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The influence of climate variability on chemical composition of European wines: a regional scale study (Italy and Slovenia)

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Abstract

Climate change is having an increasing influence on vine phenology and grape composition, affecting vinifications, wine chemistry and the quality of productions. Wine grape cultivation provides a good test case for measuring indirect impacts mediated by changes in agriculture, because viticulture is sensitive to climate and is concentrated in Mediterranean climate regions that are global biodiversity hotspots. Moreover, on a regional level and on a shorter time scale, the seasonal weather conditions modify the quality of yields determining the final properties of wine.

In the present research, we studied wines from Italy and Slovenia with the purpose of differentiating them by the different vintages, which are supposed to be influenced by temperature and rain during each year's growing season. Specific chemical techniques were used, in particular mass spectrometry (ICP-MS) and isotopic mass spectrometry (IRMS), both of which are usually employed to detect wine adulterations and to establish the geographical provenance of wines. In particular, we investigated the relationship between macro- and micro-elements, Rare Earth Elements and stable isotopes (δ^{13}C, δ^{18}O, $(D/H)_I$, $(D/H)_{II}$). Soils and grapes analyses were then included to understand how the cycle of elements could be modified up to the final products. The datasets were examined via statistical techniques to show their relation to weather conditions as well as their mutual connection.

The results for four vintages of wine showed strong affinity to temperature and precipitation while wines from the two countries were not clearly geographically differentiated.

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Abbreviations

- **CNR-IDPA**: National Research Council - Institute for the Dynamics of Environmental Processes.
- **CRA-VIT**: Consiglio per la Ricerca in Agricoltura, Centro per la ricerca in Viticoltura. Sede di Conegliano.
- **CRM**: Certified Reference Materials.
- **FA**: Factor Analysis.
- **ICP-MS**: Inductively Coupled Plasma Mass Spectrometry.
- **ICP-SFMS**: Inductively Coupled Plasma Sector Field Mass Spectrometry.
- **IPCC**: Intergovernmental Panel on Climate Change.
- **IRMS**: Isotope Ratio Mass Spectrometry.
- **OIV**: International Organization of Vine and Wine.
- **PCA**: Principal Component Analysis.
- **PDO**: Protected Designation of Origin.
- **REE**: Rare Earth Element.
- **SNIF-NMR**: Site-specific Natural Isotopic Fractionation Nuclear Magnetic Resonance.
- **TE**: Trace Elements.
- **WS**: Working Standard.

Preface

The idea of this PhD project was prompted by three circumstances: firstly, the needs of our region's steadily expanding wine market; secondly, the considerable progress made by wine chemistry in the last years; and finally, the current effects of climate change on wine production, which are focusing our attention on the necessity to find new proxies to protect our high-quality products.

This PhD project was possible thanks to the partnership between Ca' Foscari University of Venice, the National Research Council - Institute for the Dynamics of Environmental Processes (CNR−IDPA), the Center for the Research on Agriculture and Viticulture (CRA−VIT) and the Jožef Stefan Institute of Ljubljana (JSI) (Slovenia).

Part I

Introduction

Chapter 1

Agriculture and Climate Change

Climate and weather have always played a crucial role in the history of human development. From the nomadic tribes of ancient times to modern agricultural civilizations, environmental and climatic conditions have been a source of benefits and limitations for humans (Jones and Webb, 2010). The advent of the Industrial Age and the massive use of fossil fuels gave rise to a slow but steady upward shift of the earth's average temperature, called "global warming" (Jones and Webb, 2010; Mozell and Thach, 2014; Nordås and Gleditsch, 2007; Solomon, 2007).

The Earth's average climate is changing: changes in global and local temperatures are modifying weather patterns, with a number of effects including an increased vulnerability of regions, economic sectors and communities.

Based on a discrete number of projections (IPCCwebsite, 2015), we can say that climate change is affecting and will affect our planet's environmental conditions. The most positive scientific predictions are talking of a 2 °C increase over the next century (Nordås and Gleditsch, 2007; Jones et al., 2005; IPCCwebsite, 2015). There is solid evidence about the effects of climate change on the northern hemisphere and in all the EU (IPCCwebsite, 2015). One can mention for example the decrease in average and seasonal rainfalls and, at the same time, more sudden heat waves, droughts, storm and floods. Most of these effects will have a negative impact on the regions, in particular on those that are already suffering from environmental or other changes. Farming will be particularly affected in southern Europe. However, some climate changes may be positive for a few northern regions. (IPCCwebsite, 2015). The reports of the Intergovernmental Panel on Climate Change (IPCC) foresee significant impacts in the short term from more frequent extreme conditions. However, the worst consequences may not be felt until 2050

(IPCCwebsite, 2015).

The importance of the climatic events in the agriculture is first of all because plants are made of organic matter, minerals and, above all, water. The relative amounts of these three components may vary, but in green plants, water is always present in the highest proportion and minerals in the lowest. The percentage of distribution of these three components follows the following order of magnitude (Mengel et al., 2001):

- Water 700 mg g^{-1} fresh matter;
- Organic material 270 mg g^{-1} fresh matter;
- Minerals 30 mg g^{-1} fresh matter.

Generally, the daily or seasonal water stress to which a plant is subjected induces a range of responses that depend on the extent of the water shortage. Among the various effects of water scarcity, one can mention stomatal closure, decreased assimilation and therefore lower growth and production of fruits. Water stress also hinders plant growth by reducing cell division and enlargement and by causing a decline in ion transport to the root surface. When human intervention is not sufficient, the plant can resort to multiple responses to tolerate water stresses. These responses range from stomatal sensibility to soil and atmosphere dehydration, changes in the elasticity of the cell wall and osmotic adjustment. In plants adapted to dry environments, anatomical and morphological changes in the leaf or in the whole plant prevent metabolic imbalance and help improve water relations. These adaptations have a cost: the overall effect is a reduction of growth to balance all the levels of resource acquisition (Pessarakli, 2010).

Vitis vinifera L. is a plant cultivated since ancient times and is of great importance for humans, both as a source of food and for cultural reasons. Products derived from *Vitis vinifera L.* are widespread all over the world. The capacity of *Vitis vinifera L.* to extract minor and trace components from the growth substrate, as well as the fate of these elements during processes occurring in the rhizosphere and their transport to the aerial parts of plants, therefore represent an interesting research topic. There are a few studies analyzing the responses to water deficits of *Vitis vinifera L.*, focusing for example on how irrigation can control the composition of wine grapes (Matthews and Anderson, 1988), their different physiological parameters and how they were affected by the introduction of artificial irrigation (Cifre et al., 2005), or the effects of transient water deficits on berry growth (McCarthy, 1997).

1.1 Wine production

Wine is not essential to human survival. However, it is one of the first and oldest human-made products. Wine production techniques have considerably evolved through the centuries, reaching very high standards of quality (McGovern et al., 2003).

Wine plays a very important role in the economy of several countries, among which Italy stands out as one of the most renowned producers. Wine is particularly connected to the culture, the economy and the environmental peculiarities of a region.

The definition of *terroir* is of great importance for our purposes. *Terroir* is how a particular region's climate, soils and aspect (terrain) affect the taste of wine. The *terroir* is not a property of the wine as such, but good wine reflects the *terroir*(s) of origin (Gladstones, 2011). In other words, a particular wine is inextricably linked to a particular *terroir*.

An interesting study of wine grapes, wine production and climate change is the one performed by Jones et al., 2005 and Jones, 2006. They took in consideration the climate-maturity groupings between phenological requirements and climate to reach the high quality wine production for different vine varieties, in different *terroir* achieving very interesting results. Their study, which is still a work in progress, was carried out in Oregon, but its overarching idea is of great interest also for our regions. Van Leeuwen et al., 2013, adapting the work of Jones et al., 2005, compared the average increase in seasonal temperatures from 1971 to 1999 and from 2000 to 2012 in three major wine-growing regions in France and Germany. Figure 1.1 was adapted from both studies and shows the differences in climatic conditions between the three regions studied by Van Leeuwen et al., 2013. The results of both works showed that, for some vines, the temperature increase will redefine the optimal temperature for the ripening of the grapes. Over the years, this could affect the ripening of the grapes in different ways, for example by anticipating the harvest period or, in extreme cases, by creating climatic conditions where the breeding of a specific variety would no longer be possible.

However, Van Leeuwen et al., 2013 concluded that the Rheingau, Burgundy, and Rhone Valley produced high-quality Müller-Thurgau, Pinot gris and Pinot noir, as well as Syrah and Viognier, although the temperatures were already beyond the maximum values in these regions. High-quality viticulture is very supported in these regions despite the temperatures rise and dry farming. This can be explained both by the evolution of consumer preferences and by the growers' implementation of adaptive strategies.

The high level of uncertainty associated to agriculture and model predictions shown by the previous considerations has been taken into account in the present PhD project.

However, there is much more to the problem of global warming and wine quality. Wine grapes are extraordinarily sensitive to temperature and the industry requires a early-warning system for the variations that all crops will have to face as global warming intensifies.

1.1.1 Weather conditions and grapes

The relation between climatic conditions and yield is well known in agriculture. So is the wide range of relationships between climatic elements and the quality of the yield. In particular, grapes can present differences in sugar content, acidity, aromatic intensity, concentration, anthocyanin extractability, tannin concentration, enzymatic activities, and so on. Temperature and rainfall are the environmental variables with the greatest influence on the growing process (Jones and Davis, 2000). Even a small variation in humidity can modify the growing of the vine and the maturation of the grape. The hydric and thermic conditions of plants also strongly influence the aromas and colors of the fruits. Sugars production depends on the photosynthesis cycle, which in optimal conditions takes places at 23-28 °C. Higher temperatures usually increase the sugar storage, but this does not imply higher quality.

Regarding the rainfall, it is scientifically known that excessive or prolonged exposure to water can lead to an incomplete maturation of the grapes and to high values of malic acid (Esteban et al., 2001; Kriedemann, 1968; Jones and Davis, 2000; Tomasi et al., 2011).

Both white and red wines vines prefer conditions of slight water scarcity during ripening (Castellarin et al., 2007; Ojeda et al., 2002).

Finally, it is fundamental to remember that, in order to understand the effects of climatic conditions on viticulture, all these atmospheric elements must be interrelated.

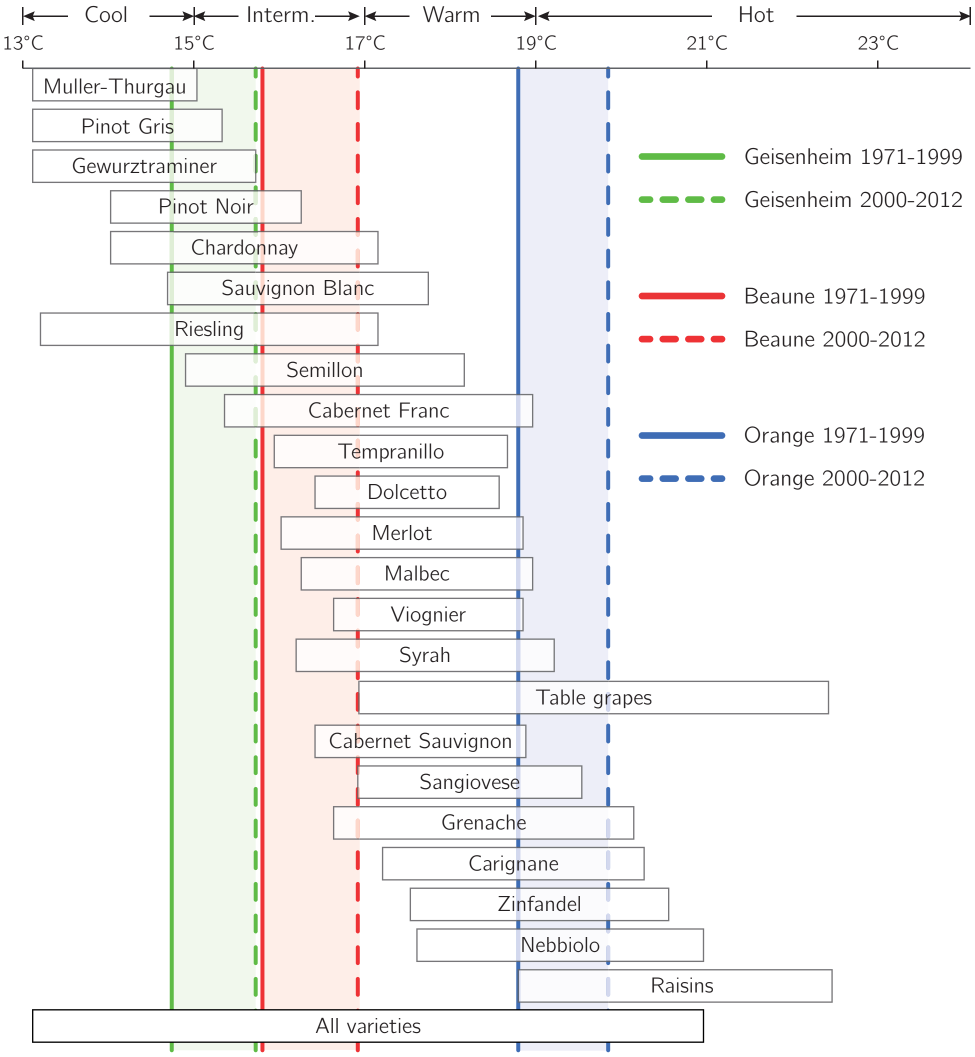


Figure 1.1: Figure adapted from Jones et al., 2005 and Van Leeuwen et al., 2013. Average growing season temperature from 1971 to 1999 and from 2000 to 2012 in Rheingau, Germany (Geisenheim station, Deutscher Wetterdienst); Burgundy, France (Beaune station); and Rhone Valley, France (Orange station). Note that Müller-Thurgau and Pinot gris, Pinot noir, as well as Syrah and Viognier are already beyond the maximum value in Rheingau, Burgundy, and Rhone Valley, respectively. The figure and research are part of a work in progress and some adjustments may be necessary. Changes of more than ± 0.2−0.6 °C are highly considered (Jones et al., 2005; Van Leeuwen et al., 2013).

Chapter 2

Aims and hypothesis

The last years have witnessed a growing number of studies, using multi-elemental and multi-isotopic analysis, on the provenance and monitoring of wines. This is due to the high standards reached by wine production, compared to other agricultural products, and to the fact that some regions have established guaranteed standards of origin and manufacture: the Protected Designation of Origin (PDO)(in Italy DOC *Denominazione di Origine Controllata*, and DOCG *Denominazione di Origine Controllata e Garantita*).

The quality of the wine is strictly related to the influence of weather conditions in the different years. This project combined two kinds of analysis, namely ICP-MS and IRMS, as adapted from the official OIV (International Organization of Vine and Wine). These were applied to yearly weather variability (T and rainfall) with the purpose of identifying the most suitable chemical techniques to explain the atmospheric conditions of vine growth and their influence on wine quality. The trace elements (TE and REE) and the stable isotope ratios (δ^{13}C, δ^{18}O, $(D/H)_I$, $(D/H)_{II}$) were measured for selected vintages produced in Italy and in Slovenia.

The partnership with the CRA-VIT allowed us to sample several vintages (2009-2010-2011-2012) of Italian wines from the Veneto Region (North of Italy). The grapevines were cultivated in different cellars of the area of Valpolicella (Verona). The harvest and wine production were then taken in charge by the CRA-VIT laboratories following a standard procedure. The standard wine production permitted a scientific comparison between the different vintages. Indeed, the reduction of variability in the winemaking process allowed us to limit human interference and to highlight environmental contributions.

Some of the wines analyzed in this project were previously used in Tomasi and Battista, 2014, a complete study of the territory, vineyards and wines of the Valpantena cellars and of the nearby area of Valpolicella.

An accurate bibliographical research (Giaccio and Vicentini, 2008; Day et al., 1994; Vystavna et al., 2014; Tardaguila et al., 1997) suggested that a joint analysis of stable isotopes and trace elements in grape musts would enable a useful distinction between wine-growing regions. This was of extreme interest for our work, and the CRA-VIT gave us access to samples of grape musts associated to the wines that we were already analyzing. The musts from the vintages 2011 and 2012 (two of the same wine vintages) also underwent the analysis of stable isotopes and trace elements.

Moreover, the work of Tomasi and Battista, 2014, which reports soil analysis of the region, gave us the idea to include the soils of the fields of Valpolicella to better understand the movements of the elements from the earth to the must and wine. Similar approaches were tried by Vystavna et al., 2014 and Piper and Bau, 2013.

The second year of the PhD was devoted to the analysis of stable isotopes at the Jožef Stefan Institute of Ljubljana. Thanks to the Slovenian Agriculture Institute, other vintages of wine (2011-2012-2013) from three Slovenian growing regions (Primorje, Posavje, Podravje) were collected.

The Slovenian wines collected were also produced following a standard procedure, like the Italian ones. This enabled a comparison between the vintages and between the two countries of origin.

Chapter 3

Study area

The next sections will be devoted to describing the zones of provenance of the wines under study. A brief description of the wines analyzed in this PhD project will also be included.

3.1 Valpolicella in Italy

Valpolicella, reported in Figure 3.1, is a famous viticultural zone of the Province of Verona, Italy. The area is known for its eponymous red wine, the Valpolicella. Winemaking in the area has existed since the ancient Greeks, and the name seems to derive from a combination of Latin and Greek terms meaning "Valley of Cellars".

Some of the Italian wines of this study were already used by Tomasi and Battista, 2014 and were made available for this work by the Agricultural Center of Italy-Viticulture (CRA-VIT). The area studied included the valleys of Valpantena, Val Squaranto, Valdonega and the higher part of Val di Mezzane. A total of about 16.500 ha were studied in Tomasi and Battista, 2014, of which 2.100 ha presented vine fields.

3.1.1 Italian wines description

Corvina grapevine, which is of great importance in the viticulture of North-Eastern Italy, is the basis of the blends of two very known and popular Italian wines, Valpolicella and Bardolino. The wines studied in this work were Valpolicella, made from Corvina vine grown using the classic system of Guyot, and three kinds of wines from the fields of Negrar (Vr), made respectively from Cabrusina, Dindarella and Boschera. This last wine is the only white wine under study.

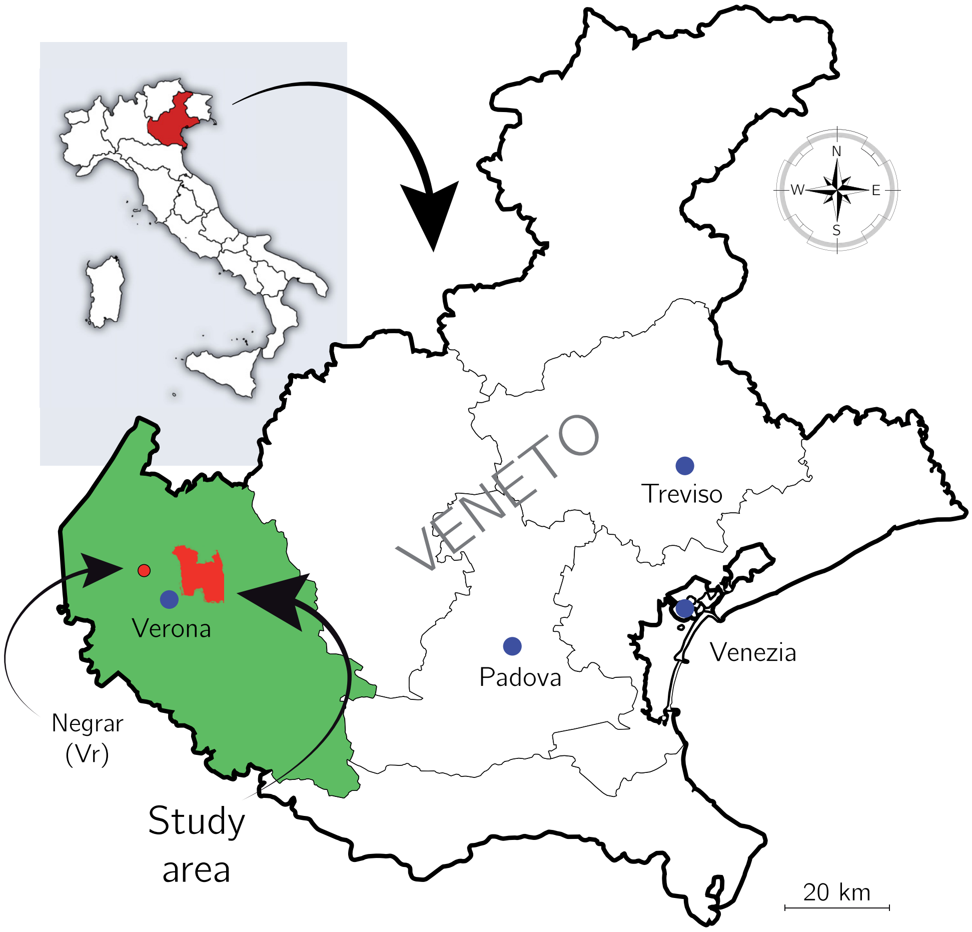


Figure 3.1: Location of the study area, Veneto region.

3.2 Sampling sites description

CRA-VIT selected the guide vineyards to be sampled in function of their environment. The sampled vines were representative of the average technical strategies of viticulture of the areas and showed no obvious signs of deficit in growth and production. Guide vineyards were chosen in a representative number to reflect the soil variability of the sub-area, taking into account the vineyard's age and the traditional breeding and production techniques. Thanks to Dott. Giuseppe Benciolini and to the CRA-VIT of Conegliano Veneto, the sampling of most interesting soils for this study was possible. In this work, the soils (identified by an acronym) are numbered from 1 to 9 according to where the sample was collected (Table 3.1).

Figure 3.2 shows the soil configuration in the studied area. Tomasi and Battista, 2014 identified four different climatic areas depending on the elevation:

- Valley bottom (AF, BF);
- Colluvial deposits (CMC, CEC);
- Average slopes (VBB, CEC, DEP, VSB);
- High slopes (VBA, VBB, VSA).

Table 3.1: Studied Soils from the 9 italian fields

Zone		**Code**	**Name**
Alto fondovalle ghiaioso		AF	1
Basso fondovalle limoso-argilloso		BF	2
Colluvi molto calcarei		CMC	3
Colluvi estremamente calcarei		CEC	4
Paleofrane e depositi di versante		DEP	5
Versanti a moderata erosione (scaglia rossa)	< 300 m	VSB	6
	> 300 m	VSA	9
Versanti fortemente erosi (biancone)	< 300 m	VBB	7
	> 300 m	VBA	8

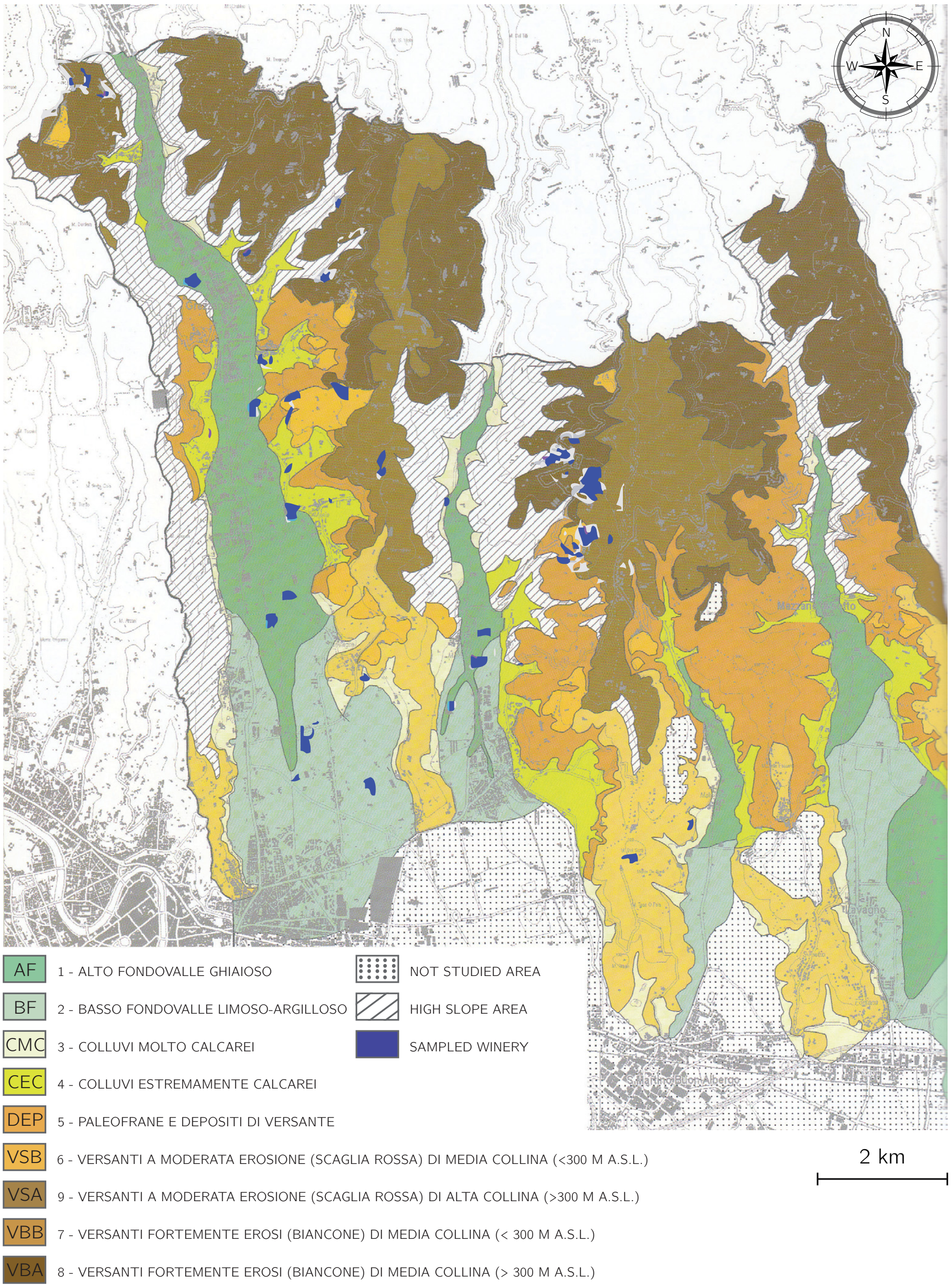


Figure 3.2: Map of soil zones in the studied area. The blue color indicates the winery, where the guide vineyards were selected (Tomasi and Battista, 2014).

3.3 Slovenian vine growing regions: Primorska, Podravje and Posavje

Slovenia has three vine growing regions that are famous for the high quality of their wine production: Primorska, Posavje, and Podravje (Figure 3.3). These three areas are characterized by very different microclimates, soil composition and viticultural traditions. The production of wine in this region dates back to the time of the Celts and Illyrians tribes, long before the Romans would introduce winemaking into the lands of Germany, France and Spain. The influence of other countries, including Italy, is evident both in the growing and in the production of wines, as well as in the terminology.

Slovenian wine production is famous for its white wines, such as Riesling, Pinot Gris, Chardonnay, Sauvignon, including native varieties such as Rebula and Zelen. Among the red varieties we can find Pinot Noir, Cabernet Sauvignon and Merlot. The most famous indigenous red is the Teran.

The Slovenian Ministry of Agriculture reported that today Slovenia has more than 40.000 wineries, which annually produce 1 million hl from the country's 24.600 ha of vineyards (Ministry-of-the-Agriculture-Forestry-and Food, 2015).

About 75 % of the country's production is white wine. Most of the country's wine production falls under the classification of premium (vrhunsko vino) wine, with less than 30 % classified as basic table wine (namizno vino).

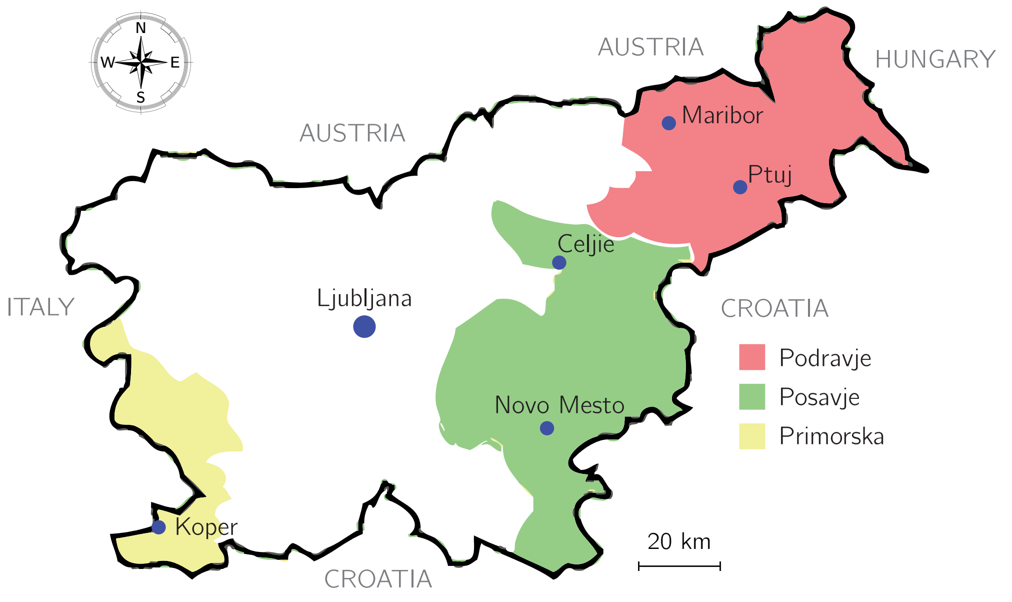


Figure 3.3: Primorska, Posavje, and Podravje winegrowing regions in Slovenia.

3.4 Precipitations and temperatures

Atmospheric conditions, such as rainfall and temperature, modify the vine growth and fruit production, with direct effects on wine quality.

We retrieved from the ISPRAwebsite, 2015 website a series of historical data about rainfall and temperature in the area of study. The "Grezzana" sampling station was selected for this study due to its position, a few km from the vine fields.

The data in Table 3.3 and 3.2 for the vintages 2009, 2010, 2011 and 2012 presented some interesting differences for the studied region. In particular, the atmospheric conditions of the year 2010 were characterized by more rainfall and lower average temperatures, while 2012, on the contrary, showed higher temperatures and less rainfall. The years 2009 and 2011 were more similar to each other.

As reported in Tomasi and Battista, 2014, the most discriminant water provision and temperature conditions occur during the growing seasons, i.e. from March to September. Figure 3.4 reports the cumulative rainfall during the growing seasons for the four vintages, evidencing their mutual relationship.

The data taken from ISPRAwebsite, 2015 and used in this study are very similar to those published by Tomasi and Battista, 2014.

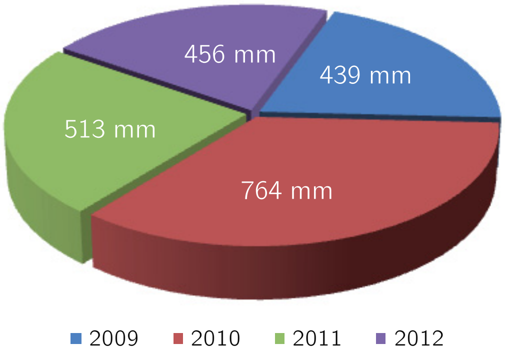


Figure 3.4: Cumulative precipitation during the growing seasons (ISPRAwebsite, 2015).

Table 3.2: Grezzana (Verona, Italy) sampling station. Maximum daily precipitation (Coordinates long: 11.01372 lat: 45.50916) (ISPRAwebsite, 2015).

Year	**Month**	**Values** (mm)	**Year**	**Month**	**Values** (mm)
2009	1	37.6	2011	1	17.2
2009	2	14.2	2011	2	23
2009	3	30.2	2011	3	35.8
2009	4	24.6	2011	4	5
2009	5	3.8	2011	5	25.4
2009	6	32.8	2011	6	83.6
2009	7	25.2	2011	7	25.8
2009	8	11.8	2011	8	7.4
2009	9	30.4	2011	9	33.8
2009	10	34.8	2011	10	73.2
2009	11	25.8	2011	11	30.8
2009	12	34.2	2011	12	10
2010	1	NA	2012	1	14.6
2010	2	27.6	2012	2	4.6
2010	3	6.4	2012	3	1.6
2010	4	13.2	2012	4	21.2
2010	5	19.2	2012	5	31.6
2010	6	35	2012	6	19.2
2010	7	63.2	2012	7	24
2010	8	104.4	2012	8	33.6
2010	9	42.6	2012	9	24.2
2010	10	39	2012	10	32.6
2010	11	48	2012	11	68.8
2010	12	32.6	2012	12	7.8

Table 3.3: Grezzana (Verona, Italy) sampling station. Average daily temperature (Coordinates long: 11.01372 lat: 45.50916) (ISPRAwebsite, 2015).

Year	**Month**	**Values** (°C)	**St. Dev.**	**Year**	**Month**	**Values** (°C)	**St. Dev.**
2009	1	3	2.9	2011	1	2.4	1.8
2009	2	5.4	2.4	2011	2	5.9	2.2
2009	3	9.2	1.9	2011	3	9.2	3.5
2009	4	14.6	1.7	2011	4	15.8	2.4
2009	5	19.9	3.4	2011	5	18.9	3.1
2009	6	21.4	2.2	2011	6	21.3	2.1
2009	7	23.9	2.3	2011	7	22.3	2.7
2009	8	25.8	2	2011	8	25.2	2.7
2009	9	21	2.1	2011	9	22.3	2.1
2009	10	14.1	4.1	2011	10	13.8	4.4
2009	11	9.5	1.2	2011	11	8.6	3.9
2009	12	3.9	3.9	2012	12	5.3	1.8
2010	1	2	2.4	2012	1	3.3	3.2
2010	2	5	2.7	2012	2	2.4	5.3
2010	3	8.1	4.2	2012	3	12.5	3
2010	4	13.3	3.2	2012	4	12.2	3.2
2010	5	16.5	2.4	2012	5	17.2	3.2
2010	6	21.5	2.8	2012	6	23.1	3.5
2010	7	25.1	2.9	2012	7	25.4	2.1
2010	8	22.2	2.7	2012	8	26.3	2.5
2010	9	17.9	2.1	2012	9	19.6	2.6
2010	10	12.2	2.9	2012	10	14.8	3.5
2010	11	9.1	3.5	2012	11	10.3	1.9
2010	12	2.3	3.5	2012	12	2.9	2.4

Figure 3.5 includes the detailed variability in the four growing seasons: average temperatures (lines), and cumulative maximum daily precipitations (bars).

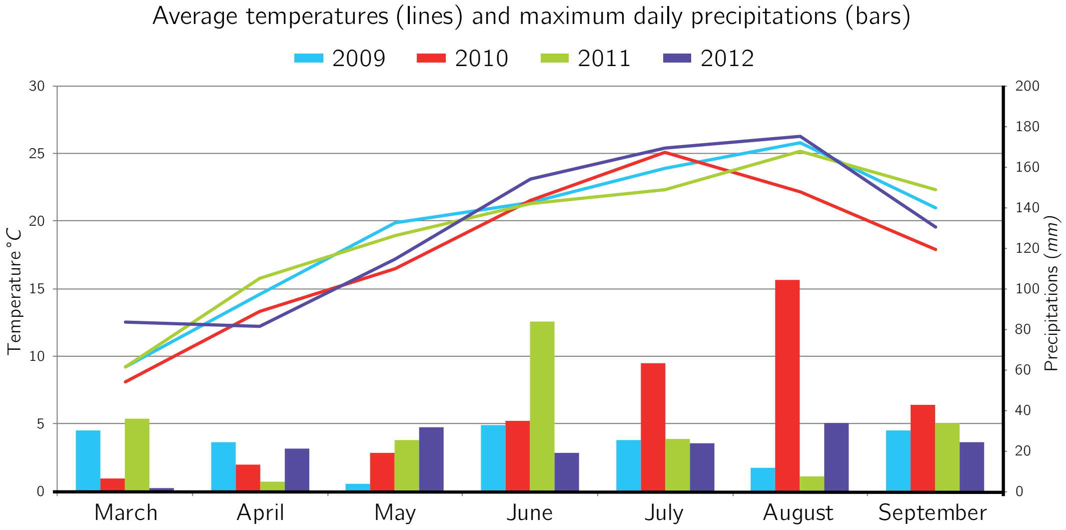


Figure 3.5: Average temperatures (lines) and cumulative maximum daily precipitations (bars) during the growing seasons. Italy (ISPRAwebsite, 2015).

Slovenian data about annual precipitations and average temperatures was reported in Table 3.4 (Ministry-of-the-Envinronment-and Spatial-Planing, 2015) for three stations: Murska Sobota, Novo mesto and Ljubljana.

Data regarding growing seasons was not available for Slovenia, we therefore reported only the annual ones. Data showed that, while temperatures were different from those of Italian regions, it was possible to evidence, similarly to Italy, a generally rainier and colder 2010 and a wormer year 2012. Year 2011 generally presented less precipitations than the others.

In Figure 3.6 is reported a graphic illustration of the variations of annual average temperatures (lines) and the annual precipitations (bars) of the three stations.

Table 3.4: Annual average temperature and annual precipitations for three Slovenian stations (Ministry-of-the-Envinronment-and Spatial-Planing, 2015).

Annual average Temperature (°C)					
Year	**2009**	**2010**	**2011**	**2012**	**2013**
Murska Sobota	10.9	10.3	10.7	11.3	11
Novo mesto	11.4	10.3	11.3	11.6	11.2
Ljubljana	11.7	10.7	11.8	12.1	11.7
Annual precipitations (mm)					
Murska Sobota	989	876	693	782	912
Novo mesto	1066	1278	834	1387	1259
Ljubljana	1406	1798	998	1339	1531

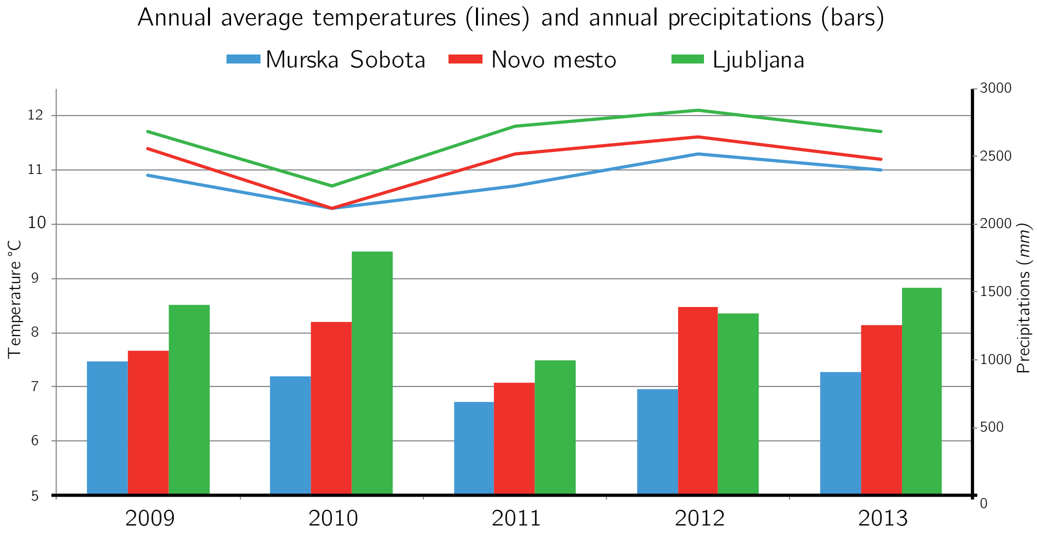


Figure 3.6: Annual average temperature (lines) and annual precipitations (bars) for three Slovenian stations (Ministry-of-the-Envinronment-and Spatial-Planing, 2015).

Chapter 4

Analytical chemistry and wine

Analytical chemistry is a very powerful instrument that has been gaining popularity in food research, and especially in the field of authentication.

Determining food authenticity is an important issue in terms of food quality control and safety. The outbreak of diseases related to foodstuffs around the world has raised consumer awareness about the quality and safety of food. This is why such aspects as the geographical origin and agricultural practices in food production have acquired great importance.

Authenticity is a quality criterion for food and food ingredients that is being increasingly emphasized in Europe as a result of legislative protection of regional foods (Gonzalvez et al., 2009).

Food authentication can address different aspects such as characterization, mislabeling, misleading origin, and adulteration. The last issue is defined as a process whereby the quality or the nature of a given substance is reduced by adding a foreign or inferior substance and by removing a vital element (Collins, 1993).

It is well known that selected minerals and trace elements in food can reflect the soil type and environmental growing conditions. This is why the evaluation of trace element content has been proposed as a means to establish the geographical origin of food samples. The diffusion of trace elements in the food chain is also one of the most important indicators of environmental conditions. Indeed, ground composition influences the presence of oligoelements in plants and has a direct impact on the organisms in the food chain.

The relationship between environment and food chain is tightly correlated to geological factors such as rock mineralogy, landscape and climate, as well as to soil chemistry. There are many different analytical approaches for the identification and quantification of trace compounds connecting a specific food product to a particular manufacturing procedure or to a specific geographic area. Moreover, different mono-elemental techniques have been employed in food authentication [flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ETAAS)]. The same goes for multi-elemental techniques [inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICPMS)] (Gonzalvez et al., 2009).

Stable isotope ratios of bio-elements have been used for over twenty years for the authentication of different foodstuffs and derivatives.

Among the methods used in authenticity control and origin assignment of foods and beverages, stable isotope ratio analysis has gained in importance for the quality assessment of wine, spirits, flavored fruit juices, honey and oil For wine analysis, the first method for analysis of stable isotope ratios to be officially adopted by the European Community was the site-specific determination of deuterium/hydrogen ratio by NMR in wine ethanol. The method was mainly used to determine the addition of beet sugar, which is illegal (Christoph et al., 2015; Roßmann et al., 1996).

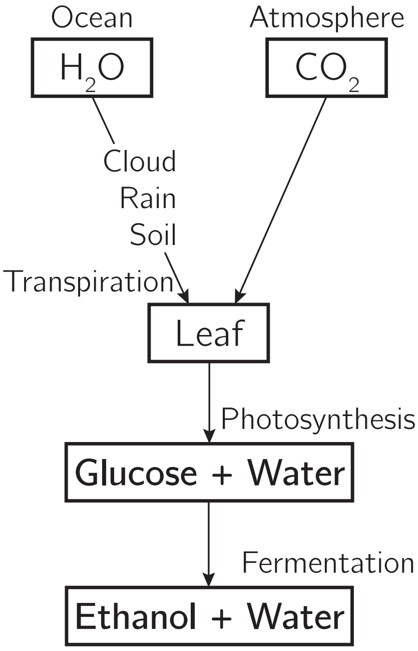


Figure 4.1: Scheme of parameters influencing the chemical composition of a plant.

For D/H results of authentic wines, which should be used as a basis for reliable comparison, European Community authorities have installed a system of data banks for all wine-producing countries within the EC (EC regulation 2676/90 from October 3, 1990), which has since increased (Christoph et al., 2015).

The results of these studies and the application of the knowledge to commercial wines highlighted the need to use not only the hydrogen isotope ratio, but also carbon and oxygen isotope data for ethanol and water (Christoph et al., 2015; Roßmann et al., 1996). Such multielement isotope analyses can give a better estimate of the nature of a natural product as compared to the analysis of the stable isotope ratio of only one element.

Environmental factors such as temperature and rain influence the physiology of the plants and contribute to modifying its overall contents of ^{13}C and ^{18}O (Martin et al., 1995). This phenomenon can be important for plants such as grape vines, which have very large foliar surfaces and evaporate a great quantity of water by transpiration. It has been shown that a large range of δ^{13}C values may be observed for ethanol from grapes harvested in typical soil and climatic conditions (Day et al., 1995). Figure 4.1 briefly reports the parameters influencing the chemical and isotopic composition of plants, leafs and related fruits (Farquhar et al., 2007; Küpper et al., 1996; Guy et al., 1993; Palliotti et al., 2010).

This argument is also interesting for its relevance to the evaluation of the nutritional quality of foodstuff and to the possible verification of adulteration. For this reason, in recent years several works were carried out to study trace element content and stable isotope ratios as indicators of the origin of food industry products (Suhaj and Korenovska, 2005; Arvanitoyannis et al., 1999).

4.1 Wine and Elemental composition

4.1.1 Trace Elements (TE)

Wine is a very complex matrix. Among the various components that contribute to its quality, characteristics and nutritional value, there are macro, micro-elements (Trace Elements) and lanthanides, whose concentrations can be quite variable (Galgano et al., 2008).

The moderate consumption of wine contributes significantly to the intake of essential elements such as Ca, Cr, Co, K, Se, Zn; however, other elements such as As, Cd, Pb and Br are known to be potentially toxic. Moreover, elements such as Al, Cu, K, Fe, Mn, and Zn may have detrimental effects on the stability of wine (Galani-Nikolakaki et al., 2002; Monaci et al., 2003; Galgano et al., 2008).

Italian regulations establish legal levels in wine only for Br (1 mg L^{-1}), Cu (1 mg L^{-1}), Pb (0,2 mg L^{-1}) and Zn (5 mg L^{-1}) [Table 4.1, *RepubblicaItaliana*, DM 29 dicembre 1986]. Accordingly, beside its toxicological implications, elemental analysis has become of interest for the chemical differentiation of wines and for the identification of their geographical origin.

A number of studies indicate that trace elements analysis provides solid criteria for describing the provenance of wines (Baxter et al., 1997; Capron et al., 2007; Gremaud et al., 2004; Greenough et al., 1997; Latorre et al., 1994; Marengo and Aceto, 2003; Šperková and Suchánek, 2005; Suhaj and Korenovska, 2005). The trace element patterns of a specific wine are regulated by the soil of origin and reflect the geochemistry of the grapevine fields (Suhaj and Korenovska, 2005).

Several studies consider environmental contamination, climatic change, vinification processes, agricultural practices, as factors that can modify the multielemental composition of wine and the relationship between wine and soil composition (Suhaj and Korenovska, 2005; Taylor et al., 2003; Thiel et al., 2004; Kment et al., 2005).

4.1.2 Rare Earth Elements (REE)

The name Rare Earth Elements (REE) refers to a total of 17 elements on the periodic table. Lanthanides have atomic numbers ranging from 57 to 71 (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu), with the inclusion of scandium (Sc) and yttrium (Y).

The adjective "rare" is quite improper in this case considering that, with the exception of the unstable Pm, the concentration of these elements in the Earth's crust is quite high. For example La, Ce and Nd are more abundant

Table 4.1: Legal levels in wines for some substances (OIV-MA-C1-01, 2015; *RepubblicaItaliana*, DM 29 dicembre 1986).

Legal levels in wine	
Arsenic	0.2 mg L^{-1}
Boron	80 mg L^{-1} (expressed as boric acid)
Bromine	1 mg L^{-1} (limit exceeded by way of exception in wines from certain vineyards with a brackish subsoil)
Cadmium	0.01 mg L^{-1}
Copper(A)	1 mg L^{-1}
Copper(B)	2 mg L^{-1} (for liqueur wines produced (A)from unfermented or (B) slightly fermented grape must)
Silver	0.1 mg L^{-1}
Lead	0.15 mg L^{-1} (for wine made starting from the 2007 harvest year)
(Excess) Sodium	80 mg L^{-1}

than Pb. Elements from Sc to Gd are called Light Rare Earth Elements (LREE), while Y, Tb, Dy, Ho, Er, Tm, Yb and Lu are also known as Heavy Rare Earth Elements (HREE). The peculiarity of these elements is to have highly similar chemical properties, and therefore they usually occur jointly.

The term "lanthanide contraction" describes a phenomenon of great decrease in the ionic radii of these elements resulting in ionic radii that are smaller than expected for the subsequent elements (McLennan and Taylor, 1985).

REE concentration can considerably vary in different soils, and the bioavailability varies depending on pH, organic matter and weathering state. The transfer from soil to plants is very low, and some studies (Brown et al., 1990; Tyler, 2004) demonstrate that lanthanides may maintain their distribution when passing from soil to plants. These studies suggest that lanthanides, well known as powerful geochemical markers, can also play an important role in the food industry as geographical markers. Moreover, they can help identify the provenance of foodstuffs, provided that their distribution in soil remains unaltered in plants growing on that soil and eventually in agricultural products obtained from those plants. The link between soil and food products, based on lanthanides patterns, has already been shown in studies on Moscato wine (Aceto et al., 2013), hazelnuts (Oddone et al., 2009) and on Hungarian paprika (Brunner et al., 2010). The use of REEs seemed promising, at least for what concerns simple production chains like those of fruits and vegetables.

Other studies, however, reported very different concentration of REEs between soil, roots, plants, fruits, and relative wines (Kment et al., 2005; Almeida and Vasconcelos, 2003). These studies evidenced that lanthanides were not helpful in the determination of the geographical origin of samples.

The normalization of REEs will be used to eliminate the "sawtooth" pattern, which is typical of the REEs and caused by the Oddo-Harkins effect, and eventually shows single element's anomalies into the studied samples (McLennan and Taylor, 1985). The normalization can be done with different geological standards such as the World Shale Average (WSA), the North American Shale Composite (NASC), the Post Archean Australian Shale (PAAS), the Upper Continental Crust (UCC) and the use of the Chondrites (Piper and Bau, 2013).

4.2 Stable isotope composition

The different isotopes of a specific element have the same number of protons but a different number of neutrons. They therefore occupy the same position in the periodic table.

Accordingly, the isotopes of an element have the same chemical properties but different masses. 61 elements in the periodic table have naturally occurring stable isotopes. Among these elements, hydrogen, carbon, oxygen, nitrogen, and sulfur have the most common isotopes analyzed in organic and biological compounds.

The terms radioisotopes or radionuclides designate isotopes subject to radioactive decay, while stable isotopes are those that have never been observed to undergo radioactive decay. For example, ^{12}C and ^{13}C are stable isotopes while ^{14}C is a radioactive isotope (Figure 4.2).

4.2.1 Isotope fractionation

Isotope fractionation is the enrichment of one of the isotopes of an element in a chemical or physical process due to the different bond energies of each isotope. The stronger bonds, and hence the slower reaction rates, are present in the heavier isotopes. There are two classes of isotope fractionation: the kinetic isotope effect and the thermodynamic isotope effect. Kinetic isotope fractionation occurs in irreversible reactions when there are different isotopes moving or reacting at different rates (Galimov, 2012). A classic example of kinetic fractionation is the evaporation of seawater to form clouds. In this

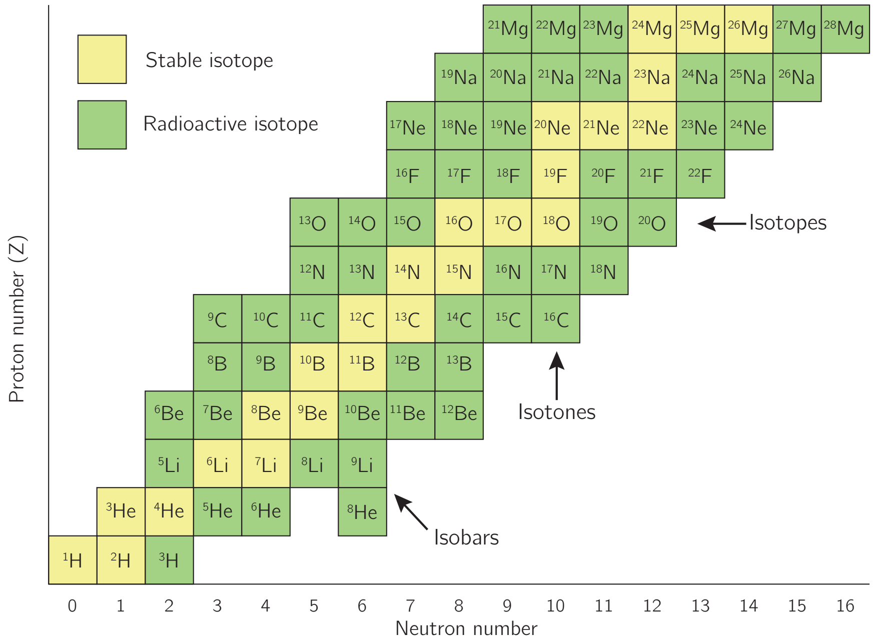


Figure 4.2: Examples of stable and radioactive isotopes for the first 30 elements in the Periodic Table of Elements.

situation, ^{16}O is enriched relative to the heavier oxygen isotopes in comparison with sea water.

It is important to mention that the naturally occurring variance in stable isotope abundance is very small.

The Oxygen cycle is the classic example of isotope fractionation. In the Oxygen cycle the ^{18}O isotope is two neutrons heavier than ^{16}O and forms a water molecule heavier by that amount. Moreover, the process of vaporization needs more energy to vaporize $H_2{}^{18}O$ than $H_2{}^{16}O$. On the contrary $H_2{}^{18}O$ liberates more energy in the condensation process. In addition, $H_2{}^{16}O$ diffuses more rapidly.

Thanks to the lower amount of energy required by $H_2{}^{16}O$ to vaporize, the first water vaporized is the one formed by the ^{16}O isotope, while the residual is enriched in $H_2{}^{18}O$ (Figure 4.3).

Rainfall precipitations are generally due to water condensation as a result of an air mass moving from a warm region to a cold region. What happens during the rainfall is that, first of all, the precipitation removes $H_2{}^{18}O$, leaving progressively more $H_2{}^{16}O$ -rich water vapor. This process is called distillation and causes precipitations characterized by lower $^{18}O/^{16}O$ as the temperature decreases. Some environmental factors can further modify the efficiency of distillation, such as the direct precipitation of ice at lower temperatures. In the case of hurricanes, the $H_2{}^{18}O$ is usually exhausted relative to the $H_2{}^{16}O$, resulting in low $^{18}O/^{16}O$ ratios. This shows how the analysis of rainfall deposited in trees after a hurricane can be useful to create a historical record of environmental climate. Furthermore, $^{18}O/^{16}O$ ratio can provides a record of ancient water temperatures. Carbon isotopes ^{12}C and ^{13}C, moreover, are usually measured as the $\delta^{13}C$ values in paleoclimate reconstructions (Kohn, 2010; VanDeVelde et al., 2013).

Thus, the analysis of the variation in stable isotope composition can provide information about the chemical, biological and geographical origins of substances.

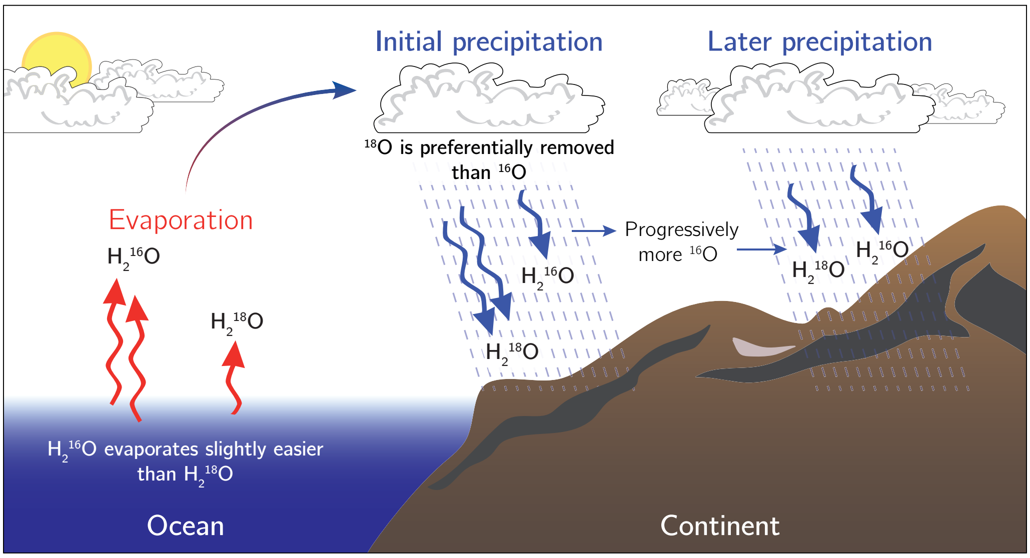


Figure 4.3: The oxygen and hydrogen cycles are the biogeochemical cycle that describe the movement of oxygen and hydrogen within their three main reservoirs: the atmosphere (air), the biosphere (the global sum of all ecosystems), and the lithosphere (Earth's crust).

Technical notation: Commonly the δ-value is the expression of stable isotope ratios (Brand and Coplen, 2012). This notation, introduced in the late 1940s, is defined as the relative difference between the isotope ratio of the sample and the international reference material. This difference is usually expressed in parts per thousand (‰, per mil) between the isotope ratio of the sample $R(\frac{^hE_c}{^lE_c})$ and the international reference material $R(\frac{^hE_{ref}}{^lE_{ref}})$ The equation for calculating $\delta^h E_{c,ref}$ is as follows:

$$\delta^h E_{c,ref} = \frac{(R(^h E_c) - R(\frac{^h E_{ref}}{^l E_{ref}})}{R(\frac{^h E_{ref}}{^l E_{ref}})}$$

The V-PDB (Vienna Pee Dee Belemnite) standard is used for stable carbon isotope analysis, while the V-SMOW (Vienna Standard Mean Ocean Water) is used for hydrogen and oxygen. The equation for stable carbon isotopes is as follows:

$$\delta^{13} C_{s,VPDB} = \frac{R(\frac{^{13}C}{^{12}C})_s}{R(\frac{^{13}C}{^{12}C})_{VPDB}} - 1$$

The next one is the equation for stable oxygen isotopes:

$$\delta^{18} O_{s,SMOW} = \frac{R(\frac{^{18}O}{^{16}O})_s}{R(\frac{^{18}O}{^{16}O})_{SMOW}} - 1$$

4.2.2 Wine and stable isotopes

In the natural environment, phenomena can lead to measurable changes in the ratio of the isotopes of a given element. The fractionation of the isotopes can be due to very different kinds of natural reaction. The determination of isotope ratios is a powerful instrument to study the provenance of vegetables, trees and fruits and therefore also of derived products such as wine (Camin et al., 2015; Camin et al., 2013; Ogrinc et al., 2009; Dordevic et al., 2013; Bizjak Bat et al., 2012). Moreover, Hydrogen and Oxygen isotope ratios heavily depend on latitude, and can be used as reliable geographic indicators (Camin et al., 2015). Carbon and Nitrogen are generally used to find information about the plant type or animal diet (DeNiro and Epstein, 1981).

Grapevine roots uptake soil water from any depths down to a few meters, but it is not clear which zone of the grape root absorbs the most water (Caldwell, 1995). However, several studies on vineyards show that the majority of roots are found in the top meter of soil (Vitic, 1981).

Plant water status depends on the availability of soil moisture and on the evaporation rate. During most of the year cycle, the grapevine experiences negative water potential created by transpiration "pull". This is not the case in early spring when root pressure is positive following rainfall or irrigation (Caldwell, 1995; Coombe, 1986).

Isotopic fractionation does not occur during water uptake by plants. Thus, the isotopic composition of plant roots and stem water can reflect the isotopic composition of local soil water and ground water (White et al., 1985; Flanagan and Ehleringer, 1991; Caldwell, 1995).

During plant respiration, the process of isotopic fractionation has been described as a process of photosynthesis by Park and Epstein, 1960 and by O'Leary, 1981. The assimilation of carbonic gas by plants occurs through two specific kinds of metabolism presenting different types of isotope fractionation:

- Calvin cycle: metabolism C_3;
- Hatch and Slack: metabolism C_4 (Hatch and Slack, 1970).

Additives derived from C_4 plants and fermentation, e.g., cane sugar and alcohol, present higher levels of ^{13}C than grapevine, which belongs to the C_3 group. As grapevine belongs to the C_3-type, its photosynthesis lacks the high water-use efficiency usually found in the photosynthesis of C_4 plants (Mullins et al., 1992; Sage and Kubien, 2007), although the maximum rates of photosynthesis in *Vitis vinifera L.* leaves are among the highest values reported for C_3 plants (Caldwell, 1995). Measuring the ^{13}C contents in wine samples enables the detection and quantification of C_4 sugars, which can be illegally added during the production.

One important study comes from de Souza et al., 2005, they found that the response to deficit irrigation varies with the environmental conditions of the particular year, the driest conditions exacerbating the differences among treatments. The highest values of $\delta^{13}C$ found in the pulp of some vines (NI vines in Castelão) compared with other ones (Moscatel) suggested different sensitivities to water deficits in the two cultivars.

The ^{18}O enrichment of grape water is known to be caused by the process of evapotranspiration. The latter is directly related to the temperature and relative humidity of the surrounding water vapor experienced by the grapes during the growing season (Bricout, 1978).

Dongmann et al., 1974 assumed, with a 95 % probability, the existence of a linear correlation between the $H_2\,^{18}O$ enrichment in the leaves and the relative humidity and temperature of the air.

Camin et al., 2015 showed the relationship between $(D/H)_I$, $(D/H)_{II}$, and $\delta^{13}C$ of wine ethanol, $\delta^{18}O$ of wine water. This relationship was visible after adding several variables describing the climate and geography to identify the production area in Italy. After collecting about 4000 wines over 11 years, and considering all the isotopic parameters, Camin et al., 2015 showed that $\delta^{18}O$, followed by $(D/H)_I$ present the strongest correlation with climate and location.

The other dominant variables were latitude, with a negative relationship and $\delta^{18}O$ and $\delta^{2}H$ of precipitation and temperature, both with a positive relationship.

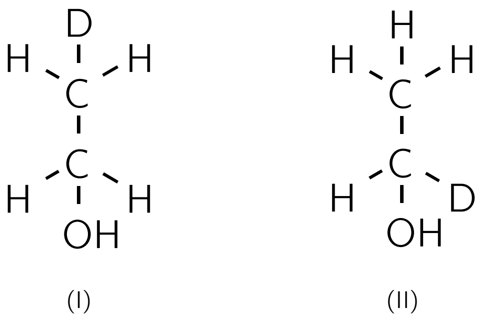
4.2.3 Deuterium in ethanol

Alcoholic fermentation, also known as ethanol fermentation, is the anaerobic pathway carried out by yeasts in which simple sugars are converted into ethanol and carbon dioxide. Yeasts generally work under aerobic conditions, i.e. in the presence of oxygen, but are also capable of functioning under anaerobic conditions, i.e. in the absence of oxygen. When no oxygen is readily available, alcohol fermentation occurs in the cytosol of yeast cells (Ribéreau-Gayon et al., 2006).

After the fermentation of must in wine, the deuterium of sugars is distributed into four molecules:

1. CH_2DCH_2OH
2. CH_3CHDOH
3. CH_3CH_2OD
4. HOD

The molecules of interest for environmental behaviors are 1 and 2 ethanol isotopomers (Figure 4.4), on the methyl and methylene sites respectively.



Methyl site *Methylene site*

Figure 4.4: Ethanol isotopomers, (I)$(D/H)_I$ and (II)$(D/H)_{II}$.

Notation Regarding the deuterium in ethanol, the fundamental definition is:

$(D/H)_I$ = the isotope ratio associated with the molecule 1;

$(D/H)_{II}$ = the isotope ratio associated with the molecule 2.

$$R = \frac{2(D/H)_{II}}{(D/H))_I}$$

R expresses the relative distribution of deuterium in molecules 1 and 2 and can be measured directly from the intensities h (peak heights) of the signals ($R=3h_{II}/h_I$).

Camin et al., 2015 reported general values for stable isotopes in European wines from different zones (Table 4.2).

Table 4.2: Values of range for stable isotopes in European wines by Christoph et al., 2015.

Wine growing zone Europe	Central Europe	Southern Europe
$\delta^{18}O$ ‰ V-SMOW water	-4 to +3	-1 to +6
$(D/H)_I$ ppm ethanol	97 to 103	99 to 105
$(D/H)_{II}$ ppm ethanol	121 to 127	125 to 132
$\delta^{13}C$ ‰ V-PDB ethanol	-30 to -27	-27 to -24

Part II

Materials and methods

Chapter 5

The wine making process

The particulars of winemaking vary from winery to winery, but the broad guidelines are always the same. The production process of the studied wines was the same for all the samples. The Italian wines were all produced in the vinery of the Centro per la Sperimentazione in Vitivincoltura of the Verona District by a standard protocol of microvinification.

A standard protocol was used also for the Slovenian wines.

5.1 Samples description

A summary table for all the samples is reported in Appendix A.

5.1.1 Italian wines

The CRA-VIT produced the vintages 2009, 2010, 2011 and 2012 from 9 fields of the Valpolicella area (Verona, Italy). Wines from one additional field, in the area near Negrar (Vr), were also used for the analysis. For this last field, wines from grapes of three different kind of plants were taken into account (Cabrusina, Boschera and Dindarella).

Furthermore, the grapes of the vintages 2011 and 2012, for the 9 fields, were added to the bank samples (Table 5.2). Because of the small amounts of 2012 samples, the analysis of TE and REE was carried out only for the vintages 2009, 2010, 2011. Table 5.1 describes the Italian wines used in this study and the analyses carried out for each sample.

Thanks to Dott. Giuseppe Benciolini's geo-pedological description of the Valpolicella area in Tomasi and Battista, 2014, we were able to carry out the sampling and analysis of soil samples. The accurate descriptions of the soil profile allowed us to select the soils in contact with the vine roots, which were the most relevant for this work. A total of 19 samples were analyzed.

A brief soil description is reported in section 3.2.

The studied soils are shown in Table 5.3 and the relative depths in 5.4.

Table 5.1: Studied wine samples produced by CRA.

Vintage	**Fields**	**Number of Samples**	**ICP-MS**	**IRMS**
2009	1-10	12	*TE* *REE*	$\delta^{13}C$ in ethanol, $\delta^{18}O$ in wine, $(D/H)_I$ & $(D/H)_{II}$ in ethanol
2010	1-10	12		
2011	1-10	12		
2012	1-10	11	not analyzed	$\delta^{13}C$ in ethanol, $\delta^{18}O$ in wine, $(D/H)_I$ & $(D/H)_{II}$ in ethanol

Table 5.2: Studied Italian must samples.

Vintage	Fields	Number of Samples	ICP-MS	IRMS
2011	1-9	9	*TE* *REE*	δ^{13}C in pulp δ^{18}O in water juice δ^{13}C in must sugar
2012	1-9	9		

Table 5.3: Studied soils from the 9 Italian fields.

Vintage	Fields	Number of Samples	ICP-MS	IRMS
2012	1-9	19	*TE* *REE*	not analyzed

Table 5.4: Studied soils from the 9 Italian fields.

Code	Field	Depth (cm)
P1A	1	0-40
P1B	1	40-80
P2A	2	0-30
P2B	2	30-60
P3A	3	0-35
P4A	4	0-20
P4B	4	20-40
P5A	5	0-50
P5B	5	50-80
P5C	5	80-100
P6A	6	0-35
P6B	6	35-75
P6C	6	75-120
P7A	7	0-30
P7B	7	30-60
P8A	8	0-30
P8B	8	30-60
P9A	9	0-40
P9B	9	40-90

5.1.2 Slovenian wines

Section 3.3 reports the three different grapevine regions studied in Slovenia. The wines were made by the Slovenian Institute of Agriculture for three vintages: 2011, 2012 and 2013 (Table 5.5). As we were unable to retrieve the same Italian vintages of wines, the only common vintage is the year 2011.

These wines comprised both red and white varieties, but for the purpose of our study knowing the grapevine of provenance is irrelevant.

Table 5.5: Studied wines produced by Agriculture institute of Slovenia in the three regions Primorska, Posavje, Podravje.

Vintage	**Regions**	**Number of Samples**	**ICP-MS**	**IRMS**
2011	3	21	TE REE	not analyzed
2012	3	22		
2013	3	21		

Chapter 6

Chemical analyses

All mass spectrometry analyses were carried out in the laboratories of Ca' Foscari University of Venice and of CNR-IDPA (National Research Council - Institute for the Dynamics of Environmental Processes).

All stable isotope analyses of wine and must were carried out in the laboratories of the Group for the Isotope Geochemistry at the Department of Environmental Sciences at the Jožef Stefan Institute of Ljubljana (Slovenia).

Wines were distilled in the laboratories of the Agriculture Institute of Slovenia in Ljubljana.

Additional analyses of D/H in wine ethanol using SNIF-NMR were performed in the laboratories of the National Institute of Chemistry of Ljubljana.

Samples conservation: Wine samples were stored into opaque glass bottles (0.75 L) right after production. These bottles, easy available on the market, were stopped by classic crown corks. The musts samples were stabilized by means adding benzoic acid and then stored into smaller dark-glass bottles (0.25 L).

All the bottles were stored at room temperature, avoiding direct sun exposure.

Soil samples were inserted in small Teflon™ cans and stored at room temperature, right before the analysis.

6.1 Mass spectrometry

6.1.1 Inductively Coupled Plasma Mass Spectroscopy

ICP-MS technique is a very sensitive and powerful analytical method used to analyze trace and ultra-trace elements. The principle of this technique is that ionized or excited atoms produced by the application of a high-temperature argon plasma to the samples are separated and identified according to their mass to charge ratios (m/z).

The main components of ICP-MS and the specific characteristics of the instruments used for this work are briefly described in the following sections (Taylor, 2001; Beauchemin, 2006). In particular, the modern ICP-MS instrument incorporates:

I. a sample introduction system;

II. a plasma source;

III. an interface region;

IV. a vacuum system;

V. an ion focusing system;

VI. a mass analyzer and a detector.

6.1.2 Instrumentation

In this PhD project samples were analyzed by two different mass spectrometry instruments. The first one was a quadrupole mass analyzer ICP-MS Agilent 7500 (Agilent Technologies) and was used for must and soil analyses.

For wine analyses, a double focusing magnetic sector field Thermo Scientific™ ELEMENT2™ (ICP-SFMS, Thermo Scientific, Bremen, Germany) was used.

In the next sections the different parts of the ICP-MS instruments will be briefly explained, along with the stages of sample analysis.

Sample introduction system This part of the instrument consists of an autosampler, a nebulizer and a spray chamber. In the sample introduction system, the liquid sample is converted to aerosol and introduced into the plasma. This is performed by a nebulizer used in conjunction with a spray chamber. The sample is pumped into the nebulizer through a peristaltic pump, which ensures a constant flow.

Figure 6.1: Picture of the ICP-MS Agilent 7500cx (left) and Thermo Element2 (right).

For this work we used the v-groove, a pneumatic nebulizer which uses the mechanical forces of the Ar gas flow to shatter the liquid of the sample and turn it into aerosol. This nebulizer is well suited to samples containing high levels of particulate matter. It therefore met our needs, as our wine samples were not filtered and may have caused obstruction of the tubes.

As the plasma discharge cannot efficiently dissociate large droplets, the main function of the spray chamber is to select the particles that can enter into the plasma itself. The aerosol is directed into the central tube of the spray chamber where the larger droplets fall out by gravity and exit through the drain tube. The finest droplets (diameter 5-10 µm) are transported into the sample injector of the plasma torch.

In the Sector Field ICP-MS (ICP-SFMS) analysis, an APEX™ (ESI, Omaha, US; Figure 6.2) desolvation unit was used as introduction system. This desolvation unit allows one to remove as many water particles as possible before the introduction into the plasma, resulting in the removal of oxides and of a few interfering species and in an improvement of the ionization efficiency.

Plasma source After going through the nebulizer and the spray chamber, the sample is carried to the torches where plasma is generated. The plasma is a highly ionized inert gas, in this case Argon (the temperature reaches 6000 to 10000 K). The torches are usually made of quartz and are connected to a radio-frequency generator.

The radio-frequency power is used to produce and maintain the plasma and is generated by means of a magnetic field induced through a water-cooled copper coil. Its main role is to control the ICP source and the generation of RF power to create a RF signal at 27 MHz. The application of the RF power creates an intense electromagnetic field. When Ar flows through the torch, some electrons are stripped from their Ar atoms. These electrons, caught up and accelerated in the magnetic field, collide with other atoms, stripping off

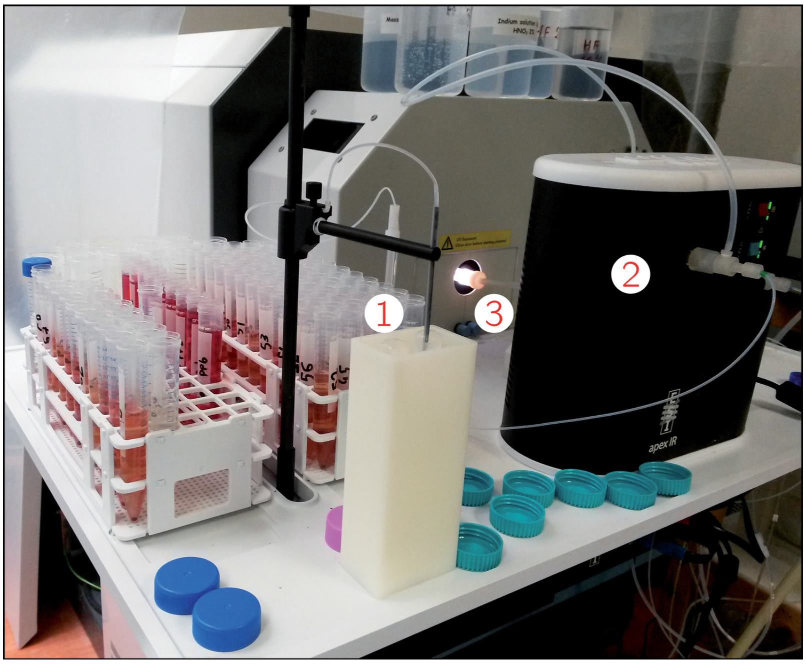


Figure 6.2: ICP-SFMS, setup for wine sample analysis : Autosampler (1), APEX™ (2), Plasma torch (3).

more electrons. The ensuing collision-induced ionization of the Ar continues as a chain reaction and breaks down the gas into Ar atoms, Ar ions and electrons: this is known as inductively coupled plasma discharge.

Interface region The mass analyzer region requires vacuum for its optimal operation, while the ICP operates at atmospheric pressure. Because of these different pressure requirements, there exists an interface region that transfers the ions from the plasma to the mass spectrometer through several steps of pressure reduction. The interface region consists of two metallic cones with small orifices: a sampler and a skimmer cone.

Vacuum system The maintenance of high-vacuum conditions is required for optimal operations. Only in vacuum conditions can one increase the length of the mean free path of the ions and reduce the background and the scattering effects that a high level of residual gas molecules would cause.

Ion focusing system The focusing system is characterized by ion lenses positioned between the skimmer cone and the mass analyzer.

The role of the focusing system is to focus and transfer to efficiently selected analyte ions from the interface region to the mass spectrometer and to reject the maximum amount of matrix components, neutral species and photons, as they tend to cause signal instability.

Mass analyzer and detector The quadrupole mass analyzer is a sequential mass filter, that is able to separate ions based on their mass to charge ratio (m/z) (Taylor, 2001). It is characterized by two pairs of parallel rods to which high frequency and different AC and DC voltages are applied. The voltages produce a dynamic hyperbolic electric field where the ions can traverse a trajectory and reach the detector, while the ions entering into an unstable trajectory can be removed by colliding with the rods.

Unlike the quadrupole mass analyzer, the sector field mass analyzer consists of two analyzers: an electromagnet (or magnetic sector) and an electrostatic analyzer.

The detectors used were electron multiplier devices, able to generate a measurable signal pulse from the impact of a single ion.

Resolution in ICP-MS The resolution of a mass spectrometer is defined as a measure of its ability to separate adjacent mass regions in the mass spectrum. Considering two peaks in the mass spectrum having a mean mass m, and the separation of the peaks is Δm, the resolving power of the mass spectrometer R, i.e. the parameter describing the resolution, is given by the equation:

$$R = \frac{m}{\Delta m}$$

Quadrupole analyzers typically are able to separate integer masses ($\frac{m}{\Delta m}$ of approximately 300-400), while sector field mass analyzers can achieve a resolution up to 10000. Accordingly, sector fields are able to resolve most polyatomic species from analytes at the same nominal mass.

Due to the different approaches for the pre-treatment of wines, musts and soils (discussed later in section 6.1.3), wines were simply diluted while musts and soils were digested in a microwave. Wines presented very low concentrations of elements, in particular regarding some REEs. We therefore decided to use a sector field instrument for these kinds of measurements.

6.1.3 Methodology

The must and wine samples were analyzed according to the OIV methods (OIV-Oeno 334-2010) "Multielemental analysis using ICP-MS" (OIV-Methods, 2015).

The starting assumption of this methodology is that wine samples do not require a preventive digestion with acids in a microwave; on the contrary, this procedure is suggested for less homogeneous matrices such as grapes and musts. For the wine samples a pre-analysis was carried out to understand the order of the elements' concentration and the dilution (water milliq and 2 % of HNO_3 ultrapure quality) required for the analysis via ICP-MS. Acid concentration in the final dilution of the samples was the same as in the internal standard.

Sample pre-treatment

Must and soil samples were dissolved in closed-pressurized digestion vessels in a microwave oven Milestone-Ethos1. To homogenize the must, we used an electric mixer before acid digestion.

Regarding musts, about 1 mL (±1.30-1.50 g) of each sample was digested in 9 mL of a acid mixture (2 mL H_2O_2, 1 mL HCl, 6 mL HNO_3) via Milestone Ehos1 Microwave (Figure 6.3 A). Aliquots of about 0.200 g of soil samples were digested adding the same acid mixture.

The programs of the two digestions are reported in Figure 6.3 B.

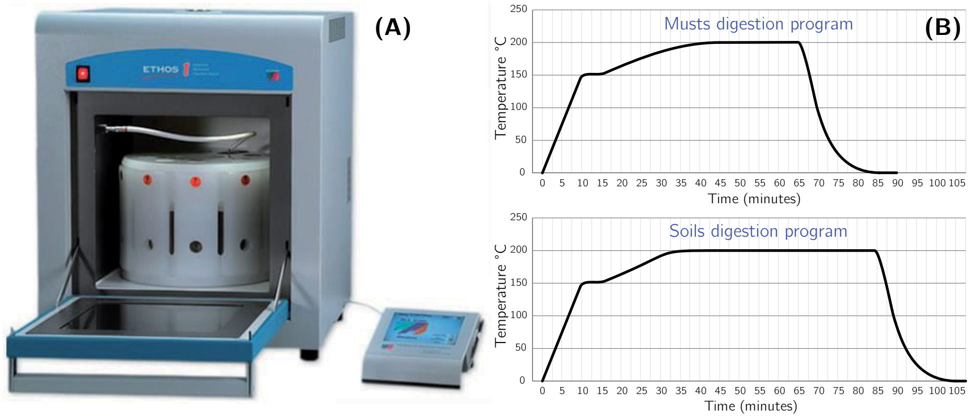


Figure 6.3: Milestone Ehos1 Microwave (A) and adapted program from OIV methodology for digestion with microwave digester for musts and soils (B).

The digested mixture, presenting no residual solid matter, was diluted with acidified milliq water (2 % HNO_3) for ICP analysis at a 1:5 ratio for both musts and soils.

The contents of the digestion vessels were quantitatively transferred into graduated 50 mL polypropylene tubes, which were filled to the mark with high-purity MilliQ water.

According to the OIV methods, wine samples were only diluted 1:9 with acidified milliq water HNO_3.

The analyses were carried out using ultrapure demineralized water with resistivity (≥ 18 MΩ), in accordance with ISO 3696.

Procedure of analysis

The procedure is the one described in the OIV methods (OIV-Methods, 2015) and widely adopted in other works.

The software of the instrument can calculate the results directly. Interpolating the calibration curves allows one to determine the concentration of the elements in the diluted samples. The following equation was used to calculate the concentration of the elements in the sample:

$$C = \frac{C_m \times V_t}{V_m}$$

Where:

- C = Concentration of the element in the sample;
- C_m = Concentration of the elements in the diluted sample;
- V_t = Final volume of the measurement solution, in mL;
- V_m = Aliquot volume of wine, in mL.

ICP setting up The concentrations of 42 elements were determined directly. In particular all the isotopes were: ^{7}Li, ^{24}Mg, ^{27}Al, ^{39}K, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{51}V, ^{52}Cr, ^{53}Cr, ^{55}Mn, ^{56}Fe, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{69}Ga, ^{75}As, ^{78}Se, ^{82}Se ^{85}Rb, ^{88}Sr, ^{89}Y, ^{107}Ag, ^{109}Ag, ^{111}Cd, ^{112}Cd, ^{114}Cd, ^{133}Cs, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{151}Eu, ^{153}Eu, ^{156}Gd, ^{157}Gd, ^{158}Gd, ^{159}Tb, ^{162}Dy, ^{163}Dy, ^{164}Dy, ^{165}Ho, ^{166}Er, ^{168}Er, ^{169}Tm, ^{171}Yb, ^{172}Yb, ^{173}Yb, ^{174}Yb, ^{175}Lu, ^{205}Tl, ^{206}Pb, ^{208}Pb, ^{232}Th, ^{238}U.

Instrumental ICP-MS parameters are described in Table 6.1. Appendix E presents an example of ICP-MS tuning.

The Agilent 7500 was equipped with an inlet system consisting of a V-groove nebulizer and a water cooled (4 °C) Scott-type spray chamber.

ELEMENT2 allows to change the mass resolution by altering the width of the entrance and exit slits in three different settings: low resolution ($\frac{m}{\Delta m}$=400),

Table 6.1: Operating conditions for Agilent 7500cx ICP-MS.

Plasma Conditions	
RF power	1500 W
RF Matching	1.74 V
Carrier gas	1.15 L/min
Make Up gas	0.1 L/min
S/C temperature	2°C
Ion lenses	
Extract 1	0 V
Extract 2	-132.5 V
Omega bias-ce	-22 V
Omega lens-ce	-0.6 V
Cell entrance	-30 V
QP focus	3 V
Cell exit	-40 V

medium resolution ($\frac{m}{\Delta m}$=4000) and high resolution ($\frac{m}{\Delta m}$=10000). The chosen isotopes for wine analyses were:

- Low resolution mode: ^{7}Li, ^{9}Be, ^{111}Cd,^{138}Ba, ^{205}Tl, ^{208}Pb, ^{238}U, ^{133}Cs, ^{23}Na, ^{85}Rb, ^{209}Bi, ^{107}Ag, ^{109}Ag, ^{139}La, ^{140}Ce, ^{141}Pr, ^{142}Nd, ^{144}Nd, ^{148}Nd, ^{147}Sm, ^{150}Sm, ^{152}Sm,^{151}Eu, ^{153}Eu, ^{155}Gd, ^{157}Gd, ^{158}Gd, ^{160}Gd, ^{159}Tb, ^{161}Dy, ^{162}Dy, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{167}Er, ^{168}Er, ^{169}Tm, ^{171}Yb, ^{172}Yb, ^{174}Yb, ^{175}Lu, ^{232}Th;

- Medium resolution mode: ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{64}Zn, ^{69}Ga, ^{205}Tl, ^{65}Cu, ^{66}Zn, ^{88}Sr, ^{44}Ca, ^{85}Rb;

- High resolution mode: ^{75}As, ^{39}K, ^{80}Se, ^{82}Se.

Regarding the introduction system of the ICP-SFMS, as reported in the previous sections, the autosampler was connected to an APEX™ (ESI, Omaha, US) desolvation unit in order to remove as much water as possible before ionization in the plasma.

Wine samples were just diluted and for the complexity of the matrix were created 50 samples sequences (+ Calibrations) for each analytical session. The connection tube from Apex to the torch threatened to clog due to the solid matter present in the samples. Thus, we removed and cleaned the tube and the Apex before starting a new analysis. The cleaning of the Apex contributed to stabilize the sensitivity of the instrument.

Calibration

The quantification of analytes was done thanks to an external calibration. Certified Multi-elemental solutions diluted in acidified Milli-Q® (Millipore Corporation) water in different concentrations were used for both ICP-MS and ICP-SFMS. Two different 10 mg L^{-1} mother solutions were prepared by diluting 1000 mg L^{-1} multi-elemental standard solutions (ULTRA Scientific, Inc.):

I. IMS-102 containing Ag, Al, As, Ba, Be, Bi, Cd, Ca, Cs, Cr, Co, Cu, Ga, Fe, In, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn;

II. IMS-101 containing Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Sc, Tb, Th, Yb, Y.

Calibrations were performed using 7 dilutions of calibration standards for each multi-elemental standard in order to cover for each element the ranges present in must, soil and wine samples. Regarding wines, the only samples that were not digested, the external calibration was prepared by diluting the multi-elemental solution in a wine sample in order to create a calibration solution similar to the wine matrices.

For must samples calibrations ranged from 0.05 to 50 µg L^{-1} (IMS-101) and from 0.05 to 500 ppb (IMS-102), for soils sample from 0.01 to 100 µg L^{-1} (IMS-101) and from 0.05 to 100 µg L^{-1} (IMS-102), and for wines from 0.01 to 100 µg L^{-1} (IMS-101) and from 0.05 to 100 µg L^{-1} (IMS-102).

In the three matrices, the element concentration levels vary greatly, but the chosen ranges of dilution for the calibration were perfectly suited to calculate the concentrations.

A 10 µg L^{-1} Rh solution (ULTRA Scientific, 1000 mg L^{-1}) was continuously mixed with the sample flow into the ICP-MS nebulizer. The Rh signal was then used as internal standard to correct any instrumental drift or plasma fluctuation.

Linear regression was used to fit the intensity of standard solutions and the y-axis intercept at zero concentration was subtracted for the calibration (average blank of the standards). For all the elements and matrices a fitting $R^2 \geq 0.99$ was obtained.

Procedural blanks and detection limits

The concentrations of the elements in ultrapure water were similar to the unspiked standard used for the calibration, and below the most diluted standard. This means that the use of ultrapure water for the sample preparation, digestion and dilution, and for blank preparation was justified. The detection limits for each element were calculated as the concentration corresponding to 3 times the standard deviation of the measurement of 7 to 10 acidified blank solutions, depending on the analyses.

6.1.4 Accuracy and reproducibility

Regarding soil samples, the Standard Reference Material 2711a, Montana II Soil was used as standard material (SRM) (Appendix B).

The mean value of precision for all the tests (10) is reported in Table 6.3. Results showed quite precise data according to the certified values. The precision was generally 10 % while some elements resulted exceeding. For example, Se precision was 71.4 % but the certified concentration was quite low and the concentration was similar.

The reproducibility of the methods was tested by digesting or by simply diluting (depending on the matrices), and by analyzing replicate samples (n=3). Replicate analyses revealed a good agreement for all elements, with standard deviations always better than 21.8 %. The results of the reproducibility tests were consistent with the precision obtained with SRM samples, indicating no significant contribution to the total uncertainty due to the matrix variability.

Regarding must and wine reference materials as standards were not commercially available. So, for wines and musts, precision was calculated with the repetition of the same samples few times during the analysis. For both the matrices precision was very good, generally of 10-15 % and better than 25 %.

In Appendix C were reported three examples of reproducibility for soil, must and wine.

Table 6.2: Summary table for ICP-SFMS and ICP-MS average blank and Detection Limits.

	ICP-SFMS			**ICP-MS**		
	Average Blank µg L^{-1}	St. dev	DL	Average Blank µg L^{-1}	St. dev	DL
Li	0.021	0.003	0.01	0.099	0.027	0.099
Be	0.003	0.001	0.0039	N.M.	N.M.	N.M.
Na	4.923	0.756	2.27	N.M.	N.M.	N.M.
Mg	N.M.	N.M.	N.M.	27.801	2.231	8.197
Al	0.233	0.024	0.07	3.289	0.303	1.112
K	0.012	0.006	0.02	49.676	1.528	5.615
Ca	0.145	0.020	0.06	47.566	3.365	12.362
V	0.005	0.001	0.004	0.011	0.002	0.009
Cr	0.010	0.002	0.01	0.068	0.004	0.021
Mn	0.034	0.018	0.05	0.098	0.001	0.005
Fe	0.104	0.023	0.07	41.268	0.803	2.952
Co	0.005	0.002	0.01	0.007	0.002	0.008
Ni	0.065	0.007	0.02	0.120	0.008	0.029
Cu	1.561	0.116	0.35	0.357	0.042	0.155
Zn	0.370	0.071	0.15	0.404	0.018	0.067
Ga	0.004	0.002	0.01	0.013	0.002	0.008
As	0.033	0.033	0.10	0.165	0.010	0.035
Se	0.217	0.046	0.46	10.851	0.399	1.465
Rb	0.031	0.016	0.05	0.024	0.008	0.029
Sr	0.019	0.004	0.01	0.543	0.035	0.128
Y	N.M.	N.M.	N.M.	29.332	20.235	0.002
Ag	0.008	0.002	6.12E-03	0.006	0.002	0.008
Cd	0.005	0.001	4.41E-03	0.005	0.001	0.005
Cs	0.003	0.001	2.88E-03	0.007	0.003	0.012
Ba	0.019	0.003	0.01	N.M.	N.M.	N.M.
La	4.46E-05	5.19E-05	1.56E-04	2.26E-03	6.39E-04	2.35E-03
Ce	2.47E-05	3.91E-05	1.17E-04	2.88E-03	7.22E-04	2.65E-03
Pr	8.23E-06	1.05E-05	3.14E-05	9.50E-04	2.71E-04	9.97E-04
Nd	3.26E-05	4.32E-05	6.06E-05	1.83E-03	3.72E-04	1.37E-03
Sm	6.86E-06	5.6E-06	1.68E-05	1.33E-03	2.60E-04	9.56E-04
Eu	3.11E-06	3.62E-06	1.09E-05	8.41E-04	2.43E-04	8.92E-04
Gd	1.34E-05	1.29E-05	1.87E-05	1.23E-03	3.38E-04	7.60E-04
Tb	1.62E-06	1.79E-06	5.38E-06	7.24E-04	2.39E-04	8.78E-04
Dy	7.98E-06	1.21E-05	3.40E-05	9.38E-04	1.37E-04	6.81E-04
Ho	1.99E-06	3.07E-06	9.22E-06	6.34E-04	1.90E-04	6.99E-04
Er	6.16E-06	9.65E-06	3.16E-05	7.95E-04	1.56E-04	5.66E-04
Tm	9.81E-07	1.38E-06	4.14E-06	5.96E-04	1.91E-04	7.02E-04
Yb	7.47E-06	1.16E-05	3.89E-05	9.78E-04	1.41E-04	4.78E-04
Lu	1.49E-06	2.42E-06	7.27E-06	5.72E-04	1.65E-04	6.06E-04
Tl	0.0045	0.0015	4.53E-03	4.50E-03	1.90E-03	0.007
Pb	0.078	0.008	0.025	0.014	0.001	0.004
Bi	0.004	0.0003	8.81E-04	N.M.	N.M.	N.M.
Th	4.67E-06	5.26E-06	1.58E-05	0.007	0.005	0.018
U	0.003	0.001	3.17E-03	0.004	0.002	0.007

N.M. = Not measured.

Table 6.3: Accuracy and precision of ICP-MS soil measurement according to SRM 2711a, Montana II Soil.

	SRM certified concentrations (µg kg^{-1})	**SRM found concentrations** (µg kg^{-1})	**Precision** (%)
V	80.7 ± 5.7	76.75 ± 2.8	4.9
Cr	52.3 ± 2.9	49.44 ± 0.7	5.5
Mn	675 ± 18	637.21 ± 17.4	5.6
Co	9.89 ± 0.18	9.28 ± 0.4	6.1
Ni	21.7 ± 0.7	20.21 ± 0.8	6.9
Cu	140 ± 2	128.24 ± 2.5	8.4
Zn	414 ± 11	376.25 ± 15.1	9.1
An	107 ± 5	102.25 ± 2.4	4.4
Se	2	3.43 ± 0.3	71.4
Rb	120 ± 3	88.84 ± 6.2	26.0
Sr	242 ± 10	231.38 ± 13.4	4.4
Ag	6	6.29 ± 0.2	4.8
Cd	54.1 ± 0.5	51.49 ± 1.4	4.8
Cs	6.7 ± 0.2	7.44 ± 0.5	11.1
La	38 ± 1	43.41 ± 1.6	14.2
Ce	70	79.52 ± 3.7	13.6
Nd	29 ± 2	27 ± 1.7	6.9
Sm	5.93 ± 0.28	6.38 ± 1	7.6
Eu	1.1 ± 0.2	1.07 ± 0.03	2.5
Gd	5	5.67 ± 0.8	13.3
Tb	0.8	0.89 ± 0.03	11.8
Dy	5	4.89 ± 0.2	2.3
Yb	3	2.29 ± 0.1	23.6
Lu	0.5	0.33 ± 0	33.8
Tl	3	2.88 ± 0.1	4.0
Th	15 ± 1	14.07 ± 0.7	6.2
U	3.01 ± 0.12	2.53 ± 0.5	15.8

6.2 Isotope Ratio Mass Spectrometry

6.2.1 Methodology

According to the OIV methods for $\delta^{13}C$ and δ^{18}O measurements (OIV-MA-AS312-06 "Determination by isotope ratio mass spectrometry ^{13}C/^{12}C of wine ethanol or that obtained through the fermentation of musts, concentrated musts or grape sugar" and OIV-MA-AS2-12 "Method for ^{18}O/^{16}O isotope ratio determination of water in wines and musts" respectively), the must and wine samples were analyzed (OIV-Methods, 2015).

6.2.2 Instrumentation

Stable oxygen isotope ratios were measured directly in using an IsoPrime™ IRMS and MultiFlow preparation system (IsoPrime, Cheadle, UK).

δ^{13}C was determined in must sugar and pulp, while δ^{13}C, SNIF-NMR analysis were performed in ethanol obtained after distillation.

Isotope Ration Mass Spectrometry

The Isotope Ratio Mass Spectrometry (IRMS) is a specific technique for isotope analysis at low enrichment and natural abundance levels. The analytic procedure is based on a few steps, the first of which is the conversion of the sample in a simple gas before introducing it into the mass spectrometer. For the most common elements, i.e. the ones of interest in this work, the gas conversions were CO_2 for both carbon isotope analysis and for oxygen isotope analysis.

The gas introduced into the spectrometer is ionized in an electron ionization source and separated by a magnetic sector. Usually the magnetic sector is set to single-field strength for the isotope analysis of one element. The ions are then collected by dedicated Faraday cups. In the case of carbon isotope analysis, IRMS instruments are built with three Faraday cups for the detection of m/z 44, 45 and 46. Each of these cups is connected to a separate amplifier, which has a different gain, so that output signals for different ions at natural abundance have similar intensity.

It is then possible to calculate the isotope ratio thanks to the peak area of the detected three ions derived from the sample and the lab working reference gas of CO_2.

A scheme of IRMS for stable carbon isotope analysis is shown in Figure 6.4.

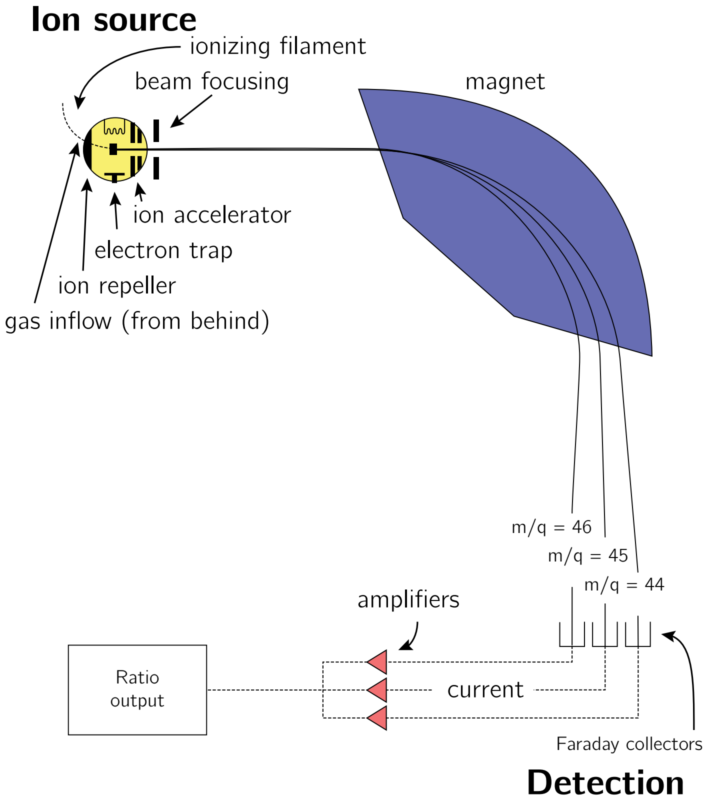


Figure 6.4: Schematic presentation of isotope ratio mass spectrometry for stable carbon isotope analysis.

Elemental analyzer

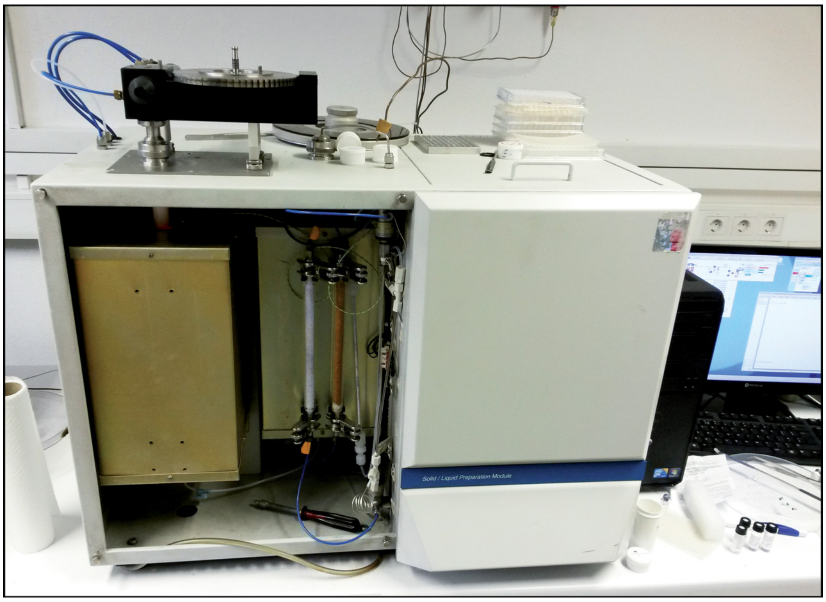


Figure 6.5: Solid/Liquid Preparation Module in the Department of Environmental Sciences, Jožef Stefan Institute (Ljubljana, SLO).

The Elemental Analyzer (EA/IRMS) with an isotope ratio mass spectrometer is a preparation system mainly used for bulk isotope analysis. There are many variants in the configurations of EA/IRMS for specific applications of isotopes analysis. However, they generally consists of an autosampler, an oxidation reactor, a reduction reactor, a water trap, a gas chromatographic column, a thermal conductivity detector, an open split and an IRMS system.

The measurement of the isotope ratio of one compound must be done with a isolation before the EA/IRMS analysis. After the conversion into gases in a high-temperature oven (around 1000 °C) and before entering the IRMS, there is the separation via gas chromatography.

The oxidation reactor column used was a quartz tube filled with catalyst of chromium oxide, copper oxide mixed with platinum, and silver wool (Figure 6.7).

The gases were carried by a gentle stream of helium into the reduction column where the excess of oxygen was removed by a filled copper at around 600 °C. This was followed by a water trap where the moisture was absorbed by a filled absorbent material. After the absorbent material, the gases were passed through a packed gas chromatographic column where they were separated.

Figure 6.6: Stable Isotope Ratio Mass Spectrometer (IRMS) in the Department of Environmental Sciences, Jožef Stefan Institute (Ljubljana, SLO).

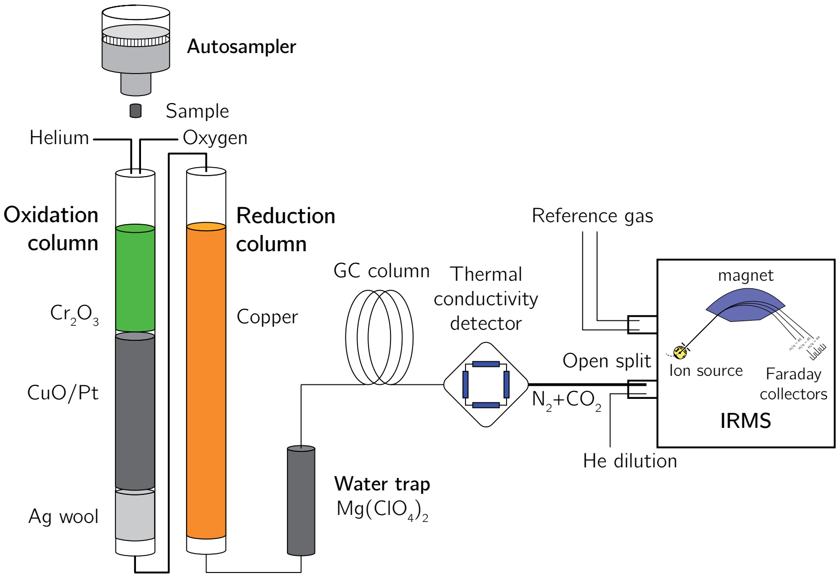


Figure 6.7: Schematic presentation of an EA/IRMS for carbon isotope analysis (Elemental Analyzer coupled with IRMS).

6.2.3 SNIF-NMR

Site-Specific Natural Isotope Fractionation by Nuclear Magnetic Resonance (SNIF-NMR) is a method mainly used to check the authenticity of wines, spirits, fruit juices, honey, sugar and vinegar (Dennis, 1998; Martin and Martin, 1991; Ogrinc et al., 2009; Košir et al., 2001). This method enables the highly precise quantification of the differences in isotopic content on each site of a molecule and the subsequent measurement of the specific natural isotope fractionation for each site of the molecule.

The SNIF-NMR method was officially adopted by the OIV and the European Union for wine analysis.

The principle of the SNIF-NMR is based on natural isotopic fractionation. For food authentication, two nuclei are normally used:

- H nuclei: ^{2}H-SNIF-NMR method was the initial application of SNIF-NMR, it measures the ratio of deuterium/hydrogen (D/H) on each site of a sample molecule, in our case of ethanol (Martin and Martin, 1991);
- Carbon nuclei: ^{13}C-SNIF-NMR method has opened new possibilities of analysis by SNIF-NMR (not used in this study).

6.2.4 Sample treatments and instrumental analysis

Different matrices were analyzed through different analytical procedures. In particular, Figure 6.8 shows a scheme of the analytical procedures for stable isotope measurements of wine and must.

Stable oxygen isotope ratios were measured using an IsoPrime™ IRMS and MultiFlow preparation system (IsoPrime, Cheadle, UK). The δ^{18}O values were measured directly in water or wine after equilibration with reference CO_2 (Epstein and Mayeda, 1953). The samples were equilibrated at 25 °C for 24 hrs before analysis.

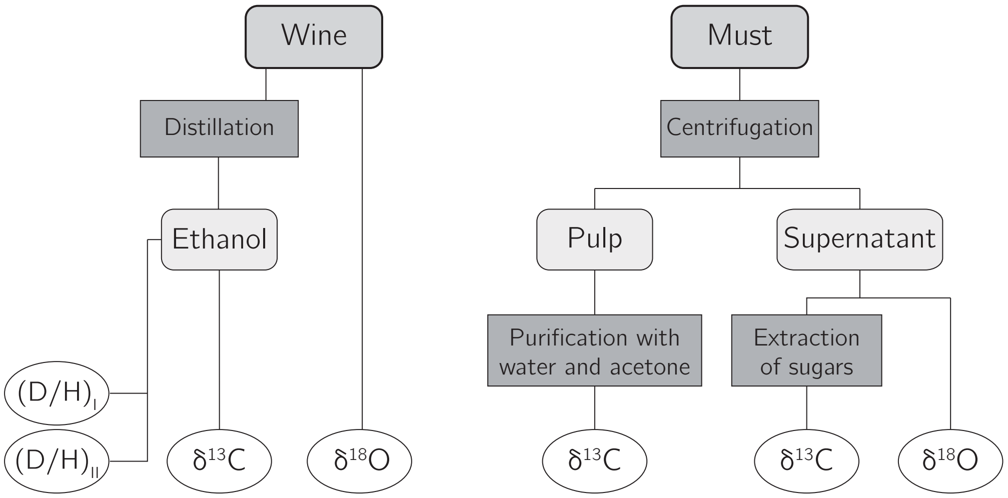


Figure 6.8: Scheme of the procedure for isotope measurements of wine and must.

Before the isotope ratio measurements approximately 5 µL of sample were inserted into tin capsules (SerCon, UK, Figure 6.9) which were already been filled with chromosorb (Chromosorb W 30–60 mesh, PDZ Europa Ltd, Northwick, Cheshire, UK). Chromosorb is an inert absorbent material useful to avoid losses of samples by means, for example, evaporation.

Wines were distilled by the Agriculture Institute of Slovenia and the distillations were analyzed of δ^{13}C and $(D/H)_I$ and $(D/H)_{II}$.

Ethanol was recovered by distillation (Automatic Distillation Control System - ADCS System, Eurofins, Nantes Cedex, France) made by Agricultural Institute of Slovenia. To control the distillate yield i.e., ≥90 %, the water content of the distillate was measured using a Karl Fischer titrator (Karl Fischer 787, Metrohm) and the alcohol grade using a densimeter (DMA-5000, Anton Paar, Austria).

δ^{13}C measurements in ethanol were performed manually using classical tin capsules according to a standard procedure described in Commission Regulation 440/2003, annex 2 (FAO, 2015). Briefly, in tin capsule we put ~1 mg of chromosorb and 1.0 mL of alcohol sample. The capsule was then closed with tweezers and put into the automatic sampler of the elemental analyzer. The analysis was performed on EA/IRMS with ANCA SL preparation system for solid and liquid samples.

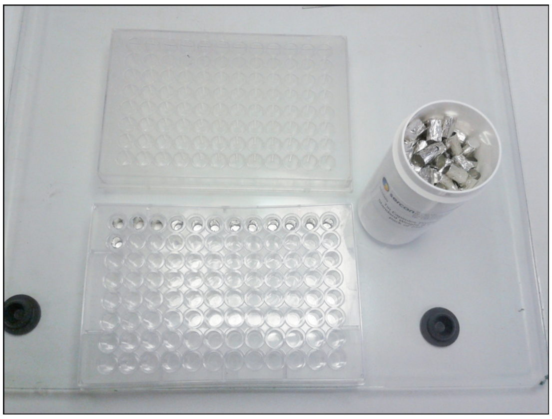


Figure 6.9: Tin capsule for isotope ratio measurements, SerCon, UK. Institute Jožef Stefan of Ljubljana (Slovenia).

$(D/H)_I$ and $(D/H)_{II}$ values were determined at National Institute of Chemistry Slovenia in Ljubljana on distillates following the standard method described in EU regulation 2676/90 for wines (EEC, 1999).

The ^2H NMR spectra were acquired using a Varian Unity plus 300 NMR spectrometer equipped with a 10 mm probe with fluorine lock. The instrument was operating for ^1H at the frequency of 299.9 MHz and at 46.0 MHz for ^2H. Was used the XWIN NMR™ 3.1 software for analysis and EUROSPEC (Eurofins, Nantes Cedex, France) software for the calculations.

The musts pre-analysis was different: the pulp was separated from the liquid fraction (supernatant) of 50 mL of juice by centrifugation (Tehtnica Centric 322a, 3200 rpm x 10 minutes). It was first resuspended in pure water, mixed and centrifuged and finally the resulting supernatant was discarded. The pulp was then washed with water and twice with acetone, moreover was dried at room temperature (Rossmann et al., 1997). Dried samples were then homogenized with a spatula and placed into tin capsules (SerCon, UK) for isotope ratio measurement of $\delta^{13}C$.

The analysis of $\delta^{18}O$ was carried out on the supernatant separated from the pulp. 5 µL of sample were inserted into the tin capsules which were already been filled with chromosorb.

The $\delta^{13}C$ (sugar) measurements was made thanks to the separation of the sugars from the supernatant and the purification by the method of Koziet et al., 1993 adapted in Bizjak Bat et al., 2012. Soluble substances remaining in the supernatant were purified by adding to the solution 2 mg of powdered calcium hydroxide while stirring well and heating it in a water bath at 90 °C for 3 minutes. The precipitate was separated by centrifugation of the hot solution (3400 rpm x 5 minutes) and the clear supernatant was decanted and acidified with 1 M sulphuric acid to pH=5 until the color of the solution became lighter. Residual calcium sulphate was then partially removed by storing the solution in a refrigerator at about 4 °C overnight.

Before the measurement, 100 µL of sample were placed into a tin capsule, already filled with chromosorb.

6.2.5 Accuracy and reproducibility

The results of stable isotope analyses in this study are reported using the δ notation as a difference in parts per mil of the isotopic ratios of $^{18}O/^{16}O$ and $^{13}C/^{12}C$ in the sample from those of the international reference materials VSMOW (Vienna Standard Mean Ocean Water) and oxygen, VPDB (Vienna Pee Dee Belemnite) for carbon (as reported in section 4.2).

The precisions of the measurements were as follows: ±0.1 ‰ for $\delta^{18}O$, ±0.2 ‰ for $\delta^{13}C$ in wine and must.

The certified reference materials (CRMs) and laboratory working standard (WS) used for stable isotope analysis are reported in Table 6.10. All δ values of CRMs are certified by the IAEA (IAEAwebsite, 2015). The δ values of laboratory working standards were determined in the Laboratory of Stable Isotope Geochemistry at the Department of Environmental Sciences (Jožef Stefan Institute of Ljubljana).

For deuterium NMR analysis of ethanol, N,N-tetramethylurea (TMU-Institute for Reference Materials) was used as the internal standard with a D/H ratio determined from the V-SMOW/SLAP scale, while a certified wine alcohol (CRM/BCR 656) from the IRMM (Institute for Reference Materials and Measurements) acted as a secondary internal standard. Each spectrum was recorded 10 times and the (D/H) ratios were obtained from the intensities of the deuterium signals corresponding to the methylene and methyl sites of ethanol and tetramethylurea.

The $(D/H)_I$ and $(D/H)_{II}$ isotope ratios are expressed in parts per million (ppm) on the international V-SMOW scale (Section 4.2.3). The average precision values of the measurements were ±0.5 ppm for $(D/H)_I$, ±0.6 ppm for $(D/H)_{II}$, and ±0.015 for R.

Figure 6.10: List of reference and working materials used for stable isotope analyses. All δ values are certified or recommended (*) values reported by the IAEA (IAEAwebsite, 2015) or IRMM (**) (IRMMwebsite, 2015). The δ values of Working Standards were determined in the Group of Stable Isotope Geochemistry at the Department of Environmental Sciences (JSI).

	Name	**Analyte**	**Material**	**δ value (‰)**
Reference Certified Materials (RCM)	VSMOW2	$\delta^{18}O$	Water	0 ± 0.02*
	SLAP2			-55.50 ± 0.02*
	GISP			-24.76 ± 0.09
	CRM/BCR 659		Water-ethanol mixture (12 vol. %)	-7.18 ± 0.02**
	IAEA CH-3	$\delta^{13}C$	Cellulose	-24.724 ± 0.041
	IAEA CH-6		Sucrose	-10.449 ± 0.033
	IAEA CH-7		Polyethylene	-32.151 ± 0.050
	NBS 22		Oil	-30.031 ± 0.043
	CRM/BCR 656		Ethanol	-26.91 ± 0.07**
Working standard	Tap water	$\delta^{18}O$	Water	-9.07 ± 0.11
	Seawater			-0.35 ± 0.11
	Snow		Snow	-19.7 ± 0.13
	UreaC	$\delta^{13}C$		-28.3 ± 0.2
	Rum		Ethanol	-13.73 ± 0.03

Chapter 7

Data analysis

The datasets were first examined via descriptive statistics using IBM® SPSS® Statistics 20 software, which provided information about minimum, maximum, mean, median, 1^{st} quartile and 3^{rd} quartile values, i.e. the most common indexes of position and data distribution.

Datasets were moreover examined via Principal Component Analysis (PCA). PCA is a very powerful method to analyze a large set of complex data and belongs to descriptive statistical techniques. The peculiarity of PCA is to reduce the dimensionality of multivariate data and to derive meaningful patterns from the information. The variables describing the samples are projected into a lower dimensional space, while retaining the maximal amount of information about the variables. For each PCA the resulting principal components for each sample are a combination of the original variables after transformation. The first principal component (PC1) describes the largest difference in the combined variables between the samples. A second principal component (PC2) describes the next largest difference, and so on.

PCA could be therefore a powerful instrument to understand the relationship between the wines under study because of capacity to reduce the complexity of the results.

Statistical data treatment, in particular PCA, was mainly done with Canoco 5, (Microsoft Windows® release) and IBM SPSS Statistics 20 software. The PCAs results were then confirmed by Factor Analyses performed on the same data, using Unistat® Statistics Software. Factor analysis (FA) is related, but not identical to PCA. It is a method used to describe the observed and correlated variability of several variables in terms of a potentially lower number of unobserved variables called factors. In particular, it reduces the set of variables in a dataset by searching for such joint variations in response to unobserved latent variables; the variables are modeled as linear combinations of the potential factors.

The principal difference between FA and PCA is that the former adopts regression modeling techniques to test a hypothesis, while PCA is a descriptive statistical technique.

Part III

Results and discussion

Chapter 8

Trace Elements and Rare Earth Elements

8.1 Wines analytical results

The analyses were carried out during the second and third year of the PhD and each set of results underwent an interpretation of the data and a comparison to the relative bibliography.

In particular, the resulted concentrations of macro-, micro-elements and lanthanides are summarized in Tables 8.1 and 8.2.

With regard to macro-elements, the most abundant ones were Na, Ca, Mn, Zn. However, the mean concentration of most elements was similar among wines of the regions considered. Among the micro-elements, the most abundant ones were Ba, Se, Ni, Cu, Cr. Moreover, some samples showed different values of concentration of As or Cd.

The ICP-MS results showed a few differences in the composition of wines from different geographical origins: in most samples there were significant differences between Italy and Slovenia regarding Li and K. The values of Li, in particular, were significantly higher in Italian wines than in all the other samples.

In all the wines, the elements of special interest due to their toxicity in case of excess had much lower values than the legal threshold limit established for Italian wines by the Italian Republic (DM 29/12/86) (Table 4.1).

The mean concentrations of macro- and micro-elements recorded in all the wines were generally lower than those reported in literature for Southern Italian wines (Interesse et al., 1985; Galgano et al., 2008).

Moreover, all the Rare Earth Elements studied in the wines had similar values to the concentrations reported in literature on wines from very different

	Li	Be	Na	Al	K	Ca
Min	0.69	<DL	515.94	77.94	152.74	404.10
1stQu	4.69	0.005	863.89	112.20	302.66	581.38
Median	4.92	0.03	1010.66	149.34	359.24	689.29
Mean	4.93	0.08	1014.88	149.11	359.92	710.68
3rdQu	5.24	0.07	1150.11	163.80	444.77	863.00
Max	6.29	0.70	1644.30	263.48	534.24	1149.49

	V	Cr	Mn	Fe	Co	Ni
Min	0.33	4.61	79.28	78.12	0.34	2.96
1stQu	0.47	12.14	269.71	217.76	0.92	9.78
Median	0.52	13.32	341.80	252.59	1.31	11.80
Mean	0.58	12.93	358.65	278.05	1.63	13.59
3rdQu	0.60	14.55	415.82	286.71	2.03	15.81
Max	1.69	16.01	627.85	942.40	6.28	35.34

	Cu	Zn	Ga	As	Se	Rb
Min	<DL	90.01	<DL	<DL	<DL	58.62
1stQu	22.96	147.92	0.00	0.00	17.29	138.72
Median	46.76	253.95	0.00	0.66	136.42	172.08
Mean	127.87	381.54	0.02	4.95	578.42	197.25
3rdQu	112.76	409.64	0.02	2.34	1356.73	244.74
Max	800.22	3393.91	0.18	64.15	1910.39	518.34

	Sr	Ag	Cd	Cs	Ba	La
Min	52.22	<DL	0.08	0.25	17.53	<DL
1stQu	127.34	0.00	0.12	0.49	56.74	4.43E-03
Median	140.94	0.00	0.15	0.70	71.50	9.96E-03
Mean	156.01	0.01	0.17	0.83	79.26	1.14E-02
3rdQu	172.11	0.00	0.19	1.08	94.15	1.33E-02
Max	306.03	0.13	0.39	2.64	188.41	3.84E-02

	Ce	Pr	Nd	Sm	Eu	Gd
Min	<DL	2.01E-03	1.04E-02	1.22E-02	3.93E-03	1.89E-03
1stQu	4.34E-02	4.12E-03	2.34E-02	2.08E-02	7.80E-03	3.30E-03
Median	5.00E-02	4.40E-03	2.73E-02	2.76E-02	1.09E-02	3.65E-03
Mean	5.18E-02	5.19E-03	2.95E-02	2.93E-02	1.16E-02	4.37E-03
3rdQu	5.41E-02	5.52E-03	3.01E-02	3.57E-02	1.40E-02	4.09E-03
Max	1.41E-01	1.64E-02	8.11E-02	5.66E-02	2.43E-02	1.27E-02

	Tb	Dy	Er	Tm	Yb	Lu
Min	<DL	1.67E-03	3.83E-03	2.12E-04	2.14E-03	3.39E-04
1stQu	3.65E-04	2.71E-03	4.58E-03	4.45E-04	3.29E-03	6.51E-04
Median	4.58E-04	3.11E-03	5.49E-03	5.45E-04	4.09E-03	7.49E-04
Mean	5.60E-04	4.18E-03	6.74E-03	7.25E-04	6.31E-03	1.32E-03
3rdQu	6.16E-04	3.82E-03	7.03E-03	6.76E-04	5.50E-03	1.05E-03
Max	1.96E-03	1.46E-02	2.32E-02	3.65E-03	4.06E-02	9.77E-03

	Tl	Pb	Bi	Th	U
Min	8.37E-02	3.30E+00	<DL	2.29E-03	<DL
1stQu	1.20E-01	4.18E+00	0.00E+00	3.55E-03	0.00
Median	1.78E-01	5.02E+00	0.00E+00	4.77E-03	0.003
Mean	2.02E-01	5.57E+00	1.24E-02	6.51E-03	0.03
3rdQu	2.36E-01	5.91E+00	0.00E+00	6.84E-03	0.03
Max	6.49E-01	1.59E+01	1.29E-01	4.54E-02	0.32

<DL = element with lower concentration than the Detection Limits.

Figure 8.1: ICP-MS results for Italian wines. Notation is in µg L^{-1}.

	Li	Be	Na	Al	K	Ca
Min	0.11	<DL	221.41	14.88	0.016	276.52
1stQu	0.92	0.01	386.11	38.89	103.09	528.55
Median	1.41	0.04	538.21	57.71	209.45	597.57
Mean	1.84	0.08	598.20	103.78	200.87	626.09
3rdQu	2.10	0.07	690.69	117.91	281.86	705.30
Max	7.23	2.11	2081.79	487.18	598.44	1375.63

	V	Cr	Mn	Fe	Co	Ni
Min	0.05	1.07	123.92	53.87	0.45	6.01
1stQu	0.21	3.74	229.90	150.91	1.29	9.94
Median	0.27	8.50	340.29	256.89	1.73	13.25
Mean	0.40	8.09	377.01	321.55	1.89	16.56
3rdQu	0.41	9.98	422.11	358.35	2.36	17.52
Max	2.07	27.07	1341.40	1576.40	7.28	96.88

	Cu	Zn	Ga	As	Se	Rb
Min	<DL	16.11	<DL	<DL	<DL	64.25
1stQu	8.23	393.04	0.00	0.00	0.14	208.41
Median	23.60	487.21	0.02	1.11	503.99	290.49
Mean	66.48	580.10	0.03	2.60	859.14	300.57
3rdQu	64.09	733.14	0.03	2.75	1363.75	367.24
Max	1035.06	2191.08	0.29	46.13	3502.33	655.74

	Sr	Ag	Cd	Cs	Ba	La
Min	45.65	<DL	<DL	0.54	10.66	<DL
1stQu	86.84	0.00	0.08	1.21	23.57	3.68E-03
Median	120.86	0.00	0.16	1.70	32.18	6.07E-03
Mean	138.58	0.01	0.23	1.92	38.13	7.86E-03
3rdQu	170.33	0.003	0.23	2.47	48.10	9.88E-03
Max	427.17	0.15	2.32	4.91	125.30	4.69E-02

	Ce	Pr	Nd	Sm	Eu	Gd
Min	2.74E-03	4.45E-04	2.44E-03	3.27E-03	1.46E-03	4.35E-04
1stQu	5.11E-03	1.60E-03	4.54E-03	7.96E-03	3.87E-03	1.22E-03
Median	8.38E-03	2.28E-03	7.31E-03	1.09E-02	5.09E-03	2.00E-03
Mean	1.43E-02	2.78E-03	1.08E-02	1.23E-02	5.66E-03	2.37E-03
3rdQu	1.86E-02	2.91E-03	1.22E-02	1.72E-02	7.08E-03	2.64E-03
Max	1.29E-01	1.43E-02	7.48E-02	2.57E-02	1.43E-02	1.16E-02

	Tb	Dy	Er	Tm	Yb	Lu
Min	3.95E-05	2.54E-04	2.40E-04	5.35E-05	5.22E-04	9.75E-05
1stQu	1.73E-04	9.19E-04	9.51E-04	1.80E-04	1.45E-03	3.13E-04
Median	2.56E-04	1.74E-03	1.80E-03	3.09E-04	2.35E-03	5.29E-04
Mean	3.56E-04	2.21E-03	2.35E-03	4.52E-04	3.77E-03	8.63E-04
3rdQu	3.65E-04	2.53E-03	3.01E-03	5.38E-04	4.36E-03	9.85E-04
Max	1.92E-03	1.25E-02	1.09E-02	1.97E-03	1.94E-02	5.18E-03

	Tl	Pb	Bi	Th	U
Min	0.10	0.07	<DL	1.77E-04	<DL
1stQu	0.21	1.21	0.00	5.10E-04	0.00
Median	0.28	2.62	0.00	8.18E-04	0.004
Mean	0.33	8.57	0.13	2.47E-03	0.02
3rdQu	0.36	5.48	0.00	1.48E-03	0.02
Max	1.43	232.95	6.64	3.81E-02	0.37

<DL = element with lower concentration than the Detection Limits.

Figure 8.2: ICP-MS results for Slovenian wines. Notation is in µg L^{-1}.

Table 8.1: Summary table from Galgano et al., 2008 results on wine samples from South Italy (Trace Elements).

	Italian regions (South)					
Elements	**Basilicata**		**Calabria**		**Campania**	
mg L^{-1}						
Al	0.893	±0.20	1.55	±0.12	1.26	±0.27
B	7.20	±1.50	6.94	±1.67	4.92	±1.03
Br	0.33	±0.13	0.39	±0.13	0.29	±0.11
Ca	83.17	±13.91	73.32	±14.34	70.27	±13.95
Fe	3.91	±1.16	3.92	±1.15	2.60	±0.99
Mg	102.35	±15.59	116.70	±16.51	88.25	±10.83
Mn	1.63	±0.48	1.53	±0.59	1.04	±0.24
K	1102.25	±226.87	1017.25	±197.15	1159.50	±193.74
Na	20.97	±10.16	28.47	±14.87	18.70	±5.32
P	170.79	±35.84	193.45	±47.46	162.21	±34.53
Rb	2.29b	±0.85	2.18	±0.65	3.79	±2.21
S	250.68	±69.47	267.82	±57.11	214.69	±50.81
Si	19.62	±8.75	25.05	±8.63	22.24	±9.35
Sr	1.34	±0.56	1.86	±0.19	1.11	±0.15
Zn	0.56	±0.19	0.72	±0.27	0.60	±0.31

countries (Interesse et al., 1985; Galgano et al., 2008; Koreňovská and Suhaj, 2005; Brescia et al., 2003).

Nevertheless, the concentration of most elements in all the wines fell in the range of European wines. As reported in Koreňovská and Suhaj, 2005, wine concentrations varied widely among different European Countries and geographical origins.

The results of this work agreed also with Roggi and Ronchi, 2001. The latter reported examples of Italian wines from Northern and central regions characterized by lower contents of Al, Cd, Li, Mn, Rb, and Sr with respect to Southern wines and islands, which are similar to the ones of Galgano et al., 2008.

Orescanin et al., 2003 found statistically significant differences in elemental composition between white and red wines. In particular, they found that all elements showed greater concentrations in red grape. In our analytical data, wines from Slovenia, which were mostly red ones, did not show general higher concentrations than the Italian ones, which were all white wines.

Potassium, generally one of the most abundant inorganic constituents (Interesse et al., 1985), ranged here only from 1.61E-5 to 0.59 mg L^{-1} (average 0.25 mg L^{-1}). These values were quite low if compared to those found in the most frequently studied wines. The differences in the values of concentration for different types of wine were not significant. However, some Slovenian samples showed very low values, while Italian ones were generally higher. The contents of this metal was supposed vary depending on the types of wine: in particular, it was supposed to present higher values in red wines than in white wines (Interesse et al., 1985). This was not the case in our results. Some studies suggest that these values could be related to the winemaking technique, as the grapes used in white tend to remain in contact with the skins for a shorter time. The results of the present work confirmed the hypothesis that geographical factors and different winemaking techniques could influence the potassium concentration.

Lithium ion is present in wines due to plant roots uptake or to storage in glass bottles (Zerbinati et al., 2000). The lithium concentrations measured in 97 wines showed very low values, ranging from 0.72E-2 to 0.11E-3 mg L^{-1} (average value 2.98E-3 mg L^{-1}). Lithium concentrations were generally higher in Italian wines than in Slovenian ones. Moreover, some Slovenian samples (1547 and 1670b) showed higher concentrations. Lithium has more homogeneous concentrations in Italian wines than in Slovenian ones. This could be due to differences in soil composition and winemaking techniques.

The International Office of Vine and Wine (OIV) progressively reduced the threshold limit value of lead in wines, which is currently set at 200 μg L^{-1}. Almeida, 2002 reported the presence of lead in wine due to two types of contamination: one is natural and soil-related, while the other results from human activity. The presence of lead can be due to atmospheric precipitation, pesticides used in the fields, specific materials used in wine production such as tinned containers, transport and store of the wine, etc. as reported in several works (Dugo et al., 2005; Marengo and Aceto, 2003; Monaci et al., 2003; Gulson, 2005).

The samples 1948, 1949, 1950 showed higher concentrations of lead. In particular, the last one was above the legislation limits, while all the other samples were below the limits. This higher concentration could be derived by a contamination during the winemaking process and not by a soil contamination because all the other wines present very low concentrations.

In conclusion, wine quality is very high for both the Countries.

8.2 Must analytical results

The measurement of Trace Elements and Rare Earth Elements concentration in musts was carried out to study the relation between wine and grapes.

The levels of elements measured in musts are reported in Tables 8.3 and 8.4. Already before the analysis, must resulted very inhomogeneous from one sample to another. Samples weighing evidenced different quantities of grape skin in some bottles, seeds and different juice density in others.

Each sample used to produce the must probably would have needed an identical number of acini and grapes to obtain a higher level of homogeneity, although the homogenization of the samples did reduce these physical differences in composition. Analytical results were very different between the two years, with concentrations very low in the vintage 2012. The comparison between the results of musts and wine samples yielded significant differences. As reported in Catarino et al., 2006, important losses of Be, Al, Mn, Co, Ni, Cu, Zn, Ga, Rb, Cd, Ba, Tl and U occur from musts to wines during alcoholic fermentation, probably due to precipitation as insoluble salts, namely sulphides. Moreover, the musts were made by just pressing and then mixing the grapes. During wine production, however, musts are filtered and the skins, seeds and other parts of the grape are macerated or removed, considerably reducing the concentrations of some elements.

The greater concentration levels of some trace elements in wines in comparison to musts can be linked to the pre-concentration and precipitation processes, but also to the use of metal-containing equipment in winemaking (Vystavna et al., 2014; González and Peña-Méndez, 2000).

These few motivations could explain why we measured very different concentration in musts from different vintages and between musts and wines.

Li		Mg		Al		K		Ca		Tl	
Min	3.1	Min	<DL	Min	<DL	Min	1351.2	Min	14442.8	Min	0.1
1stQu	10.2	1stQu	3086.1	1stQu	0.0	1stQu	3390.2	1stQu	77500.6	1stQu	0.2
Median	24.0	Median	5102.7	Median	11900.7	Median	4988.7	Median	92901.2	Median	0.4
Mean	21.1	Mean	6045.5	Mean	21098.1	Mean	4921.8	Mean	115483.8	Mean	0.4
3rdQu	27.1	3rdQu	7791.6	3rdQu	22539.3	3rdQu	6585.8	3rdQu	192867.3	3rdQu	0.6
Max	44.7	Max	27269.0	Max	234245.0	Max	7833.8	Max	214685.7	Max	0.9

V		Cr		Mn		Fe		Co		Ni	
Min	18.0	Min	16.7	Min	377.7	Min	<DL	Min	4.8	Min	17.8
1stQu	37.0	1stQu	29.2	1stQu	518.4	1stQu	2422.2	1stQu	7.9	1stQu	26.9
Median	88.0	Median	82.5	Median	716.1	Median	4740.7	Median	16.3	Median	53.0
Mean	82.1	Mean	74.9	Mean	775.1	Mean	5336.7	Mean	16.6	Mean	54.1
3rdQu	101.8	3rdQu	93.7	3rdQu	991.8	3rdQu	6426.8	3rdQu	22.3	3rdQu	73.1
Max	176.0	Max	158.5	Max	1382.6	Max	14087.4	Max	43.6	Max	124.6

Cu		Zn		Ga		As		Se		Rb	
Min	23.5	Min	33.2	Min	3.5	Min	1.9	Min	1.0	Min	13.6
1stQu	34.1	1stQu	44.5	1stQu	8.7	1stQu	3.9	1stQu	1.8	1stQu	31.8
Median	42.6	Median	63.4	Median	22.5	Median	7.8	Median	2.1	Median	59.7
Mean	49.9	Mean	62.9	Mean	20.7	Mean	9.0	Mean	2.1	Mean	62.5
3rdQu	52.6	3rdQu	74.7	3rdQu	28.4	3rdQu	13.6	3rdQu	2.4	3rdQu	91.3
Max	117.8	Max	100.8	Max	42.0	Max	22.4	Max	3.2	Max	113.1

Sr		Y		Ag		Cd		Cs		La	
Min	73.9	Min	10.4	Min	<DL	Min	0.2	Min	0.8	Min	11.3
1stQu	132.6	1stQu	23.5	1stQu	0.08	1stQu	0.3	1stQu	2.3	1stQu	23.0
Median	194.3	Median	27.1	Median	0.1	Median	0.4	Median	3.8	Median	45.5
Mean	239.6	Mean	32.1	Mean	0.1	Mean	0.4	Mean	4.4	Mean	46.1
3rdQu	366.4	3rdQu	40.9	3rdQu	0.2	3rdQu	0.5	3rdQu	6.4	3rdQu	63.5
Max	425.4	Max	62.6	Max	0.3	Max	0.7	Max	8.9	Max	92.5

Ce		Pr		Nd		Sm		Eu		Gd	
Min	15.1	Min	2.0	Min	7.9	Min	1.5	Min	0.3	Min	1.6
1stQu	24.1	1stQu	4.3	1stQu	16.1	1stQu	3.1	1stQu	0.7	1stQu	3.4
Median	82.9	Median	10.0	Median	36.4	Median	7.0	Median	1.6	Median	6.7
Mean	74.5	Mean	9.7	Mean	36.2	Mean	7.0	Mean	1.6	Mean	6.8
3rdQu	98.4	3rdQu	14.0	3rdQu	52.6	3rdQu	1.0	3rdQu	2.2	3rdQu	9.6
Max	155.7	Max	19.8	Max	74.0	Max	14.0	Max	3.9	Max	13.9

Tb		Dy		Ho		Er		Tm		Yb	
Min	0.2	Min	1.2	Min	0.2	Min	0.7	Min	<DL	Min	0.5
1stQu	0.4	1stQu	2.7	1stQu	0.5	1stQu	1.6	1stQu	0.2	1stQu	1.3
Median	0.8	Median	4.5	Median	0.8	Median	2.3	Median	0.2	Median	1.7
Mean	0.8	Mean	4.7	Mean	0.9	Mean	2.5	Mean	0.3	Mean	1.9
3rdQu	1.2	3rdQu	6.6	3rdQu	1.2	3rdQu	3.3	3rdQu	0.3	3rdQu	2.4
Max	1.8	Max	9.6	Max	1.8	Max	4.9	Max	0.6	Max	3.7

Lu		Pb		Th		U	
Min	<DL	Min	4.4	Min	1.3	Min	0.3
1stQu	0.2	1stQu	9.2	1stQu	2.7	1stQu	0.6
Median	0.2	Median	20.4	Median	6.5	Median	0.9
Mean	0.2	Mean	18.8	Mean	6.8	Mean	0.9
3rdQu	0.3	3rdQu	27.3	3rdQu	10.1	3rdQu	1.1
Max	0.5	Max	33.6	Max	14.9	Max	1.8

<DL = element with concentrations below the Detection Limits.

Figure 8.3: Summary table for ICP-MS results on grape samples, year 2011. Notation is in µg L^{-1}.

Li		Mg		Al		K		Ca		Tl	
Min	0.2	Min	3.1	Min	11.9	Min	585.8	Min	24.8	Min	<DL
1stQu	0.3	1stQu	3.3	1stQu	21.4	1stQu	649.8	1stQu	26.9	1stQu	0.0
Median	0.4	Median	4.9	Median	23.0	Median	748.6	Median	80.0	Median	0.0
Mean	0.5	Mean	7.7	Mean	21.9	Mean	742.1	Mean	85.2	Mean	0.0
3rdQu	0.5	3rdQu	12.2	3rdQu	25.7	3rdQu	825.2	3rdQu	119.9	3rdQu	0.0
Max	1.5	Max	13.3	Max	30.4	Max	1006.6	Max	163.6	Max	0.1

V		Cr		Mn		Fe		Co		Ni	
Min	0.3	Min	2.3	Min	2.5	Min	1962.4	Min	1.4	Min	0.9
1stQu	0.4	1stQu	2.8	1stQu	3.0	1stQu	2277.8	1stQu	1.6	1stQu	1.1
Median	0.5	Median	2.8	Median	3.4	Median	3191.9	Median	1.7	Median	1.7
Mean	4.4	Mean	347.6	Mean	3.5	Mean	3485.4	Mean	1.8	Mean	2.3
3rdQu	8.7	3rdQu	4.5	3rdQu	3.9	3rdQu	4260.2	3rdQu	2.0	3rdQu	1.9
Max	11.0	Max	1667.7	Max	4.9	Max	5959.0	Max	2.6	Max	7.4

Cu		Zn		Ga		As		Se		Rb	
Min	1.9	Min	1.5	Min	0.2	Min	0.5	Min	0.2	Min	0.1
1stQu	3.2	1stQu	2.6	1stQu	0.2	1stQu	0.8	1stQu	1.5	1stQu	0.2
Median	4.2	Median	3.1	Median	0.4	Median	1.1	Median	7.7	Median	0.4
Mean	5.4	Mean	3.8	Mean	0.7	Mean	5.9	Mean	6.9	Mean	0.4
3rdQu	5.5	3rdQu	5.1	3rdQu	1.1	3rdQu	9.9	3rdQu	10.3	3rdQu	0.4
Max	14.7	Max	6.6	Max	1.3	Max	19.1	Max	20.4	Max	1.3

Sr		Y		Ag		Cd		Cs		La	
Min	0.04	Min	<DL	Min	<DL	Min	<DL	Min	<DL	Min	<DL
1stQu	0.05	1stQu	0.07	1stQu	0.04	1stQu	0.0	1stQu	0.0	1stQu	0.0
Median	0.06	Median	0.08	Median	0.05	Median	0.2	Median	0.0	Median	0.1
Mean	0.4	Mean	0.07	Mean	0.06	Mean	0.2	Mean	0.1	Mean	0.1
3rdQu	0.7	3rdQu	0.09	3rdQu	0.07	3rdQu	0.4	3rdQu	0.1	3rdQu	0.1
Max	0.8	Max	0.11	Max	0.1	Max	0.8	Max	0.2	Max	0.1

Ce		Pr		Nd		Sm		Eu		Gd	
Min	<DL	Min	<DL	Min	<DL	Min	<DL	Min	<DL	Min	<DL
1stQu	0.1	1stQu	0.01	1stQu	0.0	1stQu	0.01	1stQu	0.0	1stQu	0.0
Median	0.2	Median	0.02	Median	0.1	Median	0.02	Median	0.0	Median	0.0
Mean	0.1	Mean	0.02	Mean	0.1	Mean	0.07	Mean	0.0	Mean	0.0
3rdQu	0.2	3rdQu	0.04	3rdQu	0.2	3rdQu	0.12	3rdQu	0.0	3rdQu	0.0
Max	0.2	Max	0.05	Max	0.3	Max	0.23	Max	<DL	Max	0.1

Tb		Dy		Ho		Er		Tm		Yb	
Min	<DL	Min	<DL	Min	<DL	Min	<DL	Min	<DL	Min	<DL
1stQu	0.0	1stQu	0.01	1stQu	0.0	1stQu	0.00	1stQu	0.0	1stQu	0.0
Median	0.0	Median	0.01	Median	0.0	Median	0.00	Median	0.0	Median	0.0
Mean	0.0	Mean	0.04	Mean	0.0	Mean	0.03	Mean	0.0	Mean	0.0
3rdQu	0.0	3rdQu	0.07	3rdQu	0.0	3rdQu	0.05	3rdQu	0.0	3rdQu	0.0
Max	0.0	Max	0.12	Max	<DL	Max	0.09	Max	0.0	Max	<DL

Lu		Pb		Th		U	
Min	<DL	Min	0.5	Min	<DL	Min	<DL
1stQu	0.0	1stQu	0.6	1stQu	0.00	1stQu	0.02
Median	0.0	Median	0.7	Median	0.02	Median	0.02
Mean	0.0	Mean	1.0	Mean	0.02	Mean	0.24
3rdQu	0.01	3rdQu	1.3	3rdQu	0.04	3rdQu	0.53
Max	0.02	Max	2.1	Max	0.05	Max	0.57

<DL = element with concentrations below the Detection Limits.

Figure 8.4: Summary table for ICP-MS results on grape samples, year 2012. Notation is in µg L^{-1}.

8.3 Soil analytical results

To understand the relation between wines and soils, the analysis of the elemental composition in soil was carried out for the 9 fields.

The analytical results of the fields are reported in Table 8.5.

The results of our research showed diverse patterns for the elements, which resulted different from the patterns of musts and wines. The first problem is that soil samples were acquired during few years and not for a specific one, then mixing together the possible variability of the vintages. Moreover, fields were not very distant from each other even if they were at different altitudes. These soils are strongly influenced by human activity, which has transformed the environment into cultivated fields, modifying the composition of the soil.

The solubility of elements, and hence their concentration in the soil solution and their bioavailability depend upon the solubility equilibrium.

However, the equilibrium is constant only under specific conditions, and solution equilibrium for soil phases is therefore not easy to understand (Kabata-Pendias, 2004).

There are a few articles of interest regarding the different fingerprints of elements in soils, musts and wines. Vystavna et al., 2014 analyzed irrigation waters, topsoil, leaves and grapes, and showed that different varieties of grape presented very different capacities to uptake nutrients and trace metals. In particular, they found more similar concentrations into soils and leaves than into soils and grapes. Orescanin et al., 2003 found greater concentrations of some element in red wines, while in the white wines trace elements were significantly lower and with different pattern of elements concentrations than soils.

Kment et al., 2005 in their study about the chemical compositions of wines and soils, showed that, statistically, the clustering of wines did not follow the clustering of soils. Moreover, they suggested that major and trace element fingerprints of wines reflect the chemistry of the soil as well as pollution and individual wine manufacturing practices.

Chopin et al., 2008 studied contaminated soils presenting elevated trace element concentrations and *Vitis vinifera L.* roots that showed low ratios of trace element accumulation. Almeida and Vasconcelos, 2003 found that multielemental composition of wine is mostly influenced by the vinification processes than by the grape and soil compositions. Moreover, those variation of element concentration should not be useful to understand wine provenance.

The analytical results of this PhD project did not take into account the cycle of uptake of nutrients, which are selected by the plant and are species-specific. Species, indeed, differ widely in their capacity to acquire nutrients from soil (Lambers et al., 2008).

Li		Mg		Al		K		Ca		Tl	
Min	3.17	Min	<DL	Min	<DL	Min	1351.26	Min	14442.88	Min	0.11
1stQu	10.24	1stQu	3086.11	1stQu	0.00	1stQu	3390.26	1stQu	77500.60	1stQu	0.24
Median	24.01	Median	5102.76	Median	11900.76	Median	4988.71	Median	92901.19	Median	0.40
Mean	21.10	Mean	6045.58	Mean	21098.13	Mean	4921.86	Mean	115483.85	Mean	0.46
3rdQu	27.15	3rdQu	7791.69	3rdQu	22539.39	3rdQu	6585.79	3rdQu	192867.28	3rdQu	0.65
Max	44.70	Max	27269.02	Max	234245.00	Max	7833.81	Max	214685.70	Max	0.94

V		Cr		Mn		Fe		Co		Ni	
Min	18.05	Min	16.70	Min	377.77	Min	<DL	Min	4.838	Min	17.82
1stQu	37.06	1stQu	29.22	1stQu	518.45	1stQu	2422.25	1stQu	7.938	1stQu	26.90
Median	88.05	Median	82.53	Median	716.16	Median	4740.74	Median	16.31	Median	53.06
Mean	82.12	Mean	74.98	Mean	775.13	Mean	5336.70	Mean	16.59	Mean	54.16
3rdQu	101.87	3rdQu	93.76	3rdQu	991.81	3rdQu	6426.78	3rdQu	22.32	3rdQu	73.17
Max	176.04	Max	158.53	Max	1382.63	Max	14087.39	Max	43.63	Max	124.58

Cu		Zn		Ga		As		Se		Rb	
Min	23.53	Min	33.28	Min	3.52	Min	1.88	Min	1.08	Min	13.58
1stQu	34.10	1stQu	44.56	1stQu	8.73	1stQu	3.89	1stQu	1.86	1stQu	31.85
Median	42.62	Median	63.44	Median	22.56	Median	7.81	Median	2.14	Median	59.71
Mean	49.96	Mean	62.93	Mean	20.76	Mean	8.98	Mean	2.12	Mean	62.52
3rdQu	52.61	3rdQu	74.79	3rdQu	28.40	3rdQu	13.67	3rdQu	2.45	3rdQu	91.35
Max	117.88	Max	100.87	Max	42.05	Max	22.43	Max	3.24	Max	113.11

Sr		Y		Ag		Cd		Cs		La	
Min	73.97	Min	10.49	Min	0.04	Min	0.20	Min	0.86	Min	11.31
1stQu	132.60	1stQu	23.56	1stQu	0.08	1stQu	0.28	1stQu	2.38	1stQu	23.06
Median	194.38	Median	27.12	Median	0.16	Median	0.41	Median	3.86	Median	45.55
Mean	239.61	Mean	32.16	Mean	0.16	Mean	0.41	Mean	4.46	Mean	46.10
3rdQu	366.41	3rdQu	40.90	3rdQu	0.20	3rdQu	0.54	3rdQu	6.42	3rdQu	63.55
Max	425.40	Max	62.66	Max	0.33	Max	0.68	Max	8.94	Max	92.53

Ce		Pr		Nd		Sm		Eu		Gd	
Min	15.18	Min	2.07	Min	7.92	Min	1.56	Min	0.37	Min	1.67
1stQu	24.16	1stQu	4.31	1stQu	16.14	1stQu	3.16	1stQu	0.73	1stQu	3.48
Median	82.94	Median	10.03	Median	36.44	Median	7.03	Median	1.63	Median	6.71
Mean	74.55	Mean	9.74	Mean	36.22	Mean	6.94	Mean	1.65	Mean	6.85
3rdQu	98.42	3rdQu	14.03	3rdQu	52.63	3rdQu	9.99	3rdQu	2.22	3rdQu	9.65
Max	155.75	Max	19.84	Max	74.02	Max	13.99	Max	3.96	Max	13.96

Tb		Dy		Ho		Er		Tm		Yb	
Min	0.22	Min	1.24	Min	0.26	Min	0.72	Min	0.09	Min	0.56
1stQu	0.48	1stQu	2.74	1stQu	0.58	1stQu	1.65	1stQu	0.20	1stQu	1.33
Median	0.87	Median	4.54	Median	0.85	Median	2.29	Median	0.28	Median	1.77
Mean	0.89	Mean	4.76	Mean	0.92	Mean	2.49	Mean	0.30	Mean	1.90
3rdQu	1.27	3rdQu	6.63	3rdQu	1.25	3rdQu	3.32	3rdQu	0.39	3rdQu	2.44
Max	1.82	Max	9.63	Max	1.85	Max	4.98	Max	0.60	Max	3.70

Lu		Pb		Th		U	
Min	0.08	Min	4.46	Min	1.31	Min	0.33
1stQu	0.19	1stQu	9.22	1stQu	2.76	1stQu	0.60
Median	0.25	Median	20.42	Median	6.51	Median	0.92
Mean	0.27	Mean	18.80	Mean	6.84	Mean	0.94
3rdQu	0.34	3rdQu	27.31	3rdQu	10.13	3rdQu	1.11
Max	0.53	Max	33.60	Max	14.93	Max	1.86

<DL = element with lower concentration than the Detection Limits.

Figure 8.5: Summary table for ICP-MS results on soil samples (TE and REE). Notation is in mg kg^{-1}.

Chapter 9

REEs normalization

Several authors have reported that some winemaking treatments, for example yeast hulls and bentonite, can increase or deplete the contents of some mineral elements, influencing the final macro-, micro- and Rare Earth Elements composition of wine (Jakubowski et al., 1999).

Some studies reported that Ce and Eu were more useful than absolute Rare Earth Elements concentration to differentiate wines from different origins (Rodushkin et al., 1999). Other studies reported that Rare Earth Elements are not suitable to differentiate wines according to provenance or the vintage (Marengo and Aceto, 2003; Pérez-Trujillo et al., 2003; Galgano et al., 2008).

The REEs studied in our analyses were normalized in relation to the UCC (Upper Continental Crust)(Rudnick and Gao, 2003) values and then related to the normalized REEs concentration of the other matrices. The normalization can eliminate the "sawtooth" pattern, which is typical of the REEs and caused by the Oddo-Harkins effect, and eventually show the anomalies of single elements in the studied samples. The UCC normalization was chosen for wine samples, drawing on other similar matrices such as plants and fruits, used in other articles, e.g., Gonçalves et al., 2013, Piper and Bau, 2013 and Lee et al., 2011.

The normalization of Italian wine samples, in Figure 9.1, showed a similar pattern distribution among the three vintages. Vintages 2009 and 2010 contained also wines made from a different field near Negrar (Vr) and some of those evidenced significantly different patterns. The red and a blue lines (samples 526 (2009) and 527 (2010) respectively) indicate two wines made from Boschera vine of the Negrar fields. The evident pattern configuration could be due to the different soil origin and wine variety of those samples. The other two vine Cabrusina and Dindarella, also produced in Negrar fields, followed the same distribution as the Corvina vines from the fields of Valpolicella.

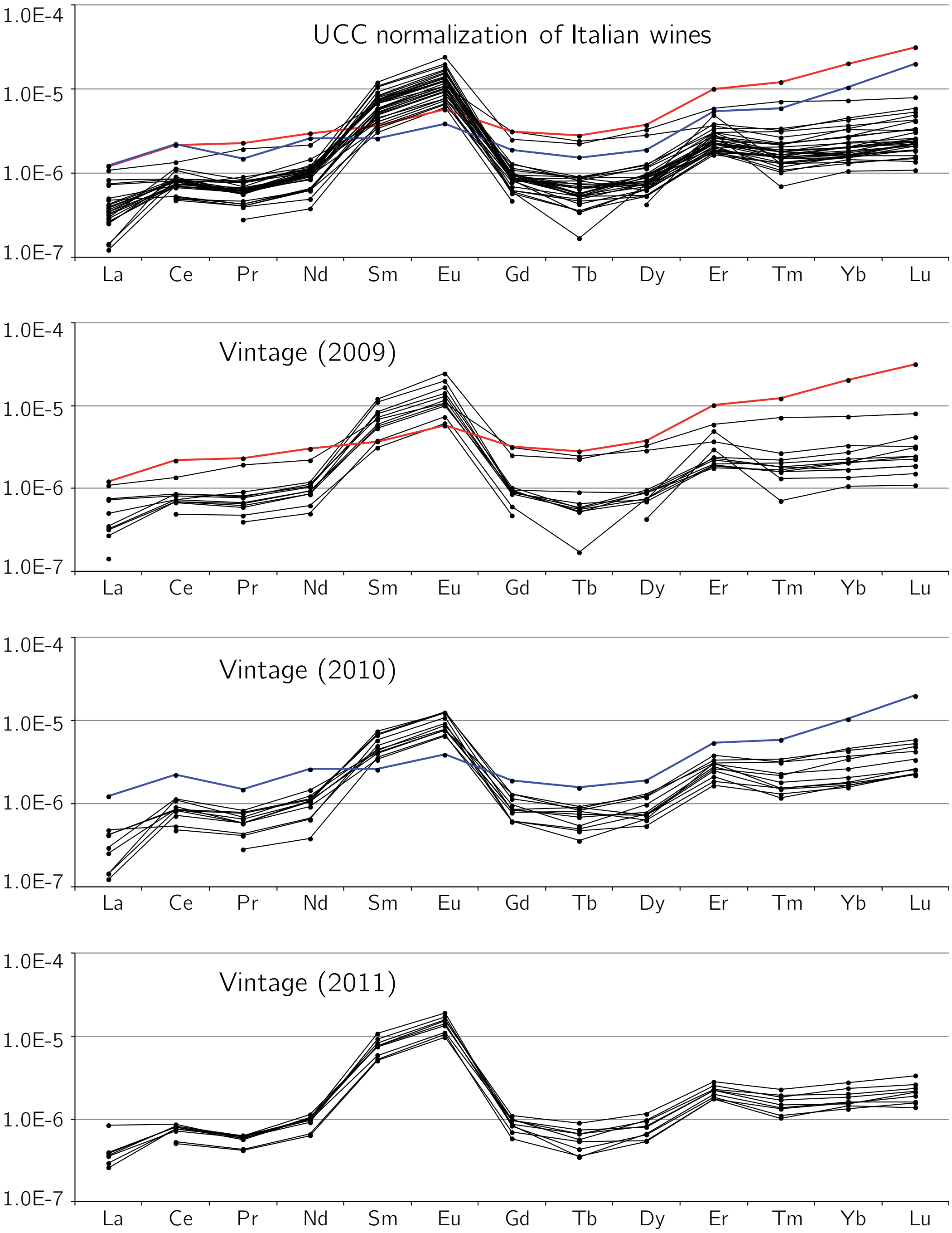


Figure 9.1: Italian wines' REEs normalization in respect to the Upper Continental Crust. Vintages 2009, 2010 and 2011.

There were no other noticeable differences between the three vintages.

characterized

Regarding the soil results, the normalization highlighted a pattern distribution with some differences in the samples. In figure 9.2 the soil normalization in respect to the UCC presented a depletion of Ce for some samples, in particular soils coming from fields 1, 2 and 3 (in red color) which can be due to a different soil composition. Soils 1 and 2 correspond to the climatic area "Valley bottom" but there is no particular evidence of the relation between REEs composition and climatic areas. Generally all the soils presented a light depletion in HREEs.

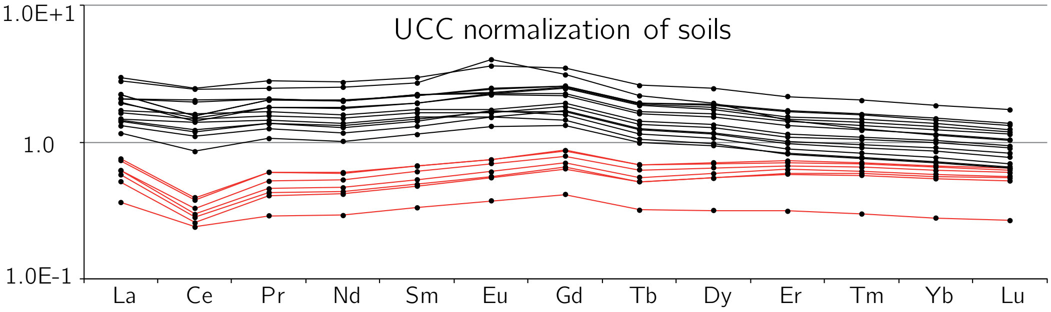


Figure 9.2: Italian soils normalization in respect to the Upper Continental Crust. Fields 1-9. Lines in red color are fields 1, 2 and 3.

The normalization in respect to UCC was done also on Slovenian wines. The samples from the Slovenian regions were more numerous than Italian ones and the pattern showed a denser disposition (Figure 9.3). The normalizations of the three vintages resulted very similar, as in the Italian samples. Wines evidenced positive anomalies for Eu and Sm, in particular vintage 2012 manifested smoother Eu picks than the other vintages. Generally all the samples showed an enrichment of HREEs.

Some samples presented quite different patterns (1695 from 2011, 1657 from 2012, and 1553 from 2013), rather difficult to interpret, but not so distant from the other samples.

The same normalization but with the three Regions separated is reported in Figure 9.4. The three different Regions manifested very similar trends, exception for a few samples. As in the previous Figures, we observed a positive anomaly of Eu and Sm and a general enrichment of HREEs.

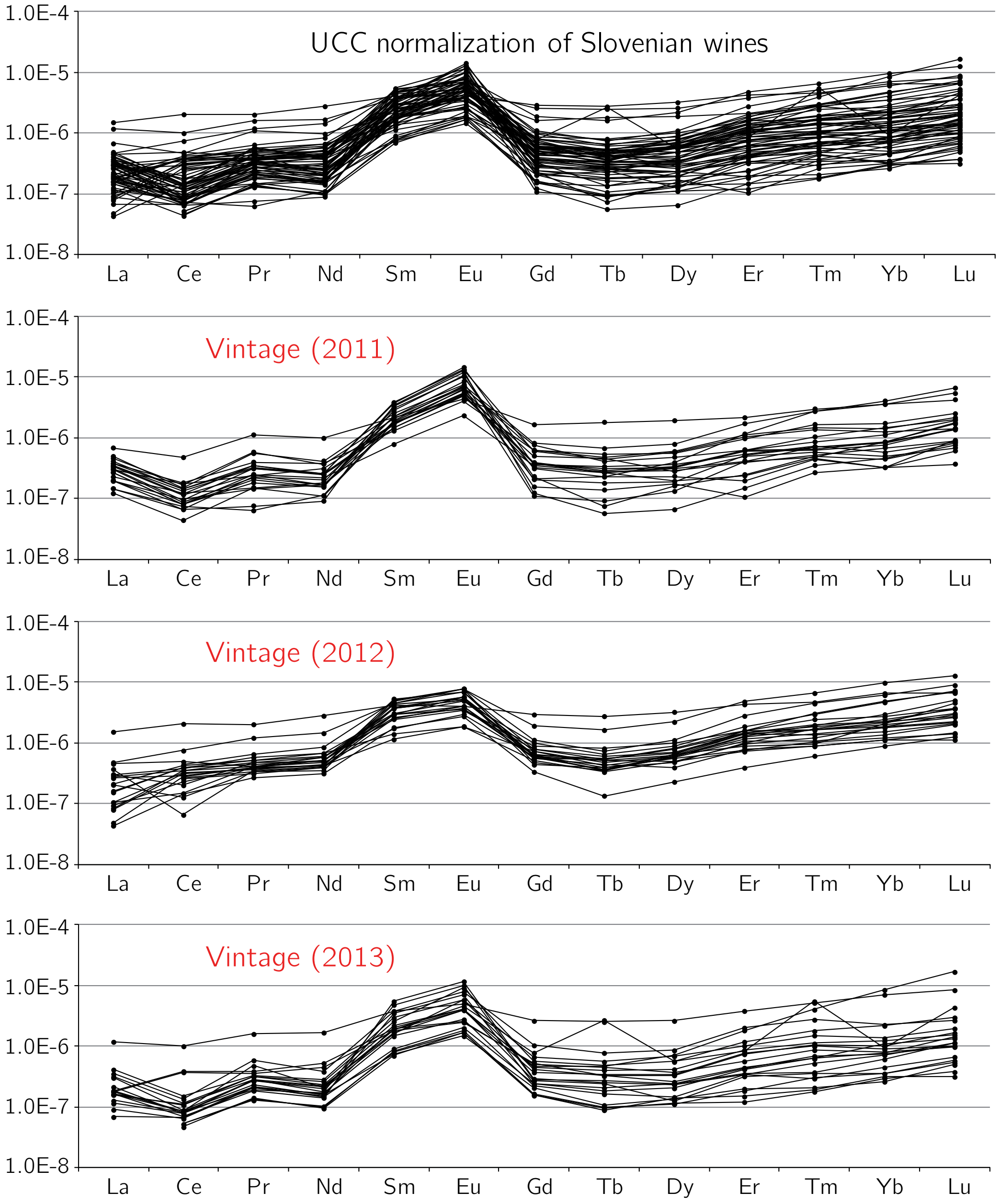


Figure 9.3: Slovenian wines' REEs normalization in respect to the Upper Continental Crust. Vintages 2010, 2011 and 2012.

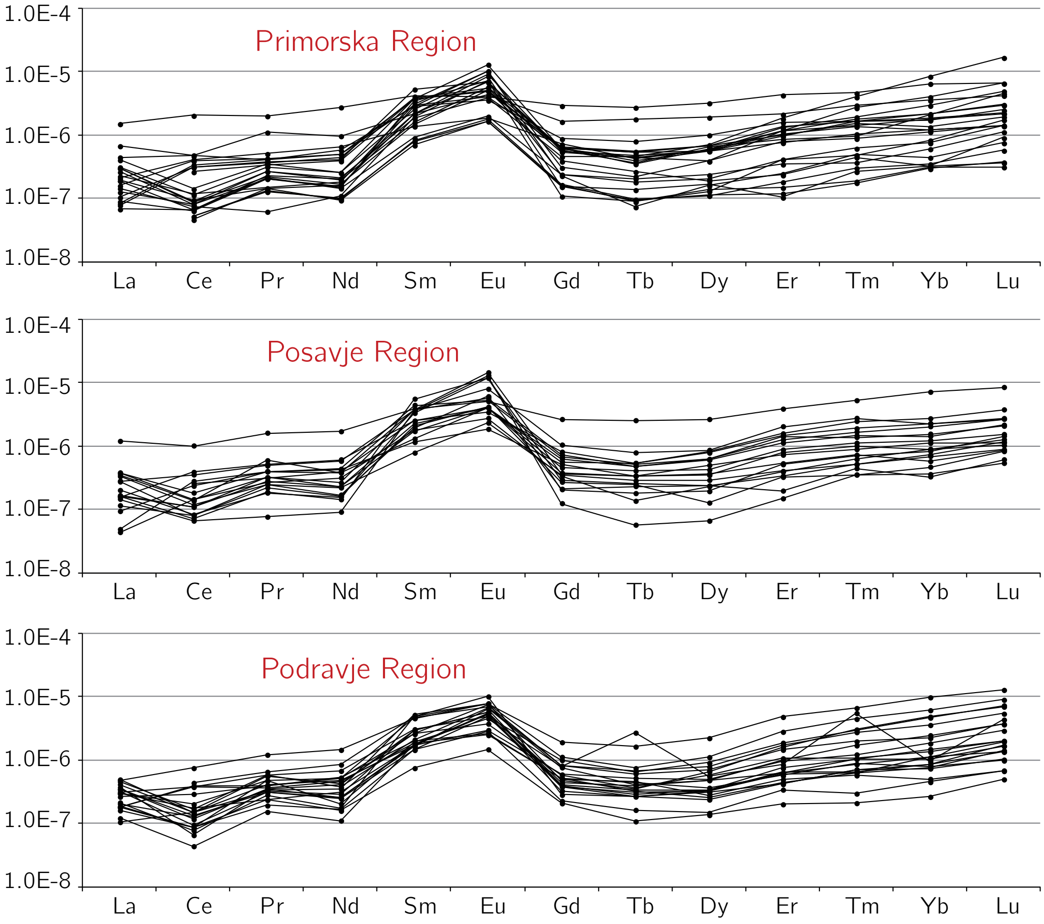


Figure 9.4: Slovenian wines' REEs normalization in respect to the Upper Continental Crust. Regions Primorska, Posavje, Podravje, respectively.

9.1 Ce and Eu

The anomaly calculation, to evidence anomalies in Eu and Ce was done with the following equations:

$$Ce* = \frac{(3Ce_n)}{2(La_n + Nd_n)}$$

$$Eu* = \frac{(2Eu_n)}{2(Sm_n + Gd_n)}$$

Equations 9.1, n indicates the element normalized in respect to UCC.

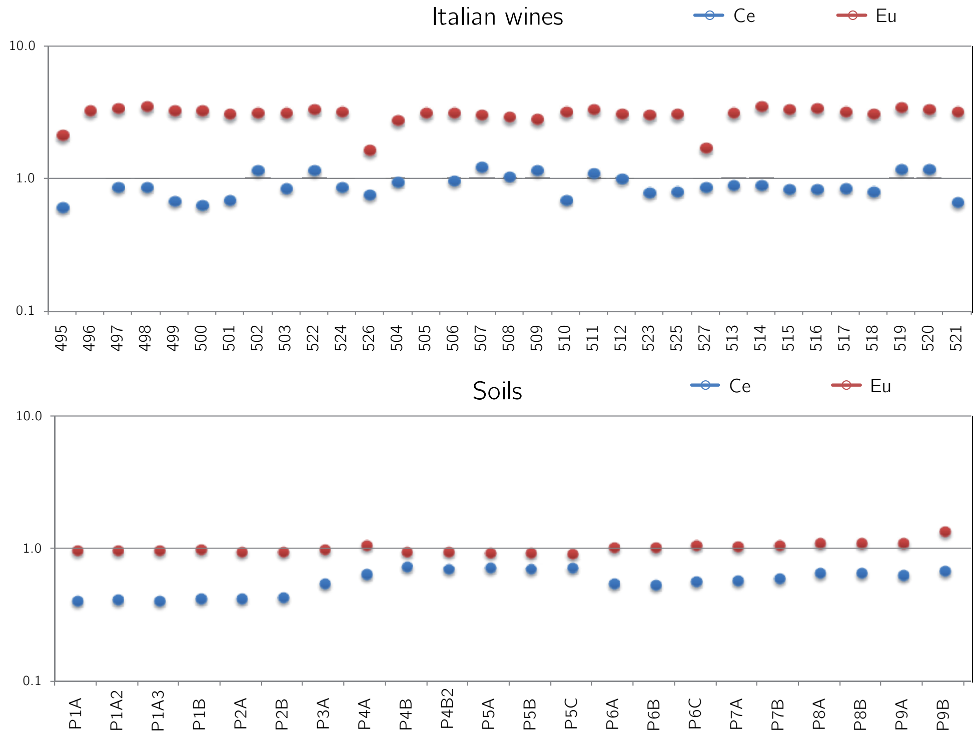


Figure 9.5: Anomalies calculation for Italian wines and soils, Ce and Eu.

The anomaly calculation for Italian wines and soils (Figure 9.5) contributed to evidence the different compositions of the two matrices.

Wines pattern showed a light positive anomaly for Eu in all the samples, with the exception of samples 526 and 527 which were separated also in this graphics. Moreover, Ce showed a quite constant pattern in the anomaly calculation results. Regarding soil samples the profile of Eu and Ce were quite similar. Ce in soils showed a slightly more negative pattern than in wines.

Slovenian samples, produced from three distant regions , showed very interesting results regarding anomaly calculations. Figure 9.6 evidenced a visible separation of vintage 2012 from the 2011 and 2013 ones. This separation was more evident in the Primorska and Posavje Regions but less visible in Podravje, which had a more similar pattern for most samples (except for sample 1674b and 1560 which were showing a more negative and a more positive results of Ce than the media of samples, respectively).

Primorska and Posavje regions were characterized by similar trends during the vintages 2011 and 2013. In 2012, they both evidenced lower results of Eu and higher results of Ce than the average results in the region. Year 2012 was a warm and dry year and the vine could have been influenced by these weather conditions, evidenced by Eu and Ce.

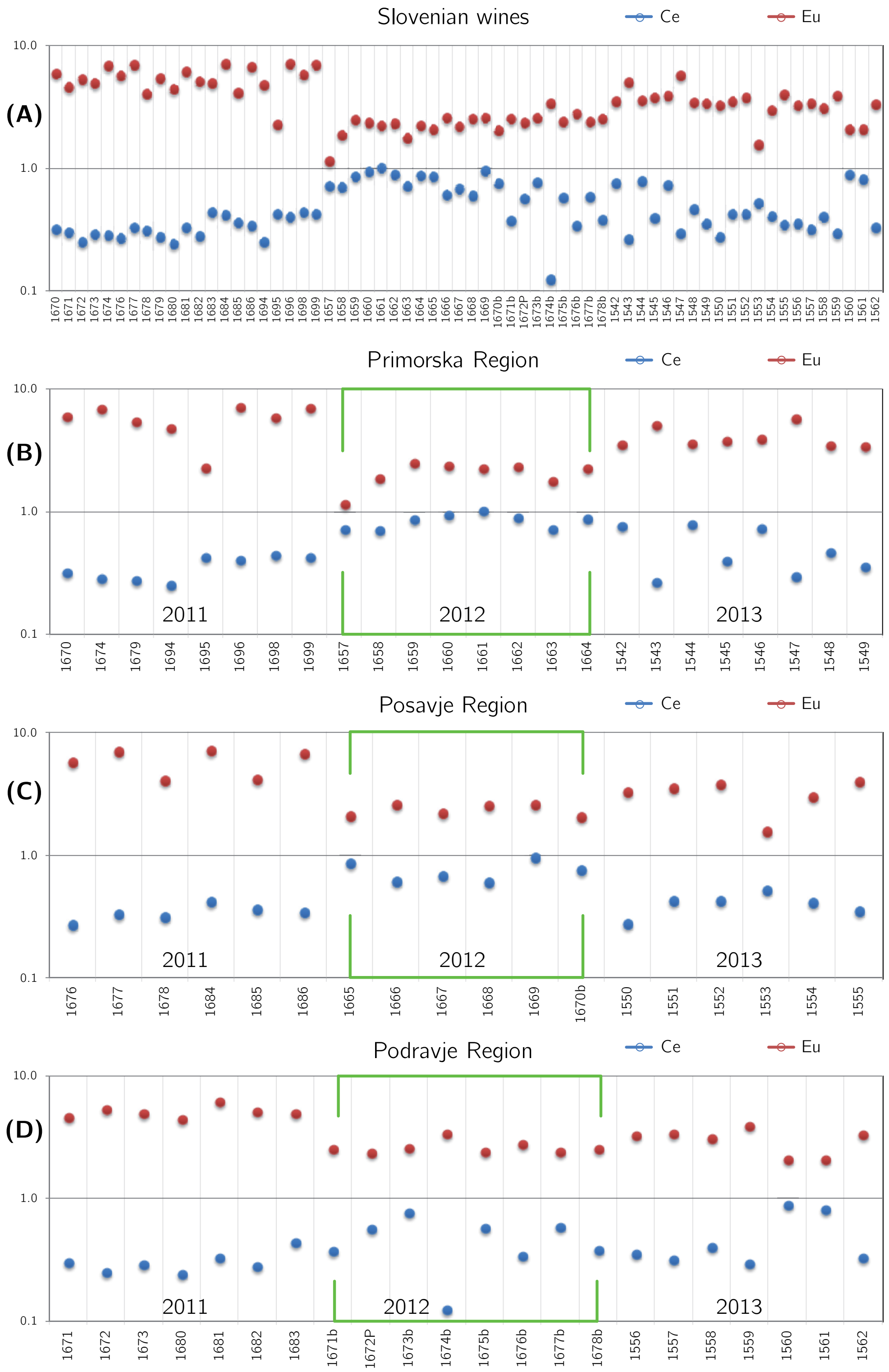


Figure 9.6: Anomalies calculation for Slovenian wines, Ce and Eu. All the samples (A), Primorska (B), Posavje (C), Podravje (D). The green bar separates vintage 2012.

Chapter 10

Stable Isotope results

10.1 Italian wines

Both δ^{18}O in wine and δ^{13}C in ethanol (Figure 10.1 A and 10.1 B respectively) resulted quite different in the four vintages, while evidencing similar trends among the nine fields (Table 10.1).

Results of δ^{18}O were comparable to the study of Caldwell, 1995 and Rossmann et al., 1999, which presented similar values for the vintages 2009 and 2011. In his work, values were generally lower for vintage 2010 and higher for vintage 2012. In particular we obtained: for the year 2009, values from 4.6 to 1.8 (±0.1) with an average of 3.7; for the year 2010, valued from 0.1 to -1.8 with an average value of -0.7; for the year 2011, values from 5.3 to 2.9 with an average value of 4.1; for the year 2012, values from 7.6 to 5.4 with an average value of 6.3.

Moreover δ^{13}C also showed lower values for the vintage 2010, but with a less evidence than δ^{18}O. In particular we obtained: for the year 2009, values from -24.0 to -27.3 (±0.1) with an average value of -25.9; for the year 2010, values from -27.6 to -28.8 with an average value of -28.2; for the year 2011, values from -26.2 to -28.1 with an average value of -27.1; for the year 2012, values from -25.3 to -26.9 with an average value of -25.9.

The values of $(D/H)_I$ and $(D/H)_{II}$ of ethanol were in the order of the ones suggested in Guyon et al., 2006 for French wines. The values shown in in Table 10.2 evidenced a diminution of $(D/H)_I$ for the vintage 2010, similar to the trend of δ^{18}O and δ^{13}C. Results of 2011 were harder to interpret because samples 3, 4 and 7 showed stable values, obtaining the same results as in 2010 (Figure 10.1 C). Results of samples 2 and 5 were even lower in 2011 than in 2010. Vintage 2012 presented higher values for some wines (1, 3, 4, 7, 8, 9), and lower one for others (2, 5, 6). Wine 5 presented the same values in

Table 10.1: C and O isotope ratio on Italian wine samples (δ^{13}C and δ^{18}O). Values are in per-mil (‰).

Wine Year	**Field**	**Code**	δ^{13}**C**	δ^{18}**O**
2009	1	495	-26.0	4.2
2009	2	496	-26.6	3.6
2009	3	497	-26.0	4.2
2009	4	498	-25.7	4.4
2009	5	499	-25.8	4.0
2009	6	500	-24.9	4.3
2009	7	501	-26.1	4.6
2009	8	502	-25.9	3.6
2009	9	503	-23.9	4.1
2009	10 (Dind.)	522	-27.3	1.8
2009	10 (Cabru.)	524	-25.2	3.2
2009	10 (Bosch.)	526	-26.8	2.5
2010	1	504	-27.8	0.1
2010	2	505	-27.6	-1.0
2010	3	506	-28.9	-0.1
2010	4	507	-28.6	0.1
2010	5	508	-28.4	-1.8
2010	6	509	-27.7	-1.3
2010	7	510	-28.7	-1.2
2010	8	511	-27.6	-0.8
2010	9	512	-28.1	-0.4
2010	10 (Dind.)	523	-28.5	-1.3
2010	10 (Cabru.)	525	-27.8	0.1
2010	10 (Bosch.)	527	-28.6	-1.4
2011	1	513	-26.2	5.0
2011	2	514	-28.1	4.1
2011	3	515	-27.6	3.8
2011	4	516	-26.9	5.3
2011	5	517	-27.1	4.5
2011	6	518	-26.2	4.4
2011	7	519	-27.7	3.1
2011	8	520	-27.1	2.9
2011	9	521	-27.1	3.8
2012	1	1479	-26.2	6.6
2012	2	1480	-26.9	5.4
2012	3	1481	-25.7	6.1
2012	4	1482	-25.3	7.6
2012	5	1483	-25.5	5.9
2012	6	1484	-25.6	7.0
2012	7	1485	-26.7	6.1
2012	8	1486	-25.6	6.1
2012	9	1487	-25.4	6.2

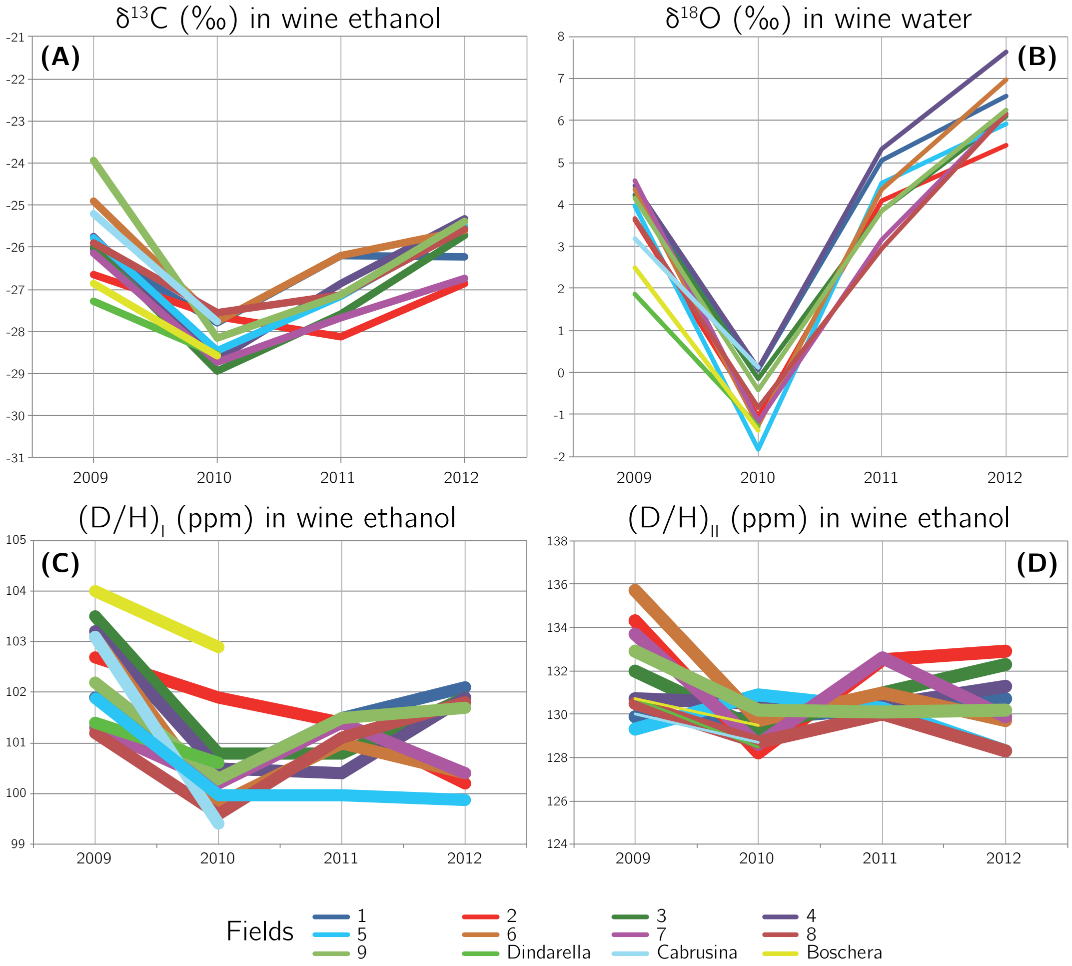


Figure 10.1: Stable isotope results for Italian wine samples. Four vintages. Dindarella, Cabrusina and Boschera were from a different field (10) near Negrar (Vr), only vintages 2009 and 2010. Error bars are graphically contained within the lines.

vintages 2010, 2011 and 2012. Moreover, Boschera vine had generally higher values than the other wines, but nonetheless presented a decreasing value during 2010.

Regarding ethanol isotopomer on methylene site $(D/H)_{II}$ the interpretation was even more difficult due to the different trends of the samples (Figure 10.1 D). Wines 2, 3, 6, 7, 8 and 9 evidenced a trend similar to the δ^{18}O and δ^{13}C with lower values in vintage 2010 while wines 1 and 4 resulted stable during the four vintages and wine 5 resulted higher during 2010.

Table 10.2: Ethanol isotopomer on methyl and methylene site $(D/H)_I$ and $(D/H)_{II}$. Results are expressed as ppm.

Wine Year	**Field**	**Code**	$\mathbf{(D/H)_I}$	$\mathbf{D(D/H)_I}$	$\mathbf{(D/H)_{II}}$	$\mathbf{D(D/H)_{II}}$	**R**
2009	1	495	101.9	±0.6	129.9	±0.6	2.55
2009	2	496	102.7	±0.5	134.3	±0.7	2.62
2009	3	497	103.5	±0.5	132.0	±0.9	2.55
2009	4	498	103.2	±0.5	130.7	±0.8	2.53
2009	5	499	101.9	±0.8	129.3	±0.7	2.54
2009	6	500	103.1	±0.3	135.7	±0.7	2.63
2009	7	501	101.3	±0.6	133.7	±0.9	2.64
2009	8	502	101.2	±0.5	130.5	±1.0	2.58
2009	9	503	102.2	±0.5	132.9	±0.9	2.60
2009	10 (Dind.)	522	101.4	±0.5	130.7	±0.9	2.58
2009	10 (Cabru.)	524	103.1	±0.8	130.0	±0.9	2.52
2009	10 (Bosch.)	526	104.0	±0.5	130.7	±0.9	2.51
2010	1	504	100.3	±0.6	129.8	±0.7	2.59
2010	2	505	101.9	±0.8	128.2	±0.9	2.52
2010	3	506	100.8	±0.5	129.3	±0.9	2.57
2010	4	507	100.5	±0.5	130.5	±0.6	2.60
2010	5	508	100.0	±0.6	130.9	±0.9	2.62
2010	6	509	99.8	±0.5	129.7	±0.8	2.60
2010	7	510	100.2	±0.5	128.6	±0.7	2.57
2010	8	511	99.6	±0.7	128.7	±0.8	2.58
2010	9	512	100.3	±0.7	130.2	±0.9	2.60
2010	10 (Dind.)	523	100.6	±0.5	128.5	±0.9	2.55
2010	10 (Cabru.)	525	100.6	±0.5	128.7	±1.0	2.56
2010	10 (Bosch.)	527	102.9	±0.7	129.5	±1.0	2.52
2011	1	513	101.5	±0.5	130.3	±0.6	2.57
2011	2	514	101.4	±0.7	132.5	±1.0	2.61
2011	3	515	100.8	±0.5	131.0	±0.7	2.60
2011	4	516	100.4	±0.7	130.4	±1.0	2.60
2011	5	517	100.0	±0.6	130.3	±0.6	2.61
2011	6	518	101.0	±0.5	131.0	±0.7	2.59
2011	7	519	101.4	±0.6	132.6	±0.6	2.62
2011	8	520	101.1	±0.6	130.0	±0.4	2.57
2011	9	521	101.5	±0.4	130.1	±0.6	2.56
2012	1	1479	102.1	±0.8	130.7	±0.9	2.56
2012	2	1480	100.2	±0.6	132.9	±0.6	2.65
2012	3	1481	101.9	±0.9	132.3	±0.8	2.60
2012	4	1482	101.9	±0.6	131.3	±0.5	2.58
2012	5	1483	99.9	±0.5	128.3	±0.9	2.57
2012	6	1484	100.4	±0.6	129.7	±0.6	2.58
2012	7	1485	100.4	±0.6	129.9	±1.0	2.59
2012	8	1486	101.8	±0.8	128.3	±1.0	2.52
2012	9	1487	101.7	±0.7	130.2	±0.9	2.56

10.1.1 Combined use of IRMS and SNIF-NMR

The combined use of IRMS and SNIF-NMR is reported in Figure 10.2 A, showing the correlation among the four vintages taking into account the $(D/H)_I$ and the $\delta^{13}C$. This combination is generally used to understand the provenance of a wine and our data were well distributed in the *X axes*, proving useful to distinguish these wines to the one coming from other environmental conditions and latitude.

Figure 10.2 B reports the correlation between the four vintages taking into account the $(D/H)_{II}$ and the $\delta^{18}O$. Both variables depend on atmospheric conditions, in particular temperature and rainfall (Camin et al., 2015; Ogrinc et al., 2001). Thus, this was further evidence of the diversity of the vintage 2010. It was not possible to distinguish the vintages 2009 and 2011, which were produced under similar atmospheric conditions, while vintage 2012 is shown in the upper part of the graphic.

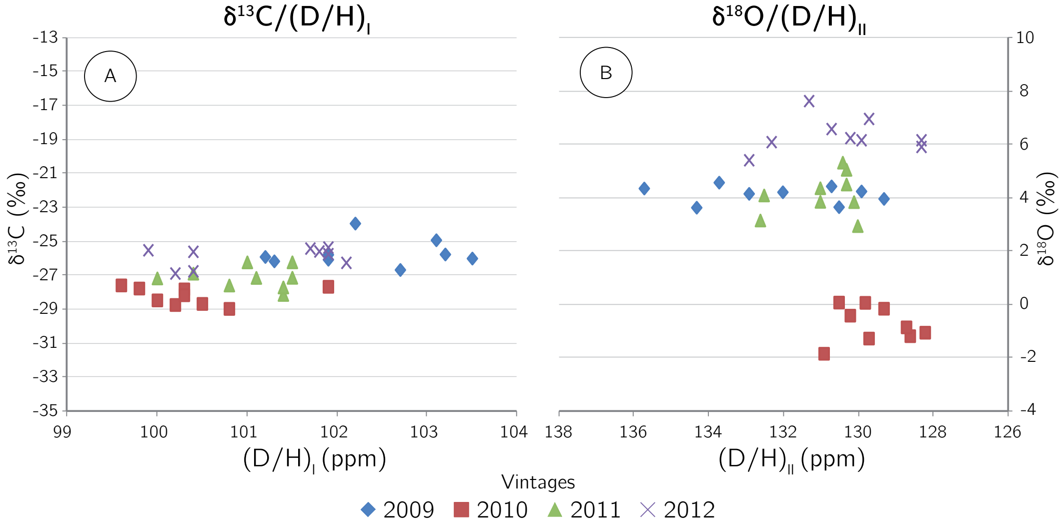


Figure 10.2: Correlation between $(D/H)_I$ and $\delta^{13}C$ (A) and between $(D/H)_{II}$ and $\delta^{18}O$ (B) of Italian wines. Error bars are graphically contained within the symbols.

10.2 Grapes

Stable isotope results for grapes confirmed the trace metals analysis and the inhomogeneity in the chemical and physical composition of the original samples, in particular for vintage 2011 (Figure 10.3).

Vintage 2012 presented values very similar to vintage 2012, with an average difference of 0.3 (‰). Samples 4 and 6 presented the greatest differences between the two matrices, 0.5 and 0.6 ‰) respectively. This could be due to a low fractionation during wine production, although the results were not very distant.

Values of δ^{18}O in water juice resulted similar to those found in other studies. Christoph et al., 2015 reported values from -1 to +6 ‰) for Southern Europe wines, while our data were characterized by generally higher values. Values are similar also to Koziet et al., 1995 for orange juice.

Values of δ^{13}C in sugar were lower than those found for δ^{13}C in pulp (in exception of field 6 of 2012).

Comparing must to wine, values are different in both δ^{13}C of sugar and δ^{13}C of pulp and is not possible to associate each must to the relative wine. This fact could be explained by the possible fractionation of δ^{13}C during wine-making process and with the same inhomogeneity of must samples. Values of δ^{13}C were similar to Bizjak Bat et al., 2012 and to Koziet et al., 1993 for orange juice. Tables 10.3 and 10.4 show the results of the measurements.

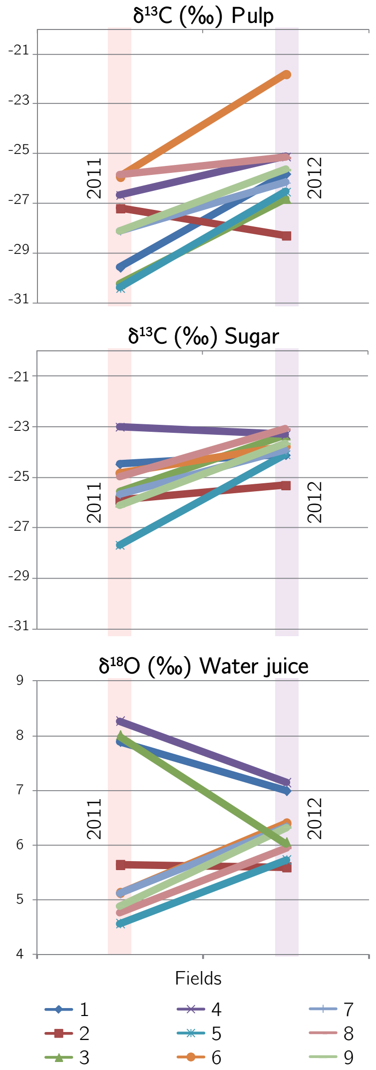


Figure 10.3: δ^{13}C on Italian must's sugar, δ^{13}C on Italian must's pulp, δ^{18}O on Italian must's pulp. Vintages 2011 and 2012. Error bars are graphically contained within the lines.

Table 10.3: Stable isotopes for 2011 grapes (Italy), average values (‰).

Field	δ^{13}C **Sugar**	δ^{13}C **Pulp**	δ^{18}O **Water juice**
1	-24.5	-29.6	7.9
2	-25.9	-27.2	5.6
3	-25.5	-30.2	8.0
4	-23.0	-26.6	8.3
5	-27.7	-30.4	4.6
6	-24.8	-25.9	5.1
7	-25.7	-28.1	5.1
8	-25.0	-25.8	4.8
9	-26.1	-28.1	4.9

Table 10.4: Stable isotopes for 2012 grapes (Italy), average values (‰).

Field	δ^{13}C **Sugar**	δ^{13}C **Pulp**	δ^{18}O **Water juice**
1	-24.1	-25.8	7.0
2	-25.3	-28.3	5.6
3	-23.3	-26.8	6.0
4	-23.3	-25.1	7.1
5	-24.1	-26.5	5.7
6	-23.7	-21.8	6.4
7	-23.9	-26.1	6.3
8	-23.1	-25.1	5.9
9	-23.6	-25.6	6.3

Chapter 11

Statistical approach

11.1 Wine

Principal Components Analysis technique is explained in Chapter 7. For each PCA, we performed a Factor Analysis for confirmation, always obtaining the same results. One FA was reported as example. Error bars were not shown in the PCA graphics because the error was even smaller than the symbols used on the images.

11.1.1 Trace Elements

PCA was performed on trace metals to find evidence of similarity/differences in wines from Italy and Slovenia.

However, taking into consideration all the macro- and micro-trace elements (and not REEs) in the PCA, no grouping of vintage was clearly visible while we could evidence a distinction between the two countries. This possible separation is shown in Figure 11.1, featuring the plot of the first and third principal components. Figures 11.2 show the plots of the PCA performed on the elements. These Figures show that, expecially in the second plot, the PC3 separated Ga, As, Ca, U and Cd to Rb, Co, Tl, Zn, Ni, Mn, Fe, Be and Sr. Some elements appeared grouped together (V, Na, Al, Pb, Cr, Ba, Cu), but this was very difficult to interpret.

The separation of the two groupings may be related both to the environmental breeding conditions and to different processes of production. Although the latter were standard processes, they may have altered the composition of elements. The first three principal components (PCs) included 76.9 % of the explained variance, with PC1 representing 51.3 % (Table 11.1).

A PCA of trace metals was done only for Italian wine samples to reduce

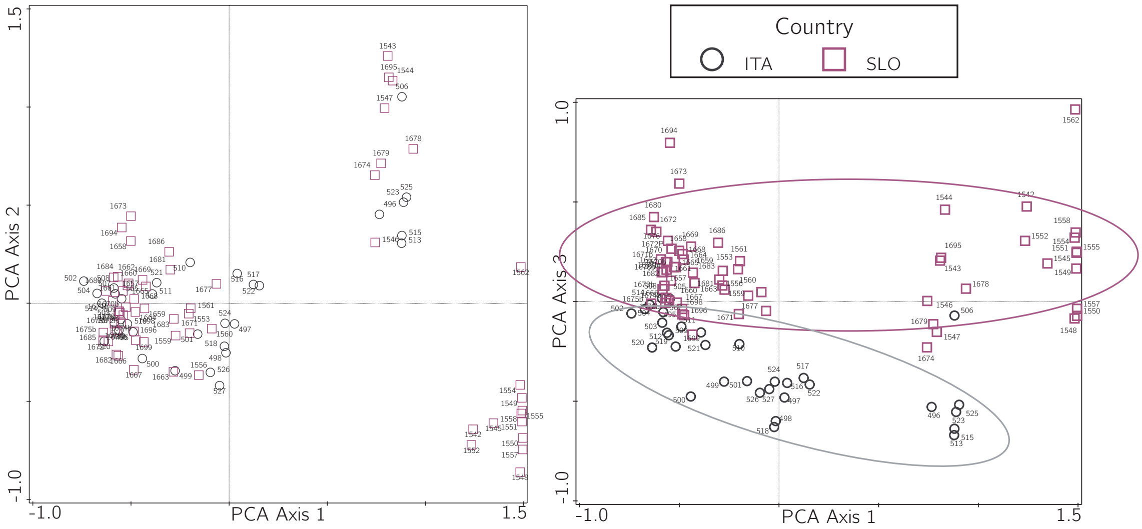


Figure 11.1: PCA on trace elements for all the wines, Principal Components 1 and 2, Principal Components 1 and 3.

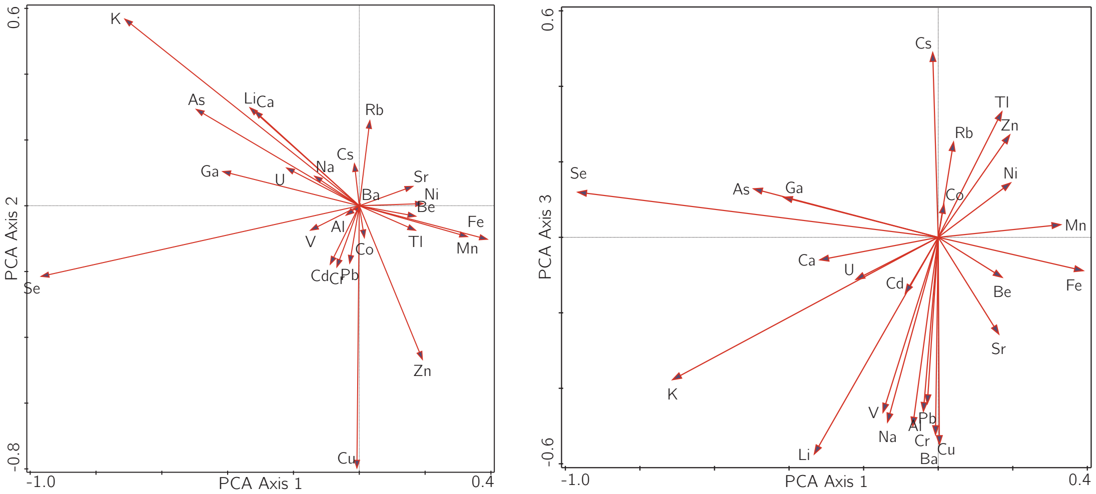


Figure 11.2: PCA on trace elements for all the wines, Plot of Principal Components 1 and 2 and Plot of Principal Components 1 and 3 for the elements.

Table 11.1: Summary table for PCA.

Statistic	**Axis 1**	**Axis 2**	**Axis 3**
Eigenvalues	0.51	0.16	0.09
Explained variation (cumulative)	51.2	67.5	76.8

the dimensionality of the data set and help in the interpretation. The PCA in Figure 11.3 evidenced a separation of vintage 2012 in PC2 but the other two vintages were mixed.

The first three principal components (PCs) included 94.36 % of the explained variance, with PC1 representing 79.9 % (Table 11.2). In this case the PCA was not able to separate the different fields of the Valpolicella area. This fact could be due to the small amount of samples from the Italian wines which were not so different and came from very close fields.

Samples from vintage 2010 seemed to be explained by elements Ca, Al, Fe, Li, V, Na, and Be which are shown in Figure 11.4.

Table 11.2: Summary table for PCA.

Statistic	**Axis 1**	**Axis 2**	**Axis 3**
Eigenvalues	0.79	0.10	0.04
Explained variation (cumulative)	79.9	89.9	94.3

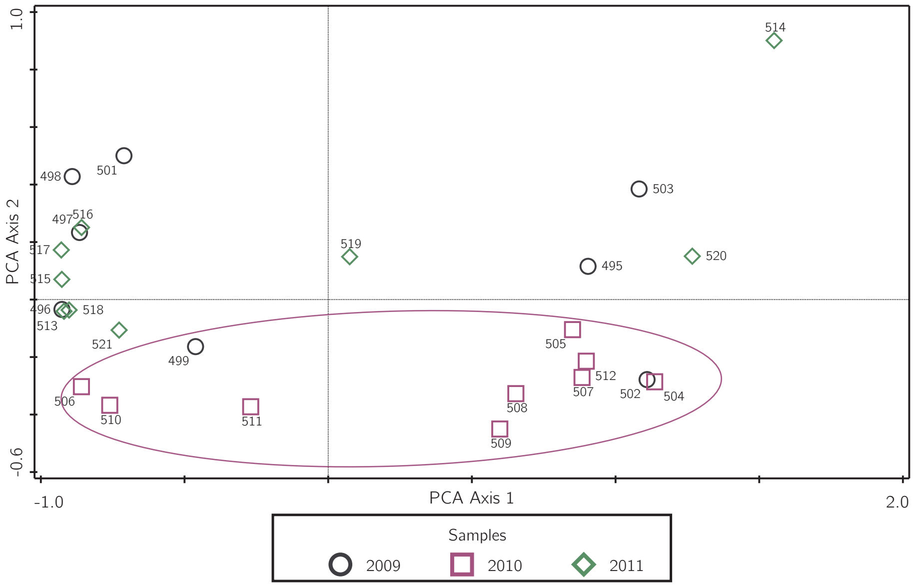


Figure 11.3: PCA on trace elements for Italian wines, PC1 and PC2. Three vintages.

Slovenian wines appeared more interesting. The PCA on trace elements for Slovenian wines, regarding the three vintages, is reported in Figure 11.5. This figure evidences a clear separation between vintage 2012 and vintage 2013 while 2011 is not consistently separated from the others. The first three

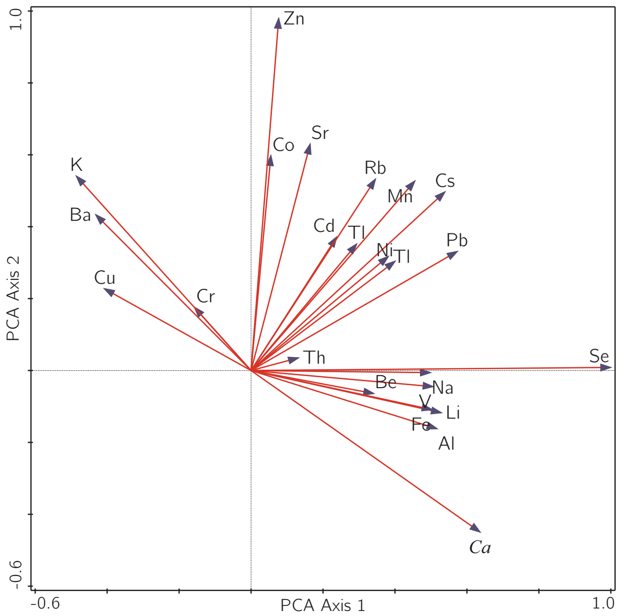


Figure 11.4: PCA on trace elements for Italian wines, PC1 and PC2 of studied elements. Three vintages.

principal components (PCs) included 87.26 % of the explained variance, with PC1 representing 62.03 % (Table 11.3).

Table 11.3: Summary table for PCA.

Statistic	**Axis 1**	**Axis 2**	**Axis 3**
Eigenvalues	0.62	0.18	0.06
Explained variation (cumulative)	62.0	80.3	87.2

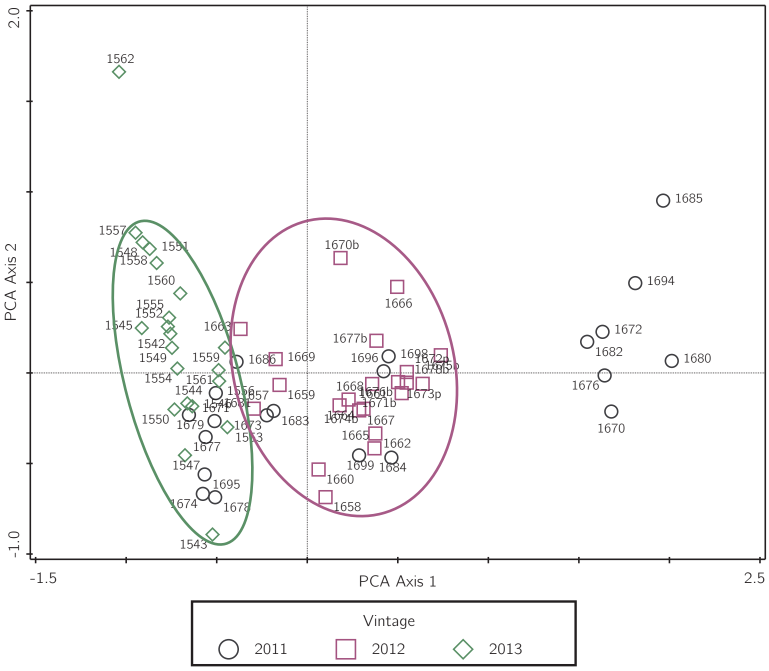


Figure 11.5: PCA on trace elements for Slovenian wines, PC1 and PC2. Three vintages.

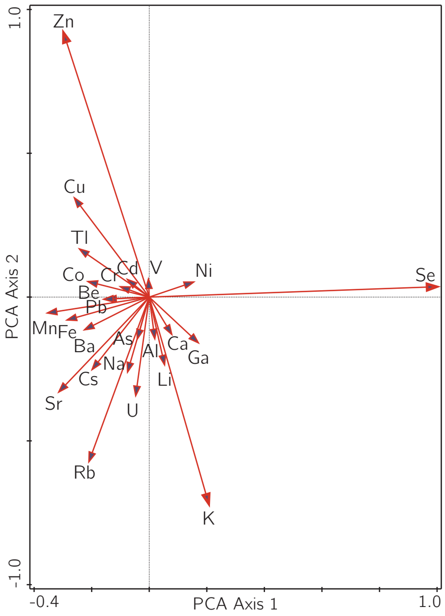


Figure 11.6: PCA on trace elements for Slovenian wines, PC1 and PC2 of studied elements. Three vintages.

11.1.2 Rare Earth Elements

Plots in Figure 11.7 summarize the relationship between the Italian and Slovenian studied wines regarding 14 REEs.

The first three principal components (PCs) included 95.05 % of the explained variance, with PC1 representing 81.20 %. The Figure shows, in both plots, a grouping of the wines from the two countries. The first and the second principal components separate the samples: the Slovenian wines are on the left of the image, and the Italian ones on the right. However, the separation is not really strong, as some samples remain out of the groupings, and others out the border of their own supposed group. A case in point can be seen in samples 526 and 527, representing the 2009 and 2010 vintages of Boschera wine. These differ from the other samples in few kind of analysis.

Figure 11.8 presents the correlation between the elements: a strong separation of Eu and Sm from the other REEs was found. Moreover Eu and Sm seem very similar.

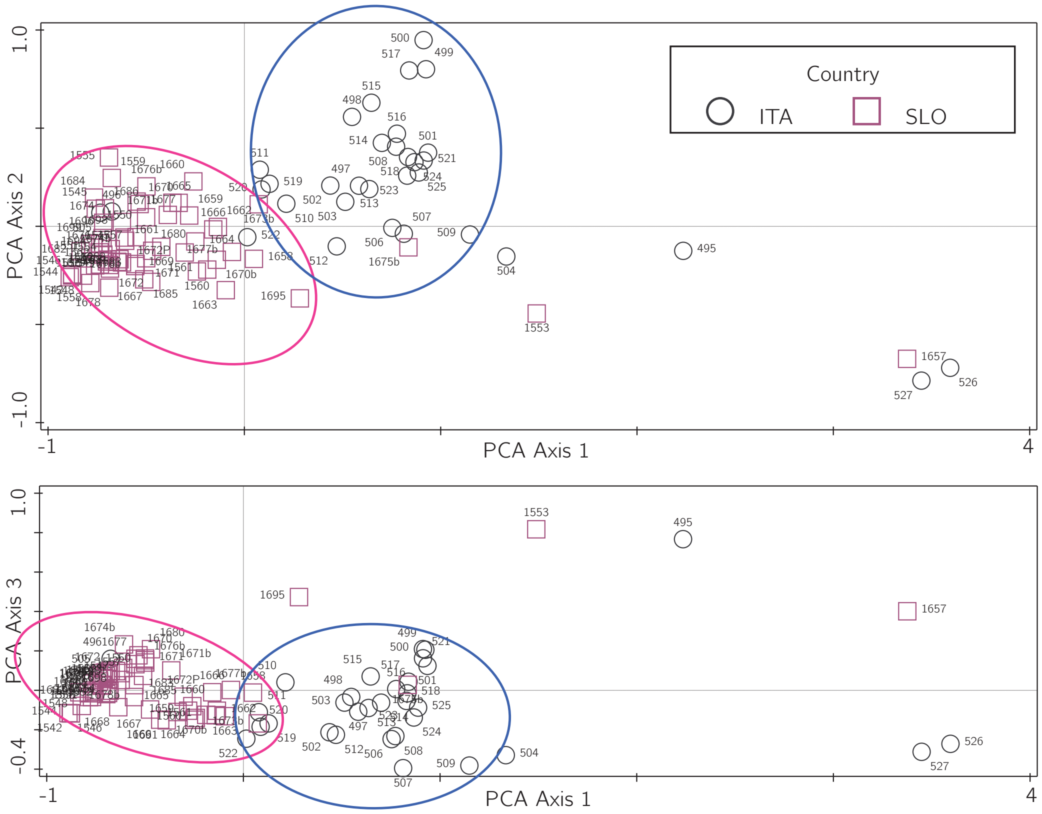


Figure 11.7: PCA on Rare Earth Elements, Italian and Slovenian wines: PC1/PC2 and PC1/PC3.

Table 11.4: Summary table for PCA.

Statistic	**Axis 1**	**Axis 2**	**Axis 3**
Eigenvalues	0.81	0.09	0.03
Explained variation (cumulative)	81.2	91.0	95.0

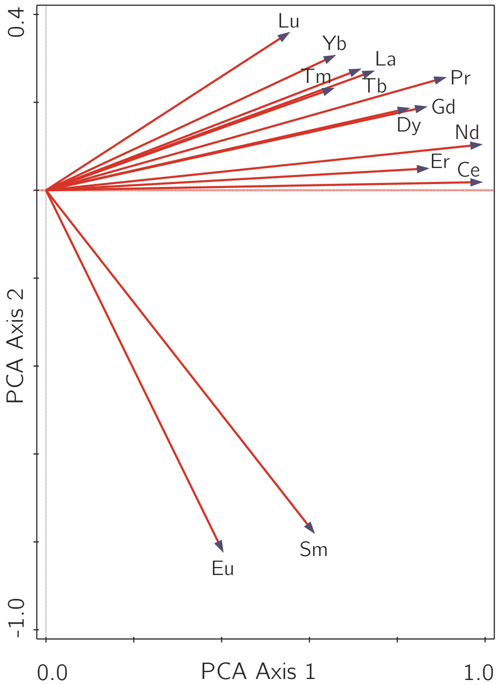


Figure 11.8: PCA on Rare Earth Elements, Italian and Slovenian wines, distribution of the elements.

Factor Analysis is reported here to underline the efficiency of the PCAs used in this work. Plot in Figure 11.9 showed the same results of Plots in Figure 11.7, regarding REEs in Italian and Slovenian wines, even if a rotation of the elements and the view has been done. The samples were disposed in the same configuration as the PCAs, obtaining the same distance from each other. It was easy to identify the Slovenian samples and the Italian ones, although some samples got mixed into the wrong group and others were outliers. It is worth noting that samples 526 and 527 were again separated from the group of the Italian samples.

Figure 11.10 confirms the different explanation by Factor1 and Factor2 of Eu and Sm compared to the remaining elements. This Figure is very similar to the one of the PCA in Figure 11.8.

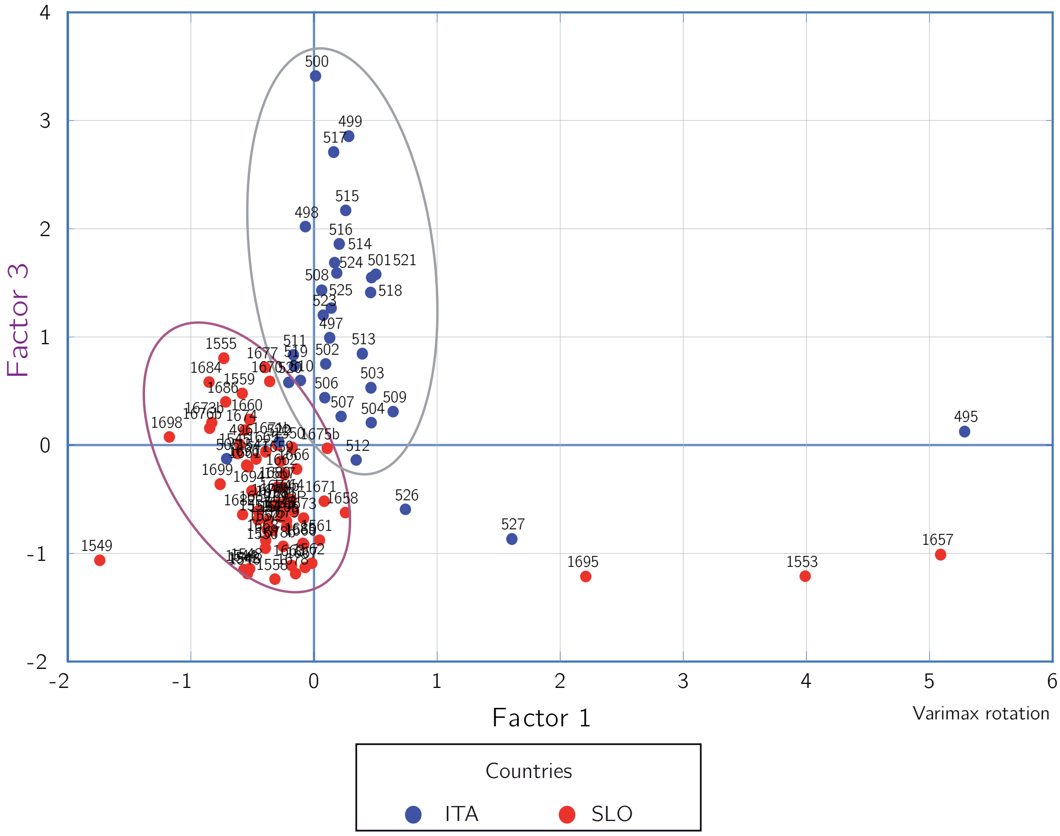


Figure 11.9: CFP on Rare Earth Elements, Italian and Slovenian wines.

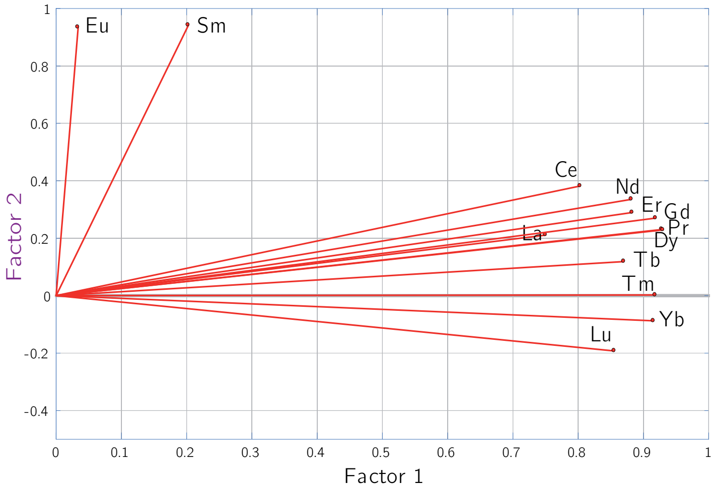


Figure 11.10: CFP on Rare Earth Elements, Italian and Slovenian wines, elements.

Italian wines

The results of the previous PCA and FA suggested to separate Italian and Slovenian wines trying to better understand the relation between the samples.

Figure 11.11 summarized the relationship among the studied Italian wines regarding the 14 REEs. The first three principal components (PCs) included 91.46 % of the explained variance, with PC1 representing 71.71 % (Table 11.5).

The plot in Figure 11.11, regarding the three years of production for Italian wine samples, evidences a difficult interpretation. Italian REEs resulted more homogeneous and PC1 was unable to separate any vintages. PC2 could explain and divide somehow vintage 2010 (below in the plot) from 2011 (above in the plot). This PCA was not useful for understanding the correlation between the samples, nor was it useful for the identification of the studied fields. However, the nature of the REEs in these samples agreed with the FA in Figure 11.10 and to the PCA in Figure 11.8, evidencing a separation of Eu and Sm, shown in Figure 11.12.

Table 11.5: Summary table for PCA for italian wines REEs.

Statistic	Axis 1	Axis 2	Axis 3
Eigenvalues	0.71	0.14	0.06
Explained variation (cumulative)	71.7	85.5	91.4

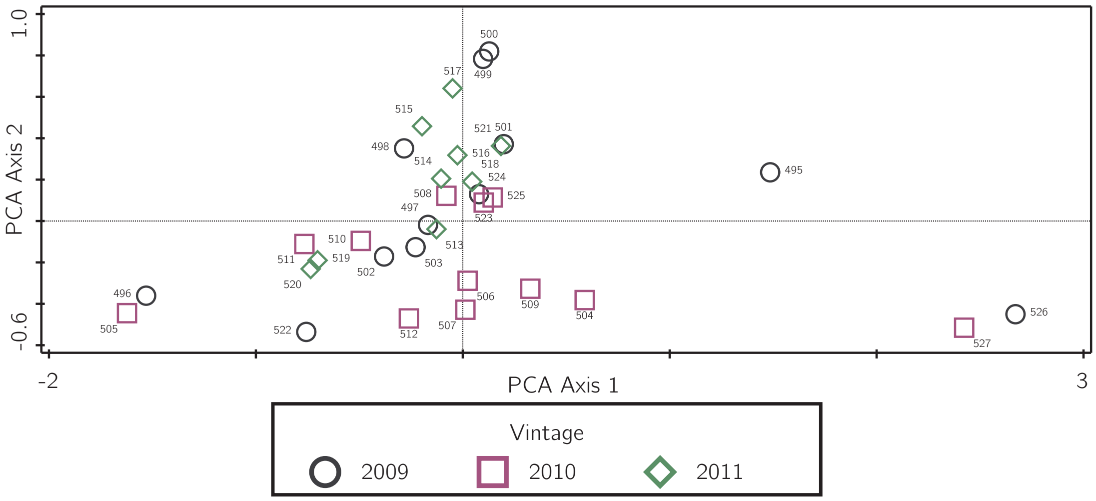


Figure 11.11: Summary table for PCA for Italian wines REEs, three vintages.

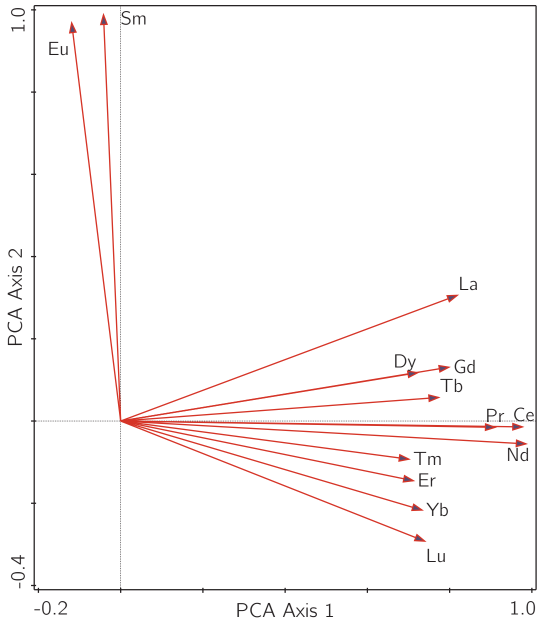


Figure 11.12: Summary table for PCA for Italian wines REEs, studied elements.

Slovenian wines

Slovenian wines resulted more interesting, evidencing a clear grouping of the vintages. Plot in Figure 11.13 summarizes the relationship connecting the Slovenian wines regarding the 14 REEs. The first three principal components (PCs) included 89.56 % of the explained variance, with PC1 representing 54.84 % (Table 11.6).

Table 11.6: Summary table for PCA for Slovenian wines REEs.

Statistic	**Axis 1**	**Axis 2**	**Axis 3**
Eigenvalues	0.54	0.23	0.11
Explained variation (cumulative)	54.8	77.8	89.5

Figure 11.13 evidenced on the first PC a grouping among the vintages of wine. The vintage 2012 is clearly separated on the right of the plot, while the other two vintages appeared on the left. Some samples in the transition zone among the two groups were found:

- 1668, 1678, and 1674 were more similar to 2011-2013 samples than 2012 samples;
- 1560 and 1561 were mixed among the 2012 samples.

Vintages 2011 and 2013 from this point of view seemed to be very similar vintages of wine. According to Brown et al., 1990 and Tyler, 2004, this can be explained as by a possible relation between with the uptake of the mineral content from the soil by the plants. As already mentioned in Section 3.4, 2012 was a warmer and drier year than 2011 and 2013, which had similar weathering condition. Moreover, we tried to graphically distinguish the three wine regions, like as it was done for the three vintages, but the PCA was unable to help in the separation. Regions seemed to be mixed together.

Plot in Figure 11.14 showed again a different behaviour of Eu and Sm which were explained by PC2, but there was also La in the same direction. Some HREEs seemed very similar (Tm, Yb, Lu).

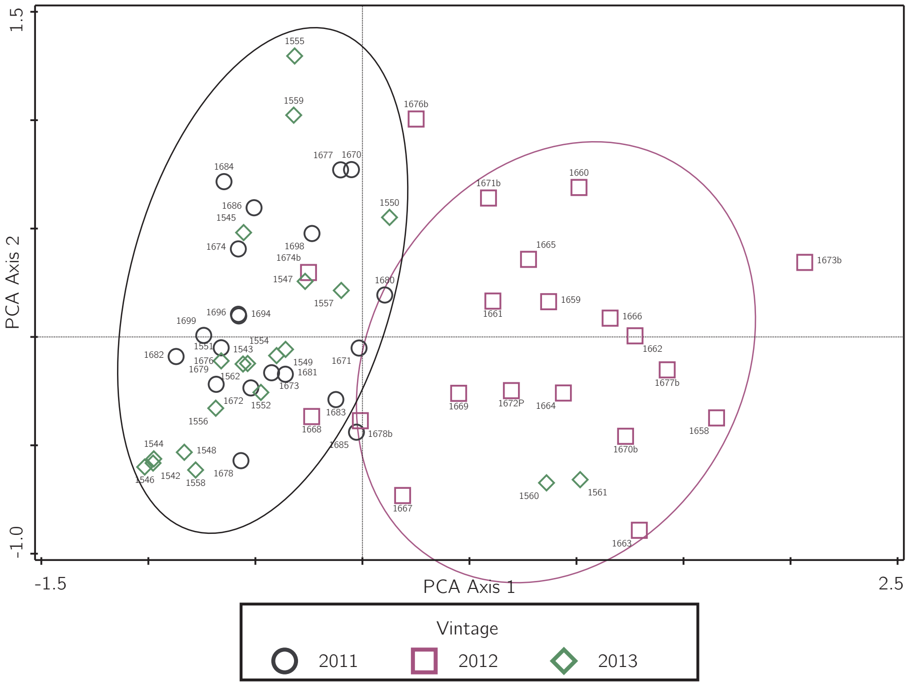


Figure 11.13: Summary table for PCA for Slovenian wines REEs, PC1 and PC2, three vintages.

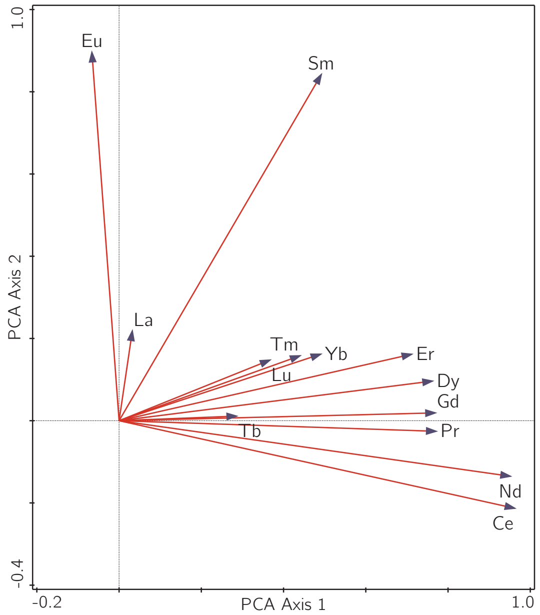


Figure 11.14: Summary table for PCA for Slovenian wines REEs, studied elements.

11.2 Grapes and soils

Analytical results of musts evidenced very different patterns, because of the inhomogeneity of the samples, and statistical analysis was not useful in the description of the dataset. The PCA described the results separating vintage 2011 to 2012, without suggesting any possible information about the nine regions.

Moreover, neither a PCA on soil results was useful to find a correlation between the samples. The soils were not clearly groped, and there was no evidence of the nine fields or of the four climatic regions.

However, statistical approach is confirming the results of the elemental analysis where the pattern composition of soils seems different of musts and wines.

11.3 Stable isotopes

PCA of stable isotopes of Italian wines was done to confirm the results proposed in section 10.1 and to take in consideration all the indexes (δ^{13}C, δ^{18}O, $(D/H)_I$ and $(D/H)_{II}$) to describe the wine vintages. Plot in Figure (11.16) showed some differences in the wines of the vintage 2010, as expected. This latter was a colder and wetter year than 2009 and 2011, reflecting the data of atmospheric events (which are reported in section 3.4). PC1 separated also vintage 2012 (in the left) from vintage 2010 which were very different. Vintage 2012 was dry and hot, as opposed to 2010. Here, stable isotopes were shown to be very sensitive to the atmospheric conditions. However, it was not possible to distinguish between vintages 2009 and 2011, which were very similar in relation to weather conditions, and some samples of 2012 were borderline.

The first three principal components (PCs) included 96.95 % of the explained variance, with PC1 representing 70.11 %.

Table 11.7: Summary table for PCA.

Statistic	**Axis 1**	**Axis 2**	**Axis 3**
Eigenvalues	0.70	0.11	0.08
Explained variation (cumulative)	70.1	88.8	96.9

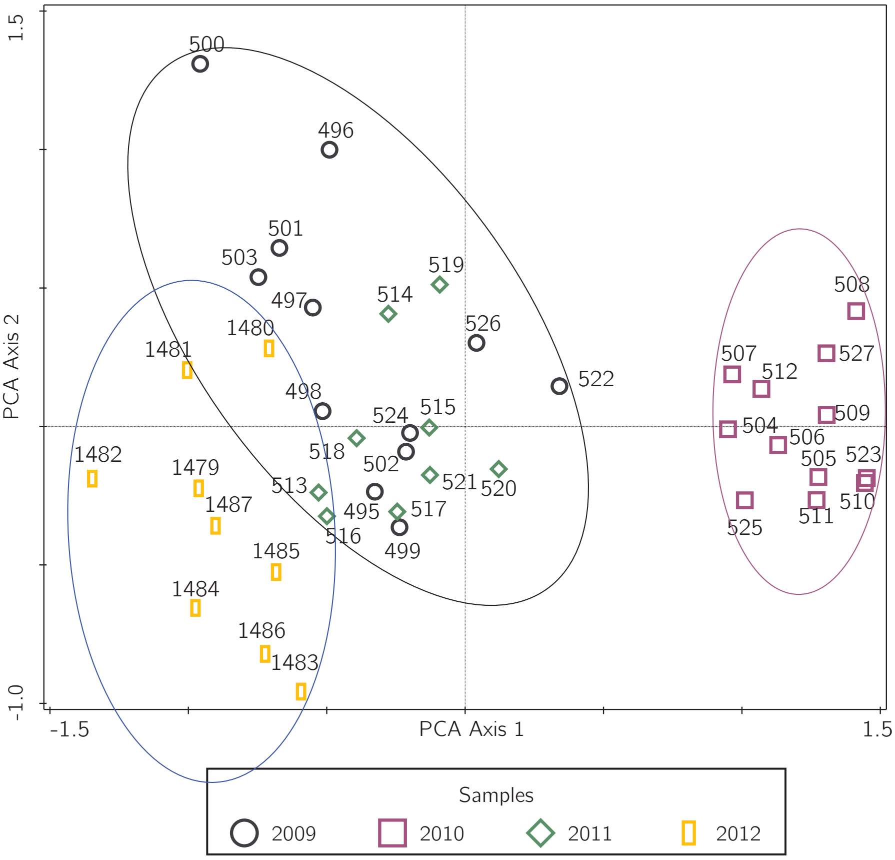


Figure 11.15: PCA taking in account the stable isotopes of the Italian wine samples: δ^{13}C in ethanol, D/H (I and II) in ethanol and δ^{18}O in water wine.

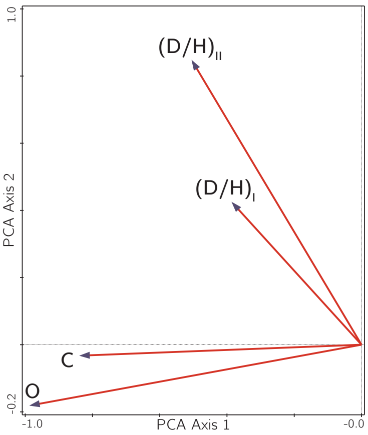


Figure 11.16: PCA taking in account the stable isotopes of the Italian wine samples: δ^{13}C in ethanol, D/H (I and II) in ethanol and δ^{18}O in water wine.

Chapter 12

Conclusions

The main aim of this PhD project, from the PhD programme in Science and Management of Climate Change, was to explore the variability of chemical and isotopic composition of wines in relation to the climate conditions. Moreover, we used techniques adopted to determine the geographical origin or the adulteration of wine products, to evidence the weather variability in wines.

The first step of the work, was to acquire vintages of wine which followed a unique method of production. This way reduced the possible human interferences and made possible to measure at best the effects of climate variability. However, the possibility to acquire wines from the markets, too much matched with the uncertainties of wine-making process, was excluded.

The aim was to investigate the more vintages as possible and the collection of a hundred samples made in different years from two countries allowed us to explore the effects of the weather variability.

The acquisition of such number of wine samples, very difficult for the short period of a PhD, was made by means of the CRA-VIT and of the Agriculture Institute of Slovenia supporting the project with four and three vintages respectively. Then, after a two years of chemical analyses, was possible to achieve a good amount of data.

Regarding Italian wines, both mass spectrometry and isotopic mass spectrometry was used to characterise the samples while was not possible to analyse Slovenian stable isotopes to confirm the results of the Italian ones.

As reported in section 3.4 for Italian vine growing zones, vintages from 2009 to to 2012 experienced different quantities of rainfall and different average temperatures during the maturity periods. Both Slovenian and Italian regions evidenced a 2010 to be more wet and colder comparing to 2012, which was warmer with less precipitation. This evidence was subsequently underlined by the results of the analyses. In particular, analytical results showed that both mass spectrometry and isotopic mass spectrometry proved powerful techniques to investigate climatic variations in wine composition.

Statistical approaches on Italian wines, considering analytical results of trace elements and stable isotope analysis, were able to separate vintage 2010 and evidence groupings for the other two years. However, the REEs were too much mixed together to evidence groupings of samples.

Studying the region of Valpolicella, Tomasi and Battista, 2014 evidenced four different climatic sub-regions that this study was not able to identify. The fields on the wine growing regions of Valpolicella resulted indeed to be of very difficult to make any differentiation. This fact may be explained first of all by an intense agricultural land use which has contribute to homogenize the elemental composition of upper soils of the vine fields. Moreover, soils resulted not connected to the relative wines. This can be justified by the fact that wines were made mixing the grapes of the two or three most representative wineries of each field.

In the case of Italian wines the weather conditions were evidenced with an important separation of stable isotopes which they confirmed to be very sensitive.

To be noticed, both isotopic and elemental analyses evidenced samples 526 and 527 as outliers. These wines were made from Boschera vine in 2009 and 2010 and were coming from a different field near Verona (Negrar). The calculation of Europium anomalies underlined the different composition of these two samples as well. Differently, Dindarella and Cabrusina wines were not showing evident dissimilarity in relation to the Valpolicella wines, while they were made in Negrar. This fact, could be due to a different sensibility to the hydrological and thermal stresses of Boschera plants, whose behaviour may be different than Cabrusina and Dindarella in the production of fruits. There is no certainties about this and a more detailed study should be performed. Another explanation could be the related to the plant variety because from Boschera vine was the only white wine studied.

Analytical results of must samples were not useful to understand the climatic variations. Both mass spectrometry and isotopic mass spectrometry techniques evidenced results of difficult interpretation. Stable isotopes ratios of wines were clearly different in 2009 and 2010, while must ones reflected the inhomogeneities of the samples.

Moreover, a possible isotopes fractionation could be evinced by δ^{13}C in must's pulp and sugar results. The data were supposed to be very similar to each other but the trends of the values resulted very often contrary. This could have been caused, in addition to the inhomogeneity of the samples, by the long time conservation of the bottles and the processes of pre-treatment during the analysis, resulting in a presumable fractionation of the isotopes.

Regarding Slovenian wines, the studied regions were characterized of three very different environments, and the elemental measurements resulted very useful. However, also in these samples was not possible to separate the different wine regions using elemental composition while the vintages were clearly evidenced.

The PCA of trace elements was helpful to distinguish two of the three vintages, but not a third, which was distributed more homogeneously in the plot. Moreover, a PCA of REEs separated vintage 2012. As expected, this technique was unable to distinguish vintages 2011 and 2013 which were reported as similar colder years from historical weather data (section 3.4).

Italian and Slovenian wines were distinguished, with the exception of few samples, by both TEs and REEs results. This separation, due to different elemental compositions, may be justified as being part of two distinct environmental and geographical belongings (*terroir*) but also to the processes of wine production, from the harvest to the bottling, which have certainly interfered and characterized the products. The amount of analysis done were not enough to correlate at best the wines from the two Countries, indeed it would been really useful being able to analyse the stable isotopes of the Slovenian wines and the soils. With the contribute of the Slovenian stable isotopes we would been able to see even better if the meteorological variations were so influential as it seems.

- According to the data collected in this study: is it possible to evidence climatic variations by means to the chemistry of wine?

The results of this work were not able to confirm the mass spectrometry and the isotopic mass spectrometry to be useful to distinguish a wine for a specific region while they were able to separate vintages growth in different weather conditions. In conclusion of the work we can furthermore suggest from our data that weather conditions showed to have more influence in the chemical composition of wines than the environmental contribution. Analytical techniques were not able to evidence the provenance while the three Slovenian growing-regions are reported to be very different while weather conditions were visible.

Moreover, one other conclusion of this PhD project is that the more is different a year in terms of weather conditions, the more the techniques of analysis can show the separation of the wines made in that year. However, easy to be supposed but confirmed by this work, has been not possible distinguish vintages produced in years of similar weather conditions.

The future work connected to this project should be the acquiring of the of trace elements and stable isotopes data of wines made in Northern Europe. The composition of the samples would be able to confirm that the climate variability affects grapes production and then wine in other latitudes.

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Part IV

Tables

Appendix A

Summary tables of samples

Table A.1: Legend of studied Italian wines, musts and soils

Italian wines

Code	**Field**	**Year of production**	**Code**	**Field**	**Year of production**
495	1	2009	513	1	2011
496	2	2009	514	2	2011
497	3	2009	515	3	2011
498	4	2009	516	4	2011
499	5	2009	517	5	2011
500	6	2009	518	6	2011
501	7	2009	519	7	2011
502	8	2009	520	8	2011
503	9	2009	521	9	2011
522	10 Dindarella (Negrar)	2009			
524	10 Cabrusina (Negrar)	2009			
526	10 Boschera (Negrar)	2009			
504	1	2010	1479	1	2012
505	2	2010	1480	2	2012
506	3	2010	1481	3	2012
507	4	2010	1482	4	2012
508	5	2010	1483	5	2012
509	6	2010	1484	6	2012
510	7	2010	1485	7	2012
511	8	2010	1486	8	2012
512	9	2010	1487	9	2012
523	10 Dindarella (Negrar)	2010			
525	10 Cabrusina (Negrar)	2010			
527	10 Boschera (Negrar)	2010			

Must

Code	**Field**	**Year of production**	**Code**	**Field**	**Year of production**
30	1	2011	165	1	2012
34	2	2011	191	2	2012
49	3	2011	171	3	2012
50	4	2011	177	4	2012
70	5	2011	180	5	2012
95	6	2011	199	6	2012
72	7	2011	204	7	2012
102	8	2011	282	8	2012
111	9	2011	274	9	2012

Soil

Code	**Field**	**Depth (cm)**	**Code**	**Field**	**Depth (cm)**
P1A	1	0-40	P6A	6	0-35
P1B	1	40-80	P6B	6	35-75
P2A	2	0-30	P6C	6	75-120
P2B	2	30-60	P7A	7	0-30
P3A	3	0-35	P7B	7	30-60
P4A	4	0-20	P8A	8	0-30
P4B	4	20-40	P8B	8	30-60
P5A	5	0-50	P9A	9	0-40
P5B	5	50-80	P9B	9	40-90
P5C	5	80-100			

Table A.2: Legend of studied Slovenian wines .

Code	**Field**	**Year of production**	**Code**	**Field**	**Year of production**
1670	Primorska	2011	1668	Posavje	2012
1671	Podravje	2011	1669	Posavje	2012
1672	Podravje	2011	1670b	Posavje	2012
1673	Podravje	2011	1671b	Podravje	2012
1674	Primorska	2011	1673b	Podravje	2012
1676	Posavje	2011	1674b	Podravje	2012
1677	Posavje	2011	1675b	Podravje	2012
1678	Posavje	2011	1676b	Podravje	2012
1679	Primorska	2011	1677b	Podravje	2012
1680	Podravje	2011	1678b	Podravje	2012
1681	Podravje	2011	1672P	Podravje	2012
1682	Podravje	2011	1542	Primorska	2013
1683	Podravje	2011	1543	Primorska	2013
1684	Posavje	2011	1544	Primorska	2013
1685	Posavje	2011	1545	Primorska	2013
1686	Posavje	2011	1546	Primorska	2013
1694	Primorska	2011	1547	Primorska	2013
1695	Primorska	2011	1548	Primorska	2013
1696	Primorska	2011	1549	Primorska	2013
1698	Primorska	2011	1550	Posavje	2013
1699	Primorska	2011	1551	Posavje	2013
1657	Primorska	2012	1552	Posavje	2013
1658	Primorska	2012	1553	Posavje	2013
1659	Primorska	2012	1554	Posavje	2013
1660	Primorska	2012	1555	Posavje	2013
1661	Primorska	2012	1556	Podravje	2013
1662	Primorska	2012	1557	Podravje	2013
1663	Primorska	2012	1558	Podravje	2013
1664	Primorska	2012	1559	Podravje	2013
1665	Posavje	2012	1560	Podravje	2013
1666	Posavje	2012	1561	Podravje	2013
1667	Posavje	2012	1562	Podravje	2013

Appendix B

Certificate of Analysis, SRM® 2711a

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2711a

Montana II Soil

Moderately Elevated Trace Element Concentrations

This Standard Reference Material (SRM) is intended primarily for use in the analysis of soils, sediments, or other materials of a similar matrix. One unit of SRM 2711a consists of 50 g of the dried, powdered soil.

Certified Values: The certified concentrations for 25 elements, expressed as mass fractions [1] on a dry-mass basis, are provided in Table 1. Certified values are based on results obtained from critically evaluated independent analytical techniques. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [2].

Reference Values: The reference values for eight constituents, expressed as mass fractions on a dry-mass basis, are provided in Table 2. The reference values are based on results obtained from a single NIST analytical method. Reference values are non-certified values that are the best estimate of the true value; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may not include all sources of uncertainty [2].

Information Values: The values for 12 elements are provided in Table 3 for information purposes only. These are non-certified values with no uncertainty assessed. The information values included in this certificate are based on results obtained from one NIST method.

Expiration of Certification: The certification of SRM 2711a is valid, within the measurement uncertainties specified, until **1 January 2019**, provided the SRM is handled in accordance with the instructions given in this certificate (see "Instructions for Use"). This certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

E.A. Mackey and R.R. Greenberg of the NIST Analytical Chemistry Division were responsible for coordination of the technical measurements leading to certification.

Statistical analyses were performed by J.H. Yen of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 22 May 2009

Robert L. Watters, Jr., Chief
Measurement Services Division

INSTRUCTIONS FOR USE

Sampling: The SRM should be thoroughly mixed by repeatedly inverting and rotating the bottle horizontally before removing a test portion for analysis. A minimum mass of 250 mg (dry mass - see *Instructions for Drying*) should be used for analytical determinations to be related to the mass fraction values in this Certificate of Analysis.

To obtain the certified values, sample preparation procedures should be designed to effect complete dissolution. If volatile elements (i.e., arsenic, mercury, selenium) will be determined, precautions should be taken in the dissolution of SRM 2711a to avoid volatilization losses.

Drying: To relate measurements to the certified, reference, and information values that are expressed on a dry-mass basis, users should determine a drying correction at the time of each analysis. The recommended drying procedure is oven drying for 2 h at 110 °C. Note that analytical determination of volatile elements (i.e., arsenic, mercury, selenium) should be determined on samples as received; separate samples should be dried as previously described to obtain a correction factor for moisture. Correction for moisture must be made to the data for volatile elements before comparing to the certified values. This procedure ensures that these elements are not lost during drying. The mass loss on drying for this material as bottled was approximately 2 %, but this value may change once the bottle is opened and the soil is exposed to air.

SOURCE, PREPARATION, AND ANALYSIS

Source and Preparation of Material[1]: The U.S. Geological Survey (USGS), under contract to NIST, collected and processed the material for SRM 2711a. Soil was collected from the top 10 cm to 12 cm of an agricultural field located near a site formerly used by a smelting plant, in east Helena, Montana. Collection was performed using a common garden spade, and the material was stored in 20 plastic-lined five-gallon buckets with snap-on lids. At the USGS, the SRM 2711a soil was dried at room temperature, disaggregated, and sieved to remove coarse material (≥2 mm). The resulting soil was ball-milled in 50 kg portions. The entire ball-milled batch of soil was transferred to a cross-flow V-blender for mixing. The blended soil was radiation sterilized prior to bottling. In the final preparation step the blended material was split into containers using a custom-designed spinning riffler, which was used to divide the material into smaller batches, and then used to apportion approximately 50 g into each pre-cleaned bottle.

Every 100th bottle was set aside for chemical analyses designed to assess material homogeneity using X-ray fluorescence spectrometry (XRF), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) at the USGS. Homogeneity assessment and sieving tests performed at NIST indicated that additional processing was needed to achieve optimum homogeneity. The material from all bottles was combined, and then ground in batches between stainless steel plates for a time sufficient to produce a powder of which ≥95%, by mass, passed through a 200-mesh (74-µm) sieve. The resulting powder was blended, and 50 g portions were dispensed into bottles using the spinning riffler. Results from analyses at NIST indicated that material homogeneity was acceptable (see below).

Analysis: The homogeneity was assessed for selected elements in the bottled material using instrumental neutron activation analysis (INAA). The estimated relative standard deviation for material inhomogeneity is ≤1 % for most elements evaluated. For antimony, magnesium, and zinc, a component for material heterogeneity (of 1 %, relative, at the 1s level) was included in the expanded uncertainties of the certified values.

Analyses of this material were performed at NIST and at the USGS (Denver, CO). Results from NIST were used to provide the certified, reference, and information values shown in Tables 1, 2, and 3, respectively. Results from the USGS were used to confirm those values. The analytical techniques used for each element are listed in Table 4; the analysts are listed in Tables 5 and 6.

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[1] Certain commercial equipment, instruments, or materials are identified in this certificate in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1. Certified Values[a,b] (Dry-Mass Basis) for Selected Elements in SRM 2711a

Element	Mass Fraction (%)	Element	Mass Fraction (mg/kg)
Aluminum	6.72 ± 0.06	Antimony	23.8 ± 1.4
Calcium	2.42 ± 0.06	Arsenic	107 ± 5
Iron	2.82 ± 0.04	Barium	730 ± 15
Lead	0.140 ± 0.001	Cadmium	54.1 ± 0.5
Magnesium	1.07 ± 0.06	Chromium	52.3 ± 2.9
Potassium	2.53 ± 0.10	Cobalt	9.89 ± 0.18
Silicon	31.4 ± 0.7	Copper	140 ± 2
Sodium	1.20 ± 0.01	Manganese	675 ± 18
Titanium	0.317 ± 0.008	Mercury	7.42 ± 0.18
		Nickel	21.7 ± 0.7
		Phosphorous	842 ± 11
		Samarium	5.93 ± 0.28
		Strontium	242 ± 10
		Uranium	3.01 ± 0.12
		Vanadium	80.7 ± 5.7
		Zinc	414 ± 11

[a] Certified values for all elements except cadmium, lead, and mercury are the equally weighted means of results from two or three analytical methods. The uncertainty listed with each value is an expanded uncertainty about the mean. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-method and within-method components of uncertainty, following the ISO Guide [3,4]. A component for material heterogeneity is incorporated into the uncertainties for antimony, manganese, and zinc. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte.

[b] The certified values for cadmium, lead, and mercury are each results from a single NIST method (see Table 4) for which a complete evaluation of all sources of uncertainty has been performed. The uncertainties for the certified values for these elements represent expanded uncertainties with a coverage factor of 2, with uncertainty components combined following the ISO Guide [4].

Table 2. Reference Values[a] (Dry-Mass Basis) for Selected Elements in SRM 2711a

Element	Mass Fraction (mg/kg)
Cesium	6.7 ± 0.2
Europium	1.1 ± 0.2
Hafnium	9.2 ± 0.2
Lanthanum	38 ± 1
Neodymium	29 ± 2
Rubidium	120 ± 3
Scandium	8.5 ± 0.1
Thorium	15 ± 1

[a] Reference values are based on results from one analytical method at NIST (see Table 4), and the uncertainties represent the expanded uncertainties, which include the combined Type A and Type B with a coverage factor of 2, following the ISO Guide [4].

Table 3. Information Values[a] (Dry-Mass Basis) for Selected Elements in SRM 2711a

Element	Mass Fraction (mg/kg)
Boron	50
Cerium	70
Dysprosium	5
Gadolinium	5
Indium	1
Lutetium	0.5
Selenium	2
Silver	6
Tantalum	1
Terbium	0.8
Thallium	3
Ytterbium	3

[a] Information values are based on results from one analytical method at NIST.

Table 4. NIST Methods Used for the Analysis of SRM 2711a

Element	Methods	Element	Methods
Ag	INAA	Mn	INAA; XRF
Al	INAA; XRF	Na	INAA; XRF
As	INAA; XRF	Nd	INAA
B	PGAA	Ni	ICP-MS; ICP-OES
Ba	ICP-OES; INAA: XRF	P	ICP-OES; XRF
Ca	INAA; XRF	Pb	ID-ICP-MS
Cd	ID-ICP-MS	Rb	INAA
Ce	INAA	Sb	ICP-MS; INAA
Co	INAA; ICP-OES	Sc	INAA
Cr	INAA; XRF	Se	CCT-ICP-MS
Cs	INAA	Si	PGAA; XRF
Cu	ICP-OES; ICP-MS	Sm	INAA(a); PGAA
Dy	INAA	Sr	ICP-OES; INAA; XRF
Eu	INAA	Ta	INAA
Fe	INAA; PGAA; XRF	Tb	INAA
Gd	PGAA	Th	INAA
Hf	INAA	Ti	INAA; PGAA; XRF
Hg	CV-ID-ICPMS	Tl	ICP-MS
In	INAA	U	ICP-MS; INAA
K	INAA; PGAA; XRF	V	INAA; XRF
La	INAA(a)	Yb	INAA
Lu	INAA	Zn	INAA; XRF
Mg	INAA; XRF		

NIST Methods of Analysis

CCT-ICP-MS	Collision cell inductively coupled plasma mass spectrometry
CV-ID-ICP-MS	Cold vapor isotope dilution inductively coupled plasma mass spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
ID-ICP-MS	Isotope dilution inductively coupled plasma mass spectrometry
INAA	Instrumental neutron activation analysis
PGAA	Prompt gamma-ray activation analysis
XRF	X-ray fluorescence spectrometry

USGS Methods of Analysis(b)

WD-XRF-2	Wavelength dispersive X-ray fluorescence spectrometry at USGS
ICP-OES-2	Inductively coupled plasma optical emission spectrometry at USGS
ICP-MS-2	Inductively coupled plasma mass spectrometry at USGS

(a)Two different INAA experiments, performed using different sub-samples and different analytical conditions, were used to provide certified and reference values for samarium and lanthanum, respectively.
(b)USGS methods were used to confirm certified, reference, or information values.

Table 5. Participating NIST Analysts:

S.J. Christopher	S.A. Rabb
R.D. Day	J.R. Sieber
S.E. Long	R.O. Spatz
E.A. Mackey	R.S. Popelka-Filcoff
A.F. Marlow	B.E. Tomlin
J.L. Molloy	L.J. Wood
K.E. Murphy	L.L. Yu
R.L. Paul	R. Zeisler

Table 6. Participating USGS Laboratory and Analysts

Laboratory	Analysts
U.S. Geological Survey Branch of Geochemistry Denver, CO, USA	M.G. Adams Z.A. Brown P.L. Lamothe J.E. Taggart S.A. Wilson

REFERENCES

[1] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*, NIST Special Publication 811 (2008); available at http://www.physics.nist.gov/Pubs/contents.html.

[2] May, W.E.; Gills, T.E.; Parris, R.; Beck, II, C.M.; Fassett, J.D.; Gettings, R.J.; Greenberg, R.R.; Guenther, F.R.; Kramer, G.; MacDonald, B.S.; Wise, S.A.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*, NIST Special Publication 260-136 (1999); available at http://www.cstl.nist.gov/nist839/NIST_special_publications.htm.

[3] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H.K.; Vangel, M.G.; Yen, J.H.; Zhang, N.F.; J. Res. Natl. Instit. Stand. Technol. 105, pp. 571-579 (2000).

[4] ISO; *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st ed.; International Organization for Standardization: Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*, NIST Technical Note 1297, U.S. Government Printing Office, Washington, DC (1994); available at http://www.physics.nist.gov/Pubs/contents.html.

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

Addendum to Certificate

Standard Reference Material® 2711a

Montana II Soil

Moderately Elevated Trace Element Concentrations

Leachable Concentrations Determined Using USEPA Methods 200.7 and 3050B

The mass fraction values contained in the NIST Certificate of Analysis for SRM 2711a represent the total element content of the material. The measurement results used to provide the certified, reference or information values are obtained from methods that require complete sample decomposition, or from nondestructive analytical methods such as instrumental neutron activation analysis or prompt gamma-ray activation analysis. Where complete sample decomposition is required, it can be accomplished by digestion with mixed acids or by fusion. For mixed-acid decomposition, hydrofluoric acid must be included in the acid mixture used to totally decompose siliceous materials such as soils and sediments.

In its monitoring programs, the U.S. Environmental Protection Agency (USEPA) has established a number of leach methods for the preparation of soils samples for the determination of extractable elements. Six laboratories participated, five of which used USEPA Method 200.7; the remaining laboratory used USEPA SW-846 Method 3050B for preparation of soil samples. All elements were determined in leachates by inductively coupled plasma optical emission spectrometry. All laboratories provided individual results from duplicate portions, and these results were averaged together to provide one result for each element from each participating laboratory. Results rejected as outliers by the USEPA Contract Laboratory Program (CLP) officials were not included. Results are summarized in Table A1. The ranges of mass fraction values, median values (to two significant figures), and the number of results included for each are given for 23 elements. The percent recovery values based on the ratios of the median values to the total element content (from the certified, reference, or information values in the Certificate of Analysis) are listed in the last column of Table A1. **Note that the certified values provided as total mass fractions in the Certificate of Analysis are the best estimate of the true mass fraction values for this material.**

This USEPA CLP Study was coordinated by C. Jones, Quality Assurance and Technical Support Program (QATS), Shaw Environmental & Infrastructure Group, Las Vegas, NV, under the direction of J. Nebelsick, USEPA, Analytical Services Branch. The participating laboratories are listed in Table A2.

Table A1. Results from Laboratories Participating in the EPA Contract Laboratory Program Study.

Element	n	Range (mg/kg)	Median (mg/kg)	Recovery (%)
Aluminum	6	9800 - 15000	13200	19
Antimony	6	2.8 - 7.2	4.9	21
Arsenic	6	81 - 110	89	85
Barium	6	170 - 200	190	25
Beryllium	6	0.73 - 1.1	0.93	--
Cadmium	6	43 - 56	47	90
Calcium	6	14000 - 17000	14000	61
Chromium	6	12 - 18	15	29
Cobalt	6	5.5 - 9.0	7.5	75
Copper	6	120 - 160	130	95
Iron	6	14000 - 18000	15000	54
Lead	6	1100 - 1400	1300	91
Magnesium	6	5000 - 6600	5700	54
Manganese	6	450 - 580	460	71
Mercury	6	6.3 - 8.3	7.4	100
Nickel	6	13 - 18	15	72
Potassium	6	3300 - 4600	3900	16
Selenium	5	1.4 - 1.9	1.7	85
Silver	6	4.0 - 6.1	5.5	89
Sodium	5	140 - 210	180	1.5
Thallium	5	0.71 - 3.1	2.1	68
Vanadium	6	24 - 34	28	36
Zinc	6	310 - 380	350	85

Table A2. List of CLP and non-CLP Participating Laboratories

A4 Scientific, Inc.
Bonner Analytical Testing Co.
Chem Tech Consulting Group
Datachem Laboratories, Inc.
Liberty Analytical Corporation
SVL Analytical, Inc.

Appendix C

Reproducibility

Table C.1: Example of reproducibility of soils, musts and wines.

	Soil P4B		Must 300		Wine 1672P	
	(mg kg^{-1})	dev.std. (%)	(µg L^{-1})	dev.std. (%)	(µg L^{-1})	dev.std. (%)
Li	44.71	2.90	1.07	32.34	1.49	3.55
Mg	5824.99	2.24	43287.34	4.10	N.M.	N.M.
Al	<DL	-	1798.37	6.51	21.03	3.18
K	7817.21	0.15	692648.02	4.12	174.59	6.33
Ca	14442.88	1.31	34807.17	6.53	701.60	4.69
Tl	0.95	2.90	0.00	-	0.39	0.08
V	175.34	0.29	3.08	8.44	0.32	6.07
Cr	149.79	1.69	2.47	9.16	8.49	0.42
Mn	699.96	5.73	247.34	6.86	171.58	3.20
Fe	<DL	-	1829.47	6.93	113.24	0.17
Co	21.18	7.11	0.52	4.22	1.17	5.53
Ni	72.43	4.25	10.50	9.91	8.70	3.47
Cu	29.78	11.18	3095.69	6.64	17.74	0.75
Zn	73.84	1.21	450.48	5.27	502.79	3.82
Ga	39.20	5.14	2.48	5.68	0.05	1.80
As	20.74	5.76	0.56	2.64	1.98	33.73
Se	2.65	11.64	0.23	3.34	4.22	44.85
Rb	113.11	2.33	166.91	10.70	284.06	1.18
Sr	73.98	9.62	56.50	3.14	179.21	2.16
Y	37.38	6.95	583.51	4.31	N.M.	N.M.
Ag	0.18	11.02	0.00150	14.14	0.02	14.15
Cd	0.52	21.76	0.04	42.43	0.17	9.91
Cs	8.95	3.53	0.07	9.18	2.28	6.34
La	64.56	0.21	1013.34	6.12	9.55E-03	3.34
Ce	127.50	1.90	1920.62	11.49	6.15E-03	4.25
Pr	14.71	0.32	215.56	12.03	2.40E-03	12.53
Nd	54.00	0.00	744.22	6.68	6.83E-03	4.36
Sm	10.55	0.32	113.20	10.18	1.22E-03	13.96
Eu	2.27	0.93	32.05	8.33	5.07E-03	5.01
Gd	9.86	2.13	137.04	6.36	1.71E-03	13.05
Tb	1.30	2.11	17.75	10.33	2.42E-04	22.79
Dy	6.75	3.29	97.52	12.09	1.36E-03	3.93
Ho	1.26	3.69	17.14	15.12	3.90E-04	10.80
Er	3.39	3.28	66.81	0.88	1.24E-03	22.53
Tm	0.42	3.96	6.99	1.75	2.79E-04	15.49
Yb	2.63	3.08	52.72	3.39	2.70E-03	13.77
Lu	0.37	3.46	3.18	1.59	5.95E-04	5.79
Pb	27.52	6.83	6.41	3.72	1.76	4.42
Th	14.94	2.52	6.35	1.78	604.79	13.44
U	1.74	0.97	505.15	0.63	0.03	0.49

N.M. = Not measured.

Appendix D

Component information for IMS-102 and IMS-101

Table D.1: Component information for IMS-102 and IMS 101 ((ULTRA Scientific, Inc.)

IMS-102		**IMS-101**	
Analyte	**Conc. µg mL**	**Analyte**	**Conc. µg mL**
aluminum	10	cerium	10
arsenic	10	dysprosium	10
barium	10	erbium	10
beryllium	10	europium	10
bismuth	10	gadolinium	10
cadmium	10	holmium	10
calcium	10	lanthanum	10
cesium	10	lutetium	10
chromium (III)	10	neodymium	10
cobalt	10	praseodymium	10
copper	10	samarium	10
gallium	10	scandium	10
indium	10	terbium	10
iron	10	thorium	10
lead	10	thulium	10
lithium	10	ytterbium	10
magnesium	10	yttrium	10
manganese	10		
nickel	10		
potassium	10		
rubidium	10		
selenium	10		
silver	10		
sodium	10		
strontium	10		
thallium	10		
uranium	10		
vanadium	10		
zinc	10		
Matrix Details			
Matrix	water with dilute nitric acid		

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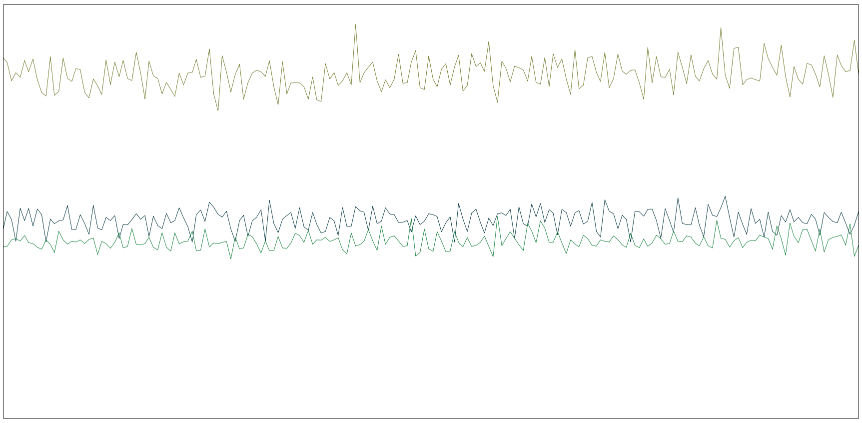
Appendix E

Example of ICP-MS tuning

Sensitivity

```
Tune File          : ATUNE.U
```



```
Integration Time:     0.1000 sec
 Sampling Period:     0.7200 sec
              n:        200
```

```
       m/z      Range      Count        Mean        RSD%
         7     50,000    25020.0     23968.1        5.05
        89     50,000    20929.0     21392.8        4.28
       205     20,000    16609.0     16642.7        4.34
   156/140          2     1.197%      1.151%       13.51
    70/140          2     1.808%      1.660%       10.47
       103     20,000    19082.0     18998.9        6.25
```

```
Tuning Parameters
===Plasma Condition===          ===Ion Lenses===                ===Q-Pole Parameters===
      RF Power   :   1500 W          Extract 1  :       0 V          AMU Gain   :    118
   RF Matching   :   1.74 V          Extract 2  : -132.5 V         AMU Offset   :    128
    Smpl Depth   :      8 mm       Omega Bias-ce  :     -22 V          Axis Gain  : 0.9989
       Torch-H   :    0.4 mm       Omega Lens-ce  :    -0.6 V        Axis Offset  :  -0.02
       Torch-V   :   -0.2 mm       Cell Entrance  :     -30 V            QP Bias  :     -1 V
   Carrier Gas   :   1.15 L/min        QP Focus   :       3 V
    Makeup Gas   :    0.1 L/min       Cell Exit   :     -40 V      ===Detector Parameters===
  Optional Gas   :      0 %                                         Discriminator  :      8 mV
Nebulizer Pump   :   0.18 rps     ===Octopole Parameters===             Analog HV  :   1780 V
   Sample Pump   :    --- rps            OctP RF  :     180 V            Pulse HV  :   1380 V
      S/C Temp   :      2 degC         OctP Bias  :      -6 V

===Reaction Cell===
 Reaction Mode   :    OFF
        H2 Gas   :      0 mL/min         He Gas   :      0 mL/min   Optional Gas   :    --- %
```

```
Generated : Apr 01, 2015 11:28:12
Printed   : Apr 01, 2015 11:28:18
```

Acknowledgments

Queste ultime poche righe le dedico per un ringraziamento a tutti coloro che hanno contribuito alla riuscita di questo lavoro.

Il mio tutor Dott. Giulio Cozzi mi ha introdotto nel grande mondo della ricerca scientifica, rendendomi persona consapevole e decisa nelle scelte che sono poi anche specchio di quella che è la vita al di fuori del lavoro. Mi ha trasmesso la sua esperienza e i ringraziamenti non possono essere mai abbastanza. Ne approfitto per ricordargli che lo sto ancora aspettando per un giro enogastronomico del Friuli-Venezia Giulia.

Il Prof. Carlo Barbante ha creduto in questo progetto di Dottorato fin dall'inizio, supportando le decisioni e le direzioni che sono state prese, mostrando massima disponibilità all'ascolto e donandomi sempre validi consigli.

La Dott.ssa Clara Turetta mi ha dato un supporto scientifico fondamentale, regalandomi la sua competenza ed esperienza. In questa tesi di dottorato c'è un grosso contributo da parte sua.

La Dott.ssa Nives Ogrinc (mi scuso se scrivo in italiano, ma so che non ha alcun problema con la lingua) ha reso indimenticabile il percorso e l'esperienza fatta a Lubiana, mostrandomi quanto sia valida la ricerca all'estero.

Questo viaggio di dottorato è stato fatto assieme a tante persone, ma sicuramente la Dott.ssa Michela Segnana è stata sempre la mia compagna di viaggio. Ci siamo conosciuti il giorno del colloquio per accedere a questo corso e finiamo questo percorso assieme. La ringrazio per essere stata una amica sempre presente nelle varie e indimenticabili vicissitudini di questi anni.
Anche se, lo so, gli amici non si ringraziano mai.

Ringrazio inoltre tutto il gruppo di ricerca del laboratorio, tutti hanno dato un contributo al mio lavoro e spero di essere stato altrettanto di aiuto nelle loro attività.
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Un caro ringraziamento a tutto il personale del Reaktor Center di Lubiana che mi ha aiutato sempre con estrema gentilezza: Bor Krajnc, Janja Vrzel, Doris Potočnik, Samo Tamše, Stojan Zigon.

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Gli amici di sempre mi hanno altrettanto supportato in questo viaggio, quindi grazie a Federico Furlanetto e a Fulvio Leschiutta.

Un enorme ringraziamento agli amici delle varie band in cui suono. Ci sono sempre alti e bassi in qualsiasi rapporto ma il confronto e l'incontro è sempre stato di enorme importanza per la mia vita umana e professionale. Senza di voi sarei una persona diversa. Tra questi, ringrazio particolarmente: Federico Baston, Davide Moras, Alessandro Jacobi, Raffaello Indri, Simone Morettin, Gabriele Gritti, Federico Salvador, Viola Roccagli, Angelo Roccagli, Giulio Venier.

I compagni di appartamento a Venezia hanno sicuramente contribuito a rendere più facile questo percorso, e li ringrazio (stappando una bottiglia): Enrico Zugno, Tommaso Fracassi, Sonila Qefa, Plutarco.

Un caro ringraziamento va anche al Prof. Arch. Luca Maria Francesco Fabris per la stupenda l'avventura in cui abbiamo portato i vini di Casarsa all'EXPO (e qui stappo la seconda bottiglia).

Dedico questo lavoro a tutta la mia famiglia, che mi ha seguito da lontano e che forse si è anche chiesta che cosa io abbia fatto negli ultimi anni. Un ringraziamento particolare a Gian Paolo Fagotto e a Paola Polo, senza i loro suggerimenti non avrei iniziato proprio questo percorso universitario, arrivando a raggiungere il Dottorato di ricerca.

Un caro pensiero di ringraziamento va anche a Chiara Tricarico che mi ha accompagnato e sostenuto in questo ultimo anno con grande pazienza.

Nella vita si incontrano molte persone che ti impreziosiscono con la loro presenza, a volte le incontri per un breve periodo di tempo, a volte ci entrano per sempre, i miei pensieri e ringraziamenti sono rivolti anche a loro, ovunque loro siano.

Questo è un punto di partenza e non di arrivo.

POLITECNICO
MILANO 1863

DIPARTIMENTO DI
ARCHITETTURA E
STUDI URBANI

Casarsa della Delizia, September 12th 2015

TO WHOM IT MAY CONCERN

UNIVERSITIES
FOR EXPO 2015
SCIENTIFIC COMMITTEE
CITY OF MILAN
Milano

Città di Casarsa della Delizia

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FRIULI VENEZIA GIULIA

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LIONS CLUB
SESTO AL REGHENA in Sylvis

STATEMENT

This is to certify that **Mr Dr Fabio Paolo Paolo**
has participated **as a lecturer** at the International Design Workshop **"Restarting from Bacchus and Dionysus, with the help of Oyamatsumi"** held in Casarsa della Delizia (Italy) and organized by the Department of Architecture and Urban Studies (DAStU) of the Politecnico di Milano under the patronage of University for Expo 2015 Scientific Committee, Regione Autonoma Friuli Venezia Giulia, ERSA, and Università IUAV di Venezia, Università degli Studi di Udine, Università Ca' Foscari Venezia, and Ordine APPC di Pordenone and in collaboration with the Lions Club of Sesto al Reghena in Sylvis.

On September 3rd, 2015 **Dr Fabio Paolo Polo** gave the lecture: *"Climate Change and Wine".*

The Workshop took place from **August 31st to September 12th 2015**
and has been validated for **4 ECTS**
by the School of Architecture and Society of the Politecnico di Milano.

Truly,

Luca Maria Francesco Fabris, prof. arch. PhD
Director
"Restarting from Bacchus and Dionysus, with the help of Oyamatsumi"

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Ca’ Foscari
Graduate
School

RELAZIONE SULL’ATTIVITÀ SVOLTA NEL CORSO DI DOTTORATO

DOTTORANDO	
(COGNOME NOME)	MATRICOLA
POLO FABIO PAOLO	824754
CORSO DI DOTTORATO	CICLO
SCIENCE AND MANAGEMENT OF CLIMATE CHANGE	28°
TUTOR (COGNOME NOME)	
COZZI GIULIO	

BREVE RELAZIONE SCIENTIFICA DELL’ATTIVITÀ SVOLTA

(max 2500 caratteri)

First year has been mainly dedicated to the PhD courses on Climate Change.

The second year of my PhD has been mainly dedicated to the research in a foreign institution.

From September 2013 I attended few meetings regarding my project and finalized to:

- Establish new collaborations for the research project;
- Find the samples to be analyzed in my study (wine, must, sediments);

The research agreement between CNR-IDPA and Jožef Stefan Institute of Lubiana allowed to me to work into the laboratories of the Reaktorski Center of Lubiana with Prof. Nives Ogrinc. The period abroad began from February the 1st and ended July the 31st 2014 and was strictly addressed to analyze wine and must samples for my PhD project.

During the period abroad I had the opportunity to practice stable isotopes analysis and mass spectrometry instruments. I improved my technical knowledge using an Elemental Analyzer (Europa Scientific) and I learned how to carry out samples pre-treatment, which is a fundamental step of the analytical methods.

The third year of my PhD has been mainly dedicated to the research in the laboratories in Venice. From September 2014 the researches have been addressed on the determination and the statistical data interpretation of Rare Earth Elements and Trace Elements in wine and must samples. The analyses have been carried out by ICP-MS through official methodologies adapted in our laboratories.

- October 2014, winner of the Best Presentation at the Ca’ Foscari Graduate School opening ceremony. Poster Title: "The influence of climate change on the quality of wine products: chemical characterization, environmental and economic impacts";

The last months have been also dedicated to the organization of an International Workshop in Casarsa della Delizia (PN). This workshop took place from August 31st to September 12th 2015. The program focused on the EXPO 2015 themes: “science and technology for agriculture and biodiversity” and “food and life styles”. The workshop “Restarting from Bacchus and Dionysus, with the help of Oyamatsumi" was under the patronage of Ca' Foscari University of Venice.

PRINCIPALI ATTIVITÀ FORMATIVE

(elencare numerando tutte le attività svolte e, per ognuna, indicare i dati richiesti)

TIPO [(indicare se Corso/Seminario/Workshp/Giornate di Studio/Convegno/Stage/Altro (specificare)]	TITOLO	SEDE	ORE/GIORNI
Courses	First year planned courses.	Palazzo Moro e San Giobbe. Venezia.	1 Year
Technical Course	DELTA V – GC-COMBUSTION III Installation and Basic Customer Training, Venice.	Venice	November 19th to 23rd, 2013
Technical Course	1st MS-WINE DAY, Mass Spectrometry and Grapes, Wine, Spirits	Bagno a Ripoli (Fi)	April 16th-17th, 2015

ATTIVITÀ DI DIDATTICA E DI RICERCA

(elencare numerando tutte le attività svolte e, per ognuna, indicare i dati richiesti)

TIPO	TITOLO
ATTIVITÀ TUTORIALI E DIDATTICO INTEGRATIVE SVOLTE (specificare)	

SEMINARI TENUTI DAL DOTTORANDO	- Seminar on “Climate Change and Wine” at the International Design Workshop “Restarting from Bacchus and Dionysius, with the help of Oyamatsumi”. The Workshop took place from August 31st to September 12th 2015. Organized by the School of Architecture and Society of the Politecnico di Milano. - INTERDOC2014, “International, Intersectoral, Interdisciplinary”. Università di Padova. Seminar: Good Practices from Italian Universities, Ca’ Foscari University of Venice. November 21st 2014, Padova.
PUBBLICAZIONI SCIENTIFICHE (indicare le opere che sono state pubblicate su riviste, miscellanee, in libri, in cataloghi etc., riportando la citazione bibliografica completa)	Recognizing different impacts of human and natural sources on the spatial distribution and temporal trends of PAHs and PCBs (including PCB-11) in sediments of the Nador Lagoon (Morocco). Giuliani S, Piazza R, El Moumni B, Polo FP, Vecchiato M, Romano S, Zambon S, Frignani M, Bellucci LG. Sci Total Environ. 2015 Sep 1;526:346-57. doi: 10.1016/j.scitotenv.2015.04.057. S. Giuliani; R. Piazza; S. Zambon; M. Vecchiato; F. P. Polo; S. Romano; L. G. Bellucci; M. Frignani *Polycyclic Aromatic Hydrocarbons in samples from the southwestern Bac Giang Province and the Cau River (northern Vietnam)* , Towards Environmental Quality Assessment in a Developing Area. The Bac Giang Province bordering the Cau River (Northern Vietnam), Bologna, S. Giuliani, M. Frignani, Giraldi Editore, pp. 91-102, Convegno: Proceedings of the workshop "Environmental Quality in the Province of Bac Giang and the Cau River Basin. Perspectives for the improvement of monitoring activities and methods", August 27th, 2013 (ISBN 9788861555860) *(Articolo in Atti di convegno)* https://iris.unive.it/handle/10278/3633699#.Vglgqvntmko F.P. Polo; L.G. Bellucci; C. Turetta; M.Vecchiato; S. Zambon; R. Piazza *STUDY OF CHEMICAL POLLUTION IN SEDIMENT CORES FROM THE NADOR LAGOON (MOROCCO). ORGANIC AND INORGANIC CONTAMINANTS* , Analitica 2012, Sesto Fiorentino (FI), Antonella Rossi, pp. 268-268, Convegno: XXIII congresso nazionale della divisione di chimica analitica della S.C.I., 16-20 Settembre, 2012 (ISBN 9788890767081) *(Abstract in Atti di convegno)* https://iris.unive.it/handle/10278/36821#.Vglg6_ntmko
PRESENTAZIONI A CONGRESSI (indicare le opere che sono state pubblicate (poster, abstracts, etc.) riportando la citazione bibliografica completa)	International Postgraduated School Conference (IPSSC) 2014, abstract, poster, and oral contribute “The influence of climate change on the quality of some Italian wine products: chemical characterization and environmental impacts, F. P. Polo, G. Cozzi, N. Ogrinc”.
ALTRE PUBBLICAZIONI	

(indicare le opere che sono state pubblicate (lavori sottomessi rapporti di ricerca, etc.) riportando la citazione bibliografica completa)	

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28/09/2015

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Luogo di nascita SAN VITO AL TAGLIAMENTO

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Dottorato di Ricerca in Scienza e Gestione dei Cambiamenti Climatici

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(data di compilazione)

Fabio Paolo Polo
(firma per esteso)

Università
Ca'Foscari
Venezia

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(Art. 47 D.P.R. 445 del 28/12/2000 e relative modifiche)

Io sottoscritto Fabio Paolo Polo

nato a San Vito al Tagliamento (prov. PN) il 26/05/1984

residente a Casarsa della D. in via dell'Aviere n. 5

Matricola (se posseduta) 824754 Autore della tesi di dottorato dal titolo:

The influence of climate variability on chemical composition of European wines: a regional scale study (Italy and Slovenia)

Dottorato di ricerca in Scienza e Gestione dei Cambiamenti Climatici

(in cotutela con ……………………………………)

Ciclo XXVIII

Anno di conseguimento del titolo 2016

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- consegno la copia integrale della tesi in formato cartaceo presso la segreteria didattica del dipartimento di riferimento del corso di dottorato ai fini del deposito presso l'Archivio di Ateneo.

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Estratto per riassunto della tesi di dottorato

L'estratto (max. 1000 battute) deve essere redatto sia in lingua italiana che in lingua inglese e nella lingua straniera eventualmente indicata dal Collegio dei docenti.

L'estratto va firmato e rilegato come ultimo foglio della tesi.

Studente: Fabio Paolo Polo matricola: 824754

Dottorato: Scienza e Gestione dei Cambiamenti Climatici

Ciclo: 28°

Titolo della tesi[1] : The influence of climate variability on chemical composition of European wines: a regional scale study (Italy and Slovenia).

Abstract:

I cambiamenti climatici in agricoltura stanno modificando sempre maggiormente le caratteristiche delle piante di viti, la composizione degli acini, influenzando la vinificazione, la microbiologia, la chimica e quindi il sapore del vino.

La coltivazione dell'uva è un ottimo esempio per misurare gli impatti diretti di questi cambiamenti in quanto risulta molto sensibile al clima ed è concentrata maggiormente in zone Mediterranee che sono caratterizzate da un'elevata biodiversità.

A livello regionale, e in periodi più brevi, la meteorologia stagionale è un importante fattore di influenza della qualità delle rese e delle proprietà finali del vino.

Nella presente ricerca, abbiamo studiato vini di Italia e Slovenia con lo scopo di differenziarli dalle diverse annate, chiaramente influenzate dalla temperatura e dalla pioggia durante le stagioni annuali di maturazione.

Sono state prese in considerazione particolari tecniche di analisi chimica, in particolare la spettrometria di massa (ICP-MS) e la spettrometria di massa isotopica (IRMS); tecniche solitamente impiegate per rilevare sofisticazioni e stabilire la provenienza geografica dei vini. In particolare, abbiamo studiato la relazione tra macro e micro-elementi, terre rare e isotopi stabili [δ^{13}C, δ^{18}O, $(D/H)_I$, $(D/H)_{II}$].

Sono stati aggiunti a queste analisi anche quelle di suoli e uva per indagare il ciclo degli elementi fino ai prodotti finali.

I set di dati sono stati esaminati mediante tecniche statistiche per mostrare la loro relazione alle condizioni climatiche e la loro reciproca connessione.

I risultati, per quattro annate di vino, hanno mostrato una forte affinità con la temperatura e le precipitazioni, ma vini dei due Paesi non sono stati chiaramente differenziati dal punto di vista geografico.

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[1] Il titolo deve essere quello definitivo, uguale a quello che risulta stampato sulla copertina dell'elaborato consegnato.

Abstract (English):

Climate change is having an increasing influence on vine phenology and grape composition, affecting vinifications, wine chemistry and the quality of productions.

Wine grape cultivation provides a good test case for measuring indirect impacts mediated by changes in agriculture, because viticulture is sensitive to climate and is concentrated in Mediterranean climate regions that are global biodiversity hotspots. Moreover, on a regional level and on a shorter time scale, the seasonal weather conditions modify the quality of yields determining the final properties of wine.

In the present research, we studied wines from Italy and Slovenia with the purpose of differentiating them by the different vintages, which are supposed to be influenced by temperature and rain during each year's growing season. Specific chemical techniques were used, in particular mass spectrometry (ICP-MS) and isotopic mass spectrometry (IRMS), both of which are usually employed to detect wine adulterations and to establish the geographical provenance of wines.

In particular, we investigated the relationship between macro- and micro-elements, Rare Earth Elements and stable isotopes [$\delta^{13}C$, $\delta^{18}O$, $(D/H)_I$, $(D/H)_{II}$].

Soils and grapes analyses were then included to understand how the cycle of elements could be modified up to the final products.

The datasets were examined via statistical techniques to show their relation to weather conditions as well as their mutual connection.

The results for four vintages of wine showed strong affinity to temperature and precipitation while wines from the two countries were not clearly geographically differentiated.

Firma dello studente