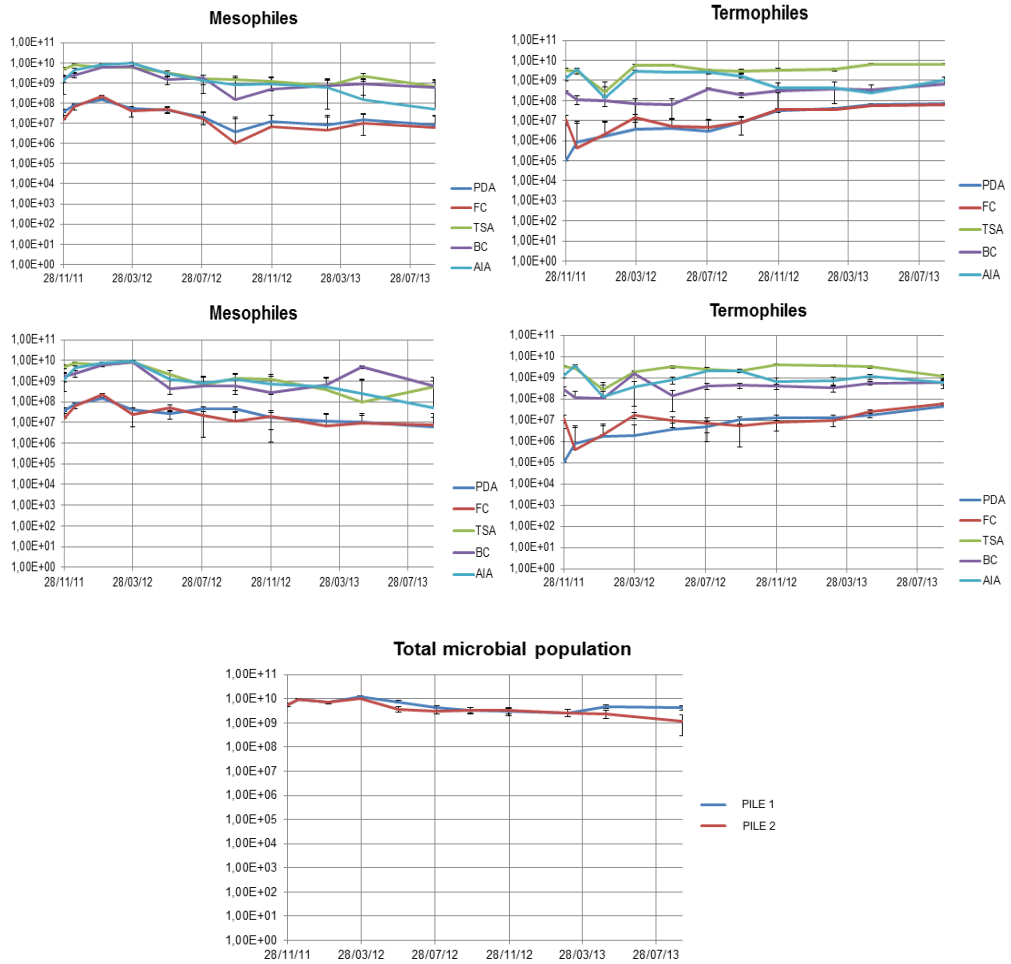


Figure 3.15 - Top: mesophilic and thermophilic microorganisms of the first pile; in the center: mesophilic and thermophilic microorganisms of the second pile, bottom: the total population of the two piles (abbreviations: PDA = Potato Destrose Agar for molds, FC = Cellulolytic Fungi; TSA = Trypticase Soy Agar for total bacteria, BC = Cellulolytic Bacteria, AIA = Actinomycete Isolation Agar for actinomycetes).

3.4.3 Analysis of microbial biodiversity (ARISA analysis)

A molecular approach to study the microbial biodiversity is represented by the ARISA technology (*Automated Ribosomal Intergenic Spacer Analysis*), a technique that in recent years has been used successfully for the study of complex microbial communities in the human and the environment field.

In this work were analyzed 36 samples of compost considered the best to represent the evolution of microflora during the process of humification. The PCR results were processed with a dedicated statistical software (Past 2.12) getting the graphs below (Figure 3.16). Certainly the most interesting aspect is the selection of specific groups of bacteria and fungi during the maturation stage of the compost (i.e. two years after the beginning of the composting process) with a more pronounced effect for fungi than bacteria, probably due to the ability of fungal species to form reproductive structures, such as spores, which have enabled them to colonize with greater speed and efficiency than bacteria the biomass. These results are certainly of great ecological importance as reveal that the mature compost obtained in this study is not only a stable environment for the microorganisms growth, but it is also a material with a function of inoculum for soil treated and able to limit the spread of mycosis (Santos M., 2007). Then the distribution in the vineyard of this composted soil improver allows to improve soil fertility on the one hand with the contribution of stable organic matter from the other hand improving the quantity and quality of the soil biomass.

3.4.4 Vegetative responses of the vines

The effects of the distribution of compost in the vineyards were quantified by measuring two significant parameters to define the nutritional status of the vines: SPAD and sprout length. The data were collected during fruit set stage and veraison stage and then statistically processed to obtain the graphs below (Figure 3.17).

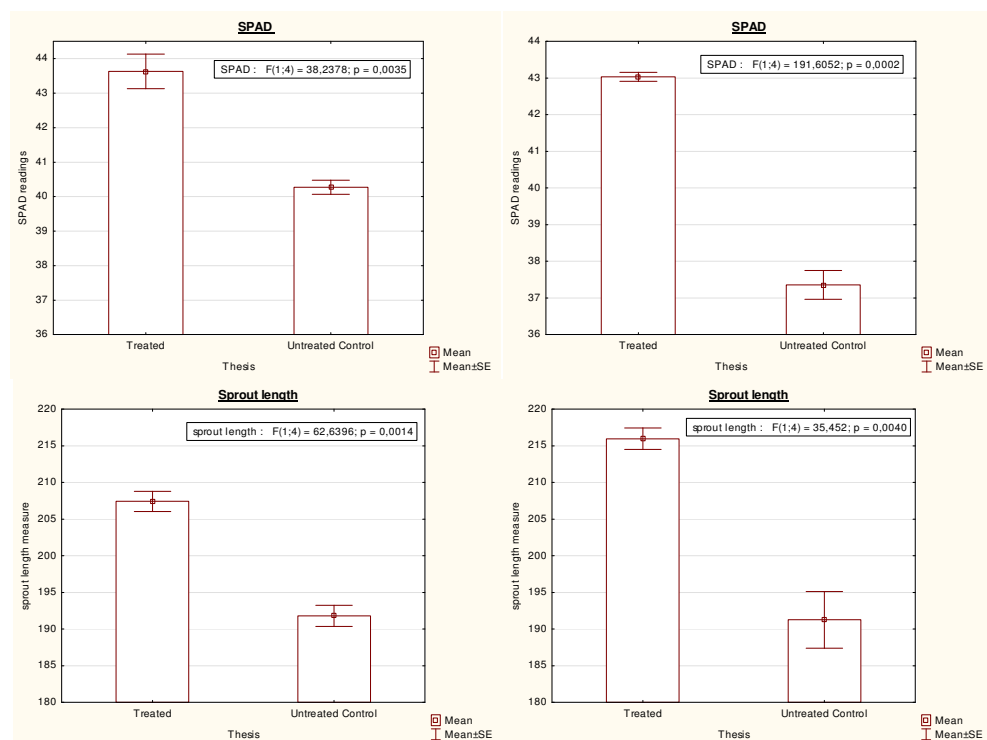


Figure 3.17 - nutritional parameters. Top: SPAD measured during the fruit set stage (on the left) and during veraison stage (on the right); Bottom : sprout length measured during the fruit set stage (on the left) and during veraison stage (on the right).

The analysis of variance (one-way ANOVA) showed large and significant differences ($p < 0.05$) between the treated thesis (the rows affected by the distribution of compost) and the untreated control. In conclusion, the compost distributed has determined an important

increase in the plants vigor without inducing, however, an excessive development of the aerial part and an increase in the chlorophyll content which usually translates into a better ability of plants to synthesize sugars which are then translocated in the berries.

3.4.5 Analysis of biodegradation of soil organic matter

The measures of degradation of cotton and silk wires have been included in the graph below (Figures 3.18) that shows a clear trend of higher fertility in the rows that have benefited from the distribution of compost. The effect seems more pronounced for the cellulolytic activity on cotton wires and less for the proteolytic activity. This result can be explained considering the massive use of antifungal products to combat downy mildew, powdery mildew and other diseases. The degradation of silk wires requires an enzymatic pattern more complex than that required by the cotton and the fungal populations in the soil, the most efficient in the performance of this action, are subject to the depressing effect of the agrochemicals.

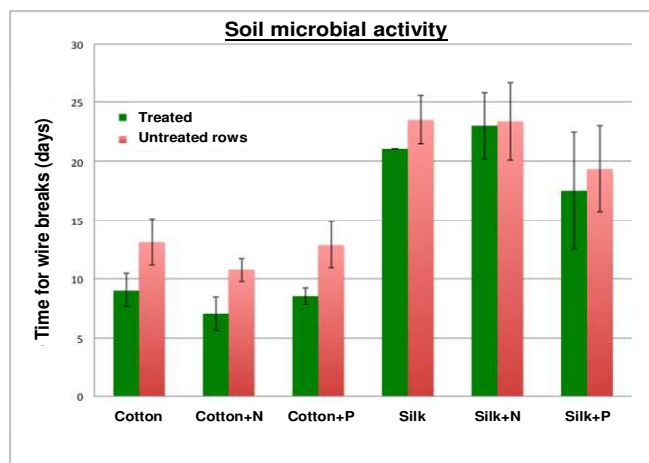


Figure 3.18 - Breakthrough times of the cotton and silk wires (untreated, treated with N and treated with P). Comparison between treated thesis and untreated control.

Chapter 4

MULTI-ELEMENT ANALYSIS : APPLICATIONS OF A MODERN CNS “VARIO MACRO”

4.1 General introduction

The modern techniques for the quantitative analysis of the main elements (C, H, N, O, S), contained in the organic and inorganic substances apply to conventional methods for combustion, which shall convert the elements of the sample to volatile molecules, which are then separated and detected automatically. These measures are very important and widely used in the process of quality control, in food analysis and environmental analysis, as well as in all laboratories of research and synthesis, for the determination of the molecular composition of new compounds.

In recent decades, the manufacturers of scientific instrumentation have proposed automated systems for general use, or designed for special applications: elemental analyzers (known as "CHN", "CHNS", "CHNOS"), able to determine in a few minutes the contents of the more important elements as C, H, N, and also of O and S, in solid and liquid organic and inorganic substances; elemental analyzers for determine the content of Total Organic Carbon (TOC), Total Inorganic Carbon (TIC) and Dissolved Organic Carbon (DOC).

The technique of thermal combustion of organic substances in the presence of catalysts, developed by German scientist J. Liebig at the first part of the nineteenth century for the carbon and hydrogen determination (Figure 4.1) and by the French scientist J.B.A Dumas for nitrogen determination (1831) (Figure 4.2) is still valid and is the basis of modern automatic elemental analyzers, which have solved the problems of dangers and duration of the original processes, enabling to increase the analysis accuracy.

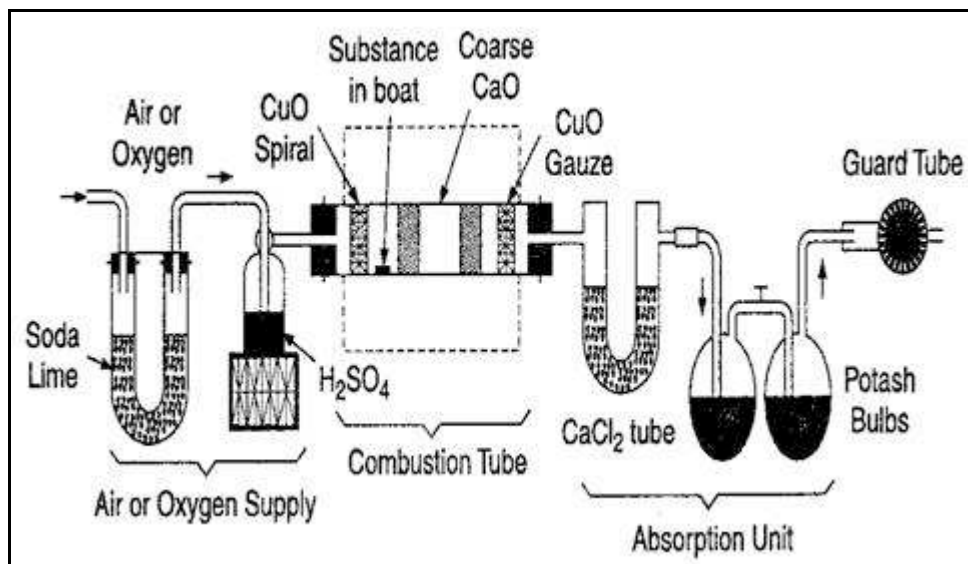


Figure 4.1 – Schematic diagram of Liebig combustion apparatus for the carbon and hydrogen measurement

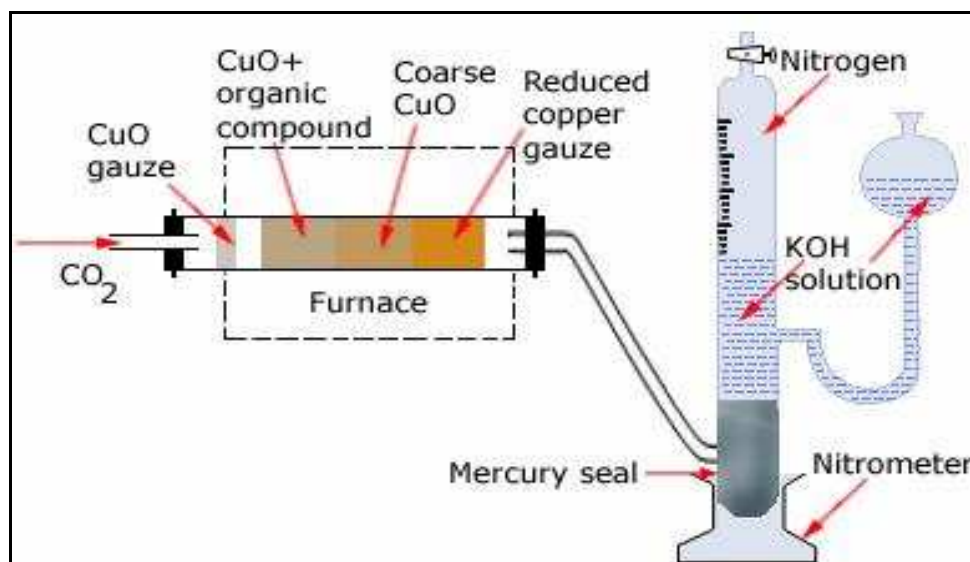


Figure 4.2 – Schematic diagram of Dumas method for nitrogen determination.

4.2 Determination of TOC in soil samples: a comparison between two different analytical methods

4.2.1 Introduction

The organic matter is the main factor of the soil quality, because it depends on the fertility, that is, the ability to support agricultural production over time.

Therefore the determination of the total organic carbon content of the soil is extremely important for the chemical and microbiological characterization of soil.

4.2.2 Aim of the study

The aim of this work was to compare two analytical methods for the determination of Total Organic Carbon (TOC) in the soil: the traditional Walkley-Black method and the method of elemental analysis.

4.2.3 Materials and methods

In the present work, it was determined the organic carbon content of 256 soil samples of different origin and composition (soils collected from the vineyards of the "BioBio" project, venetian soils kept to the ARPAV service soils of Treviso, organic soils from the Veneto south plain).

The chemical analysis of soil samples were performed following the procedures outlined in the Ministerial Decree of September 13, 1999: Approval of the "**Official Methods of soil chemical analysis**", published on October 21, 1999 and the subsequent amendments

published on March 25, 2002 by the Ministry of Agriculture and Forestry.

Walkley-Black method

The method provides that the organic carbon is oxidized to carbon dioxide (CO_2), under standardized conditions, with a solution of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) in the presence of sulfuric acid (H_2SO_4). The speed of the reaction is favored by rising temperature consequent to the abrupt dilution of the acid. After a set time, the reaction is stopped by the addition of an appropriate amount of ultrapure water and potassium dichromate which has not reacted is determined by titration with a solution of iron (II) sulfate heptahydrate ($\text{FeSO}_4 \times 7\text{H}_2\text{O}$). The end point of the titration is determined by the addition of an appropriate redox indicator. The sample was prepared by transferring into a 250 mL flask 0,5 g of ground and sieved to 500 μm (0,5 mm).

With a burette were transferred to the flask 10 mL of the solution ($0,1667 \text{ moles} \times \text{L}^{-1}$) of potassium dichromate and then were added to 20 mL of sulfuric acid [96% ($\rho = 1,835$)], making leach slowly along the inner walls of the flask in order not to over-heat the mixture. The flasks were stirred with caution preventing soil particles adhere to the walls of the container. The samples were covered with a glass and allowed to stand for 30 minutes.

The reaction was stopped by adding 200 mL of ultrapure water previously cooled in a refrigerator and the volumetric titration was performed by adding in the Erlenmeyer flask, 10 mL of phosphoric acid (H_3PO_4) [85% ($\rho = 1,695$)] and 0,5 mL of the redox indicator. The flask was placed on the magnetic stirrer and the whole thing was titrated with

the solution (0,5 moles x L⁻¹) of iron (II) sulfate heptahydrate until the color changes from blue to green.

The solutions of ferrous salts are not stable due to the oxidation of iron (II) by oxygen. This oxidation process occurs, although slowly, also on the salt in the solid state. Therefore, for each series of analyzes, it was necessary to check the exact title of the solution (0,5 moles x L⁻¹) of iron (II) sulfate.

The method involves treating a known amount of dichromate solution in the same way of the sample to perform the correction concerning the possible partial decomposition of the and simultaneously the control that the decomposition was not excessive.

With burette were then taken and transferred into Erlenmeyer conical flask of 250 mL, 10 mL of the solution (0,1667 moles x L⁻¹) of potassium dichromate.

Subsequently were added 20 mL of sulfuric acid, making leach slowly along the inner walls of the flask to not over-heat the mixture. The flasks were covered with a glass disk and left to rest for 30 minutes.

The reaction was stopped by adding 200 mL of ultrapure water previously cooled in a refrigerator and , in succession, were added 10 mL of phosphoric acid and 0,5 mL of the redox indicator. The flask was placed on the magnetic stirrer and titration was carried out with the solution (0,5 moles x L⁻¹) of iron (II) sulfate heptahydrate until the indicator changes from blue to green.

The organic carbon content is expressed in g x kg⁻¹. For the calculation we used the formula:

$$C = \frac{3}{2} \times \frac{(B-A)}{1000} \times \frac{M_{Fe(II)}}{6} \times 12 \times \frac{1000}{M} \times 1,30$$

C = organic carbon content, expressed as $\text{g} \times \text{kg}^{-1}$;

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

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

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

M Fe (II) = effective molarity of the iron (II) sulfate solution;

12 = atomic mass of carbon, expressed in $\text{g} \times \text{mole}^{-1}$;

1,30 = empirical correction factor that takes into account the partial oxidation (70%) of organic carbon;

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

Elemental analysis method

The different elemental analyzers commercially available work essentially based on the Dumas method (1831).

The original analytical method is based on the complete and instantaneous oxidation of the sample for flash combustion which determines the conversion of all organic and inorganic substances in the gaseous products. The combustion gases are conducted, in a stream of helium, on a suitable catalyst ($\text{CuO} + \text{Pt}$), to complete the oxidation of carbon compounds not completely oxidized (CO) to carbon dioxide (CO_2), and then on a layer of copper to remove the excess oxygen and reduce nitrogen oxides (NO_x) to molecular nitrogen (N_2). Subsequently, the separation of N_2 , CO_2 and SO_2 , is done by temporarily blocking the SO_2 and CO_2 on specific traps while N_2 is immediately sent to a thermal conductivity detector.

In this study was carried out a first determination of total carbon on a 300 mg soil sample sieved to 0,5 mm and a new determination on 100

mg of the same sample treated by removal of the organic fraction for combustion in a muffle furnace (550 °C for 2 h); the value obtained from the difference between the first analysis (total carbon, organic and inorganic) and the second (only inorganic carbon) represents the Total Organic Carbon (TOC).

4.2.4 Results and discussion

The 256 TOC values determined by the Walkley-Black method (official method) were compared with those obtained by the second method based on a new procedure discussed in this study: the data comes from the difference between two determinations, the second of which is carried out after a pass into the muffle furnace. In this way it avoids the use of HCl for the carbonates destruction required by ISO 10694.

As shown in Figure 4.3, the correlation coefficient (R^2) of 0,98 and the regression line with a slope equal to 0,82 indicate a close correspondence of the two methods, confirmed by the regression coefficients close to unity both for the samples with TOC values between 0% and 5%, both for the samples with a TOC content between 5% and 40%.

Even for the samples with a TOC content between 5% and 15% the regression line has a coefficient equal to 0,92 and a slope of 0,90.

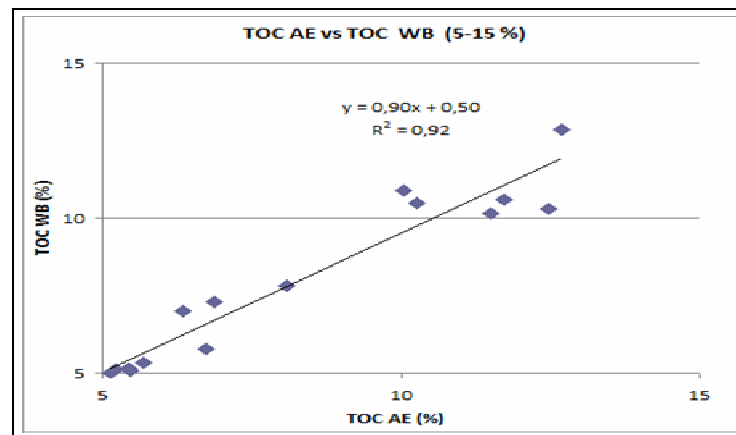
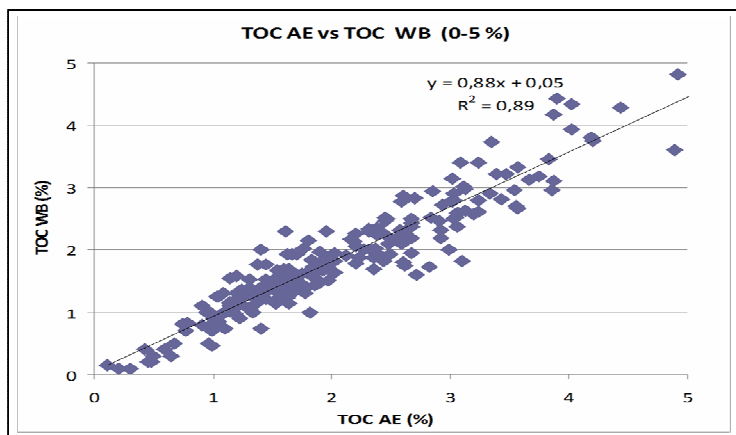
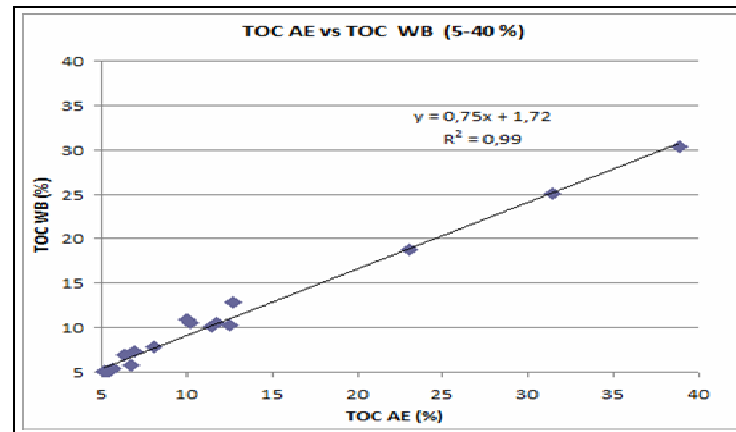
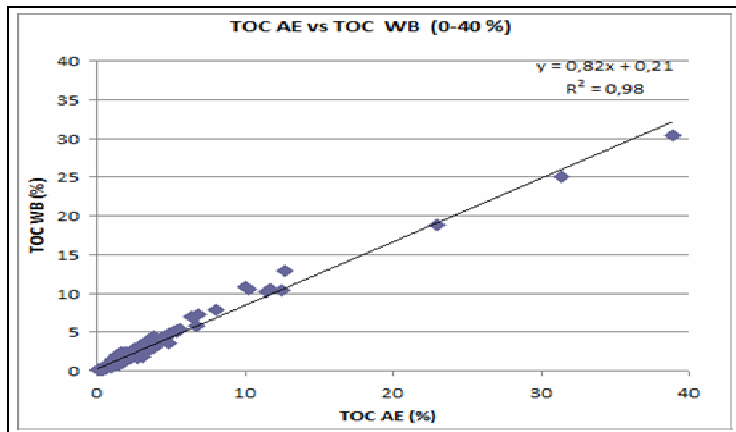


Figure 4.3 – Correlation between TOC determined by Walkley-Black method (TOC WB) and TOC obtained by elemental analysis (TOC AE).

4.2.5 Conclusions

The results show that the elemental analysis method modified respect to ISO 10694, is presented as a viable alternative to the Walkley-Black method for accuracy of the analytical result, for reduced analysis time and for less risks for the operator, who must not use chemical reagents such as potassium dichromate ($K_2Cr_2O_7$), which according to Regulation no. 1272/2008 is classified as a substance responsible for:

- germ cell mutagenicity, category 1B, H340;
- carcinogenicity, category 1B, H350;
- reproductive toxicity, category 1B, H360FD;
- toxicity to the respiratory tract, H334.

4.3 Determination of TOC in rock samples: chemical analysis of organic matter from the Noric-Rhaetic period

4.3.1 Introduction

The goal of this work was to proceed to the chemical characterization of Pignola-Abriola section belonging to the paleogeographic domain of the *Lagonegro Basin*, currently outcropping in the area of the Southern Apennines, about 100 km from Potenza. The Lagonegro Basin was formed during the Permian, when the land had gathered to form a single continent, called *Pangaea*, surrounded by a unique and very extensive ocean, called *Panthalassa*. Along the eastern margin of Pangea, close to equatorial latitudes, there was a deep gulf, known as the *Tethys Ocean*, while along the western margin was present the Lagonegro Basin (Amodeo F., 1999).

The succession outcropping in the Lagonegro Basin in its lower portion includes the formation of limestones with flint and the formation of Siliceous Shale, respectively constituted by pelagic carbonates and siliceous deposits.

4.3.2 Materials and methods

The samples were statistically selected so as to represent the section of Pignola-Abriola for its entire extent (about 60 m), with an accuracy of about 15 cm. The 95 rock samples obtained were processed in the laboratory for the subsequent elemental analysis: for each rock sample were obtained about 300 mg of powder by grinding in a mortar (Figure 4.4) or for drilling with minidriller, until reaching a granulometry extremely thin. The selection of the sample rate to analyze was carried

out in a way that was representative of the interior portion of the rock samples collected. The samples thus prepared were then dried in an oven at about 30 °C for one night, so as to remove any moisture present in the material.



Figure 4.4: on the left: tools for the grinding of the samples: spatula, mortar with pestle, test tube, rock samples. on the right: the powdered samples.

From each dried sample were obtained three subsamples:

1. 100 mg of powder weighed into tin foil and subjected to elemental analysis for the determination of total C;
2. 100 mg of powder weighed into silver foil, which have been placed in a muffle furnace (Heiri O. *et al.*, 2001) for 2 hours at a temperature of 550 °C. With this step it is possible to determine the inorganic carbon content;
3. 100 mg of powder were treated with diluted hydrochloric acid (10% HCl) to remove the inorganic carbon. Subsequently, the samples were analyzed for determine TOC content.

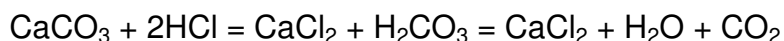
For the determination of total carbon were weighed 100 mg of powder inside of tin vessels containing about 50 mg of tungsten trioxide (WO_3 , oxidizing agent). The 95 obtained capsules were analyzed using an elemental analyzer CNS "vario MACRO" produced by Analysensysteme Elementar GmbH (Germany). This tool is fully automatic and allows a

rapid quantitative analysis of carbon, nitrogen and sulfur in different materials (solids or liquids). A second aliquot of 100 mg for each of the 95 samples was weighed within silver vessels placed in muffle furnace for 2 hours at a temperature of 550 °C. The use of silver foil is required by the temperature reached inside the muffle (Ag is resistant up to 960 °C while Sn melts at around 230 °C). With this step it was possible to remove the organic carbon without affecting the carbonates (CaCO₃), which represent the only component containing carbon that remained in the sample. With the subsequent CNS analysis it was therefore possible to quantify the inorganic carbon content.

The two obtained values (expressed as a percentage of the sample weight) allowed to calculate the Total Organic Carbon (TOC) as the difference between the Total Carbon (TC) measured with the first analysis and the Inorganic Carbon (IC) obtained by analyzing the second aliquot of the same sample.

$$\text{TOC}\% = \text{TC}\% - \text{IC}\%$$

A third sub-sample was prepared for the assessment of Total Organic Carbon (TOC) by the standard method, that involves the acid attack of carbonate rock samples (i.e. carbonates, limestone, marl, dolomite) using diluted hydrochloric acid (10% HCl) (eg, Schlager and Jenkyns, 1976). The reaction that develops is the following:



At this point the only carbon component that remained in the acidified sample is the organic one, therefore the next CNS elemental analysis has allowed to directly quantify the Total Organic Carbon (TOC).

4.3.3 Results and discussion

Based on the involved lithologies, has been possible to find a difference between the TOC values obtained with the standard method of acidification and those obtained by the muffle furnace method ($\Delta\text{TOC}\%$). In particular, the black shale, marl and marly limestones are lithologies that have values less than $\Delta\text{TOC}\%$, then follow the silicified limestones and dolomitized limestones. These latter show a discrepancy of approximately 2%, with the TOC obtained by the standard method which is higher than the figure obtained by the muffle furnace method. This is likely due to the fact that dolomite, being a double carbonate of calcium and magnesium $[\text{CaMg}(\text{CO}_3)_2]$, is more hardly attacked by hydrochloric acid. Consequently, not all of the inorganic carbon is consumed during the acidification reaction and the values measured by the CNS are therefore the sum of the real value of the TOC present in the sample plus the residual inorganic carbon.

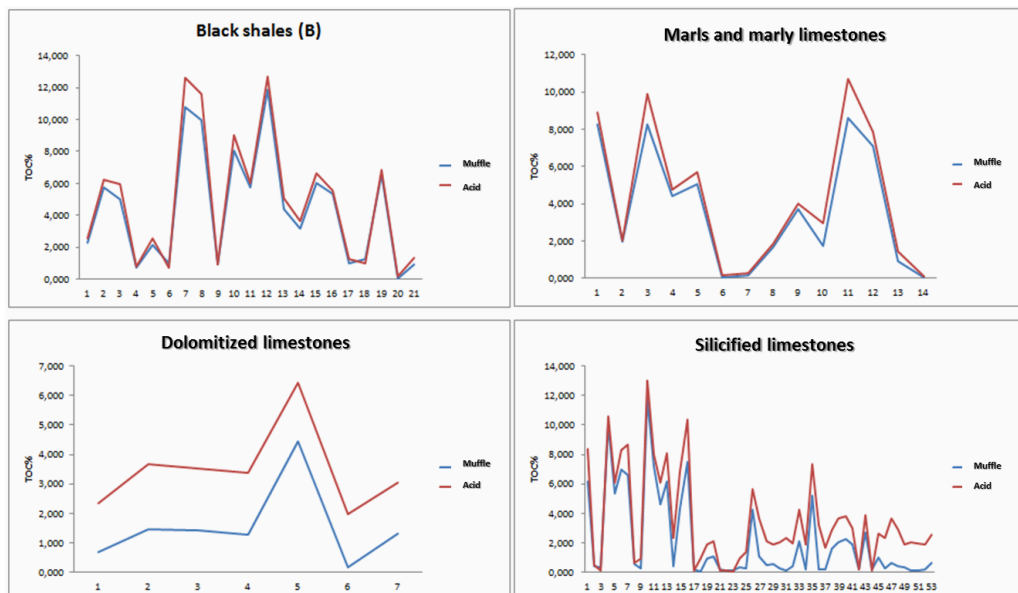


Figure 4.5 – Comparison between TOC measured with the two methods according to the different lithologies.

Furthermore, it was noted a general increase of $\Delta\text{TOC}\%$ with increasing of organic matter content (TOC). Also this phenomenon is most visible in silicified limestones and marls and marly limestones. Black shale and dolomitized limestone not show this behavior (Figure 4.5).

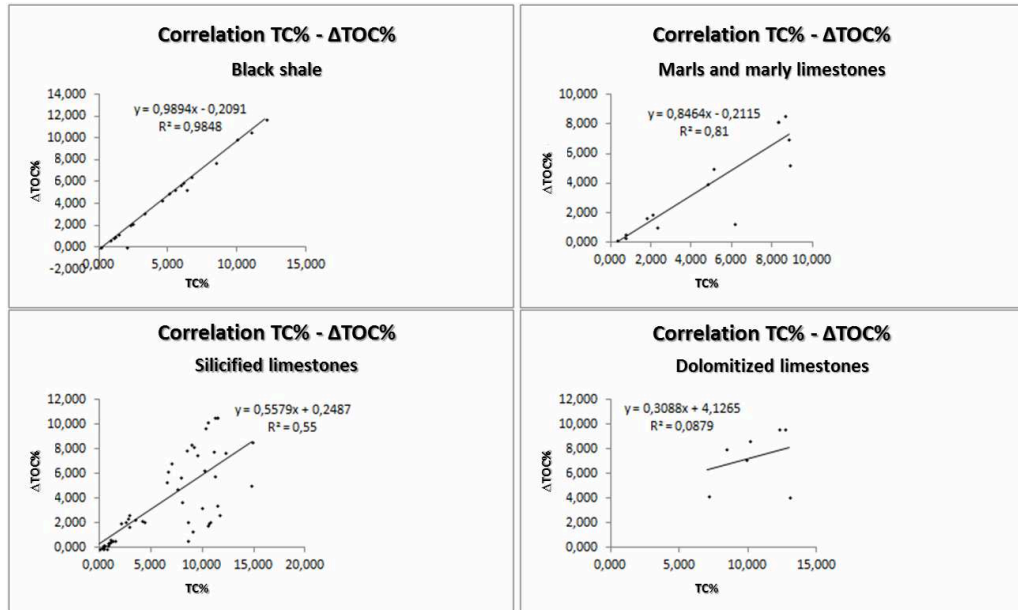


Figure 4.6 - correlation between the $\Delta\text{TOC}\%$ and $\text{TC}\%$ of each lithology.

To assess the possible correlation between the two analytical methods, it was calculated the coefficient of determination (R^2), which allows to establish whether there is a systematic relationship between two variables. The coefficient of determination R^2 is defined as follows:

$$R^2 = \frac{\sum_{i=1}^n (\hat{y}_i - \bar{y})^2}{\sum_{i=1}^n (y_i - \bar{y})^2} = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2}$$

y_i are the observed data;

\bar{y} is their average;

\hat{y}_i are estimated from the data obtained from the regression model.

In the first equation, the numerator is the deviance (sum of squared deviations from the mean) of the data estimated by linear regression: thus provides a measure of the dispersion of data than the average.

The denominator, however, is the deviance of the collected data (in our case, the measured values of TOC CNS) and thus provides a measure of the dispersion of the measured data than the average.

In the second equation, the numerator represents the residual sum of squares, namely the dispersion between the observed data and those obtained with the linear regression.

The coefficient of determination R^2 varies between 0 and 1: when is zero means that the model does not explain at all the data, when it takes the value 1 means that the model explains the data perfectly.

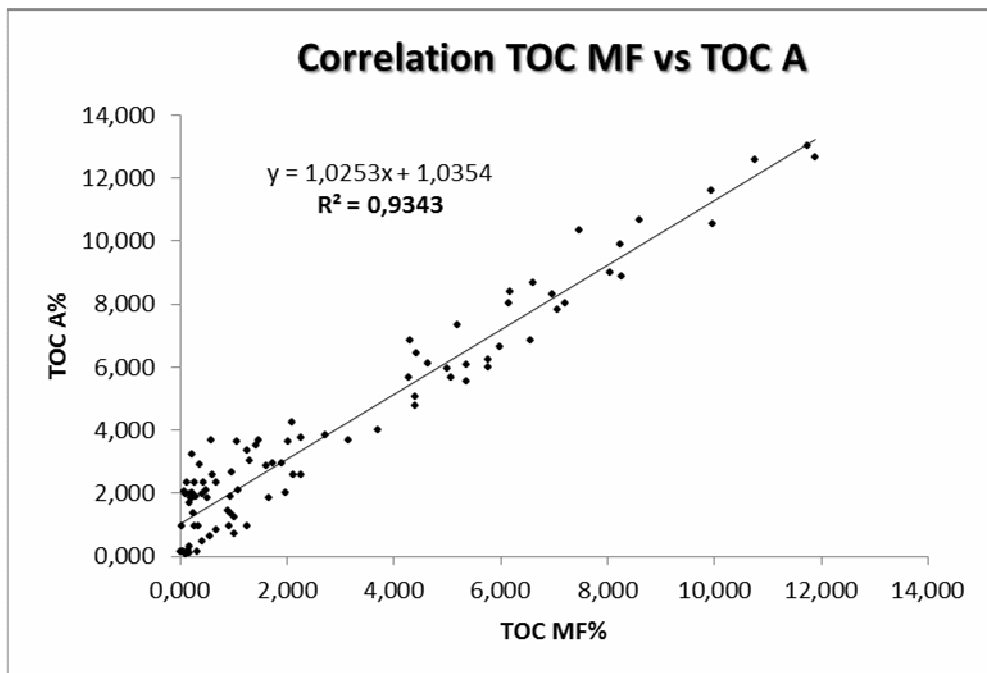


Figure 4.7 – Correlation between TOC determined by acidification method (TOC A) and TOC obtained by muffle furnace method (TOC MF).

As shown in Figure 4.7, the correlation coefficient R^2 is highly significant (equal to 0,93) demonstrating a close correspondence between the two analytical methods.

In order to establish the existence of a possible relationship between the two analytical methods was calculated the covariance that provides a measure of how much two variables vary together, then of their addition.

The covariance is defined as follows:

$$\sigma_{x,y} = \frac{1}{n} \sum_i (x_i - \bar{x})(y_i - \bar{y})$$

n represents the total number of the collected data (in our case is equal to 95); x_i and y_i are the values assumed by two variables (in our case, the values of TOC obtained by the two methods);

\bar{x} and \bar{y} respectively represent the average of the variable X and variable Y.

The covariance is positive if the two variables fluctuate in agreement (ie, when both exceed the average value), negative if they undergo oscillations divided (when one of the two variables does not exceed the average value), equal to zero if they undergo fluctuations independent.

In this case, the covariance was positive (equal to 10,085) proving the existence of a positive relationship between the two analytical methods which are therefore employees between them.

The standard deviation σ^2 is a dispersion index of the experimental measurements, that is, an estimate of the variability of a data population. Can therefore be considered as an estimate of the accuracy of an experimental method. Therefore, it was decided to consider this statistical index to calculate the Pearson correlation index, which provides a measure of the correlation degree between two variables.

The standard deviation σ^2 is defined as:

$$\sigma^2 = E[x^2] - (E[x])^2$$

E represents the expected value, (i.e. the average of the data).

The calculation has provided for the two analytical methods a standard deviation equal to 3,153 for the muffle furnace method and equal to 3,344 for the acidification method. It is immediately notice as σ^2 and therefore the accuracy of the two methods is almost comparable.

The correlation index of Pearson ($\rho_{x,y}$) expresses the strength of the linear correlation between two variables, in particular is defined as the linearity between their covariance and the product of their respective standard deviations:

$$\rho_{x,y} = \frac{\sigma_{x,y}}{\sigma_x^2 \sigma_y^2}$$

$\sigma_{x,y}$ is the covariance between the two variables;

σ_x^2 and σ_y^2 are the two standard deviations.

The Pearson correlation coefficient $\rho_{X, Y}$ always takes values between -1 and +1:

$$-1 \leq \rho_{x,y} \leq +1$$

$\rho_{x,y} > 0$, the variables x and y are said to be directly related, or positively correlated;

$\rho_{x,y} = 0$, the variables x and y are said to be uncorrelated or independent;

$\rho_{x,y} < 0$, the variables x and y are said to be inversely correlated or negatively correlated.

For the direct correlation is further distinguished:

$0 < \rho_{x,y} < 0,3$ weak correlation;

$0,3 < \rho_{x,y} < 0,7$ moderate correlation;

$0,7 < \rho_{x,y} < 1$ strong correlation.

The Pearson index $\rho_{x,y}$ is equal to 0,967, then confirming the existence of a strong correlation between the two analytical methods applied.

4.3.4 Conclusions

The TOC analysis was performed by comparing the standard method of acidification with the muffle furnace method. The first involves the elimination of inorganic carbon from the analyzed rocks by reaction with diluted hydrochloric acid (10% HCl). The second, however, allows to remove the organic fraction of the carbon by heating in a muffle furnace at 550 °C for 2 h . The comparison between the two analytical methods performed on powdered samples of 100 mg shows the excellent correspondence between the two methods . The appropriate statistical evaluations were performed in compliance with UNI CEI EN ISO/IEC 17025 (2005) and report a coefficient of determination R^2 of 0,934 and a Pearson index $\rho_{x,y}$ equal to 0,967. Both results are highly significant and indicate clearly that the two analytical methods are equivalent. The muffle furnace method, however, has several advantages over the standard approach, as it avoids the use of acidic substances dangerous to humans and the environment and harmful for the quartz components of CNS elemental analyzer and it is also potentially applicable to non-carbonate lithologies.

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