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DISTRIBUTION OF METALS IN SEVERAL VINEYARDS OF NORTH ITALY: FROM SOIL TO WINE

Tesi di laurea in: Biogeochimica

RELATORE:
Prof. Enrico Dinelli

CORRELATORI:
Dott. Denis Zannoni
Dott. Nicolas Greggio

CONTRORELATORE:
Prof. Alessandro Buscaroli

PRESENTATO DA:
Enrico Tombini

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Abstract

(Italian)

Al giorno d'oggi viviamo in regioni densamente popolate e questo porta a molti problemi di tipo ambientale. Fra tutte le sostanze inquinanti che le attività umane originano, i metalli meritano attenzione perché possono essere potenzialmente tossici per la maggior parte degli esseri viventi. Abbiamo studiato quale destino hanno *Cd*, *Cr*, *Cu*, *Fe*, *Mn*, *Ni*, *Pb* e *Zn* nei vigneti, analizzando campioni di pianta, vino e suolo. I siti sono stati scelti in considerazione del tipo di vino prodotto, del tipo di agricoltura (biologica e di tipo tradizionale) e dell'ubicazione geografica. Abbiamo preso vigneti che coltivano la stessa varietà d'uva, cioè lo stesso vitigno, il Trebbiano. Abbiamo investigato 5 vigneti che si trovano in provincia di Ravenna: due sulle sponde della Valle del Lamone, una nell'area dei depositi di argine vicino alla città di Ravenna, poi una azienda agricola vicino a Lugo ed un'altra vicino a Bagnacavallo in zone interfluviali. Abbiamo svolto nei siti una caratterizzazione molto dettagliata dei suoli, includendo l'analisi di: pH, conduttività elettrica, tessitura, calcare totale e contenuto stimato di dolomia, calcare attivo, ferro estratto con ammonio ossalato, indice del potere clorosante (IPC), azoto totale e carbonio organico, potassio assimilabile fosforoso assimilabile e capacità di scambio cationico (CSC). Poi abbiamo determinato le varie componenti, i minerali principali e i metalli in traccia, e fatto una estrazione con DTPA per determinare la frazione biodisponibile.

Tutti i siti hanno un terreno adatto all'agricoltura, con già dei buoni livelli di sostanze nutritive, per cui non sono necessari forti apporti di fertilizzante, ma un vigneto che si trova in collina soffre di clorosi ferrica, per l'alto livello di calcare attivo, resa evidente dalle foglie ingiallite. Abbiamo trovato suoli con molta silice e poco ossido di calcio che confermano il substrato marnoso arenaceo, poi altri suoli hanno più ossido di calcio e più ossido di alluminio che confermano il substrato argilloso marnoso.

Abbiamo trovato alcune criticità, come concentrazioni alte di Cromo, specialmente nell'azienda agricola vicino a Lugo, e abbiamo trovato differenze tra vigneti biologici e di tipo tradizionale: questi ultimi hanno un più alto arricchimento di alcuni metalli (Rame e Zinco) nei suoli.

Ogni metallo si accumula differentemente nelle varie parti della pianta. Abbiamo trovato differenze tra piante di collina e di pianura: sembra che la vite accumuli secondo un certo schema. I metalli si accumulano maggiormente nella corteccia, poi nelle foglie o ogni tanto nelle radici. Sembra che le piante cerchino di rimuovere metalli in eccesso, accumulandoli nelle cortecce.

Due vini hanno troppo acido acetico ed una azienda agricola di tipo tradizionale produce un vino con contenuto di Zinco sopra il limite di legge.

Abbiamo visto valori alti rispetto ad ambienti incontaminati, ma è consigliabile approfondire lo studio per collegarli ai loro apporti antropici.

1. INTRODUCTION

Nowadays we live in densely populated regions, with extensive industrialization and intensive farming that may lead to diffuse degradation, create pollution and other environmental issues. Human activities can steal soil with loss of its important functionality or bring in it high levels of pollutants and toxic elements: this means danger to environment and health too.

Contamination of soil may determine knock-on effects, on aquifers or other bodies of water, on plants by absorption, on animals by drinking and feeding and on humans by feeding. Many are the examples of organisms with concentrations of toxic substances higher than those normally found in the environment: when it occurs, we call the process bio-accumulation or bio-magnification, if it comes from the diet (Mann, 2011 [35]; Bini et Bech, 2014 [10]). Among all pollutants that human activities create, heavy metals are relevant because they are potentially toxic for most of living beings: they tamper with the normal metabolism of plants, animals and humans, bringing serious symptoms or even death in extreme cases.

It is clearly a risk for us, so we must try to defend ourselves and the first step we have to start from is, of course, finding those metals and determine where they are more abundant, then follow their distribution and fate in the environment. This thought leads to this study, that has been applied to the vineyard environment.

1.1. PURPOSE

The study was developed in a University Campus (Ravenna) located in the Emilia-Romagna region in Italy, so is naturally concerned with local interests due to a characteristic product and focused on a common agricultural activity in the area. We thought it would be interesting to analyse vineyards from the surroundings, chosen for their common aspects, like typical grape variety, but even for their differences, that makes them interesting too.

The studied sites were chosen as representative of different cultivation techniques and different geographical settings (plain area and hilly sites) that commonly host vineyard crops.

As a basic information, important for the description of the sites, we investigated in the detail the physico-chemical properties of the soils at each site, investigating the bulk chemical composition as well as tracing the more mobile forms of selected elements (*Cd, Cr, Cu, Fe, Mn, Ni, Pb* and *Zn*). This information provided the basis for the next steps of the study.

We investigated the fate of metals within the plant so we also included the analysis of different plant tissues, enabling the evaluation of metal retention in the plant. As a final information we also analysed the metal content of the wine representing one final product of this agricultural practice.

1.2. VINEYARD

A vineyard is the plantation of *Vitis vinifera* L., a grapevine native of the Mediterranean Region, known from the Neolithic Age and now spread all over the world for its important role in human race nutrition. It numbers thousand of varieties and it can be used for the production of wine, table grape or raisin.

The plant develops from roots, passing through a trunk with an external bark layer, two or three arms, older than two years lignified shoots, no-lignified shoots (that are between two and one years old), then to green shoots, tendrils, leaves, and clusters of grapes joined together with a rachis (as shown in ***Figure 1***).

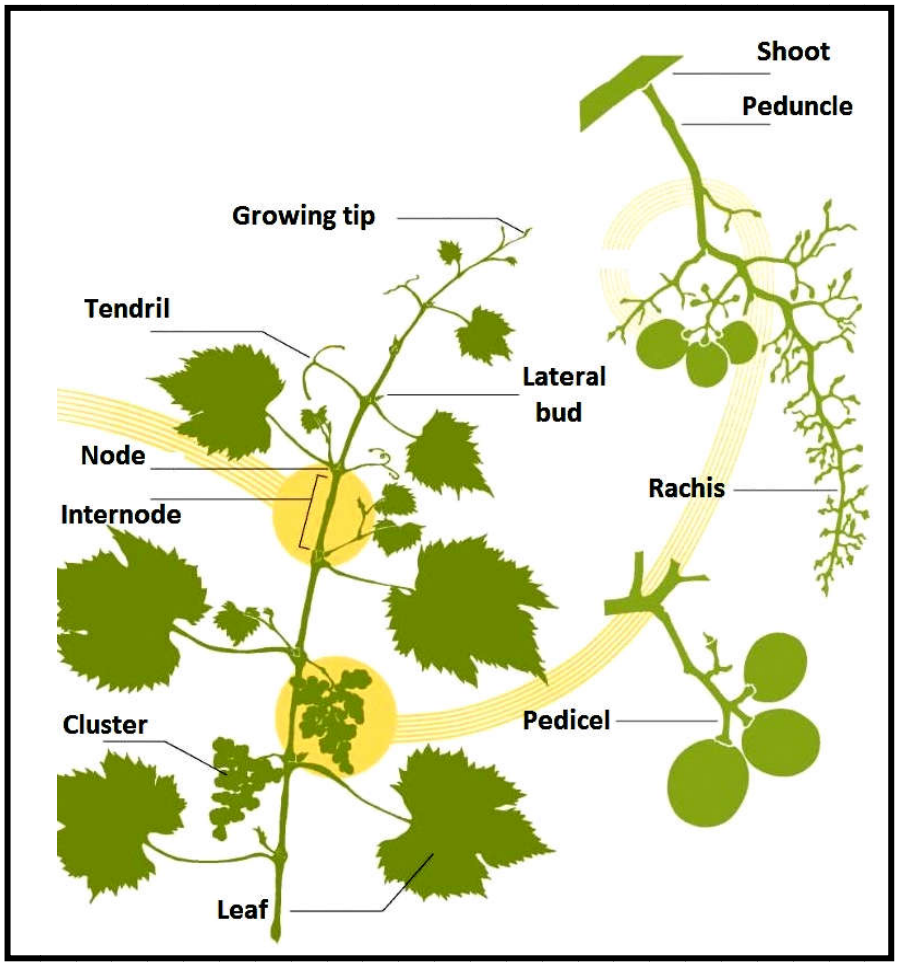

Figure 1. Upper parts of Vitis vinifera L.

It prefers climatological situations that can be found on hilly areas and sheltered places, where there is no springtime hoarfrost. They do not like soils with high pH, active carbonate, clay or where there is stagnation or a high water table, so marly soils (a mixture of calcium carbonate and clays) are one of the best bases for vineyards. As we can notice in Viticulture maps, as the one drawn up by "*Civiltà del Bere"* (in the Annex.1), Italy is a very good territory for grapevine cultivation from the North to the South: there are crops in the Aosta Valley, in the central part of Piedmont, a bit in Lombardy, on the Garda Lake banks, in the Adige Valley, in most parts of Veneto, in the south of Friuli-Venezia Giulia, entirely in Liguria and Tuscany and a bit in Umbria, in Emilia-Romagna at the foot of the Apennines, a lot in Marches and Latium, in the east of Abruzzo and a bit in Molise, in several places in Campania, along all Apulia, some parts of Calabria and Basilicata, and in a large part of Sardinia and Sicily.

From 2005 (one of the best crops ever), Italy had a fluctuating but clear decrease in wine production, but 2015 has been a year with a propitious climate for grapevines in Italy, so the harvest has been very good: the production reached 48,9 million hectolitres, more than France, with its 46,6 million hectolitres, and Spain, with 36,6 million hectolitres (*ISTAT/EU*); this amount is almost the same as ten years ago (***Figure 2***).

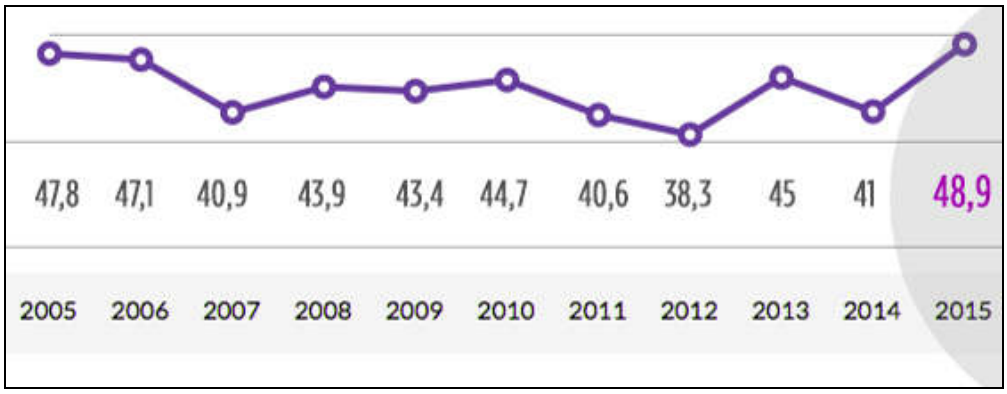

Figure 2. Evaluation of Italian wine production (million of hectolitres – ISTAT)

The business of wine in Italy reaches yearly about 10 thousand millions euros, of which 55% comes from exportation and 45% from internal market.

1.3. DISEASES AND TREATMENTS

Grapevines, like all fruit trees, are sensitive and weak: so they need help in keeping healthy and in giving a good production. Many solutions are available in trying to reach this goal, and they can be divided in fertilisations, antiparasitic or fitosanitarian treatments and different expedients, like pruning and pre-plantation choices, for example finding the best geographical location, taking the proper variety and doing the right grafting. All these strategies have a great influence because it's known that a precautionary approach is surely the best.

Grapevines grow even on poor land: they don't need high levels of nutrients, so a weak fertilisation is enough, usually it's necessary only a compensation of consumptions (Sbaraglia et Lucci, 1994 [47]). For example a vineyard that produces one hundred quintal every hectare, needs about 50 kg/ha of *N*, *25* kg/ha of P_2O_5, 80 kg/ha of K_2O and 30 kg/ha of *MgO*.

Many diseases, with a large variety of symptomatology, menace grapevines and usually must be fought on different fronts because fungicides often are not enough. One of the most widespread is a eukaryote called Peronospora that can be identified by clear stains on the upper face of leaves, by white spots on the other side and effects on clusters; in this case copper is suggested.

Another fearful illness is the Powdery Mildew ("Oidio" in Italian) originated by different agents (*Unciunula necator*, *Erisiphe necator*, *Oidium tuckeri* and others) that causes white bushes on leaf, shoot and rachis: the mildew on grapes becomes grey and makes them rot; the cure is Sulphur.

Then the Esca (by *Phaemoniella chlamydospora*, *Pharemonium alcophilum* and *Fomitiporia* mediterranea) affects the inner part of trunks and causes red colouration of leaves (as shown in ***Figure 3***).

Figure 3. Leaf of grapevine with Esca

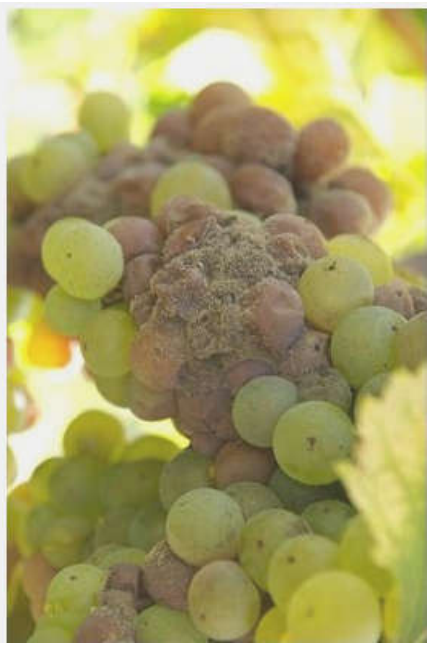

Figure 4. Grapes of grapevine with Botrytis

The only solution is to remove infected branches, burn them and protect the cuts. To avoid *Phomopsis Viticola*, that causes grape cancer/dead arm ("Escoriosi" in Italian) turning wood black, caution is necessary with fertilisation of Nitrogen. A bug named *Scaphoideus titanus* is also an important menace that leads to leaves yellowing (Flavescence dorée). Lastly a mention should be given to the *Botrytis cinerea*, a fungus turning fruits to ash-appearance (***Figure 4***).

1.4. ORGANIC FARMING

To fight these diseases nowadays there is a practise that is slowly replacing conventional agriculture: the organic farming. It has been defined in terms of legislation at EU level with a first regulation, the EEC 2092/91, replaced later by the Reg. 834/07 and at Italian level with the DM 18354/09. Organic farming has appreciable efficacy only when there is a moderate disease pressure, is more expensive and requires more attention and professionalism, but is based on a management philosophy which proposes however to reduce the number of interventions to avoid side effects. An example of this process is given by Copper that is active against Peronospora, but may have collateral action on Dead arm, and by Sulphur that is active against Powdery Mildew, but sometimes increases Botrytis and Dead arm. The term "organic" refers to a method of cultivation and breeding that admits only the use of natural substances that are present in nature, excluding the use of synthetic chemical compounds, with some exceptions (because usually organic farming is based on a defense with chemicals, such as copper and sulphur).

It improves quality of products on four different aspects: the healthiness, the environmental impact, the absence of Genetically Modified Organisms (GMOs) and the guaranteed control system and certification. Not using synthetic chemicals, is generally safer under the sanitary point of view and several research studies show that the nutritional value of organic products is often superior to the those of conventional products: in particular a greater presence of antioxidants was frequently noted (Lairon, 2009 [29]). Furthermore several studies tell us that sometimes organic products taste better than conventional, but not always and so each product type should be treated separately. (Fillion, 2002 [21]).

Conventional agriculture creates many problems: erosion and loss of soil fertility, loss of biodiversity, high energy consumption, production of greenhouse gases (the contribution of agriculture is estimated at 7%), and so pollution of air, water and soil. Organic farming has instead proved to be able to offer solutions through the application of the UE Regulation, or even through more restrictive rules adopted voluntarily by farmers. Organic farming, in fact, minimises the release of waste into soil, air and water, preserves the natural fertility of the soil, consumes less energy and protects biodiversity. In 1991, before the UE Regulation for organic farming, GMOs were much less common than they are today. Fortunately the international movement for organic agriculture, which wanted and promoted that regulation, understood the uncertainties and risks inherent to the use of

GMOs in agriculture, that now is more and more clear. Organic farming means developing a model of production that avoids the dismissal of waste in soil, water and air, using these resources in a model of development that can be used for long periods of time and is not doomed to last along the line. It preserves the natural fertility of soil, protecting the sensitive agronomic ecosystem and the biodiversity, even saving energy resources.

Many are the expedients of organic farming: it is possible to select species resistant to diseases, rotate the crops, use natural fertilisers, or cultivate two plants, where the first is hated by pests of the second one and, at the same time, the second is hated by pests of the first. These techniques can be enough, but sometimes it becomes necessary to set a protection from pests with treatments: authorised substances are listed by the European Regulation 2092/91 in the chapters A and B of the annex II (the "positive list" - Annex.2).

The most widespread permitted products for fertilisation are: manure, household waste processed into compost, wood ash, peat and some minerals of rocks.

For plant protection there are four types of products: there are substances from plants and animals, such as, for example, beeswax, gelatin, lecithin, mint oil, pine oil, caraway oil, pyrethrins, rotenone and quassia; then the micro-organisms (bacteria, viruses and fungi) for integrated pest management, only after the approval of the supervisory authority; substances used in traps such as ammonium phosphate, metaldehyde, pheromones, pyrethrum, pyrethroids and the Iron(III) phosphate; finally the substances from traditional use, such as copper sulfate, ethylene, soft potassium soap, potassium alum, lime sulfur, paraffin oil, mineral oils, potassium permanganate and sulfur.

Organic farming is the only form of agriculture controlled in accordance with European and national laws. It does not rely, therefore, on the manufacturer's self-declaration but on a uniform Control System throughout the European Union. The farmers who want to start organic production will notify their intention to the Region and one of the authorized inspection bodies. The inspectors carry out the first examination with specialized technicians who examine the company and view the different plots, checking the various land records, warehouses, stables and any other corporate structure. If the inspection shows compliance with the legislation, the company is permitted in the control system, and can start the conversion: a period of detoxification of the land, that, depending on the previous use of chemicals and crops, can last two or more years. Only on conclusion of this conversion period, the product can be marketed as organic. The authorized inspection bodies provide several inspections per year, even by surprise, and take samples to be

analysed. The farms that produce organically must even document each step of the production with registers prepared by the Specific Italian Ministry, which ensures full tracking. In Italy there are nine authorities that can make inspections and certification of organic production: they are recognized by a decree of the Ministry of Agriculture and Forestry, and are subjected, in turn, to the control of the same ministry and regions. Here are the names and their identification code: ICEA or Institute for Ethical and Environmental Certification, IT ICA (ex-AIAB); BIOAGRICERT or Bioagricoop, IT BAC; BIOS, IT BIO; C.C.P.B or. Control Consortium Organic products, EN CPB; CODEX, EN CDX; ECOCERT Italy, EN ECO; I.M.C. or Mediterranean Institute of Certification, IT IMC; QC&I or International services, IT QCI; SUOLO E SALUTE (Soil and Health), IT ASS; BIOZERT, EN BZ BZT. In Italy products that obtain the approval, can show a symbol (*Figure 5*) to be recognized as such.

Even a biodynamic agriculture exists. It looks at earth, plants and animals as parts of a whole. They have to find harmony, and help each other. It tries to cure plants as homeopathy does with human beings. The fertiliser used is the compost, but even eight substances are permitted: one made from manure, one from quartz sand, one from nettle, one from camomile, one from yarrow, one from valerian, one from oak bark and one from dandelion. Even biodynamic agriculture is subject to regulation. To use that name it has to obtain both the organic certification and the Demetra International one.

Figure 5. Organic farming logo

1.5. SOIL

Soil is a fundamental resource for life, the medium in which air, water and plants interact: it represents the centre of a net of biogeochemical equilibriums between substances. Soil has a number of functions: it's a buffer, a store for water, a home for many living beings and a filter for nutrients. It consists in the surface layer that covers Earth's crust and it develops from the geological substratum (the parent rock) that is broken and modified by physical and chemical weathering processes and by the biological action. A vertical section of soil, called profile, with clear differences among the layers develop if

pedogenesis has enough time to operate. From the surface layer downwards five main layers can be recognized:

- the organic horizon (O) on top with animal or plant remains (mainly leaves forming the litter) not yet decomposed;
- the topsoil (A) constituted by organic compound, humus, peat and so called humic substances (humic acids, humin and fulvic acids) where little animals live and short roots take hold;
- the subsoil (B), fine sediments where roots penetrate even if it's poor in nutrients;
- the parent material (C), a mixture of fine sediments and clasts whose composition reflects the geology of bedrock;
- the bedrock (R), a substratum of consolidated parent rock.

Pedogenesis depends on many factors (climate, morphology, involved chemical compounds, time, biological processes) and may give origin to many different types of soil. The International Union of Soil Sciences (IUSS) has developed an international standard taxonomic classification, identifying 32 reference groups.

Instead of a name, a better description can be made by giving a list of parameters. The first, a physical one, is the texture, an agronomic classification according to the proportion of three grain-size classes (sand, silt and clay) which are defined by particle size. Every agricultural institute usually uses its own classification, putting their borders of each class, but the one described in ***Figure 6*** given by the United States Department of Agriculture (USDA [53]) is internationally accepted.

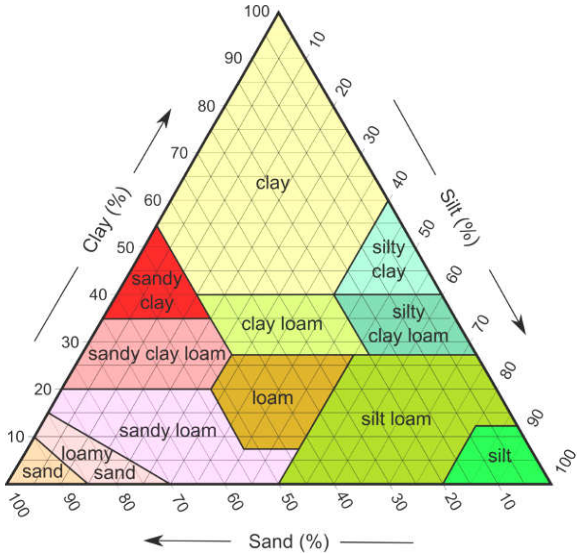

Figure 6. Triangle plot of USDA classification

In this classification the sediments of a diameter of 2 mm or more are removed; sands have particles with a diameter from 0,05 to 2,0 mm, keeping soil permeable and properly aerated; silts have a diameter from 0,002 to 0,05 mm, increase available water capacity of soil and give compactness; clays have the smallest particles, with a diameter of no more than 0,002 mm and are very useful in keeping nutrients, but may bring to waterproofing if too abundant. The best texture for farming is composed by a 50-70% of sand, a 30-50% of silt and a 10-25% of clay (USDA, 1993 [53]).

Then there are many chemical parameters: the soil reaction (pH - linked with assimilability of nutrients by the plant), the electrical conductivity (EC), the total carbonate content, the active carbonate, iron by ammonium oxalate extraction, total organic carbon, total nitrogen, ammoniacal nitrogen, available Calcium, Magnesium, Potassium and Sodium, the cation-exchange capacity (CEC), available Phosphorus and available Potassium. Elaborating these values, other important information can be obtained: for example, the ratio between the active carbonate and the squared value of iron by ammonium oxalate extraction gives an indicator of potential occurrence of iron deficiency chlorosis, when there are values over 100. Another important ratio is between carbon and nitrogen, because high levels (over 14) mean that organic matter is not decomposing (Barbiroli et al., 2000 [9]).

Another description method is to list the percentages of the most common compounds: Silica, Titanium dioxide, Aluminium oxide, Ferric oxide, Manganese dioxide, Magnesium Oxide, Calcium oxide, Potassium oxide, Sodium oxide, Phosphorus pentoxide and the LOI. The last variable is the “loss on ignition”, the percentage of weight that the soil has lost after a heating at 950°C in a furnace for at least 16 hours. It represents the organic matter, carbonates, hydrates and hydroxides.

1.6. LITHOLOGY

Soil is the result of many chemical, physical and microbiological processes that affect the substratum, so its composition can be strictly linked to the lithological background, if the weathering processes are not intense. The origin of a rock affects its mineralogical composition and its chemical composition. During mineral formation, either in the igneous or in the metamorphic and even in some sedimentary rocks, substitution between elements is possible in the crystal lattice, without changing its structure and properties. Such

substitutions can happen if the replacing elements have a comparable ionic radius, same charge (but also exceptions are possible) and similar electronegativity. In general, the substitution, called isomorphic, happens when the difference between the radius of the elements involved is lower than 15%. For example *Ni* can replace the *Mg* or *Fe* in olivines, *Co* can replace *Mg* in pyroxenes and Cr^{3+} can replace Fe^{3+} in various clay minerals (Essington, 2015 [19]).

In the sedimentary rocks the content of trace elements can depend on their types. In Carbonate rocks (like limestones) the content of trace elements is generally low and generally related to possible substitution with *Ca* and *Mg*. So for example *Fe*, *Mn*, *Sr* and *Cu*, *Cd*, *Zn*, can have relatively high concentration, depending on the environmental condition during formation or on the diagenetic history. Clastic rocks (sandstones, siltstones, shales) can have various metal contents depending on the type of grains present. In general the highest concentrations of a large number of elements are observed in shales, that are mostly composed of clays that can host many elements through substitution in their lattice but that are also characterized by high surface area and can have important absorption properties. In ***Table 1*** there are the average values of some metals and metalloids in different types of rocks (Alloway, 2013 [5]).

Table 1. Values of some metals and metalloids in different types of rocks (Alloway, 2013)

	Igneous rocks			***Sedimentary rocks***		
mg/kg	***Granites***	***Basalts***	***Ultramaphic rocks***	***Limestone***	***Sandstones***	***Shales***
Ag	0,04	0,1	0,06	0,12	0,25	0,07
As	3	0,7	0,7	1,5	0,5	13
Ba	600	330	5	90	300	550
Cd	0,1	0,2	0,05	0,1	0,03	0,25
Co	4	45	110	0,1	0,3	20
Cr	10	250	2300	5	35	100
Cu	12	90	40	6	2	45
Mn	400	1500	1200	15	100	850
Mo	1,5	1,2	0,3	0,3	0,3	2
Ni	5	130	2000	5	2	70
Pb	20	4	0,05	5	10	22
Sb	0,3	0,2	0,1	0,15	0,05	1
Sn	3,6	0,9	0,3	0,3	0,6	5
U	4	0,5	0,02	1	1,3	3,2
V	70	260	80	15	20	130
Zn	50	100	60	40	20	100

1.7. METALS

More significant elements from the environmental and eco-toxic point of view are: *As, Hg, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sn, Zn* e *Se* (Salomons et Förstner, 1984 [46]). Speaking of toxic metals very often refers to heavy metals. With a chemical approach, the definition of heavy metals concerns a series of elements of the periodic table that have a density higher than 5 g/cm^3 (Lapades, 1974 [31]). They are characterized by having a cationic behaviour and different states of oxidation depending on the conditions of the reduction potential (Eh) and pH. They are naturally present in the Earth's crust, but their concentration in soil has increased because of the contamination. Sources of contamination are various: industrial activities, such as mines and the mineral foundries, the use of fertilisers and antiparasitics, gas emissions, and the production of energy and fuel (Kabata-Pendias, 2011 [28]). Heavy metals have a strong affinity for sulphur, usually form hydrates that are rather insoluble and have a strong attitude to form complexes. They are not subjected to processes of decomposition or microbial metabolization, so they therefore remain in the soil until they are transported by some chemical, physical or biological mechanism in another environmental compartment.

In this study we have chosen to investigate in detail eight metals: Cadmium, Chromium, Copper, Iron, Manganese, Nickel and Zinc.

Cadmium (*Cd*) geochemical behaviour is very similar to that of Zinc: both have similar atomic structure and electronegativity, and tend to form sulphides. Cadmium is obtained as a by-product from the minerals of Zinc and Lead. The average concentration of Cadmium in the Earth's crust is about 0,15 ppm. In igneous and metamorphic rocks, Cadmium has almost always the same value, but in sedimentary ones shows higher variability; sediments formed in anoxic environments, rich in organic matter, such as phosphorite or black shales, can contain hundreds of ppm (Alloway, 2013 [5]). The pollution by Cadmium has increased in recent decades, in parallel with its industrial use. Lead, Copper and Mercury have a long time utilization history, but Cadmium found an industrial use only in the last 50 years. The main anthropogenic inputs (Kabata-Pendias, 2011 [28]) come from extraction and processing in mine and steelworks, from the accumulation of wastes containing Cadmium (burning of plastics, disposal of batteries), from spreading of sewage sludge, burning of fossil fuels and from production of artificial phosphorus fertilisers. Generally Cadmium is enriched in the surface horizon, since the organic matter tends to hold it; however, contrary to Copper and Lead, it shows a higher

mobility and moves rapidly in depth, depending on climatic conditions and soil characteristics (Botes, 2004 [12]). In nature there are very few *Cd* minerals: the most common are greenockite [*CdS*] and otavite [$CdCO_3$]. Natural sources of this element can be usually found in minerals containing Zinc such as sphalerite [*ZnS*], in which *CdS* is a significant impurity (3%). An alternative comes from the refining of Copper, and Lead. Its concentration is higher in sedimentary rocks and increases with decreasing sand fraction, because cadmium is generally associated with smaller particles (Alloway, 2013 [5]).

Chromium (*Cr*) is available in nature in three different oxidation states (II, III, VI), and is fundamental for human life: an alimentation too poor of it may lead to metabolic disease, but at the same time the absorption of large quantities causes kidney damage (De Vivo et al., 2004 [17]). The risk associated to its presence is due to Chromium(VI), that is very toxic, carcinogenic, but it is not easy to distinguish the percentage of the three oxidation states from the total concentrations. When released into air or water, it has the tendency to deposit in sediments (De Vivo et al., 2004 [17]).

Copper (*Cu*) is strongly absorbed by the particles of sediment, in particular on the organic fraction (humic and fulvic acids) and is a trace element essential for human health (Alloway, 2013 [5]). Contact with too much copper can cause serious problems such as irritation to the nose, mouth and eyes, provoking even dizziness, vomit and diarrhoea. An accumulation in the body can lead to anomalies to the normal function of the nervous system, to the liver and kidneys (De Vivo et al., 2004 [17]). The ingestion of its salts such as copper sulphate, can even cause death. The concentration of copper in the soil varies from 20 ppm of sandy soils to 100 ppm of clay soils. Contents higher than 100 ppm are anomalous. When a certain quantity reaches the soil, it stays there, and doesn't reach water table because it sticks to organic matter. The solubility, the mobility and the bioavailability of copper are closely related to the pH. The copper does not decay in the environment, because it bio-accumulates in plants and biomagnifies in animals. Few plants can grow in soils with little copper, but its compounds are often used for the control of pests and are accepted also by organic farming (De Vivo et al., 2004 [17]), so sewage sludge coming from factory farms is usually rich, as well as zinc, in copper, in the form of hydrogen sulphate, oxychloride or chelate.

Iron (*Fe*) is the most abundant metal on the planet. It exists mainly as iron oxides, hematite or magnetite. It allows animals to breath because it is an essential constituent of haemoglobin and for plants it is even basic to photosynthesis, so there is no danger when it

is abundant. The real problem occurs when there is low adsorption of iron, that can lead, for example, to human anaemia (Alloway, 2013 [5]).

Manganese (*Mn*) presence in soils is usually due to the parent rock, but can be also the effect of the agricultural practice of fertilisation with sulphate or manganese monoxide in case of natural deficiency. The mobility of manganese is strongly influenced by the pH and redox potential (Botes, 2004 [12]).

Nickel (*Ni*) is a by-product of many industries, from home heating to fertilisation. The concentration of nickel present in the Earth's crust is of an average of 80 ppm and varies considerably according to the type of rock. The mobility increases with decreasing pH, which also contributes to the precipitation of various compounds. Nickel is essential in small amounts, but when absorption is too high it can be a danger to human health because it is carcinogenic to the respiratory system (De Vivo et al., 2004 [17]). It is usually absorbed by sediments but when the soil is acid, it becomes more mobile and often reaches the water table. It can be found in minerals such as biotite, augite, hornblende, but these are unstable and easily suffer the effects of weathering. In sedimentary rocks, concentrations range between 5-90 ppm, with the highest levels in shales, and in ultramafic rocks they reach 2000 ppm (Alloway, 2013 [5]). There are two main nickel deposits exploitable also from the commercial point of view: laterites, where the main minerals are nickeliferous limonite [$(Fe,Ni)O(OH)$], garnierite and deposits of sulphides, containing pyrrhotite, pyrite [FeS_2], chalcopyrite [$CuFeS_2$] and pentlandite [$(Fe,Ni)_9S_8$] (Alloway, 2013 [5]).

Lead (*Pb*) is not an essential element for any living being, and tends to accumulate especially in soils and sediments. Its bioavailability is not reduced over time. It is in the Earth's crust at a concentration of 14,8 ppm and Pb^{2+} can easily replace the K^{+} in silicates and Ca^{2+} in carbonates. In silicate rocks its presence increases in proportion of *Si* (Alloway, 2013 [5]). *Pb* is readily emitted from high temperature processes such as coal burning or the use of leaded petrol in cars, and frequently results in considerable concentration in surface soils (Alloway, 2013 [5]) . The main source of *Pb* is galena [*PbS*], but it is abundant also in anglesite [$PbSO_4$] and cerussite [$PbCO_3$].

Zinc (*Zn*) is a transition metal present in all soils with a background concentration between 10 and 100 ppm (Alloway, 2013 [5]). It is potentially dangerous: high levels may negatively influence the activity of microorganisms and earthworms (De Vivo et al., 2004 [17]). Zinc alloys were known even two thousand years ago, but only in the last 20 years its production has doubled. Its air diffusion has lead to large pollution areas coming from

industry. It can be extracted from sphalerite [ZnS], smithsonite [$ZnCO_3$] and hemimorphite [$Zn_4Si_2O_7(OH)_2 \cdot (H_2O)$]. It is rather common in many rock types since it can replace Fe^{2+} and Mg^{2+} in ferromagnesian minerals. High concentrations are common in basic rocks, such as basalt, while lower in metamorphic rocks and sandstones too (Alloway, 2013 [5]). A great amount of the zinc normally present in soils is due to natural phenomena such as volcanic activity and forest fires. The use of inorganic fertilisers and the addition of manure may contribute to a local increase in the concentrations of Zinc. Other pollutants come from cosmetics and metal alloys (like brass).

1.8. METALS AND PLANTS

According to the importance for vegetable organisms, an element can be considered (Pilon-Smits, 2009 [40]):

- essential: when, in its absence, the plant cannot complete its own vital cycle, when the function of such an element cannot be developed by any other element and when it is directly involved in the metabolism of the plant;
- beneficent: when it can compensate the toxic effects of other elements or it can replace mineral nutriments in some other less specific function such as the maintenance of the osmotic pressure;
- toxic: when even in very low concentrations it can reduce the growth of the plant damaging its metabolism.

The graph in ***Figure 7*** (Alloway, 2013 [5]) shows typical dose-response curves for essential and non-essential trace elements in crops.

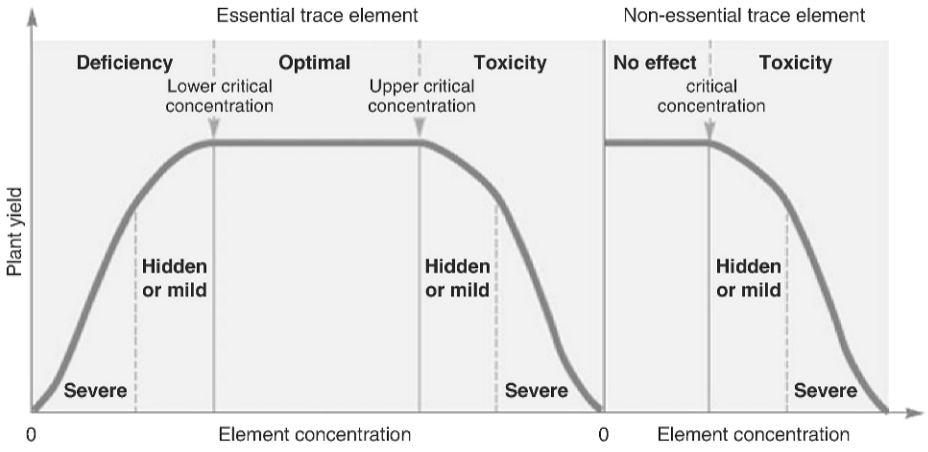

Figure 7. Typical dose-response curves in crops (Alloway, 2013)

The concept of toxicity must not be seen in absolute terms, because if some elements like *Hg* and *U* are always toxic for the vegetable organisms, there are others, including essential elements, that can provoke harmful effects. The toxicity for the plant is linked to high concentrations: in fact all the elements over a certain threshold become toxic (Freedman, 1995 [22]).

The total content of a metal in soil, is not indicative enough of how dangerous it is; the harmfulness of the heavy metals depends on their availability and mobility. The forms assumed by the elements in the ground can be divided in the followings fractions (Violante, 2002 [56]):

- soluble fraction: immediately available elements, that are present as ions, molecules or chelates in solution;
- exchangeable fraction: elements that are easily available because they are bound to the exchange surfaces;
- available reserves: elements that are bound in minerals, in simple organic forms or in difficultly reached positions;
- non-available reserves: elements that are in structures that cannot be easily altered

In the ground the concentration of a metal that is in solution, is a good indicator of its availability, because only the soluble fraction, or the portion that can be readily solubilised, is really available (Violante, 2002 [56]). The available fraction in soils is what the plants extract from the ground with nutrients, through their roots, through a process of uptake. However plants can assume some elements, not only from the ground, but even through the leaf apparatus from atmospheric depositions (Schreck et al., 2012 [48]) even if the roots remain the main way of uptake of trace elements in the vegetation (Adriano, 2001 [1]).

Mobility is the ability of an element to migrate in the different layers of soil. The most dangerous forms are those that can be easily moved. Plants are the greatest channel of diffusion of the heavy metals, because an element can be brought into the food chain if the plant uptakes it.

The characteristics of the soil that condition the mobility and the presence of metals, are listed in ***Table 2***.

Table 2. Main characteristics responsible for presence and mobility of metal in soils

Physical characteristics	Chemical characteristics
texture;	abundance and characteristics of humic substance;
structure.	abundance and characteristics of clay fraction;
	abundance of oxides and hydroxides of Fe and Mn;
	CEC; pH; Eh.

There are several analytical techniques to determine the concentration of a pollutant that could potentially be available, even if there is a dispute on the best methodology to its measurement (Ajmone-Marsan et al., 2008 [2]). An attempt to harmonize such methodic, has been made with the international protocol ISO 17402:2008 denominated "*Soil quality: requirements and guidance for the selection and application of method for the assessment of bioavailability of contaminants in soils and soils materials*". This protocol explains how to measure the availability of metals as well as metalloids, organic-metal complexes and other pollutants, using the common methodic of the extraction: a separation of one or more substances from a matrix (the soil in our study), through a treatment with solvents.

Every solvent, the extracting substance, has a different behaviour in terms of efficiency in the extraction of a specific fraction of metals. According to the solvent used, the extraction of a metal can be total or partial, so the choice of which method to use depends on the result we want to get from the analysis, that can be the measurement of the content of a single metal, or of a group of metals. The efficiency of the extraction is actually conditioned by the composition of the reagent we use and by the characteristics of the soil. In *Table 3* there is the list of the possible extracting methods.

Table 3. Soil extracting methods for the available part

Kind of extracting substance	***Chemical compounds***
Weak	Simply water
	Saline aqueous solutions with $CaCl_2$, $Ca(NO_3)_2$, magnesium salts or $BaCl_2$
Reducing	$C_6H_7NaO_6$, $Na_2S_2O_4$, etc...
Weak acid	Citric or acetic diluted acids
Chelating (strong complexing)	EDTA, DTPA, NTA
Salt and acid combined	$(NH4)_2C_2O_4$ + $(COOH)_2$, CH_3COONa + CH_3COOH
Diluted acid	HNO_3, HCl, HCl+H_2SO_4
Strong concentrated acid	HNO_3, HCl, HCl+HF, Acqua regia (3HCl+HNO_3)

Aqua regia leads to a pseudo-total extraction because it eats carbonates, a great part of sulphur minerals, some silicates, clay minerals, salts and hydroxides (Salminen et Tarvainen, 1997 [45]) and brings an over-evaluation of the available part for plants. Methods of extraction that use chelating agents, such as Ethylene Diamine Tetraacetic Acid (EDTA) by Lakanen et Ervio (1971) and Diethylene Triamine Pentaacetic Acid (DTPA) by Lindsay and Norwell (1969) represent some good methods that probably best fit what happens in the environment.

The Italian normative offers a practical guide of extracting methods in the section XII of the D.M. 13/09/99 called *"Metodo Ufficiale di Analisi Chimica del suolo"* drawn up by the Italian Society of Soil Science (SISS).

1.9. REGULATIONS

As said before, soil has a fundamental role in environmental health and the effects of its degradation are always irreversible, so it is protected by regulations. The European Commission, with the COM (2002) 179, identifies the possible damages to soil: the erosion, the loss of organic matter, the local and the widespread contamination, the waterproofing, the compacting, the loss of the biodiversity, the increase of salinity and the hydrogeological disarrangement instability (with landslides and floods).

In Italy soil defence had its first regulation with the L.183/1989, but the real interest in controlling pollution comes from the DM.471/1999. Finally in the D.lgs. 152/2006 we have a table (Annex 4 from the Chapter 5 of Part IV) with the limits over which an intervention is needed: for example in ***Table 4*** there are the limits for the chemical elements in the inorganic section of the same Annex 4.

Table 4. Soil limits for the chemical elements from the D.lgs. 152/2006

SOIL LIMITS	For industrial use (ppm)	For open green spaces and residential use (ppm)	Difference of the two uses, as ratio
As	50	20	150%
Be	10	2	400%
Cd	15	2	650%
Co	250	20	1150%
tot-Cr	800	150	433%
Cr VI	15	2	650%
Cu	600	120	400%
Hg	5	1	400%
Ni	500	120	317%
Pb	1000	100	900%
Se	15	3	400%
Sb	30	10	200%
Sn	350	1	34900%
Tl	10	1	900%
V	250	90	178%
Zn	1500	150	900%

Wine in Italy and in Europe is a great business and has an important role in nutrition, as part of the Mediterranean diet. To avoid commercialisation of wine that could be dangerous for human health, about thirty years ago in Italy the DM 29/12/1986 introduced a first regulation. The law describes the forbidden processes in grapevine culture and in vinification, gives the maximum levels of certain physical and chemical parameters of the final product and tells how to obtain them. Then, recently, the European Union wrote the regulation for its territory with the Commission Regulation (EC) n°606/09. However there is also an accredited institution, the International Organisation for Grapes and Wine (OIV) that gives suggestions on the matter. Actual limits to the elements that are most interesting for this study are listed in ***Table 5***.

Table 5. Limits of some wine values in Italian Regulation and OIV

Limits (mg/L)	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Volatile acidity (CH_3COOH)	
Italy	-	-	1,00	-	-	-	0,20	5,00	**red wine**	**white wine**
									1,08	1,20
OIV	0,01	-	1,00	-	-	-	0,15	5,00	**all wines**	
									1,20	

2. MATERIALS AND METHODS

2.1. GRAPE VARIETY

As stated in the introduction we tried to limit the possible uncontrolled variable represented by the metabolic behaviour of plants choosing one type of grapevine. We focused on the Trebbiano that, among white wines, is a widespread variety in Italy, particularly in Romagna. There are regions where Trebbiano is typical, so we have the Tuscan one, the Abruzzese, from Soave, the Giallo, the Spoletino, the Romagnolo and the Modenese. Many are the DOCs (*Denominazione di Origine Controllata*) that need this variety in their winemaking and, in the region where the vineyards we study are, almost all use it.

In the map of wines of the Emilia-Romagna region (***Figure 8***) (*Civiltà del Bere*, 2004 [16]) we find the following DOC e DOCG (*Denominazione di Origine Controllata e Garantita*) wines that use Trebbiano for their production: the Colli Piacentini, the Colli of Scandiano and of Cimossa, the Colli Bolognesi, the Colli of Imola, the Colli of Faenza, the Colli of Romagna, the Trebbiano of Romagna and the Bosco Eliceo.

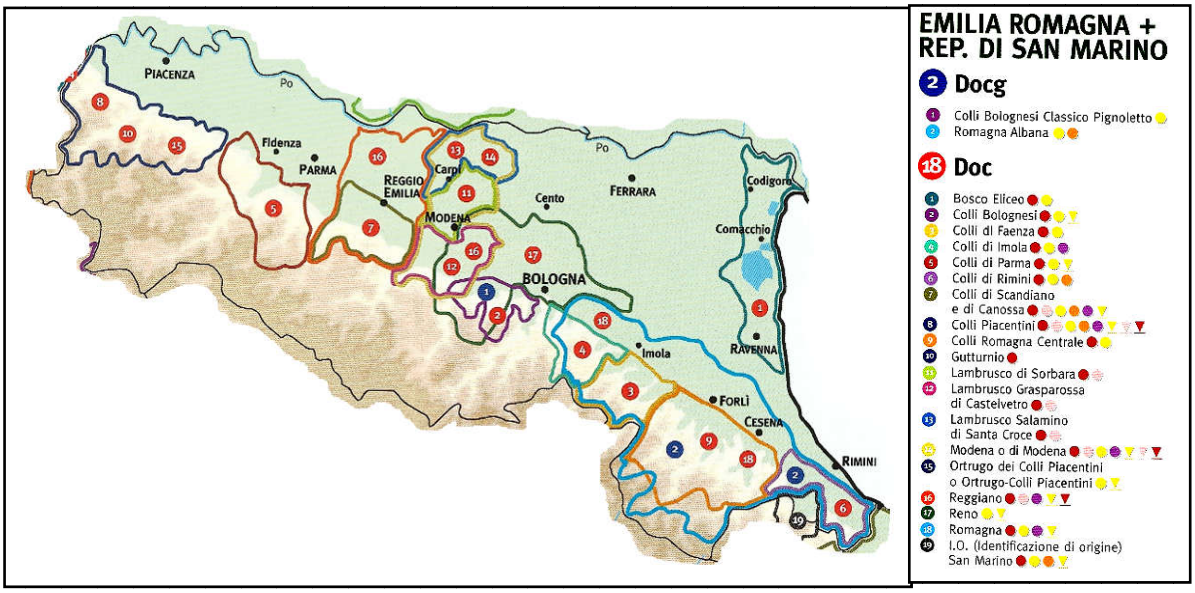

Figure 8. DOC and DOCG wines in Emilia-Romagna (Civiltà del Bere, 2004)

2.2. STUDY AREA

We searched in the Emilia-Romagna region, farms fitting to our needs and we found five of them that allowed us to take samples. In the study we call them with the following acronyms: MA, AB, MF, DZ and NG.

In *Figure 13* there is the location of the 5 vineyards, in *Table 9* there are their geographic coordinates and in *Table 6* their main features are summarised.

Table 6. Main characteristics of investigated vineyards

	Municipality	Geographic context	Geologic context	Soil texture or lithological substratum	Grape type	Agricultural scheme
MA	Brisighella	Hill	Valley slope	Marl-sandstone	Trebbiano	Conventional
AB	Brisighella	Hill	Valley slope	Marl-sandstone	Trebbiano	Organic and biodynamic
MF	Lugo	Lowland	Interfluve	Silt loam	Trebbiano	Organic
DZ	Bagnacavallo	Lowland	Interfluve	Silty clay	Trebbiano	Conventional
NG	Ravenna	Lowland	Riverbank	Sandy loam	Trebbiano	Conventional

MA (*Figure 9*) is on the right side of the Lamone Valley. The farmer also cultivates in the immediate proximities other fruit plants, such as kiwis. It adopts traditional techniques of fertilisation and phytosanitarian treatments.

Figure 9. MA vineyard

AB (*Figure 10*) is on the left side of the Lamone Valley. The owner treats the vineyard with a biodynamic philosophy and so also with organic methods.

Figure 10. AB vineyard

MF (*Figure 11*) is a farm that is found in the lowland of Lugo and from about ten years ago applies organic farming.

Figure 11. MF vineyard

DZ (*Figure 12*) is a farm located in the lowland, near Bagnacavallo. The owner gave us the list of the products used for the care of the vineyard with the annual quantities per hectare employed. We have consulted the card of every product to get the percentages of the active ingredients and of the other components, noting their Chemical Abstracts Service (CAS) reference. From there we have found the percentages of the elements of

greatest interest and therefore also determined the quantities for every single plant through the density of plantation. In the

Table 7 there are the main features of the DZ crop.

Figure 12. DZ vineyard

Table 7. Main DZ vineyard features

planting pattern:	1 m (between plants) x 4 m (between rows)

vineyard density	
2500	plants/ha

Cu	**K**	**Mn**	**Na**	**P**	**Zn**	
5663	512	1621	3,58	749	4057	g/ha
2,27	0,205	0,65	0,001434	0,300	1,62	g/plant

NG, like DZ, adopts conventional schemes and it is located in the lowland, but it grows on different deposits of river-bank. Also NG gave us the list of the employed products, so we also found the quantities of each element for it (*Table 8*).

Table 8. Main NG vineyard features

planting pattern:	3 m (between plants) x 2 m (between rows)

vineyard density	
1670	plants/ha

Cu	**K**	**Mn**	**Na**	**P**	**Zn**	
5951	70298	1644	346,4	11332	3831	g/ha
3,56	42,1	0,98	0,21	6,79	2,29	g/plant

More information about these vineyards are in the Geographic Information System (GIS) of Regione Emilia-Romagna: in the *Table 6* there is the information we obtained concerning the soil type ([41]), the lithologic substratum ([44]) and the geological landscapes ([43]). The map of the landscapes is in *Figure 13*.

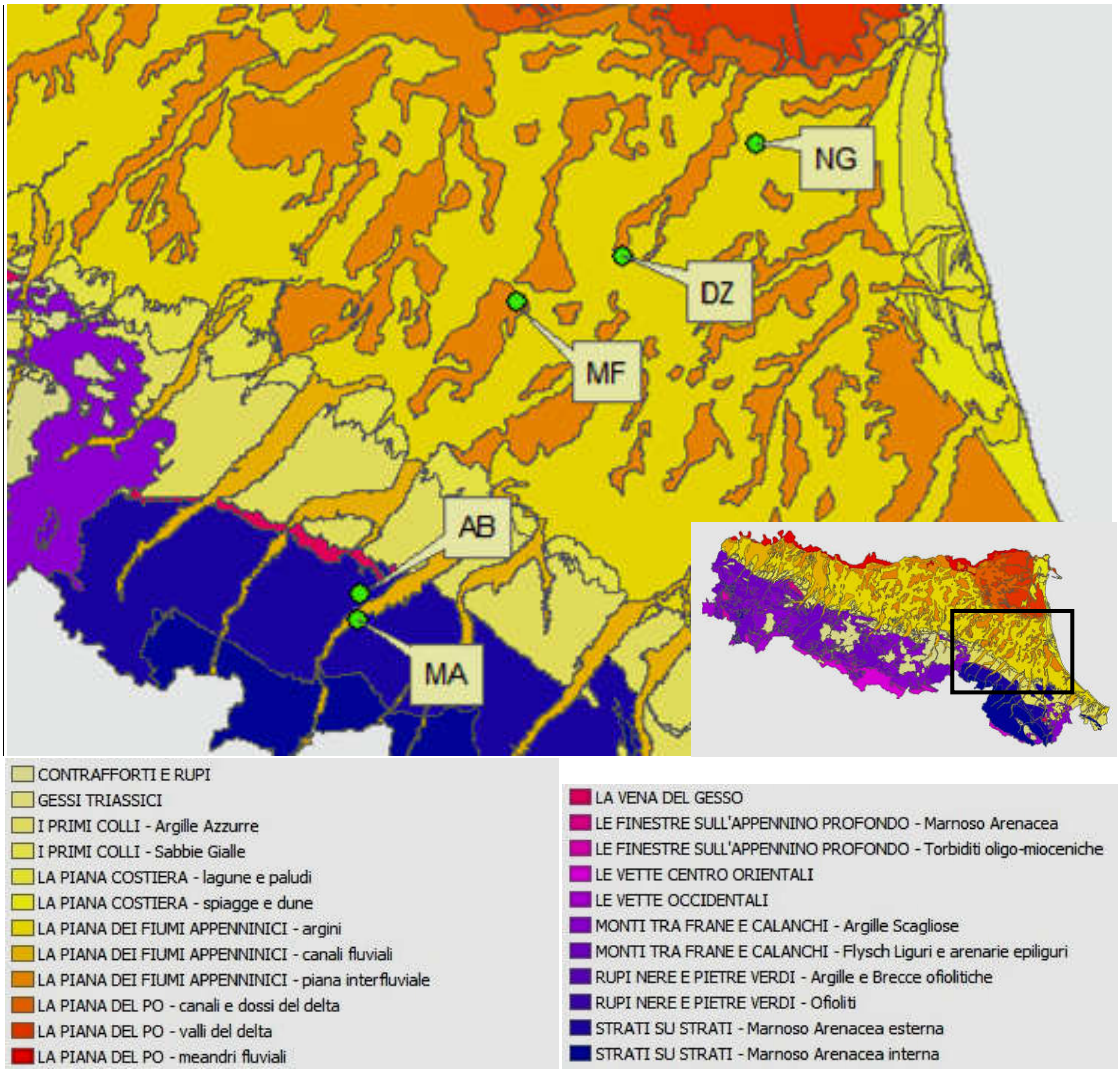

Figure 13. Geological landscapes with the location of the vineyards

With the *Figure 13* we can see that NG is in the middle of the area (the yellow one) with river-bank deposits. MF is in the interfluve area (the orange one) and DZ is just on the border of the area, so they have the typical deposits. AB and MA are located on the marl-sandstone (in the blue area).

2.3. COLLECTION OF SAMPLES

The collection of samples started in September 2014. We asked the owners if they had a wine that was exclusively produced with grapes from their vineyard, but only AB, DZ and NG provided it. Then for each farm we selected 3 plants to sample, in distant points, to have more representativeness of the vineyard in its whole.

For every point we sampled the topsoil, from the first 10 cm of depth, the subsoil, at a distance of more than 60 cm from the ground, then from the plant leaves, green shoots, one

year-old shoots, lignified shoots, bark, roots, grape clusters (even if for MF only in one point and for NG without choosing the plant they came from), trunk for DZ and NG. To reduce the amount of samples to be analysed, however we thought to consider only two points of each vineyard and not all the parts of the plant from every farm but only some of them. In *Table 9* there are the coordinates of the sampling points we chose.

Table 9. Geographic coordinates of the sampling points

	WGS84 (GPS)				
	MA1	**AB1**	**MF1**	**DZ1**	**NG 2**
E	11,724024	11,725578	11,882636	11,979952	12,107155
N	44,193671	44,211077	44,398235	44,426602	44,498090
	MA2	**AB2**	**MF3**	**DZ3**	**NG 3**
E	11,723486	11,726210	11,880531	11,980386	12,108527
N	44,193389	44,210927	44,399143	44,427252	44,497053

2.4. CHEMICAL AND PHYSICAL SOIL ANALYSES

Now we have 4 soil samples (two points and two different depths) for each vineyard. We considered one of the four samples to characterize the vineyards for some of their chemical and physical features. For these analyses we followed methods described in the Italian D.M. 13/09/1999, that usually have references in the ISO methods (Giandon et Bortolami, 2008 [25]), with some exceptions due to the available equipment. We chose to find several parameters of each sample: pH, EC, pH, texture, total carbonate, an estimated content of dolomite, active carbonate, iron from ammonium oxalate, the Iron Deficiency Chlorosis Index (IDCI), total nitrogen and the organic carbon, Cation Exchange Capacity (CEC), available *Mg*, *K*, *Na*, *Ca* and phosphorous.

The value of **hydrogen-ion activity (pH)** comes from an aqueous extract with a 1:2,5 ratio, obtained from shaking 50 ml of deionised water with 20 g of soil, through a direct measurement with the Mettler Toledo SG2 pH-meter until the value that the instrument was displaying was stable.

For the **electric conductivity (EC)** we used the same aqueous extract with 1:2,5 ratio and we measured it directly through the Delta Ohm Hd 8706 Conductivity-meter. Internationally the standard reference method of it is an aqueous extract obtained from

saturated soil paste (ECsp) that has been elaborated by the U.S. Salinity Laboratory Staff (1954 [52]) of the USDA. However the measure of the electric conductivity with saturated soil paste can be estimated even by aqueous extracts, with different dilutions. Sbaraglia et Lucci (Sbaraglia et Lucci, 1994 [47]) proposes that:

$$ECsp = 4 \cdot EC_{(1:2,5)}.$$

For the **texture**, the grain-size analysis, we followed the indications of the method described in the D.M. 11/05/1992 at the annex 6. The method is based on the Stokes' law which explains that the particles fall with a speed proportional to their surface. The equation is:

$$v = 2\,(\rho_s - \rho_l)\,g\,r^2 / 9\eta_l\,;$$

where v is the precipitation speed, ρ_s and r^2 are the density and the surface of the particle, ρ_l and η_l are the density and the viscosity of the liquid of sedimentation and g is the gravitational acceleration. We measure, with the Bouyouces' hydrometer, the density of the suspension of around 50 g of soil in 1 L of a dispersing solution with 5 g/L of sodium hexametaphosphate $(NaPO_3)_6$ at different times previously determined, from the beginning of the sedimentation. Every grain-size class needs a certain period to deposit, before which time it is found in suspension and it contributes to increase the density of the same suspension. Then we found the percentages of the three macro-categories of sediments (sand, silt and clay) with proper equations.

The **total carbonate** has been determined through a De Astis calcimeter even if the official method of the D.M. 13/09/1999 recommends a Dietricht Fruehling calcimeter. The method finds the content of $CaCO_3$ from the measure of the volume of the CO_2 produced by the reaction of soil with an excess of *HCl*. We took quantities of between 1 and 2 g of soil and made them react with 5 mL of *HCl* to 18,5%. The results are expressed in g/kg and calculated from the volume in mL of CO_2 produced in the first minute of reaction, multiplied for the mol/L of CO_2, for the g/mol of $CaCO_3$, adjusted with the temperature (t), the pressure (P), the tension of steam (φ) to the temperature t and the right conversion on unities of measure. So the complete formula is this:

$$C(g/kg) = V\ (mL) \cdot \frac{1\ mol}{22{,}26\ L} \cdot \frac{273{,}15\ K}{273{,}15\ K + t(°C)} \cdot \frac{P\ (mmHg) - \varphi\ (mmHg)}{760\ mmHg} \cdot \frac{1\ L}{1000\ mL} \cdot \frac{100{,}08\ g\ CaCO_3}{mol} \cdot \frac{1}{g_{campione}} \cdot \frac{1000g}{kg}$$

where φ is the tension of steam to the temperature t.

Moreover with this method we can obtain an assessment of the content of **dolomite** [$MgCa(CO_3)_2$] measuring the volume produced after the first minute of reaction of the sample. We can use the same equation used for the carbonate replacing the molecular weight of the $CaCO_3$ with the dolomite one (184,39) and, considering the different stoichiometric ratio of the reaction, dividing it by 2.

Also for the **active carbonate** determination we followed the D.M. 13/09/1999: we took 5 g of soil, put them in 125 mL of ammonium oxalate solution (0,1 mol/L) and let the suspension react, without heating, for 2 hours. After filtering the suspension we took 10 mL to titrate with potassium permanganate ($KMnO_4$) the ammonium oxalate that did not react. Then we took an extraction from 5 g of soil with 125 mL of ammonium oxalate solution (0,2 mol/L) and analysed the *Fe* in Flame Atomic Absorption Spectroscopy (FAAS). Linking the **iron from ammonium oxalate** and the active carbonate we can obtain the **Iron Deficiency Chlorosis Index (IDCI)** (Juste et Pouget, 1972 [28]) with this equation:

$$\text{IDCI} = A \cdot 10^3 / B^2$$

where

A = active carbonate content, in g/kg;

B = iron from ammonium oxalate extraction, in mg/kg.

For the **total nitrogen** and the **organic carbon** we took 15-20 mg of soil, we added 20-40 µL of *HCl* (10%) and put them in a heater. The dry sample ends in the elemental analyzer, an instrument that uses a combustion technique to obtain volatile substances, separates them with frontal chromatography and through a detector returns the weight percent of some compounds.

For the **ammoniacal nitrogen**, first we extracted 20 g of soil with 200 mL of *KCl* (2 mol/L) then the suspension was distilled according to the Kjeldahl method and titrated with a solution of HSO_4 (0,005M).

For the **Cation Exchange Capacity (CEC)** first we took 2,5 g of soil and 30 mL of a solution with 100 g/L of barium chloride ($BaCl_2 \cdot 2H_2O$) and 22,5 mL/L of triethanolamine (TEA) [$N(CH_2OHCH_2)_3$] (98%) buffered at pH 8,2. In the soil the *Ba* in excess replaces the *Ca*, the *Mg*, the *K* and the *Na*. We discarded the part in solution and we washed the soil with deionised H_2O. We added 25mL of solution of magnesium sulphate ($MgSO_4 \cdot 7H_2O$) (5 cmol/L) and *Mg* replaces *Ba*. The last step of the method consists in the titration with ethylenediaminetetraacetic acid (EDTA) (2,5 cmol/L) of the *Mg* remaining in 10 mL of solution. The formula used for returning to the value of CEC is:

$$CEC = \frac{\left(V_B - V_A \cdot \frac{(25+B-A)}{25}\right) \cdot M \cdot 1000}{m \cdot 1000} \cdot \frac{25}{10} \cdot 2$$

where
CEC = cation exchange capacity, in cmol(+)/kg;
VA = volume of the EDTA solution used in the titrate of the sample, in mL;
VB = volume of the EDTA solution used in the titrate of a blank, in mL;
A = sample + tare;
B = sample + tare + residual H_2O;
25 = mL of $MgSO_4$ solution;
M = concentration of the EDTA solution, in cmol/L;
2 = ratio between molecular weight and equivalent weight of Mg;
m = weight of soil used.

Also for **available Mg, K and Na** we take 2,5 g of soil and let them react with 50 mL of a solution with 100 g/L of barium chloride ($BaCl_2 \cdot 2H_2O$) and 22,5 mL/L of triethanolamine (TEA) [$N(CH_2OHCH_2)_3$] (98%) buffered at pH 8,2. We filtered the solution and analysed it with FAAS. The **available Ca** comes from the difference between the CEC and the values of *Mg*, *K* and *Na* in cmol(+)/kg.

The **available phosphorus (P)** has been obtained with the Olsen method. We took 2 g of soil to which we added 0,5 g of active coal and 40 mL of a solution (0,5 mol/L) of sodium bicarbonate with pH 8,5. The $NaHCO_3$ makes the ions Ca^{2+}, Al^{3+} and Fe^{3+} decrease because they fall (as carbonate, aluminate ions and iron hydroxides) and the solubility of the phosphorus from calcium phosphate, aluminium and iron phosphates increases. A volume of the solution is mixed with a series of reagents and analysed with a spectrophotometer.

In the ***Table 10*** are summarised all methods used in the study.

Table 10. Chemical and physical analyses methods

Parameter	Soil	Solution	Reagent/Extractor	Analyser	D.M. 13/09/1999	ISO
pH	20g	50mL		Mettler Toledo SG2	III.1 Method	
EC	20g	50mL		Delta Ohm Hd 8706	IV.1 Method	11265:1994
Texture	50g	1000mL	$(NaPO_3)_6$ (5 g/l)	Bouyouces Hidrometer	D.M. 11/05/1992, VI Annex	
Total carbonate	1-2g	5mL	HCl (18,5%)	De Astis calcimeter	V.1 Method	10693:1995
Estimated dolomite	1-2g	5mL	HCl (18,5%)	De Astis calcimeter	V.1 Method	
Active carbonate	5g	125mL	$[(NH_4)_2C_2O_4 \cdot H_2O]$ (0,1 mol/L)	Titration with KMnO4	V.2 Method	
Fe by NH_4-ox.	5g	125mL	$[(NH_4)_2C_2O_4 \cdot H_2O]$ (0,2 mol/L)	FAAS	V.2 Method	
Tot. N	15-20 mg	40µL	HCl (10%) + calore	Elementary analyzer	VII.1 Method	
Org. C	15-20 mg	40µL	HCl (10%) + calore	Elementary analyzer	VII.1 Method	
NH_3-N	7g	70mL	KCl (2mol/L)	Distillation and titration with HSO_4 (0,005M)	XIV.4 Method	
CEC	2,5g	25mL	$BaCl_2$ + TEA + $MgSO_4$ (5cmol/L)	Titration with EDTA	XIII.5 Method	11260:1994
Mg, K, Na	2,5g	50mL	$BaCl_2$ + TEA	FAAS	XIII.5 Method	13536:1995
Available P	2g	40mL	$NaHCO_3$ (0,5mol/L)	Spectrophotometer	XV.3 Method	11263:1994

2.5. BULK CHEMICAL COMPOSITION

The analysis of the total concentrations of the soil samples has been obtained with the X-ray fluorescence (XRF) technique. It gives us an accurate and not destructive analysis of a lot of chemical elements. It records the radiation coming from the sample when it is hit by the X-ray produced by the instrument. The analysis is developed in a structure of the BiGeA Department of the Università di Bologna with a Panalytical Axios 4000 spectrometer.

We ground the soil, then we took about 3 g of it and 8 g of boric acid, to form the pellets used in the analysis. The XRF gives us the major components, expressed as weight percentage (wt%): silica (SiO_2), titanium dioxide (TiO_2), aluminum oxide (Al_2O_3), iron oxide (Fe_2O_3), magnesium oxide (MgO), calcium oxide (CaO), sodium oxide (Na_2O), potassium oxide (K_2O), phosphoric anhydride (P_2O_5), to which we integrated the Loss on Ignition (LOI) results. These last values come from a method described by Heiri et al. (2001) which consists in the heating at 950°C. The difference between the weight of the sample before and after heating gives us the organic matter, carbonates, hydrates and hydroxides in soil. Moreover XRF technique give us values of 28 trace elements expressed

in ppm: *As, Ba, Ce, Cl, Co, Cr, Cs, Cu, Ga, Hf, La, Mo, Nb, Nd, Ni, Pb, Rb, S, Sm, Sn, Sr, Ta, Th, U, V, Y, Zn* and *Zr*.

The limits present in the D.lgs 152/2006 are results of an aqua regia extraction and refer to pseudo-total concentrations. Taraškevičius et al. (2013 [49]) examined several laboratories which did analysis of soils with aqua regia extraction and which found the total contents too; the study compared the results and found the median value of the degrees (%) of aqua regia extractability (***Table 11***). The results are not the same, the analysis methods are different, so we cannot compare our result with the D.lgs 152/2006 limits.

Table 11. Median value of aqua regia extractability (%) (Taraškevičius et al., 2013)

As	Be	Cd	Co	Cr tot	Cr VI	Cu	Hg	Ni	Pb	Sb	Se	Sn	Tl	V	Zn
82,4%	52,2%	93,8%	85,9%	55,9%		90,6%	97,6%	88,6%	78,7%	53,0%	34,6%	56,7%		61,0%	89,5%

2.6. AVAILABLE METALS IN SOIL

As said before, to obtain the available part of metals the best choice is to make an extraction with chelating agents. The D.M.13/09/1999 suggests a solution with diethylene triamine pentaacetic acid (DTPA) 1,97 g/L, 1,46 g/L of barium chloride and 14,92 g/L triethanolamine (TEA) buffered at pH 7,3 with *HCl*. This method comes from the proposal of Lindsay and Norwell (1969) and is adopted by the ISO 14870:2001.

For the extract preparation we used 40 ml of DPTA solution and 20 g of soil. We shook the suspension for at least 2 hours, then we centrifuged it for 20 minutes at 3000 rpm and filtered with the Whatman 589/3. The solution has been acidified at 2% with HNO_3 to keep pH< 2 and avoid metal precipitates, then in some cases we diluted them from ten to one hundred times.

2.7. VEGETABLES

First of all, we split all the samples in two parts and washed the one we will analyse. The washing was done with deionized water to try to eliminate the particles coming from other matrixes (atmosphere, soil and treatments) and not from the metabolism of the plant. The grapes, once washed, were temporarily frozen, while the other parts of the plant, were left to dry for a few days. Once they were dry, we weighed them, heated them for one

entire day at 65°C and weighed once again to find how much fresh weight is dry residue and how much moisture. In the *Table 40* there are the averages of the residual weight percentages for those parts. The averages are on 15 samples for each part.

The dry samples were ground and mixed to make them uniform. We put 0,2 g in vessels with 1 mL of H_2O_2 (30%) and 6 mL of HNO_3 (60%), the same reagent of Alagić et al. (2014), following the indications of Milestone (Milestone acid digestion cookbook for Microwave laboratory system MLS 1200 Mega, 1996 [37]) for digestion of vegetable parts. Vessels were closed with safety covers, set on the Milestone HPR 100/10 S Rotor and the blockage put in the Milestone MLS 1200 Mega microwave digestion system (*Figure 14*) which applies the plan described in *Table 12*.

Figure 14. Milestone MLS 1200 Mega microwave digestion system with HPR 100/10 S Rotor

Table 12. Digestion programme

Watts	250	0	250	400	700	300	Ventilation
Minutes	1	1	5	5	5	5	30

The digestion system can contain ten vessels. In every digestion round we introduced two blank vessels, obtained by the same process, using a solution of 1 mL of H_2O_2 (at 30%) and 6 mL of HNO_3 (at 60%), but without any sample inside. Once the digestion was complete, the suspension did not show any trace of vegetable parts. The solutions so obtained were filtered with the Whatman 589/3 (with a mesh size of 2,5µm) and then added with deionised water to a volume of 50 mL. After each digestion round, the equipment was cleaned, by refilling the 10 vessels with the same solution of H_2O_2 and of

HNO_3, and put in the microwave digestion system and processed with the plan described in ***Table 13***.

Table 13. Washing programme

Watts	250	400	600	300	Ventilation
Minutes	5	5	3	5	10

The grapes were processed in a different way, for many reasons. First of all it was not possible to dry them by heating because of their high sugar content that would lead to a caramelisation phenomenon so we decided to freeze-dry them. The samples were washed, frozen, weighed, put in the lyophilizer for two and a half days and then weighed once again to determine the weight loss. The lyophilizer brings frozen samples to low pressure, near to the vacuum, to extract its moisture even at a low temperature, with sublimation of ice. Unfortunately grapes have structures designed to retain water so we would always find a bit of moisture: in many cases the lyophilizer cannot eliminate it completely and anyway grapes will retake it through air contact. So, once the portion of sample we need was collected for the analyses, the remaining was put in a heater at 105°C for half a day, to determine the weight loss. This amount gives us the percentage of residual moisture in the freeze-dried samples and permits us to adjust the results with the dilution of water. In the ***Table 40*** there are the averages of the residual weight percentages for grapes after freeze-dry (Grapes-I) and after heating (Grapes-II). The averages are on 9 samples.

Then for the digestion process we took a bigger amount of sample, and used open Teflon vessels. We put 0,5 g of ground and uniformed freeze-dried grapes with 10 mL of HNO_3 (at 60%) and warmed it on a hotplate, adding 2 mL of H_2O_2 (at 30%) little by little. When the volume was reduced to 5 mL we took it off the plate, refilled it with a bit of deionised water, filtered the solution and brought it up to 50 mL.

2.8. WINE

For wine analysis, as other studies have done before (Galani-Nikolakaki et al. 2002 [23]; Vystavna et al., 2013 [57]; Alkış et al., 2014 [4]; Lara et al., 2005 [32]), we worked on the volume of the sample and not on the dry weight residue. The digestion process was similar to the one used by (Vystavna et al. 2013 [57]): we took an open vessel, adding the same amount of HNO_3 and H_2O_2 used for grapes. Starting with 50 mL of wine, we put

them in a 100 mL beaker and warmed it in bain-marie, adding 10 mL of HNO_3 (at 60%) and 2 mL H_2O_2 (at 30%) little by little, because the reaction could be very fierce. We let the solution reduce to 5 mL and then repeated the procedure adopted for grapes.

In *Table 14* the treatments used for analysis of different arrays are summarised.

Table 14. Digestion treatments

	Starting sample	Final solution volume	Reaction		Wash	
			H_2O_2	HNO_3	H_2O_2	HNO_3
Wine	50 mL	50mL	2 mL	10 mL		24h at 10%
Grapes	0,5 g	50mL	2 mL	10 mL	2 mL	6 mL
Other parts of grapevine	0,2 g	50mL	1 mL	6 mL	1 mL	6 mL

Moreover we searched other features of wine: volatile acidity and, just for a descriptive reason, the hydrogen-ion reaction (pH), electrical conductivity and alcoholic content. For hydrogen-ion reaction and electrical conductivity we just immersed the pH-meter and the conductivity-meter in wine. To measure the alcoholic content we distilled wine, then weighed the pycnometer (a glass container with standard volume) filled once with deionised water and another time with distilled wine. The differences between the two densities (related to the temperature) permit us to calculate the alcohol volume percentage.

Lastly we chose to determine the volatile acidity (the content of acetic acid - CH_3COOH) that gives the vinegar taste, because there is an Italian law establishing a limit value for this parameter in wine. To do this, we distilled wine with hot vapour and titrated the solution so obtained with *NaOH* (10 N) and phenolphthalein as indicator: we found the concentration knowing that 1 mL of *NaOH* reacts with 0,006 g of acetic acid.

2.9. EQUIPMENT

Solutions obtained by the extraction of soil with DTPA were analysed by Flame Atomic Absorption Spectrometry (FAAS) or, when concentrations of metals were low, by Graphite Furnace Atomic Absorption spectrometry (GFAAS) with the Perkin Elmer 100 AAnalyst. *Cu, Fe, Mn* and *Zn* were investigated with FAAS, while *Cd, Cr, Pb* and *Ni* with GFAAS. All grapevine parts and wine were analysed in FAAS for *Cu, Fe* and *Zn*, in GFAAS for *Cd* and *Pb* and with Inductive Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) for *Cr, Mn* and *Ni* (*Table 15*).

Table 15. Techniques of analysis for each metal

	DTPA extracts	Grapes, plant parts and wines
Cd	GFAAS	GFAAS
Cr	GFAAS	ICP-AES
Cu	FAAS	FAAS
Fe	FAAS	FAAS
Mn	FAAS	ICP-AES
Ni	GFAAS	ICP-AES
Pb	GFAAS	GFAAS
Zn	FAAS	FAAS

For all the three analysis techniques mentioned above, it has been necessary to build calibration curves with standard solutions, to get the concentrations of our samples back.

We could use for all the analysis a linear curve (of first degree) because the R^2 was at least 0,99.

2.10. DETECTION LIMITS AND BLANKS

A blank is a sample that does not contain analyte. It may be the pure solvent or it may be a complex sample matrix (Hibbert, 2007 [26]). The pure solvent, that we will call 0-solution, is usually used to establish a baseline for the instrument, while a method blank is used to detect errors due to the matrix or to contaminations in preparation and in containers. For the analysis of available metals in soils, wine, grapes and other vegetable parts we created some method blanks, i.e. replications of extractions done on specimens without any sample, to verify if and how much the process affects the results. The results of the samples have been adjusted by the blanks' ones.

Detection limit (DL) is the lowest concentration detectable by the measuring instrument, so different from blank, and it is usually known, because it is an intrinsic characteristic of the instrument. If the manufacturer does not give it or if it depends on many variables, as in FAAS, GFAAS and ICP-AES analyses, we can estimate it. According to the IUPAC recommendation, DL is the mean value of the blank plus three times its standard deviation (Lajunen et Perämäki, 2004 [30]).

2.11. UNCERTAINTY

All measurements, however careful and scientific, are subject to some uncertainties (Taylor, 1997 [50]). The uncertainty is attributable to experimental errors, is the difference between a measurement and the true value or between two measured values, and the errors are described by two diverse variables: the precision and the accuracy.

Precision is a word used to describe the spread of a number of results (Hibbert, 2007 [26]). It can be expressed in two ways (Taylor, 1997 [50]), with the same measure unit of the result (and in the following chapters this kind of error will be mentioned with ER) or as fractional uncertainty or uncertainty ratio (further mentioned with $ER_{r\%}$). It is an indicator of variability of replicate measurements, and can depend on sensitivity of the measuring instrument. If this instrumental parameter is unknown, like in FAAS, GFAAS and ICP-AES, precision can be established on second instance using the standard deviation (sd) (Taylor, 1997 [50]) of replicates on the same, standard sample. In this study, we did not execute many sample replicates, but we have obtained many measures of the 0-solution, so we decided to utilise them for the precision calculation. Indeed they could be considered replicates and 0-solution can always be considered the same, standard sample too. Besides, when we examined replicates of certified values, their standard deviation confirmed to be very similar to the 0-solution one. However we took as ER, three times the standard deviation, to have a greater confidence. Now it is clear that in this study, the DLs and the ERs for available soils, vegetables and wines have the same values, but it does not mean it always happens. The detection limit and the absolute uncertainty express different things, but however uncertainty cannot be obviously higher than DL. Then if the searching value does not come from a direct measure, its error must be obtained from the calculation of propagation of uncertainties of direct measurements. So, if "q" is the result of two direct measures "x" and "y", its absolute uncertainty is the product of "q" with the sum of the fractional uncertainties of "x" and "y" (Taylor, 1997 [50]). We adopted these techniques to obtain ERs of chemical and physical soil analyses.

The second variable that causes uncertainty in measurement is the **accuracy**. It represents the distance of the average of our results from the real value. We cannot know what is the real value but we can rely on the certified materials.

We find the accuracy of our results by measuring certified materials. The certified values are those that an accredited authority give as real. The authority obtains these values by crossing several laboratory results. Accuracy can be expressed in different ways. We

can give it as a percentage of my result on the certified value, and I will mention it as $A_{r\%}$, or as the relative bias $B_{r\%}$, which is the difference between my result and the certified one, divided by the certified value (Van Reeuwijk L.P., 1998 [54]). The accuracy for spikes (replicates of the samples with the addition of a known amount of multi-standard solution) can be expressed as recovery (mentioned as $R_{\%}$), i.e. difference of spiked sample with unspiked one divided by the quantity added. (Van Reeuwijk L.P., 1998 [54]).

For the available metals in soil, the accuracy has been evaluated through the certified material NCSDC85102a, provided by the China National Analysis Centre for Iron and Steel. In the ***Table 36*** there are the certified values of the material for the metals we selected.

For grapes and other plant parts we made use of the certified material IAEA-359, a preparation of Cabbage (Brassica oleracea var. Sabauda) coming from the International Atomic Energy Agency, whose contents of selected metals are reported in ***Table 37***.

Wine is a complex substance, a suspension, and we could not use the IAEA-359, so we chose to prepare some spikes, replicates of the samples with the addition of a known amount of multi-standard solution, like Alkış et al. (2014 [4]) did too. The additional concentrations of the metals we selected are written in the ***Table 38***.

The difference between the spike value and its relating sample enabled us to verify how wine suspension has influence on correctness of the results.

2.12. DISPLAY OF RESULTS

The results concerning the eight metals in plants and in soils as available parts are shown in tables also with the total concentrations of soils and the wines too, for a better comparison. Total concentrations of Cadmium are absent because it is too difficult to obtain with XRF. The total values of iron and manganese elements come from their compounds in the main components. Values of vegetable parts below the detection limit are indicated with a "<". The results in the tables are also shown with diagrams, to have a fast visual comprehension. The values below the detection limit in the diagrams are assumed to be DL/2, as suggested by APAT protocol (APAT, 2006 [7]). In diagrams, available metals in the superficial and deep soils are shown with the average of the two results, because it is assumed that much of the area explored by the root is between these two depths, and the y-axis of concentration is shown with a logarithmic scale.

2.13. EF, BCF, TF

The results of soil and plant concentrations have been processed with the help of some indicators: the Enrichment Factor (EF), the Biological Concentration Factor (BCF) and the Translocation Factor (TF).

The **enrichment factor** represents the increase in a place of concentration of an element from the reference environment background. In many cases, it was used to detect the contribution of anthropogenic emissions (Blaser et al., 2000 [11]). For topsoil would be made by comparison with the deeper horizons (Blaser et al., 2000 [11]; Bourenanne et al., 2010 [13]). Our enrichment factor of an *X* metal is:

$$\mathrm{EF} = \frac{X_{\ topsoil}}{Al_2O_{3\ topsoil}} \Big/ \frac{X_{\ subsoil}}{Al_2O_{3\ subsoil}}$$

The **biological concentration factor** is used to evaluate the accumulation capability of a metal by a plant species and has been defined by the ratio between concentration in plant and in soil (Yoon et al., 2006 [59]). There are studies (Malik et al., 2010 [34]) in which soil concentration is referred to the total contents, but we chose to refer to the available part. The choice came from our thoughts that in this way the factor would better reflect the real accumulation of the plant. Barbafieri (2011 [8]) has the same idea and calculates a bio-concentration factor from the ratio of concentration in roots and on the available part of soil obtained from the sum of aqueous, KNO_3 and EDTA extractions. Our biological concentration factor is:

$$\mathrm{BCF} = C_{roots} / C_{soil\text{-}av\text{-}\bar{x}}$$

where C_{roots} is the roots content and $C_{soil\text{-}av}$ is the average of topsoil and subsoil concentrations from DTPA extraction.

The **translocation factor** expresses the capability of a plant to transfer metals from its lowest parts to the upper ones. It is defined by the ratio of concentration of a metal in leaves and in roots (Yoon et al., 2006 [59]):

$$\mathrm{TF} = C_{leaves}/C_{roots}$$

Plants that present both the biological concentration and the translocation factors over 1 can be considered great accumulators, while plants with BCF>1 and TF<1 are good phytostabilisers (Pandey et al., 2015 [39]).

3. RESULTS

3.1. SOILS

In *Table 16* there are the chemical and physical analyses of soil results.

Table 16. Chemical and physical characteristics of soils

	MA1 >60	AB1 0-10		DZ3 0-10	NG3 0-10	ER	
Reaction	8,37	7,51		7,23	7,52	0,01	PH
EC 1:2,5 at 25°C	0,124	0,262		0,232	0,195	0,001	dS/m
	MA1 0-10	**AB1 0-10**	**MF2 0-10**	**DZ3 0-10**	**NG3 0-10**	**ER**	
Sand 2000-50 µm	33,4%	15,1%	6,1%	25,2%	68,2%	3,4%	%
Silt 50-2 µm	49,5%	64,0%	60,5%	35,9%	23,2%	3,4%	%
Clay < 2 µm	17,2%	20,9%	33,3%	38,9%	8,6%	3,4%	%
Texture USDA	**SiL**	**SiL**	**SiCL**	**CL**	**SL**		
Total carbonate	245	209	61	104	154	4	g/kg
Estimated dolomite	15	7	0	0	0	4	g/kg
Active carbonate	65	94	28	28	21	1	g/kg
Active/Total carbonate	27%	45%	45%	27%	14%	2%	%
Fe by NH_4-oxalate	54	29	62	52	109	5	mg/kg
IDCI	**22**	**113**	**7**	**10**	**2**		
Org. C	13	15	23	13	12	2	g/kg
Tot. N	1,5	1,9	2,9	1,6	1,3	0,4	g/kg
NH_3-N	0,01	0,02	0,18	0,05	0,01	0,005	g/kg
Org. C/Tot. N	**8,5**	**8,1**	**7,8**	**7,9**	**9,1**		
CEC	13,9	16,1	28,2	18,6	10,1	0,17	cmol(+)/kg
Ca	12,5	14,4	25,0	14,6	9,1	0,2	cmol(+)/kg
Mg	0,8	1,1	2,4	3,0	0,5	0,005	cmol(+)/kg
K	0,7	0,6	0,8	0,8	0,4	0,02	cmol(+)/kg
Na	0,02	0,01	0,06	0,15	0,04	0,005	cmol(+)/kg
Avail.-P	16	6	32	16	30	3,5	mg/kg
Avail.-K	264	223	325	327	162	2	mg/kg

Soil pH goes from moderately alkaline to slightly alkaline (USDA,), due to the carbonate substratum. The Electrical Conductivity shows that the level of soluble salts are good because they have values lower than 0,4 dS/m (Barbiroli et al., 2000 [9]). In AB and

MA silt is more abundant, MF and DZ have a relevant presence of clay and NG confirms to be sandy, as we already clearly felt to the touch.

Calcium carbonate is present in all the vineyards soils, from slightly to moderate on hills of MA and AB, where there are also traces of dolomite.

The active carbonate interacts negatively with the solubility of macronutrients (*P, S*) and of some micronutrients (Barbiroli et al., 2000 [9]), so in MF and AB, where it represents a significant percentage of total carbonate, plant uptake problems may occur. The Iron Deficiency Chlorosis Index (IDCI) (Juste and Poget, 1972 [27]) confirms this risk: in AB it has a very high value (>100) that could explain the many yellow leaves. In the other vineyards, IDCI should not mean problems, even if it must be monitored in MA and DZ because its values are >10, in general in the high region of the warning range.

The organic carbon content is in line with the typical agricultural soils of the area, indicated by the regional soil map of the Regione Emilia-Romagna (2015 [42]), however, in MF it is maybe too high (0,23%) for a vineyard. In NG, soil is unique, it is a sandy loam, so is subject to more mineralization processes and the good organic carbon content is explainable by amendment. Total nitrogen follows the trend of organic carbon. The ratio *C/N* is always below 10, the optimal ratio of a stable and well humified organic matter in the soil. This means an excess of total *N* available for bacteria which increase the mineralization of organic matter (Ge et al., 2013 [24]; Barbiroli et al., 2000 [9]). Nitrogen is abundant for both bacteria and plants in mineral form (ammonia and nitrate), but is likely to be lost by leaching (Vigil et Kissel, 1991 [55]).

According to a qualitative evaluation of Barbiroli et al. (2000 [9]) for this type of agricultural soils, CEC goes to middle-low values in NG to high values in MF where the organic carbon and the fine grain content are greater and can affect the cation exchange capacity. Due to the calcareous nature of the soils, cation exchange capacity is mainly formed by *Ca*, followed by *Mg* and *K*, with only traces of sodium. Differences between available phosphorus levels of the vineyards are great, because they depend mainly on the fertilisation adopted. Available potassium for this kind of agricultural soil (Barbiroli et al., 2000 [9]) is good everywhere and soil does not need to be fertilised.

The bulk chemical composition of the sampled soils is presented in ***Table 17***.

Table 17. Bulk chemical composition of vineyard soils

Soils	MA1		MA2		AB1		AB2		MF1		MF3		DZ1		DZ3		NG2		NG3		DL	
	0-10	>60	0-10	>60	0-10	>60	0-10	>60	0-10	>60	0-10	>60	0-10	>60	0-10	>60	0-10	>60	0-10	>60		
SiO2	39,06	30,55	53,49	60,60	41,99	36,66	39,91	58,57	52,37	52,73	49,14	47,84	53,35	50,27	52,83	49,86	53,30	52,76	51,03	52,69		
LOI	19,88	23,22	12,22	7,11	19,13	22,05	19,95	7,96	14,96	13,01	15,93	17,21	13,25	13,78	13,00	13,93	11,45	14,52	11,45	13,10		
CaO	18,98	29,35	5,11	2,27	14,67	18,46	16,55	2,33	6,40	7,48	7,45	8,56	8,72	11,64	8,83	12,14	15,17	16,63	17,24	16,37		
Al_2O_3	10,64	7,72	15,05	16,30	11,30	10,66	11,10	16,29	13,14	13,37	13,24	12,99	12,14	12,19	12,56	12,08	9,37	7,77	9,51	8,53		
Fe_2O_3	3,73	2,53	5,90	5,91	3,99	3,98	4,16	6,41	5,27	5,27	5,70	5,43	4,30	4,30	4,46	4,13	2,93	1,85	2,89	2,29		
MgO	3,86	3,73	3,49	3,17	4,79	4,52	4,44	3,85	3,67	4,14	4,41	4,17	3,97	3,91	4,01	3,92	3,50	2,66	3,61	3,05	0,01	g/hg (%)
K_2O	2,25	1,59	2,73	2,50	2,57	2,43	2,46	2,70	2,37	2,24	2,47	2,26	2,29	2,15	2,38	2,20	2,26	2,06	2,27	2,09		
Na_2O	0,75	0,69	0,89	1,08	0,78	0,54	0,63	0,96	0,81	0,83	0,67	0,63	1,02	0,92	0,96	0,93	1,32	1,38	1,29	1,37		
TiO_2	0,49	0,37	0,69	0,79	0,50	0,47	0,49	0,72	0,64	0,65	0,65	0,64	0,58	0,58	0,60	0,56	0,39	0,24	0,38	0,32		
P_2O_5	0,21	0,10	0,26	0,09	0,18	0,13	0,22	0,10	0,24	0,16	0,23	0,15	0,26	0,15	0,24	0,14	0,22	0,08	0,22	0,09		
MnO	0,15	0,14	0,16	0,17	0,10	0,09	0,10	0,12	0,13	0,12	0,13	0,12	0,13	0,11	0,13	0,11	0,09	0,06	0,10	0,08		
Ce	43	39	61	69	37	55	43	65	50	59	66	68	45	53	47	48	31	27	40	30		
La	28	22	42	42	26	32	31	43	36	38	32	43	28	27	31	35	22	10	16	11		
Nd	23	14	29	30	27	21	25	30	24	24	31	30	21	24	26	26	14	10	13	15		
Sc	7	3	10	8	9	9	10	11	13	13	15	4	9	11	8	8	8	12	9	10	2	mg/kg (ppm)
Sm	3,6	2,9	4,1	4,0	4,0	3,9	3,8	4,6	4,1	4,1	4,3	4,5	3,5	3,7	3,6	3,7	3,1	2,7	3,0	2,7		
Y	26	19	31	32	25	23	22	32	26	27	27	27	24	24	25	24	18	13	16	16		
As	9	7	10	9	9	10	9	11	9	9	9	9	11	8	10	8	9	8	7	7		
Ba	354	280	450	439	375	371	336	471	389	395	382	390	387	366	393	358	371	296	329	296		
Cl	49	63	32	44	36	57	39	45	31	50	20	43	37	40	45	40	41	41	31	45		
Co	9	5	17	18	12	12	12	19	17	16	18	17	12	12	12	13	7	5	7	6		
Cr	123	85	209	208	150	163	158	234	508	107	228	102	163	167	173	165	108	67	106	76		
Cs	7,5	2,1	8,0	8,5	3,8	4,1	7,7	9,7	8,4	4,9	7,3	4,4	5,9	5,7	9,2	2,8	6,3	4,3	7,6	<		
Cu	172	17	138	20	76	32	59	26	221	77	277	128	168	34	137	33	216	48	193	30		
Ga	12	9	16	16	15	15	15	19	15	16	17	17	13	14	14	14	10	9	10	10		
Hf	7,0	4,4	5,9	5,1	3,1	2,1	4,0	4,0	4,7	4,1	5,5	3,5	3,5	3,8	8,1	2,8	5,5	2,1	3,9	2,6		
Nb	11	9	13	14	12	12	12	14	13	13	14	15	11	12	11	12	9	7	8	8		
Ni	43	27	70	67	55	61	55	87	64	66	71	71	51	53	53	52	33	24	31	27		
Pb	18	11	24	22	19	23	20	25	26	27	25	23	25	18	24	20	31	20	21	17	2	mg/kg (ppm)
Rb	67	42	107	112	88	84	85	116	103	100	108	105	90	82	97	82	74	65	69	66		
S	300	140	250	70	320	110	290	60	320	220	312	190	250	140	230	140	340	130	320	130		
Sn	8,3	<	7,1	6,0	2,8	7,4	6,0	6,6	6,4	5,8	5,5	8,0	5,5	4,7	2,0	<	4,7	4,4	3,8	2,3		
Sr	243	262	133	112	268	383	295	132	168	188	191	224	210	232	206	237	263	291	271	289		
Ta	3,5	3,0	3,9	3,2	3,6	3,4	3,4	3,8	3,6	3,4	3,7	3,6	3,9	3,4	3,5	3,3	3,7	3,2	3,8	3,7		
U	2,0	2,0	2,0	2,0	3,0	3,0	2,2	2,3	2,0	2,1	2,8	2,9	2,1	2,1	<	2,0	<	<	<	<		
V	69	51	89	88	82	91	85	102	83	87	100	102	67	77	74	77	44	33	43	39		
W	2,0	<	<	<	2,1	<	1,9	2,7	2,5	<	2,7	2,3	2,1	<	<	<	<	<	<	<		
Zn	78	42	99	70	71	79	73	81	115	90	127	103	128	74	123	74	99	35	90	38		
Zr	144	114	174	206	132	116	120	163	154	156	137	140	154	160	160	159	120	87	103	104		

Major components (*SiO_2, Al_2O_3, Fe_2O_3* and *CaO*) in soil show relevant heterogeneity on hills. Percentages of MA1 are different from MA2, while concentration in the superficial sample of AB1 is similar to the deep sample and superficial sample of AB2 too,

but deep values of AB2 are different. On the plain there is more homogeneity, due to similarity in sedimentary process and particle origins. Differences on hills are probably due to the intensive action of weathering. In MA1, AB1 and upper AB2, levels of *CaO* and little silica (SiO_2) are in accordance with calcareous-marlstone characteristics and the LOI is reasonable too. MA2 and deeper AB2 have high levels of Al_2O_3 that are compatible with argillaceous-marlstone, but the high level of silica with low *CaO* and LOI refer to the standard marl-sandstone, maybe with a decarbonating process. The sandy soil of NG shows, in comparison with MF and DZ, lower levels of alumina and iron oxide, because they are usually linked to clay.

In the other major components (*MgO,* K_2O, Na_2O, TiO_2, P_2O_5 and *MnO*) we cannot see great differences between the sites.

MF1 and MF3 are the sites where the highest values of a series of elements are found, *Cr* in the first instance, but also *Cu*, *Ni*, *V* and *Zn*.

Ce presents heterogeneous values both between locations and within sites, however in NG we have the lowest values, while in MF and MA the highest values are recorded, similar to the average concentration in the earth's crust (60 ppm) (Kabata-Pendias, 2011 [28]).

The concentrations of *Cr* almost everywhere are over the 150 ppm so we should effect an aqua regia extraction to confirm that they do not exceed the limit of the D.Lgs. 152/06. In depth values are certainly variable but seem to be slightly lower, so probably there is a diffuse anthropogenic supply.

In all the sites *Cu* shows higher values in the highest layer than in depth. The sites have very variable concentrations, both in the highest layer and in depth (maximum in MF and minimum in AB). However in the same site, at the same depth, the concentrations are similar.

La in the earth's crust has a concentration of 30 ppm (Kabata-Pendias, 2011 [28]) and most of the vineyards have the same level, but in NG it is lower, it is closer to the Pineta S.Vitale one (Cidu et al, 2013 [15]).

There are appreciable differences of *Ni* values between the sites, but not inside too. Concentrations in lowlands are similar in the two sampling points at the two depths, on the contrary in hilly sites there is more variability. This reflects the differences in substrate inside the sites. In particular, the presence of *Ni* is greater where percentages of silica, alumina and iron oxides are greater, and percentages of oxides of *Ca* are lower, in other

words where it is assumed that the substrate is a marl-clay, since the *Ni* is more concentrated in shales (Alloway, 2013 [5]). Overall, values are similar to those observed in the soils of the area (Menichetti, 2014 [36]).

The values of *Pb* do not show significant differences between the sites.

The *S* is more abundant in surface horizons presumably due to treatments with copper sulfate used against mildew. On hills, there are differences in subsoils, probably because of the heterogeneous substrate.

All values of *Sn* are above 1 ppm, the limit of the D.Lgs. 152/06, but it is referred to an aqua regia extraction and it is was a mistake (Caridei, 2013 [14]) removed with the law n. 116 of the 11/08/2014.

For *V* there aren't differences between the upper and deep horizons. NG has the lowest values.

NG has low values of *Y*, like those of Cidu et al. (2013 [15]) while the others are superior to the average value of the earth's crust (33 ppm) (Kabata-Pendias, 2011 [28]).

3.2. WINES

The results of the wines analyses are in ***Table 18***.

Table 18. Characteristics of the wines

Wine	AB	DZ	NG		ER
Alcohol	10,26	10,37	10,46	%(V/V)	0,01
pH	3,54	4,05	3,44	pH	0,01
EC at 25°C	2,315	2,496	1,766	ds/m	0,001
CH_3COOH	1,86	0,61	1,33	g/L	0,02
Cd	0,0007	0,0011	0,0022	mg/L	0,0028
Cr	0,016	0,011	0,009		0,004
Cu	0,044	0,073	0,174		0,024
Fe	0,94	5,12	1,16		0,160
Mn	0,442	0,897	0,381		0,027
Ni	0,018	0,049	0,030		0,005
Pb	0,0060	0,0831	0,0484		0,0004
Zn	0,14	2,09	12,20		0,04

NG doesn't respect the Italian Regulation limit of Zn concentration in wine: it's value is more than double. *Pb* and *Cu* values are far below the limits. AB and NG exceed the limits for white wines of volatile acidity, but it could be due to the bad conservation, because wine produces acetic acid on contact with the air.

3.3. VEGETABLES AND RELATED RESULTS

For a better comprehension and comparison, in the following chapters there are the diagrams of concentrations in vegetable parts with the average of available metals in topsoil and subsoil. In the same chapters, tables report the numeric results. There are also total contents of the metals we investigated and the results from the wine.

CADMIUM

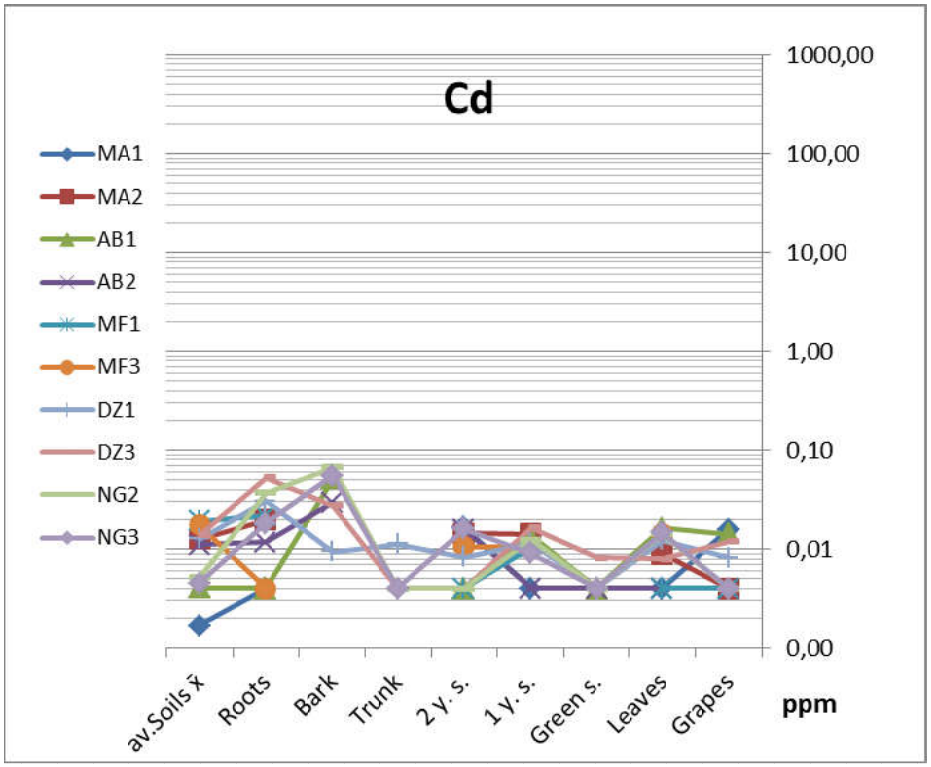

Figure 15. Diagram of Cadmium available in soils and present in plant parts

Table 19. Cadmium present in wines, in plant parts and available and total fractions in soils

Cd			MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2/4*	NG3/5*	DL
mg/L	**Wine**		-		0,0007		-		0,0011		0,0022		**0,0028**
mg/kg	**Grapes***		0,016	<	0,014	<	<	-	0,008	0,012	<	<	**0,008**
	Leaves		<	0,009	0,016	<	<	0,014	0,012	0,008	0,014	0,015	
	0 y. s.		-	-	<	<	-	-	<	0,008	<	<	
	1 y. s.		<	0,014	0,014	<	0,01	0,011	0,011	0,017	0,012	0,009	
	2 y. s.		0,016	0,014	<	0,016	<	0,01	0,008	<	<	0,016	
	Trunk		-	-	-	-	-	-	0,011	<	<	<	
	Bark		-	-	0,051	0,03	-	-	0,009	0,028	0,067	0,056	
	Roots		<	0,02	<	0,012	0,022	<	0,031	0,053	0,037	0,018	
	av.Soil	**0-10**	0,033	0,038	0,038	0,062	0,06	0,04	0,07	0,08	0,034	0,037	**0,001**
		>60	0,003	0,025	0,008	0,022	0,038	0,035	0,025	0,028	0,01	0,009	
	Soil	**0-10**	-	-	-	-	-	-	-	-	-	-	**-**
		>60	-	-	-	-	-	-	-	-	-	-	

Looking at *Table 19* and *Figure 15* we see that available *Cd* is higher in superficial than in deep soil, probably due to human activity. In vegetables *Cd* is more abundant in bark and in roots, while in the rest of the plant it is scarce.

CHROMIUM

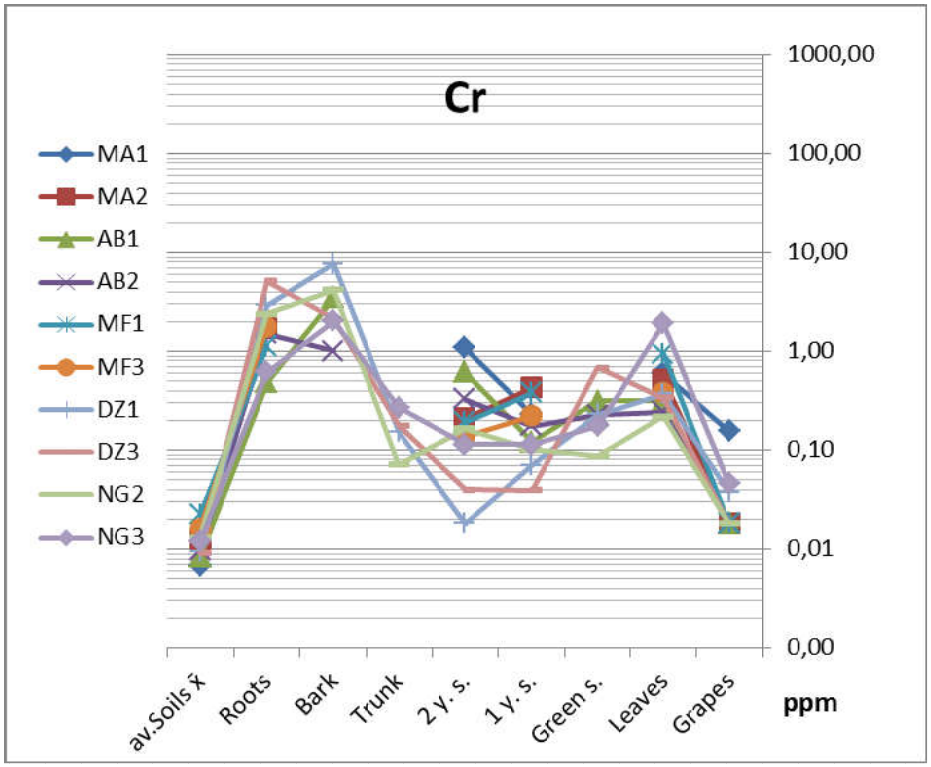

Figure 16. Diagram of Chromium available in soils and present in plant parts

Table 20. Chromium present in wines, in plant parts and available and total fractions in soils

Cr			MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2/4*	NG3/5*	DL
mg/L	**Wine**		-		0,016		-		0,011		0,009		**0,004**
mg/Kg	**Grapes***		0,155	<	<	<	<	-	0,038	<	<	0,045	**0,036**
	Leaves		0,598	0,526	0,316	0,242	0,949	0,383	0,368	0,336	0,219	1,926	
	0 y. s.		-	-	0,313	0,225	-	-	0,230	0,684	0,087	0,176	
	1 y. s.		0,229	0,433	0,121	0,176	0,380	0,224	0,070	0,039	0,100	0,114	
	2 y. s.		1,084	0,208	0,618	0,328	0,191	0,135	<	0,040	0,165	0,114	
	Trunk		-	-	-	-	-	-	0,153	0,178	0,072	0,271	
	Bark		-	-	3,484	1,000	-	-	7,740	2,143	4,165	2,040	
	Roots		1,952	1,733	0,486	1,502	1,142	1,774	2,912	5,164	2,395	0,615	
	av.Soil	**0-10**	0,008	0,010	0,010	0,011	0,029	0,021	0,008	0,008	0,019	0,018	**0,002**
		>60	0,006	0,016	0,007	0,009	0,015	0,011	0,010	0,011	0,008	0,006	
	Soil	**0-10**	123	209	150	158	508	228	163	173	108	106	**2**
		>60	85	208	163	234	107	102	167	165	67	76	

Looking at *Table 20* and *Figure 16* we see that in depth values are certainly variable but seem to be slightly lower. Indeed we observe in *Table 21* that in some sites a superficial

enrichment (EF > 2), especially in MF where this may be due to a point-like anthropogenic contamination.

Table 21. Enrichment factor of Chromium in soils

Cr	MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2	NG3
EF	1,4	1	0,9	0,7	4,8	2,2	1	1	1,6	1,4

Although MF registers a superficial enrichment, its available part of *Cr* is similar to that found in other sites. Compared with other analyses (Negro, 2013 [38]) on agricultural soils of the area of Apennine origin and vineyards of other Italian regions (Piemonte, Toscana and Emilia) (Dimartino, 2015 [18]), the average of available and total ratios (%) are a little bit higher.

From EF it seems that in AB and DZ, *Cr* hasn't anthropogenic origin, but the ratios to the Al_2O_3 (displayed in ***Table 22***) are > 11,5 so higher than the average concentration of these types of sediments (Amorosi et Sammartino, 2007 [6]). Further investigation would be necessary.

Table 22. Ratio of Chromium and Aluminium oxide in subsoils

Cr / Al_2O_3	MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2	NG3
>60	11,0	12,8	15,3	14,4	8,0	7,8	13,7	13,7	8,7	8,9

Chromium is an essential element, so plants uptake it and the high BCF (displayed in ***Table 23***) on available metals confirms that. The highest contents are in roots and in the bark.

Table 23. Bio-concentration factor of Chromium in vineyards

Cr	MA1	MA2	AB1	AB2	MF2	MF3	DZ1	DZ3	NG2	NG3
av.BCF	283,6	136,4	58,5	153,7	51,4	111,4	313,3	561,8	181,0	52,0

COPPER

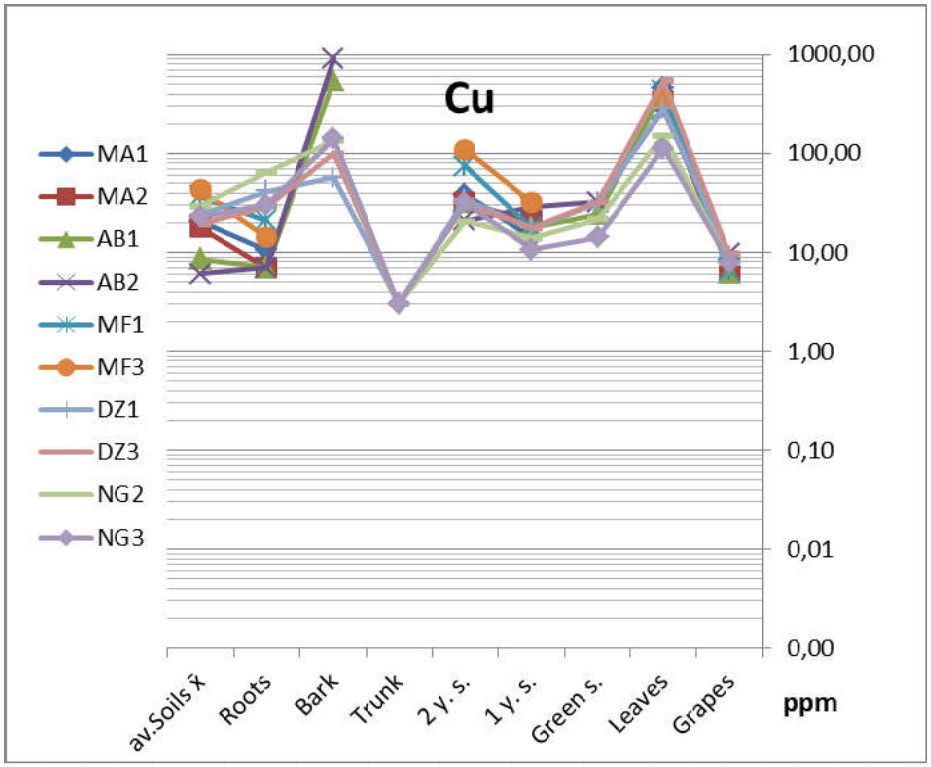

Figure 17. Diagram of Copper available in soils and present in plant parts

Table 24. Copper present in wines, in plant parts and available and total fractions in soils

Cu			MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2/4*	NG3/5*	DL
mg/L	**Wine**		-		0,044		-		0,073		0,174		**0,024**
mg/kg	**Grapes***		8,2	6,6	6,2	9,6	6,5	-	9,3	9,6	7,9	7,9	**6,0**
	Leaves		458,2	336,9	442,7	277,4	438,9	357,9	288,9	541,4	153,1	113,6	
	0 y. s.		-	-	24,5	32,1	-	-	32,1	32,2	21,5	14,1	
	1 y. s.		13,9	20,9	17,8	28,7	17,5	31,8	17,5	17,7	13,9	10,7	
	2 y. s.		38,6	31,7	31,6	20,8	74,6	110,7	34,5	31,0	20,7	31,8	
	Trunk		-	-	-	-	-	-	<	<	<	<	
	Bark		-	-	550,8	913,9	-	-	57,0	100,2	136,0	141,5	
	Roots		10,5	6,9	7,1	7,1	20,8	14,1	42,0	29,3	64,0	30,6	
	Av.Soil	**0-10**	40,93	35,07	16,27	11,60	59,96	60,86	44,57	35,07	49,53	41,86	**0,08**
		>60	0,43	1,20	0,75	0,61	13,49	25,11	3,41	3,96	9,98	4,23	
	Soil	**0-10**	172	138	76	59	221	277	168	137	216	193	**2**
		>60	17	20	32	26	77	128	34	33	48	30	

Looking at *Table 24* and *Figure 17* we see that the available *Cu* is really more abundant in the superficial layer than in depth. This can be seen by pure available values and by the ratios with total concentrations (displayed in *Table 25*).

Table 25. Percentage of available copper in topsoils

Cu av. %	MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2	NG3
0-10	23,84	25,43	21,35	19,55	27,17	22,0	26,55	25,6	22,95	21,67

In the superficial layer we can observe that these ratios are almost the same (20-27%) and are not affected by total concentration. The fact indicates that a substantial part of the *Cu* is present in its mineral form, not crystallized, which has an anthropogenic origin and can be easily attacked by the acids of roots. The available *Cu* in lands where there are grapevine plantations is one order of magnitude higher than in other cultivations (Toselli et al., 2009 [51]).

In plants there is a higher concentration of *Cu* in the leaves and bark: in the leaves variability is more limited while in bark it is linked to the site. Trunks and grapes have the lower concentrations while other parts have intermediate ones. Shoot values seem to depend on the site they belong to. Despite bark being a highly heterogeneous matrix, it allows us to appreciate the differences that exist between the sites. In AB the concentration of *Cu* in bark is significantly higher (of an order of magnitude) than in DZ and NG. In the leaves NG presents lower values than all other sites. In roots there are differences between sites but also within the same site, with the exception of AB, and generally in the hills concentrations are lower than in lowland. In AB, low root concentration corresponds to the low value in superficial soil of available *Cu*, but this doesn't happen in the other sites.

In NG and DZ the bio-concentration factor (BCF) with the average of the two depths, is < 1 if calculated on the total, while it is > 1 if it is calculated on the bio-available (as in ***Table 26***). Compared with the available concentrations in soils and roots, bark of AB has the largest accumulation.

Table 26. Bio-concentration factor of Copper in vineyards

Cu	MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2	NG3
av.BCF	0,51	0,38	0,83	1,16	0,57	0,33	1,75	1,50	2,15	1,33

Values of *Cu* in all the wines are below the limits set by Italian law and those recommended by the OIV. NG is the site that has highest values in wine and in roots too.

The Translocation Factor (shown in ***Table 27***) is high on hill and organic vineyards, while in DZ and NG it is low.

Table 27. Translocation factor of Copper

Cu	MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2	NG3
TF	43,8	49,0	62,7	39,3	21,1	25,3	6,9	18,5	2,4	3,7

IRON

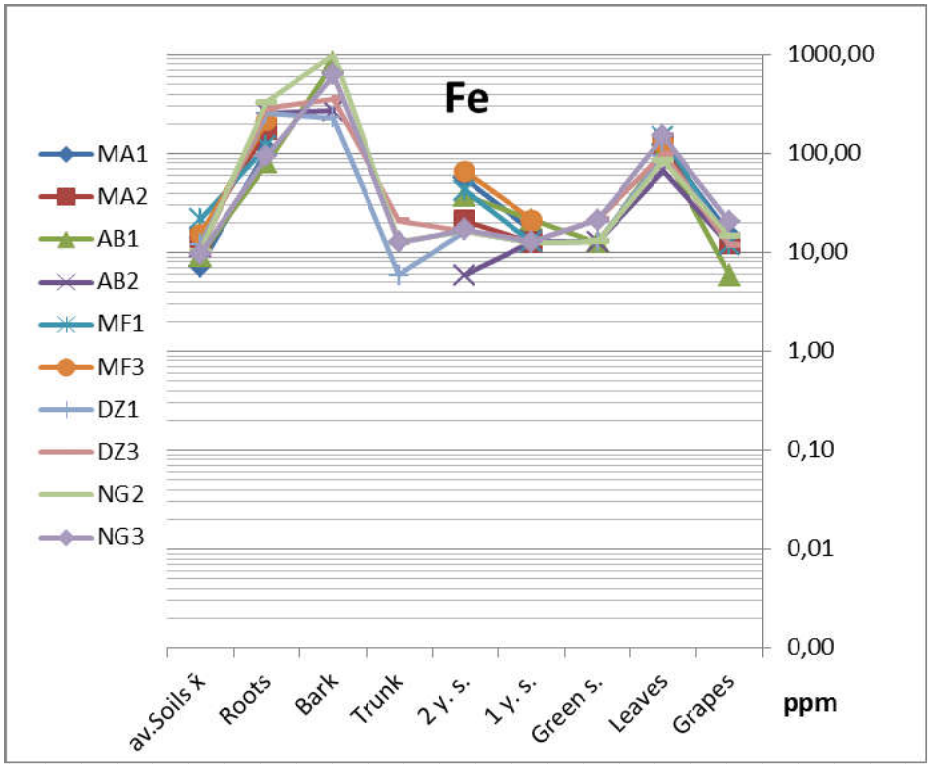

Figure 18. Diagram of Iron available in soils and present in plant parts

Table 28. Iron present in wines, in plant parts and available and total fractions in soils

Fe			MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2/4*	NG3/5*	DL
mg/L	**Wine**		-		0,94		-		5,12		1,16		**0,16**
mg/Kg	**Grapes***		15,3	12,2	<	11,9	12,1	-	14,3	11,8	14,6	20,5	**11,7**
	Leaves		121,8	127,0	103,3	67,7	146,3	129,5	94,6	101,1	85,9	149,7	
	0 y. s.		-	-	12,6	12,9	-	-	12,8	21,5	12,9	21,2	
	1 y. s.		16,6	12,5	21,4	12,9	12,6	21,2	12,6	12,7	12,5	12,8	
	2 y. s.		56,2	21,0	37,7	<	42,4	66,1	16,5	16,5	16,5	16,9	
	Trunk		-	-	-	-	-	-	<	20,9	12,8	12,5	
	Bark		-	-	844,4	268,6	-	-	230,6	360,3	1001,9	637,5	
	Roots		112,5	176,8	80,1	256,9	120,1	215,1	254,8	287,8	331,7	94,3	
	Av.Soil	**0-10**	9,7	15,9	12,3	12,2	14,4	12,6	10,2	9,2	12,7	11,9	**0,4**
		>60	4,8	12,6	6,1	10,1	28,8	17,4	13,5	11,0	7,6	7,2	
	Soil	**0-10**	26061	41262	27911	29100	36852	39832	30065	31197	20493	20192	**2**
		>60	17726	41339	27835	44851	36838	37982	30090	28901	12943	16042	

Looking at *Table 28* and *Figure 18* we see that, in general terms, the concentrations are similar to those already found in soils not contaminated of the same area (Menichetti, 2014 [36]). The concentrations of available Fe are highly variable between sites and within

them, especially in the deep horizons where MF reaches the highest concentration. However those values are within the range observed in other areas of vineyard soils even outside Italy, as indicated by Fabani et al. (2009 [20]) and Yagmur et al. (2014 [58]). The percentages of available *Fe* on total quantities have a greater homogeneity in high horizons, while in depth there is a greater difference between minimum and maximum values. High content of available *Fe* does of course not mean high percentage quantity on the total *Fe*.

According to Sbaraglia et Lucci (1994 [47]) and Lindsay et Norwell (1978 [33]) a critical minimum of *Fe* is indicated to 4,5 mg/kg for some plants and it is observed that the concentration here is higher for all. However in the MF we observe the highest values with a gradient increasing in depth, and this is strange, it is in contrast with most of the other sites.

In vegetable parts the greatest concentration is observed in bark, in second instance in the roots and in the third instance in the leaves, while in the other plant parts we have lower values (of one order of magnitude). The two points of DZ have similar trends between the parts while those of NG and AB have a great variability.

Roots show significant differences between individual samples in the site (except DZ and MA) making it difficult to make comparisons between sites. Values seem at random but we can notice that the lowest concentration occurs in AB1 where the soil has the highest IDCI (iron deficiency chlorosis index).

Iron seems to have an inverse correlation concerning roots and leaves (as displayed in ***Table 29***).

Table 29. Iron in roots, in leaves and the correlation

Fe (ppm)	MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2	NG3
Roots	113	177	80	257	120	215	255	288	332	94
Leaves	122	127	103	68	146	129	95	101	86	150
Correlation	**-0,68**									

The translocation factor (displayed in ***Table 30***) is variable, but in general low.

Table 30. Translocation factor of Iron

Fe	MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2	NG3
TF	1,08	0,72	1,29	0,26	1,22	0,60	0,37	0,35	0,26	1,59

MANGANESE

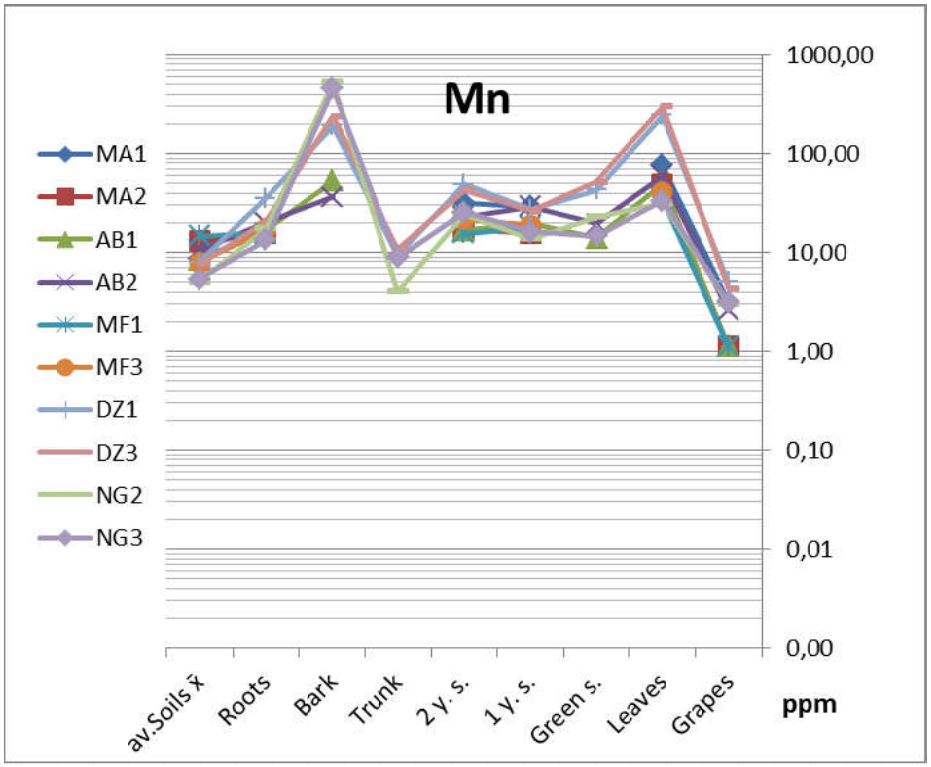

Figure 19. Diagram of Manganese available in soils and present in plant parts

Table 31. Manganese present in wines, in plant parts and available and total fractions in soils

Mn			MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2/4*	NG3/5*	DL
mg/L	**Wine**		-		0,44		-		0,90		0,38		**0,03**
mg/kg	**Grapes***		3,13	<	<	2,59	<		4,97	4,38	3,05	3,14	**2,25**
	Leaves		76,08	49,03	49,78	59,79	35,01	41,13	244,61	300,29	31,18	33,48	
	0 y. s.				14,07	19,56			42,99	51,89	22,96	14,70	
	1 y. s.		29,09	15,82	19,35	28,92	17,98	18,88	26,87	26,23	13,78	16,19	
	2 y. s.		31,85	16,90	16,77	22,78	15,57	21,73	48,98	43,22	24,02	25,60	
	Trunk								9,32	10,62	4,03	8,78	
	Bark				53,25	36,31			190,88	239,23	526,62	453,98	
	Roots		15,80	15,48	16,75	20,04	15,85	17,10	35,17	20,89	17,44	13,45	
	av.Soil	**0-10**	12,51	14,42	12,23	12,08	12,66	8,10	8,10	8,47	5,93	6,42	**0,05**
		>60	4,77	11,43	4,48	9,01	16,60	7,76	7,69	6,73	4,23	4,31	
	Soil	**0-10**	1181	1249	809	809	1006	991	970	970	735	743	**77,4**
		>60	1115	1329	718	894	942	908	824	828	470	646	

Looking at *Table 31* and *Figure 19* we see that, in terms of total contents of *Mn* we cannot notice any difference in relation to different mineralogical compositions. This also happens for other elements, such as *Fe*, to which *Mn* is often associated.

The available *Mn* is placed in the optimal range (Barbiroli et al., 2000 [9]. There is a greater variability in depth than in the superficial layer, both between different sites and within sites. The available concentrations are usually higher in the superficial layer than in depth, especially in the hills. Compared to Yagmur et al. (2014 [58]) we have on average lower available values. AB has only a little *Mn* in all plant parts, even in the bark.

NG in the bark has high concentrations and DZ has intermediate results, but DZ in leaves has values definitely higher than the other vineyards.

The BCF and the TF show that grapevine is an accumulator for *Mn*, but in grapes there are low values.

NICKEL

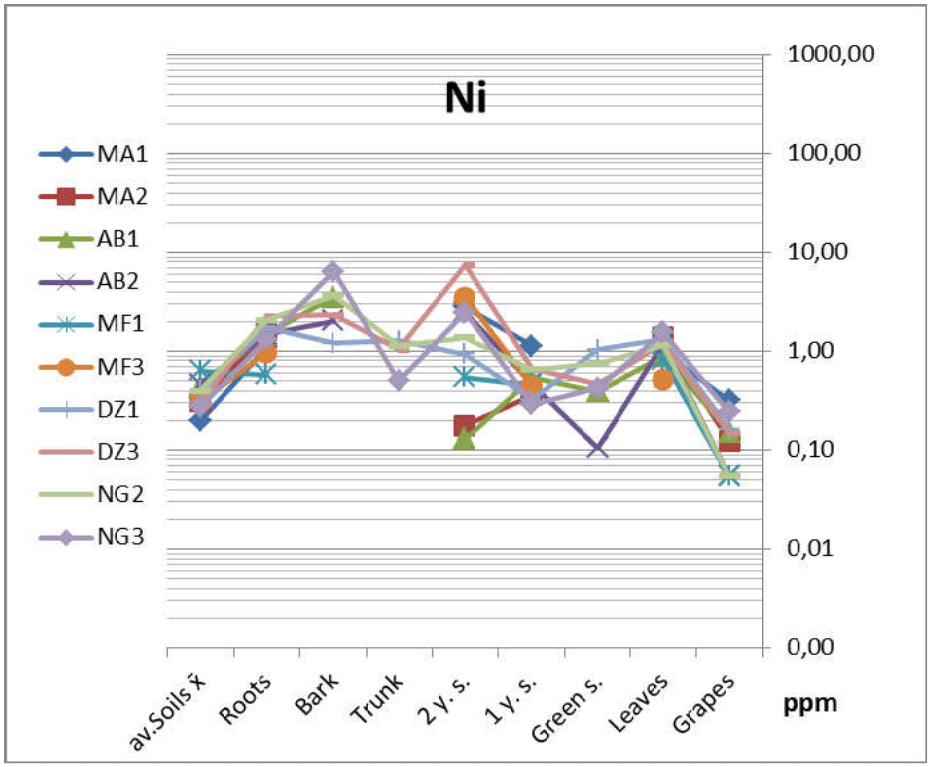

Figure 20. Diagram of Nickel available in soils and present in plant parts

Table 32. Nickel present in wines, in plant parts and available and total fractions in soils

Ni			MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2/4*	NG3/5*	DL
mg/L	**Wine**		-		0,018		-		0,049		0,030		**0,005**
Mg/Kg	**Grapes***		0,319	0,124	0,153	<	<	-	0,163	0,150	<	0,243	**0,110**
	Leaves		0,984	1,378	0,895	1,207	0,819	0,521	1,309	1,210	1,152	1,560	
	0 y. s.		-	-	0,394	0,106	-	-	1,042	0,463	0,742	0,426	
	1 y. s.		1,128	0,361	0,537	0,469	0,456	0,445	0,322	0,663	0,657	0,291	
	2 y. s.		2,814	0,177	0,130	2,513	0,548	3,560	0,932	7,513	1,383	2,463	
	Trunk		-	-	-	-	-	-	1,280	1,034	1,143	0,502	
	Bark		-	-	3,530	2,047	-	-	1,200	2,319	3,712	6,479	
	Roots		1,097	1,601	1,490	1,479	0,583	0,975	1,759	2,249	2,053	1,309	
	Av.Soil	**0-10**	0,308	0,480	0,726	0,721	0,631	0,376	0,322	0,316	0,624	0,355	**0,002**
		>60	0,087	0,140	0,148	0,203	0,633	0,244	0,469	0,283	0,170	0,192	
	Soil	**0-10**	43	70	55	55	64	71	51	53	33	31	**2**
		>60	27	67	61	87	66	71	53	52	24	27	

Looking at *Table 32* and *Figure 20* we see that the available *Ni* is on average higher in the high horizons than in depth except in DZ. In MA and AB (hill sites) there are the greatest differences according to the depth. The values are generally similar to those

observed in soils from marl and sandstone formations (Menichetti, 2014 [36]) or from Tuscany and Piedmont vineyards (Dimartino, 2015 [18]) and are slightly lower than those from other Romagna ones. The values are however much lower than toxicity levels (20 ppm, Barbiroli et al., 2000 [9]) and than those in vineyards outside Italy (Fabani et al., 2009 [20]). The percentage available on total *Ni* is within the same range as those found in soils of a non-contaminated zone (Menichetti, 2014 [36]).

Ni is the metal with the most interesting behaviour in vegetables. The highest value registered is not in bark but in a 2 year old shoot, where for other metals we usually have low levels. Also trunks, that usually have the lowest values, here have concentrations similar to young shoots.

In roots the concentration is higher than the available part in soil: this means there is bio-concentration.

Leaves, that usually are one part of the plant with the highest concentration, here have intermediate values. The translocation factor (displayed in ***Table 33***) is low.

Table 33. Translocation factor of Nickel

Ni	MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2	NG3
TF	0,8	0,9	0,6	0,8	1,4	0,5	0,7	0,5	0,6	1,2

LEAD

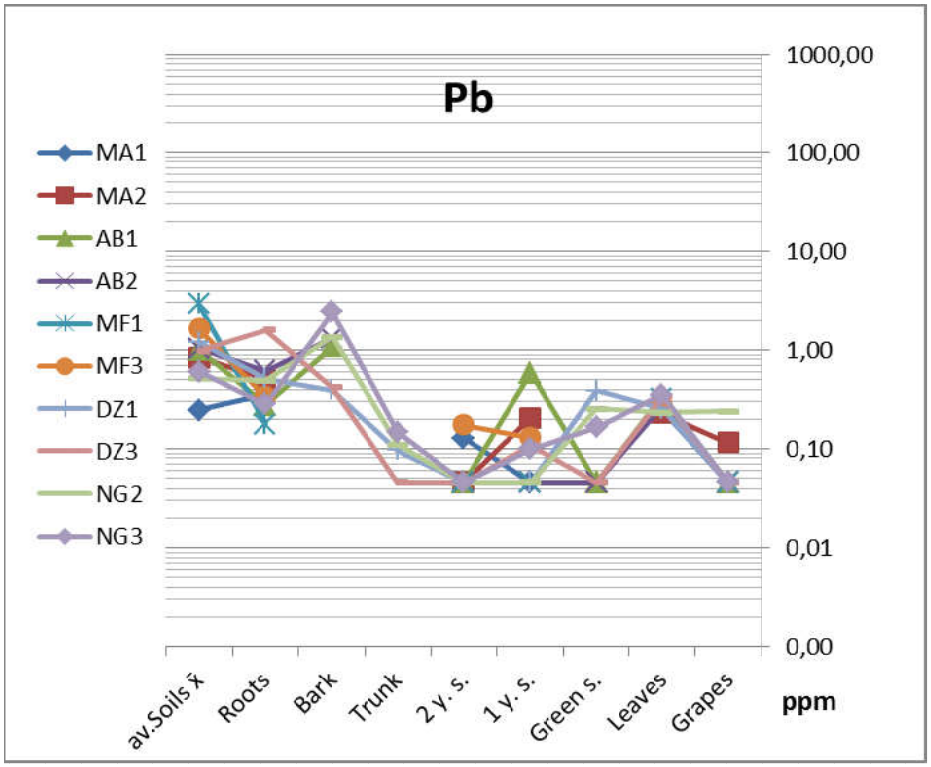

Figure 21. Diagram of Lead available in soils and present in plant parts

Table 34. Lead present in wines, in plant parts and available and total fractions in soils

Pb			MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2/4*	NG3/5*	DL
mg/L	**Wine**		-		0,0060		-		0,0831		0,0484		**0,0004**
Mg/Kg	**Grapes***		<	0,116	<	<	<	-	<	<	0,239	<	**0,092**
	Leaves		0,249	0,229	0,344	0,273	0,321	0,278	0,255	0,336	0,232	0,353	
	0 y. s.		-	-	<	<	-	-	0,386	<	0,255	0,164	
	1 y. s.		<	0,205	0,593	<	<	0,129	<	0,109	<	0,099	
	2 y. s.		0,129	<	<	<	<	0,176	<	<	<	<	
	Trunk		-	-	-	-	-	-	0,097	<	0,110	0,149	
	Bark		-	-	1,082	1,314	-	-	0,391	0,417	1,358	2,449	
	Roots		0,356	0,507	0,280	0,619	0,177	0,340	0,515	1,599	0,488	0,285	
	av.Soil	**0-10**	0,64	0,99	0,56	0,61	2,28	1,48	2,15	1,59	0,80	1,44	**0,01**
		>60	0,12	0,79	1,15	1,18	3,13	1,69	0,89	0,76	0,42	0,33	
	Soil	**0-10**	18	24	19	20	26	25	25	24	31	21	**2**
		>60	11	22	23	25	27	23	18	20	20	17	

Looking at *Table 34* and *Figure 21* we can see that the available *Pb* in soils is very heterogeneous but with low concentrations when compared with the levels of toxicity indicated by Barbiroli et al., (2000 [9]) (10 ppm as attention level and 20 ppm as toxicity

level). In MF soils have a high available fraction of *Pb* comparing it with the total. Bark is the plant part with the highest concentration, followed by roots. *Pb* is a non-essential metal and concentrations in vegetables parts are low compared with the soil ones. The values of *Pb* in wine are all below the limits of national legislation and the OIV recommendations.

ZINC

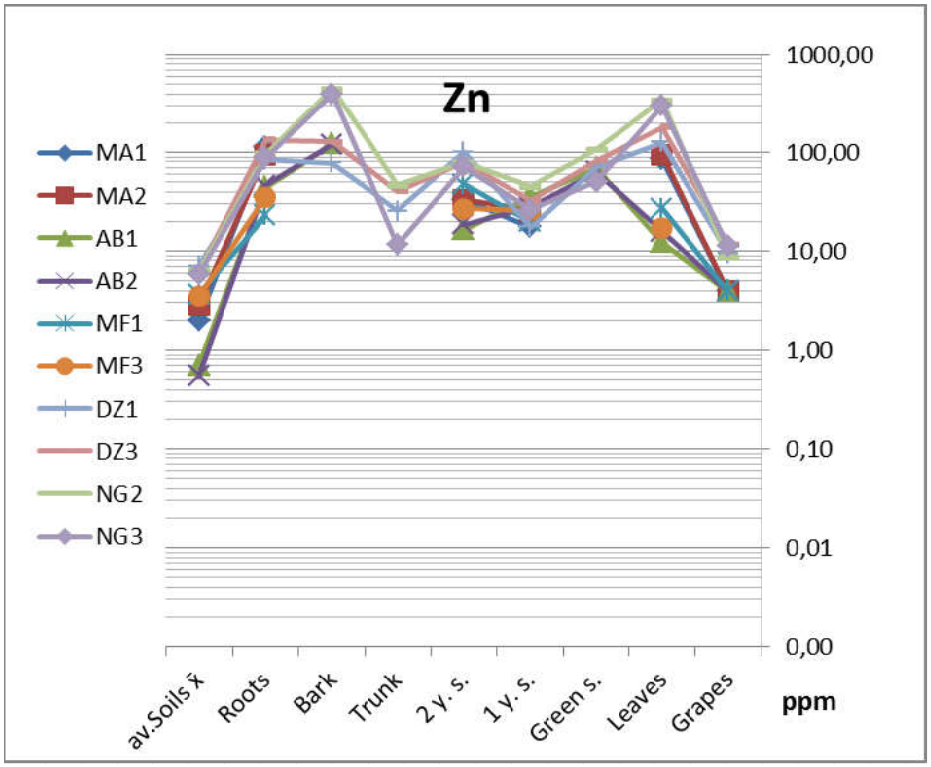

Figure 22. Diagram of Zinc available in soils and present in plant parts

Table 35. Zinc present in wines, in plant parts and available and total fractions in soils

Zn			MA1	MA2	AB1	AB2	MF1	MF3	DZ1	DZ3	NG2/4*	NG3/5*	DL
mg/L	**Wine**		-		0,14		-		2,09		12,20		**0,04**
mg/Kg	**Grapes***		<	<	<	<	<	-	9,4	11,6	8,5	11,1	**7,8**
	Leaves		85,7	94,3	12,3	15,7	27,0	17,3	126,5	180,4	340,8	300,5	
	0 y. s.		-	-	69,1	63,0	-	-	70,3	82,8	106,2	50,3	
	1 y. s.		17,5	25,6	36,1	27,7	19,8	26,0	17,4	32,1	44,8	26,3	
	2 y. s.		33,4	33,6	16,4	18,7	47,5	27,0	100,1	79,6	79,6	71,8	
	Trunk		-	-	-	-	-	-	25,3	40,6	46,4	11,5	
	Bark		-	-	121,6	120,9	-	-	77,8	127,9	448,2	400,2	
	Roots		113,9	94,5	44,2	46,6	22,9	35,6	83,8	133,9	95,9	88,5	
	av.Soil	**0-10**	3,75	5,35	1,15	0,93	5,82	5,06	13,46	11,17	11,93	11,36	**0,03**
		>60	0,22	0,33	0,26	0,16	1,52	1,89	0,55	0,92	0,67	0,24	
	Soil	**0-10**	78	99	71	73	115	127	128	123	99	90	**2**
		>60	42	70	79	81	90	103	74	74	35	38	

Looking at *Table 35* and *Figure 22* we see that the available *Zn* in soil is lower than in roots, so there is bio-concentration. The variability within the sites is little and makes us appreciate the differences among the 5 vineyards. The largest concentrations of *Zn* are

observed in the bark, followed by the leaves, the green shoots and roots, while in the grapes we have the lowest concentrations. This trend is particularly evident in NG, DZ and MA, while in AB and MF we have a lower foliar accumulation.

In general terms, the values for various parts of the plants in the sites with conventional farming are higher in concentration of *Zn*, especially in NG.

Wine in NG exceeds the concentration limit set by Italian law and by the OIV, when we already have the highest bark and foliar concentrations. However the great differences in wine between the diverse sites are not noticed in plant parts, so they could depend on the vinification process.

3.4. UNCERTAINTY

In ***Table 36***, ***Table 37*** and ***Table 38*** there are the average values of our results for the certified materials and for the differences from the spikes. Then there are the errors coming from three times the standard deviation and consequently our precision and accuracy for all the types of samples: soil, vegetable and wine.

Table 36. NCSDC85102a and uncertainty in available part of soils

	NCS DC 85102a								
	Cd	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**	
Certified value	0,040	0,025	1,17	55,0	17,3	0,27	1,7	1,08	mg/kg
ER	0,003	0,025	0,07	7,0	2,50	0,030	0,20	0,09	
Our result	0,042	0,022	1,20	40,7	14,6	0,26	1,4	0,89	
ER	0,001	0,002	0,08	0,4	0,05	0,002	0,01	0,03	
ERr%	2,0%	7,4%	6,6%	0,9%	0,3%	0,9%	0,4%	3,4%	
Ar%	105%	87%	102%	74%	84%	95%	84%	82%	
Br%	5%	-13%	2%	-26%	-16%	-5%	-16%	-18%	

Table 37. IAEA-359 and uncertainty in vegetable parts

	IAEA-359								
	Cd	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**	
Certified value	0,120	1,30	5,67	148,0	31,90	1,050	*N/A*	38,6	mg/kg
ER	0,005	0,060	0,18	3,9	0,60	0,050	*N/A*	0,7	
Our result	0,128	0,83	5,24	150,2	27,62	0,638	0,63	38,8	
ER	0,008	0,036	5,96	11,7	2,25	0,110	0,09	7,8	
ERr%	6,2%	4,3%	113,8%	7,8%	8,1%	17,3%	14,5%	20,2%	
Ar%	107%	64%	92%	101%	87%	61%	*N/A*	100%	
Br%	7%	-36%	-8%	1%	-13%	-39%	*N/A*	0%	

Table 38. Spike additions and uncertainty in wine

	Standard addition in spikes								
	Cd	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**	
Certified value	0,020	0,020	0,20	2,0	0,20	0,020	0,20	2,0	mg/L
ER	0,001	0,001	0,01	0,1	0,01	0,001	0,01	0,1	
Our result	0,0196	0,0200	0,2004	2,17	0,175	0,0205	0,0854	1,53	
ER	0,0028	0,0044	0,0238	0,16	0,027	0,0053	0,0004	0,04	
ERr%	14,1%	21,9%	11,9%	7,4%	15,4%	25,8%	0,4%	2,8%	
R%	98%	100%	100%	108%	88%	103%	43%	76%	

The tables show that precision of the DTPA extraction never exceeds 7,5%, and the accuracy $A_{r\%}$ never goes below 74% so all results can be considered good.

Among vegetable results we can report a lack of precision for low concentrations of *Cu* (up to a 113,8% on the certified value), but our samples have higher concentrations and the accuracy seems to be however very good. Measures of *Cr* and *Ni* are precise enough but inaccurate because they have $A_{r\%}$ values below 64%. We report our incapability in determining *Pb* accuracy in vegetable digestion, due to lack of a certified value, and *Pb* results are a little imprecise.

In wine, the error on reference solutions is 12,5% on average therefore measures are imprecise, but are accurate, with an exception: *Pb*, even if it is precise, it has a $R_{\%}$ that shows inaccuracy (43%).

3.5. SUMMARY

DISTRIBUTION OF METALS

In *Table 39* we summarized the average results of the three factors studied (the Enrichment Factor, the Biological Concentration Factor and the Translocation Factor). We indicate with a "-" when the factor is absent or weak in the vineyards, "+" when it is clearly present and "++" when it is strong.

Table 39. Average results of EF, BCF and TF

	EF	BCF	TF
Cd	*N/A*	+	-
Cr	-	++	-
Cu	++	-/+	++
Fe	-	+	-
Mn	-	+	+
Ni	-	+	-
Pb	-	-	-
Zn	+	++	+/-

Cd is bio-concentrated by our plants, but it does not translocate. *Cr* does not seem to be enriched, but is strongly concentrated, however it does not translocate. *Cu* is strongly enriched in soils, for anthropogenic supplies, it is not generally bio-concentrated in plants, but it is concentrated a little in the two conventional farms of lowland. The translocation for *Cu* is strong because we have high values in leaves. *Fe* and *Ni* do not seem to be enriched, are weakly bio-concentrated in plants and do not translocate. Grapevines seem to be accumulators for *Mn* because both BCF and TF are present. *Pb*, fortunately, does not seem to be enriched and does not translocate. Lastly there is enrichment of *Zn* in soils, it is strongly concentrated by plants and generally seems to be translocated in conventional and lowland farms.

PLANT PARTS

After the drying process, we found, for each part on average, the residual weights displayed in *Table 40*.

Table 40. Residual weights on average for each part after drying process

Residual weight percentage	Roots	Bark	Trunk	2 y. s.	1 y. s.	Green s.	Leaves	Grapes-I	Grapes-II
	48,4%	80,7%	58,3%	71,2%	66,6%	33,7%	34,6%	22,9%	18,4%

As expected, grapes lose much of their weight, more than the other parts, followed by the green shoots, the leaves, the roots, the trunk, the one year old shoots, the two year old shoots and lastly the bark.

Metals usually seem to accumulate in bark, then in leaves or sometimes in roots. Plants probably try to remove metal excess storing it in bark. In leaves there are sometimes high concentrations due to foliar uptake. In roots there are usually high concentrations when the absorbed metal is important for the plant.

We cannot see general connections between concentrations of metals in wine and its vineyard, we can just note that NG has the highest values of *Zn* (over the limit of law) and also has high values in soil and in plants.

VINEYARDS

In MA we do not notice abnormal values, just an enrichment of *Cu* and *Zn*.

AB has generally the lowest values, high values only for *Cu* in bark. Wine of AB has a volatile acidity that does not respect the limit of the Italian law.

MF generally has high values. In this vineyard there is a high enrichment of chromium (up to five times that of other vineyards). MF in soil has the highest value of *Cu*, but its enrichment is lower than in other vineyards. *Pb* in soil is higher than in other vineyards. Its two years old shoots have the highest concentrations of *Cu* and *Fe*.

DZ has the lowest concentrations of *Cr* in the two years old shoots. On the other hand it has much more *Mn* in leaves than other vineyards.

NG plants concentrate the *Cu*, but have low concentrations in leaves. NG has the highest concentrations in bark for *Mn*, *Ni*, *Pb* and *Zn* and in leaves for *Zn* (with high TF). *Zn* is over the limit in wine for the Italian law.

The chemical and physical analyses of soils simply confirm in all vineyards what we expected, because they confirm the characteristics of the substratum and do not need fertilisation. We only note an iron deficiency chlorosis in AB.

On hill plants there seems to be a similar behaviour concerning the metal concentrations in their different parts, even if MA has values slightly higher than AB. TF for *Cu* and BCF for *Zn* is higher on hills than in lowland.

In lowland concentrations in plants are similar inside each vineyard. These concentrations are generally higher than on hills.

Between vineyards with organic farming and with conventional ones, we noticed a difference: organic farms have a lower Enrichment Factor for *Cu* and *Zn*. Conventional vineyards of lowland have in topsoil a high percentage of available *Zn* (about 10%).

4. CONCLUSIONS

With this study we found some critical situations, such as the concentration of chromium in soils in the organic farm of lowland and the value of zinc in the wine in a conventional lowland farm. Then we found differences between organic vineyards and conventional ones: the conventional ones have higher enrichment of some metals in soils. We also found differences in the contents of metals between hill plants and lowland plants: the plant behaviour concerning the concentrations of metals seems to have a pattern.

A consequence of this study would be the research of the reasons why there are high concentrations of chromium in soils, especially when related to aluminium oxide and compared with the background values.

We then suggest extending the study to the parts of the plants we did not analyse for all sites and increase the number of investigated sampling points to reach statistical consistency.

Lastly to complete the work we would suggest comparing the concentrations in the vineyards to the amount of treatments they were subjected to.

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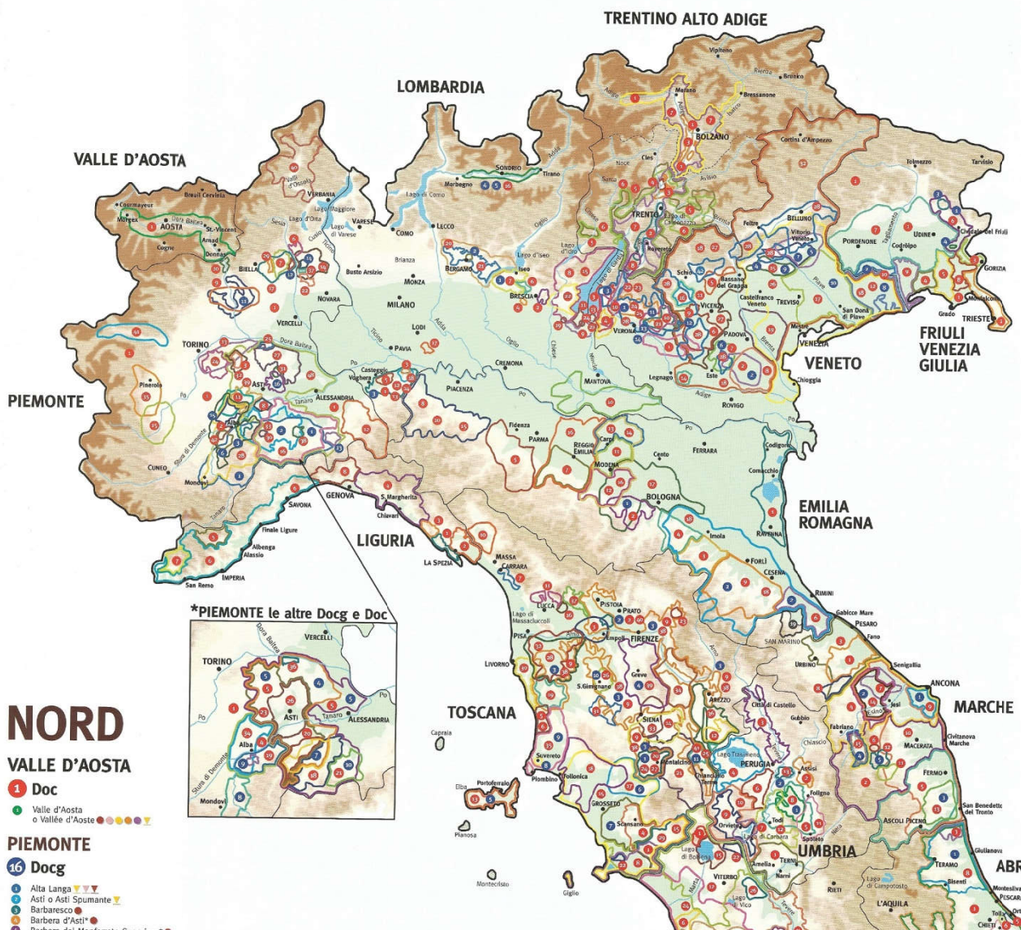
6. ANNEXES

LA CARTA ENOGRAFICA DI
Civiltà del bere

Doc e Docg d'Italia



CENTRO

EMILIA ROMAGNA + REP. DI SAN MARINO

2 Docg

- Colli Bolognesi Classico Pignoletto
- Romagna Albana

18 Doc

- Bosco Eliceo
- Colli Bolognesi
- Colli di Faenza
- Colli di Imola
- Colli di Parma
- Colli di Rimini
- Colli di Scandiano e di Canossa
- Colli Piacentini
- Colli Romagna Centrale
- Gutturnio
- Lambrusco di Sorbara
- Lambrusco Grasparossa di Castelvetro
- Lambrusco Salamino di Santa Croce
- Modena o di Modena
- Ortrugo dei Colli Piacentini o Ortrugo-Colli Piacentini
- Reggiano
- Reno
- Romagna
- I.G. (identificazione di origine) San Marino

MARCHE

5 Docg

- Cònero
- Castelli di Jesi Verdicchio Riserva
- Offida
- Verdicchio di Matelica Riserva
- Vernaccia di Serrapetrona

15 Doc

- Bianchello del Metauro
- Colli Maceratesi
- Colli Pesaresi
- Esino
- Falerio
- I Terreni di Sanseverino
- Lacrima di Morro o Lacrima di Morro d'Alba
- Pergola
- Rosso Cònero
- Rosso Piceno o Piceno
- San Ginesio
- Serrapetrona
- Terre di Offida
- Verdicchio dei Castelli di Jesi
- Verdicchio di Matelica

TOSCANA

11 Docg

- Brunello di Montalcino
- Carmignano
- Chianti
- Chianti Classico
- Elba Aleatico Passito o Aleatico Passito dell'Elba
- Montecucco Sangiovese
- Morellino di Scansano
- Suvereto
- Val di Cornia Rosso o Rosso della Val di Cornia
- Vernaccia di San Gimignano
- Vino Nobile di Montepulciano

41 Doc

- Ansonica Costa dell'Argentario
- Barco Reale di Carmignano
- Bianco dell'Empolese
- Bianco di Pitigliano
- Bolgheri
- Bolgheri Sassicaia
- Candia dei Colli Apuani
- Capalbio
- Colli dell'Etruria Centrale
- Colli di Luni *(anche in Liguria)*
- Colline Lucchesi
- Cortona
- Elba
- Grance Senesi
- Maremma Toscana
- Montecarlo
- Montecucco
- Monteregio di Massa Marittima
- Montescudaio
- Moscadello di Montalcino
- Orcia
- Parrina
- Pomino
- Rosso di Montalcino
- Rosso di Montepulciano
- San Gimignano
- Sant'Antimo
- San Torpè
- Sovana
- Terratico di Bibbona
- Terre di Casole
- Terre di Pisa
- Val d'Arbia
- Val d'Arno di Sopra o Valdarno di Sopra
- Val di Cornia
- Valdichiana Toscana
- Valdinievole
- Vin Santo del Chianti
- Vin Santo del Chianti Classico
- Vin Santo di Carmignano
- Vin Santo di Montepulciano

ABRUZZO

1 Docg

- Montepulciano d'Abruzzo Colline Teramane

8 Doc

- Abruzzo
- Cerasuolo d'Abruzzo
- Controguerra
- Montepulciano d'Abruzzo
- Ortona
- Terre Tollesi o Tullum
- Trebbiano d'Abruzzo
- Villamagna

UMBRIA

2 Docg

- Montefalco Sagrantino
- Torgiano Rosso Riserva

13 Doc

- Amelia
- Assisi
- Colli Altotiberini
- Colli del Trasimeno o Trasimeno
- Colli Martani
- Colli Perugini
- Lago di Corbara
- Montefalco
- Orvieto *(anche nel Lazio)*
- Rosso Orvietano o Orvietano Rosso
- Spoleto
- Todi
- Torgiano

LAZIO

3 Docg

- Cannellino di Frascati
- Cesanese del Piglio o Piglio
- Frascati Superiore

27 Doc

- Aleatico di Gradoli
- Aprilia
- Atina
- Bianco Capena
- Castelli Romani
- Cerveteri
- Cesanese di Affile o Affile
- Cesanese di Olevano Romano o Olevano Romano
- Circeo
- Colli Albani
- Colli della Sabina
- Colli Etruschi Viterbesi o Tuscia
- Colli Lanuvini
- Cori
- Est! Est!! Est!!! di Montefiascone
- Frascati
- Genazzano
- Marino
- Montecompatri Colonna o Montecompatri o Colonna
- Moscato di Terracina o Terracina
- Nettuno
- Orvieto *(anche in Umbria)*
- Roma
- Tarquinia
- Velletri
- Vignanello
- Zagarolo

BASILICATA

1 Docg

- Aglianico del Vulture Superiore

4 Doc

- Aglianico del Vulture
- Grottino di Roccanova
- Matera
- Terre dell'Alta Val d'Agri

MOLISE

4 Doc

- Biferno
- Molise o del Molise
- Pentro d'Isernia o Pentro
- Tintilia del Molise

NORD

VALLE D'AOSTA

1 Doc

- Valle d'Aosta o Vallée d'Aoste

PIEMONTE

16 Docg

- Alta Langa
- Asti o Asti Spumante
- Barbaresco
- Barbera d'Asti
- Barbera del Monferrato Superiore*
- Barolo
- Brachetto d'Acqui o Acqui*
- Dogliani*
- Dolcetto di Diano d'Alba o Diano d'Alba*
- Dolcetto di Ovada Superiore o Ovada*
- Erbaluce di Caluso o Caluso
- Gattinara
- Gavi o Cortese di Gavi
- Ghemme
- Roero
- Ruchè di Castagnole Monferrato

42 Doc

- Piemonte *(è presente in tutta la regione dove ci siano zone di produzione)*
- Alba
- Albugnano
- Barbera d'Alba*
- Barbera del Monferrato*
- Boca
- Bramaterra
- Calosso
- Canavese
- Carema
- Cisterna d'Asti
- Colli Tortonesi
- Collina Torinese
- Colline Novaresi
- Colline Saluzzesi
- Cortese dell'Alto Monferrato
- Coste della Sesia
- Dolcetto d'Acqui*
- Dolcetto d'Alba*
- Dolcetto d'Asti*
- Dolcetto di Ovada*
- Fara
- Freisa d'Asti*
- Freisa di Chieri
- Gabiano
- Grignolino d'Asti*
- Grignolino del Monferrato Casalese
- Langhe
- Lessona
- Loazzolo
- Malvasia di Casorzo d'Asti o Casorzo o Malvasia di Casorzo
- Malvasia di Castelnuovo Don Bosco
- Monferrato
- Nebbiolo d'Alba*
- Pinerolese
- Rubino di Cantavenna*
- Sizzano
- Strevi
- Terre Alfieri
- Valli Ossolane
- Valsusa
- Verduno Pelavergа o Verduno

** Con l'asterisco si indicano le zone Docg e Doc presenti sul particolare ritagliato della cartina, che andrebbero sovrapposte a quella grande, ma che per chiarezza abbiamo dovuto separare*

LOMBARDIA

5 Docg

- Franciacorta
- Moscato di Scanzo o Scanzo
- Oltrepo Pavese (Metodo Classico)
- Sforzato di Valtellina o Sfursàt
- Valtellina Superiore (Grumello, Inferno, Maroggia, Sassella, Valgella)

22 Doc

- Bonarda dell'Oltrepo Pavese
- Botticino
- Buttafuoco dell'Oltrepo Pavese o Buttafuoco
- Capriano del Colle
- Casteggio
- Cellatica
- Curtefranca
- Garda *(anche in Veneto)*
- Garda Colli Mantovani
- Lambrusco Mantovano
- Lugana *(anche in Veneto)*
- Oltrepo Pavese
- Oltrepo Pavese Pinot grigio
- Pinot nero dell'Oltrepo Pavese
- Riviera del Garda Bresciano o Garda Bresciano
- Rosso di Valtellina o Valtellina Rosso
- San Colombano al Lambro o San Colombano
- Sangue di Giuda dell'Oltrepo Pavese o Sangue di Giuda
- San Martino della Battaglia *(anche in Veneto)*
- Terre del Colleoni o Colleoni
- Valcalepio
- Valtenesi

SARDEGNA

1 Docg

- Vermentino di Gallura

47 Doc

- Cannonau di Sardegna*
- Monica di Sardegna*
- Moscato di Sardegna*
- Sardegna Semidano*
- Vermentino di Sardegna* **(è presente in tutta la regione dove ci siano zone di produzione)*
- Alghero
- Arborea
- Cagliari
- Campidano di Terralba o Terraiba
- Carignano del Sulcis
- Girò di Cagliari
- Malvasia di Bosa
- Mandrolisai
- Moscato di Sorso-Sennori o Moscato di Sorso
- Nasco di Cagliari
- Nuragus di Cagliari
- Vernaccia di Oristano

LIGURIA

8 Doc

- Cinque Terre
- Cinque Terre Sciacchetrà
- Colli di Luni *(anche in Toscana)*
- Colline di Levanto
- Golfo del Tigullio-Portofino o Portofino
- Pornassio o Ormeasco di Pornassio
- Riviera ligure di Ponente
- Rossese di Dolceacqua o Dolceacqua
- Val Polcèvera

TRENTINO ALTO ADIGE

8 Doc

- Alto Adige o dell'Alto Adige-Südtirol o Südtiroler
- Casteller
- Lago di Caldaro o Caldaro-Kalterersee o Kalterer
- Teroldego Rotaliano
- Trentino
- Trento
- Valdadige *(anche in Veneto)*
- Valdadige Terradeiforti o Terradeiforti
- Valdadige *(anche in Veneto)*

FRIULI VENEZIA GIULIA

4 Docg

- Colli Orientali del Friuli Picolit
- Lison *(anche in Veneto)*
- Ramandolo
- Rosazzo

10 Doc

- Prosecco *(anche in Veneto) (è presente in tutta la regione dove ci siano zone di produzione)*
- Carso o Carso-Kras
- Collio Goriziano o Collio
- Friuli Annia
- Friuli Aquileia
- Friuli Colli Orientali
- Friuli Grave
- Friuli Isonzo o Isonzo del Friuli
- Friuli Latisana
- Lison Pramaggiore

VENETO

14 Docg

- Amarone della Valpolicella
- Bagnoli Friularo o Friularo di Bagnoli
- Bardolino Superiore
- Colli Asolani Prosecco o Asolo Prosecco
- Colli di Conegliano
- Colli Euganei Fior d'Arancio o Fior d'Arancio Colli Euganei
- Conegliano Valdobbiadene Prosecco o Conegliano Prosecco o Valdobbiadene Prosecco
- Lison *(anche in Friuli Venezia Giulia)*
- Montello Rosso
- Piave Malanotte o Malanotte del Piave
- Recioto della Valpolicella
- Recioto di Gambellara
- Recioto di Soave
- Soave Superiore

28 Doc

- Arcole
- Bagnoli di Sopra o Bagnoli
- Bardolino
- Bianco di Custoza
- Breganze
- Colli Berici
- Colli Euganei
- Corti Benedettine del Padovano
- Gambellara
- Garda *(anche in Lombardia)*
- Lessini Durello o Durello Lessini
- Lison Pramaggiore *(anche in Friuli Venezia Giulia)*
- Lugana *(anche in Lombardia)*
- Merlara
- Montello-Colli Asolani
- Monti Lessini o Lessini
- Piave
- Prosecco *(anche in Friuli Venezia Giulia)*
- Riviera del Brenta
- San Martino della Battaglia *(anche in Lombardia)*
- Soave
- Valdadige
- Valdadige Terradeiforti *(anche in Trentino-Alto Adige)*
- Valdadige Terradeiforti o Terradeiforti Valdadige *(anche in Trentino-Alto Adige)*
- Valpolicella
- Valpolicella Ripasso
- Venezia
- Vicenza
- Vigneti della Serenissima o Serenissima

PUGLIA

4 Docg

- Castel del Monte Bombino nero
- Castel del Monte Nero di Troia Riserva
- Castel del Monte Rosso Riserva
- Primitivo di Manduria Dolce Naturale

28 Doc

- Aleatico di Puglia *(è presente in tutta la regione dove ci siano zone di produzione)*
- Alezio
- Barletta
- Brindisi
- Cacc'e mmitte di Lucera
- Castel del Monte
- Colline Joniche Tarantine
- Copertino
- Galatina
- Gioia del Colle
- Gravina
- Leverano
- Lizzano
- Locorotondo
- Martina o Martina Franca
- Matino
- Moscato di Trani
- Nardò
- Negroamaro di Terra d'Otranto
- Orta Nova
- Ostuni
- Primitivo di Manduria
- Rosso di Cerignola
- Salice Salentino
- San Severo
- Squinzano
- Tavoliere delle Puglie o Tavoliere
- Terra d'Otranto

CALABRIA

9 Doc

- Bivongi
- Cirò
- Greco di Bianco
- Lamezia
- Melissa
- Sant'Anna di Isola Capo Rizzuto
- Savuto
- Scavigna
- Terre di Cosenza

SICILIA

1 Docg

- Cerasuolo di Vittoria

23 Doc

- Sicilia *(è presente in tutta la regione)*
- Alcamo
- Contea di Sclafani
- Contessa Entellina
- Delia Nivolelli
- Eloro
- Erice
- Etna
- Faro
- Malvasia delle Lipari
- Mamertino di Milazzo o Mamertino
- Marsala
- Menfi
- Monreale
- Noto
- Pantelleria
- Riesi
- Salaparuta
- Sambuca di Sicilia
- Santa Margherita di Belice
- Sciacca
- Siracusa
- Vittoria

SUD

CAMPANIA

4 Docg

- Aglianico del Taburno
- Fiano di Avellino
- Greco di Tufo
- Taurasi

15 Doc

- Aversa o Aversa Asprinio
- Campi Flegrei
- Capri
- Casavecchia di Pontelatone
- Castel San Lorenzo
- Cilento
- Costa d'Amalfi
- Falanghina del Sannio
- Falerno del Massico
- Galluccio
- Irpinia
- Ischia
- Penisola Sorrentina
- Sannio
- Vesuvio

SICILIA

1 Docg

- Cerasuolo di Vittoria

23 Doc

- Sicilia *(è presente in tutta la regione)*
- Alcamo
- Contea di Sclafani
- Contessa Entellina
- Delia Nivolelli
- Eloro
- Erice
- Etna
- Faro
- Malvasia delle Lipari
- Mamertino di Milazzo o Mamertino
- Marsala
- Menfi
- Monreale
- Noto
- Pantelleria
- Riesi
- Salaparuta
- Sambuca di Sicilia
- Santa Margherita di Belice
- Sciacca
- Siracusa
- Vittoria

Legenda

DOCG Il cerchio blu indica la zona di un vino Docg

DOC Il cerchio rosso indica la zona di un vino Doc

TIPOLOGIE DI VINO PER OGNI DENOMINAZIONE

- Rosso
- Rosato o Chiaretto
- Bianco
- Passito, Vin Santo o Liquoroso
- Novello
- Spumante Bianco
- Spumante Rosato
- Spumante Rosso

ALLEGATO II
modificato dal Reg. CEE 1535/92, dal Reg. CEE n. 2608/93,
dal Reg. CEE 2381/94 e dal Reg CE 1488/97

Parte A
PRODOTTI PER LA CONCIMAZIONE E L'AMMENDAMENTO

Condizioni generali applicabili a tutti i prodotti:
- impiego consentito solo se sono soddisfatti i requisiti dell'allegato I
- impiego consentito solo in conformità delle disposizioni della normativa concernente l'immissione in libera pratica e l'utilizzazione dei prodotti interessati applicabile in agricoltura generale nello Stato membro in cui il prodotto è utilizzato."

Nome	Descrizione, requisiti di composizione, condizioni per l'uso
Letame	Prodotto costituito dal miscuglio di escrementi animali ed a materiali vegetali (lettiera). Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo. Indicazione delle specie animali. Proveniente unicamente da allevamenti estensivi ai sensi dell'art. 6, paragrafo 4 del Regolamento CEE n. 2328/91 del Consiglio, modificato da ultimo dal Regolamento CE n. 3669/93.
Letame essiccato e deiezioni avicole disidratate	Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo. Indicazione delle specie animali. Proveniente unicamente da allevamenti estensivi ai sensi dell'art. 6, paragrafo 4, del Regolamento CEE n. 2328/91.
Deiezioni animali, composte, inclusa la pollina ed il letame	Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo. Indicazione delle specie animali. Proibiti se provenienti da allevamenti industriali.
Escrementi liquidi di animali (liquame, urina, ecc.)	Impiego previa fermentazione controllata e/o diluizione adeguata. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo. Indicazione delle specie animali. Proibiti se provenienti da allevamenti industriali.
Rifiuti domestici trasformati in compost	Compost di rifiuti domestici separati selettivamente all'origine. Solo rifiuti vegetali e animali. Prodotto in sistema di raccolta chiuso e sorvegliato approvato dallo Stato membro. Concentrazioni massime in mg/kg di materia secca: cadmio 0.7; rame 70; nickel 25; piombo 45; zinco 200; mercurio 0.4; cromo (totale) 70; cromo (VI) 0*. Solo per un periodo che termina il 31 marzo 2002. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo. *= (limite di determinazione)
Torba	Impiego limitato all'orticoltura (colture orticole, floricole,arboricole, vivai).
Argille (perlite, vermiculite, ecc.)	
Residui di fungaie	La composizione iniziale del substrato dev'essere limitata ai prodotti del presente elenco.
Deiezioni di vermi (Vermicompost) e di insetti	
Guano	Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Miscela composta di materiali vegetali	Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
I prodotti o sottoprodotti di origine animale citati di seguito: - farina di sangue - polvere di zoccoli - polvere di corna - polvere di ossa, anche degelatinata - nero animale (carbone animale) * - farina di pesce - farina di carne - pennone - lana - peli e crini - prodotti lattiero caseari	Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.

Pellami	Concentrazione massima in mg/kg di materia secca di cromo (VI): 0 * *= (limite di determinazione)
Borlande ed estratti da borlande Prodotti e sottoprodotti organici di origine vegetale per la fertilizzazione (ad es.: farina di panelli di semi oleosi, guscio di cacao, radichette di malto, ecc.)	Escluse le borlande estratte con sali ammoniacali.
Alghe e prodotti a base di alghe	Se ottenuti direttamente mediante: i) processi fisici comprendenti disidratazione, congelamento e macinazione. estrazione con acqua o con soluzione acide e/o alcalina. fermentazione. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Segatura e trucioli di legno	Legname non trattato chimicamente dopo l'abbattimento.
Cortecce compostate	Legname non trattato chimicamente dopo l'abbattimento.
Cenere di legno	Legname non trattato chimicamente dopo l'abbattimento.
Fosfato naturale tenero	Prodotto definito dalla direttiva 76/116 CEE del Consiglio, modificata dalla direttiva 89/284 CEE. Tenore di Cadmio inferiore o pari a 90 mg/kg di P_2O_5.
Fosfato allumino-calcico	Prodotto definito dalla direttiva 76/116 CEE modificata dalla direttiva 89/284 CEE. Tenore di Cadmio inferiore o pari a 90 mg/kg di P_2O_5. Impiego limitato ai terreni basici (pH>7.5).
Scorie di defosforazione	Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Sale grezzo di potassio (ad es. kainite, silvinite, ecc.)	Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Solfato di potassio che può contenere sale di magnesio	Prodotto ottenuto da sale grezzo di potassio mediante un processo di estrazione fisica e che può contenere anche sali di magnesio. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Carbonato di calcio di origine naturale (ad es. creta, marna, calcare macinato, litotamnio, knaerl, creta fosfatica, ecc.)**	
Carbonato di calcio e magnesio di origine naturale (ad es. creta magnesiaca, calcare magnesiaco macinato, ecc.)	
Solfato di magnesio (ad es. kieserite)	Unicamente di origine naturale. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Soluzione ci cloruro di calcio	Trattamento fogliare su melo, dopo che sia stata messa in evidenza una carenza di calcio Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Solfato di calcio (gesso)	Prodotto definito dalla direttiva 76/116/CEE modificata dalla direttiva 89/284/CEE. Unicamente di origine naturale.
Fanghi industriali provenienti da zuccherifici	Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo. Solo per un periodo che termina il 31 marzo 2002.
Zolfo elementare	Prodotto definito dalla direttiva 76/116/CEE modificata dalla direttiva 89/284/CEE. Unicamente di origine naturale.
Oligoelementi	Oligoelementi inclusi nella direttiva 89/530/CEE. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Cloruro di sodio	Unicamente salgemma. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Farina di roccia	

*Il prodotto "carbone animale" riportato nell'allegato II, parte A del regolamento CEE n. 2092/91 prima dell'entrata in vigore del presente regolamento può essere utilizzato alle condizioni di applicazione precedenti fino a smaltimento delle scorte esistenti e comunque non oltre il 30.9.2000 (Reg. CE n.1073/200).

Parte B
ANTIPARASSITARI

1. Prodotti fitosanitari

Condizioni generali applicabili per tutti i prodotti composti o contenenti le sostanze attive appresso indicate:
- impiego in conformità ai requisiti dell'allegato I;
- soltanto in conformità delle disposizioni specifiche della normativa sui prodotti fitosanitari applicabile nello Stato membro in cui il prodotto è utilizzato (ove pertinente)*.

I - Sostanze di origine vegetale o animale

Nome	Descrizione, requisiti di composizione, condizioni per l'uso
Azadiractina estratta da *Azadirachta indica* (albero del neem)	Insetticida. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
(*) Cera d'api	Protezione potature.
Gelatina	Insetticida.
(*) Proteine idrolizzate	Sostanze attrattive. Solo in applicazioni autorizzate in combinazione con altri prodotti adeguati del presente allegato II, parte II.
Lecitina	Fungicida
Estratto (soluzione acquosa) di *Nicotiana tabacum*	Insetticida. Solo contro gli afidi in albero da frutti subtropicali (ad es. aranci, limoni) e in colture tropicali (ad es. banani). Utilizzabile solo all'inizio del periodo vegetativo. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo. Utilizzabile soltanto durante un periodo che termina il 31 marzo 2002.
Oli vegetali (ad es. olio di menta, olio di pino, olio di carvi)	Insetticida, acaricida, fungicida e inibitore della germogliazione.
Piretrine estratte da *Chrysantemum cinerariaefolium*	Insetticida
Quassia estratta da *Quassia amara*	Insetticida, repellente
Rotenone estratto da *Derris spp, Loncho carpus spp* e *Thephrosia spp.*	Insetticida Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.

** = in alcuni Stati membri i prodotti contrassegnati con asterisco non sono considerati prodotti fitosanitari e non sono soggetti alle disposizioni della legislazione in materia di prodotti fitosanitari.*

II - Microrganismi utilizzati nella lotta biologica contro i parassiti

Nome	Descrizione, requisiti di composizione, condizioni per l'uso
Microrganismi (batteri, virus e funghi), ad es. *Bacillus thuringiesis, Granulosis virus*	Solo prodotti non geneticamente modificati ai sensi della Direttiva 90/220/CEE del Consiglio *

*= GU n. L 117 del 8.5.1990

III - Sostanze da utilizzare solo in trappole e/o distributori automatici

Condizioni generali:
- le trappole e/o i distributori automatici devono impedire la penetrazione delle sostanze nell'ambiente e il contatto delle stesse con le coltivazioni in atto;
- le trappole devono essere raccolte dopo l'utilizzazione e riposte al sicuro.

Nome	Descrizione, requisiti di composizione, condizioni per l'uso
(*) Fosfato di diammonio	Sostanza attrattiva. Soltanto in trappole.
Metaldeide	Molluschicida. Soltanto in trappole contenenti un repellente per specieanimali superiori.

Feromoni	Sostanze attrattive; sostanze che alterano il comportamento sessuale. Solo in trappole e distributori automatici.
Piretroidi (solo deltametrina o lambdacialotrina)	Insetticida. Solo in trappole con sostanze specifiche attrattive. Solo contro *Bacrocera oleae* e *Ceratitis capitata wied.* Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo. Solo per un periodo che termina il 31 marzo 2002.

** = in alcuni Stati membri i prodotti contrassegnati con asterisco non sono considerati prodotti fitosanitari e non sono soggetti alle disposizioni della legislazione in materia di prodotti fitosanitari.*

IV - Altre sostanze di uso tradizionale in agricoltura biologica

Nome	Descrizione, requisiti di composizione, condizioni per l'uso
Rame, nella forma di idrossido di rame, ossicloruro di rame, solfato di rame (tribasico), ossido rameoso	Fungicida Solo per un periodo che termina il 31 marzo 2002 Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
(*) Etilene	Sverdimento delle banane
Sale di potassio di acidi grassi (sapone molle)	Insetticida
(*) Allume di potassio (Kalinite)	Prevenzione della maturazione delle banane
Zolfo calcico (polisolfuro di calcio)	Fungicida, insetticida, acaricida. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Olio di paraffina	Insetticida, acaricida.
Oli minerali	Insetticida, fungicida. Solo in alberi da frutta, viti, olivi e colture tropicali (ad esempio banani). Solo per un periodo che termina il 31 marzo 2002. Necessità riconosciuta dall'organismo di controllo o dall'autorità di controllo.
Permanganato di potassio	Fungicida, insetticida Solo in alberi da frutta, ulivi e viti
(*) Sabbia di quarzo	Repellente
Zolfo	Fungicida, acaricida, repellente

= In alcuni Stati membri i prodotti non sono considerati prodotti fitosanitari e non sono soggetti alle disposizioni della legislazione in materia di prodotti fitosanitari.

2. Prodotti per la lotta contro i parassiti e le malattie nei locali di stabulazione e negli impianti:

Prodotti elencati nella sezione 1
Rodenticidi