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► **To cite this version:**

Simon Blotevogel. Study of elemental transfers and biogeochemical mechanisms in the soil-plant-wine continuum using isotopic and biochemical tracers. Agricultural sciences. Université Paul Sabatier - Toulouse III, 2017. English. NNT : 2017TOU30373 . tel-02307009

HAL Id: tel-02307009

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THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par : *l'Université Toulouse 3 Paul Sabatier (UT3 Paul Sabatier)*

Présentée et soutenue le (29/11/2017) par :

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Etude des transferts élémentaires et mécanismes biogéochimiques dans le continuum sol-plante-vin par l'utilisation de traceurs isotopiques et biochimiques.

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Abstract

In western Europe, soil is of primordial importance for wine making. Soil qualities are often discussed and an influence on wine flavor is frequently inferred. However, evidence for a role of soil chemistry on wine composition and taste is scarce, but mineral nutrition of grapevine plants is one possible way of influence. This thesis approaches the complex question of elemental cycling between soil and plant through the use of geochemical tracers in vineyard environments. A combination of traditional tracers such as elemental ratios and mass balances as well as innovative tools such as Cu isotope analysis and electron paramagnetic resonance (EPR) are used on different observation scales.

The influence of soil type on wine elemental composition was investigated using over 200 wine samples from France, Germany, Italy and Spain. Results of chemical analysis were then statistically linked to environmental conditions. At this scale, elemental contents of wine vary depending on soil type (calcareous or not), meteorological conditions, and wine making practice.

To determine which soil properties can influence the chemical composition of grapevine plants, two vineyard plots on contrasted soils in Soave (Italy) were examined. Soil forming mechanisms were studied along two catenas and subsequently linked to elemental composition of grapevine plants and biochemical markers of plant health. Even though soil morphology was different, complex soil forming processes led to similar geochemical properties of soils. Differences in plant chemical compositions between soil types are small compared to inter-individual and inter-annual variability. However the use of Sr isotopic ratios allows the determination of the pedological origin of plants.

In a next step, the fate of Cu pesticides in vineyard soils was studied. Cu-based pesticides have been used for almost 150 years in European vineyards and Cu accumulates in soils, so that the fate of Cu and its ecotoxicological implications are of growing importance. Therefore Cu mobility in vineyard soils and transfer to grapevine plants were investigated using stable Cu-isotope ratios and EPR-measurements.

Isotope ratios of Cu-based fungicides vary largely between products, covering Cu isotope ratios in soils reported in literature and thus making source tracing impossible. However, Cu isotope ratios are useful for tracing biogeochemical mechanisms of Cu transport in soils. The vertical transport of Cu in different soil types was investigated in the Soave vineyard using a combination of mass balance calculations, kinetic extractions and $\delta^{65}\text{Cu}$ -isotope ratios. Results suggest that Cu can be transported to depth even in carbonated environments. Besides it is shown that heavy organic-bound Cu

is lost from carbonated soil columns likely caused by dissolution of Cu carbonates by organic matter.

Finally, Cu transfers in the soil-plant continuum (including the compartments: soil, soil solution, roots and leaves) were studied in a greenhouse experiment. At this scale, organic matter appears to be crucial for Cu mobility and more important than total Cu content. Elemental contents of soil solutions, not only for Cu, vary over time, likely due to plant action. Cu accumulated in plant roots, but transfer to shoots was tightly regulated. Isotopic fractionation between soil and soil solution appears to depend on soil type and in most cases is constant over time. There was a tendency for lighter isotope ratios in soil solutions at the end of the experiment in some samples, however the inverse trend was not observed. Furthermore, Cu-isotope fractionation during plant uptake and translocation processes appear to depend on Cu contamination levels.

To conclude, the results of this thesis suggest that geochemical methods can become relevant tools to answer viticultural questions and can help further understanding of mechanisms of elemental cycling in vineyard environments.

Keywords: *vineyard soil; terroir effect; elemental transfer; copper; Cu-isotopes; soil solution; plant uptake*

Résumé

Dans l'ouest de l'Europe, une grande importance est accordée à la composante sol dans l'élaboration des vins. Les propriétés du sol sont souvent mises en avant et leur influence sur le goût du vin est fréquemment décrite. La nutrition minérale est la principale voie par laquelle le sol peut avoir une influence sur la vigne et par conséquent sur le vin qui en est issu. Dès lors, ce travail de thèse appréhende la question plus large des transferts élémentaires entre le sol et la plante à travers de l'utilisation d'outils géochimiques en milieu viticole. Une combinaison de techniques impliquant des indicateurs classiques tels que les rapports élémentaires ou des bilans de masses ainsi que des traceurs plus innovants comme les rapports isotopiques du Cu et la résonance paramagnétique électronique (RPE) est utilisée.

Dans un premier temps, l'influence du sol sur la composition élémentaire du vin a été étudiée dans plus que 200 vins provenant d'Allemagne, d'Espagne, de France, et d'Italie. Les résultats chimiques ont ensuite été liés statistiquement à des paramètres environnementaux. A cette échelle, le type de sol (classé comme « calcaire » ou « non calcaire ») et les conditions météorologiques apparaissent comme des critères discriminants.

Par la suite, deux parcelles viticoles de Soave (Italie) aux sols contrastés ont été étudiées afin de déterminer l'influence du type du sol sur les vignes. Même si les sols présentent des propriétés pédologiques différentes, une pédogénèse complexe a induit des propriétés géochimiques similaires. Dans les vignes, les traceurs élémentaires et biochimiques montrent que la variabilité inter-annuelle et inter-individuelle est plus grande que la différence liée au sol lui-même. Néanmoins, il est possible de déterminer quel est le sol considéré par l'étude des rapports isotopiques du Sr.

Parmi tous les nutriments et éléments toxiques généralement étudiés pour leur mobilité dans les sols, le cuivre a été suivi dans la dernière partie de cette thèse, essentiellement du fait de son épandage toujours massif comme fongicide en viticulture. En effet, les pesticides cupriques comme la « bouillie bordelaise » sont utilisés depuis environ 150 ans en viticulture. Le Cu s'accumule dès lors dans les sols et les conséquences écotoxicologiques en font un sujet de plus en plus sensible. Parmi les pesticides analysés dans notre étude, les rapports isotopiques du Cu varient autant entre les différents fongicides que dans les sols étudiés dans la littérature, rendant ainsi impossible l'utilisation de ces isotopes comme traceurs d'origine du Cu dans les sols.

Cependant, les isotopes du Cu peuvent être utilisés afin de suivre les transformations biogéochimiques qui s'opèrent dans le sol. Le transport vertical du Cu a également été étudié sur les parcelles viticoles de Soave présentant des sols contrastés. Des calculs de bilan de masse montrent que le Cu peut être entraîné en profondeur, et ce même en milieu calcaire. Les rapports isotopiques indiquent que du Cu lourd est perdu dans les sols calcaires, suggérant alors un transport associé à la matière organique.

A plus petite échelle, les transferts du Cu entre le sol, la solution de sol et la vigne ont été examinés en conditions contrôlées. Dans cette expérience menée en serre, le transport du Cu semble également contrôlé par la matière organique. Les teneurs élémentaires varient fortement avec le temps dans les solutions de sol. Le Cu s'accumule dans les racines et reste peu transloqué vers les feuilles. Le fractionnement isotopique entre le sol et la solution de sol semble dépendre du type de sol et demeure constant dans le temps pour la plupart des échantillons. Dans certains sols, une tendance vers des rapports isotopiques plus légers a été mise en évidence en fin d'expérimentation. Au final, les rapports isotopiques dans les différents compartiments des plantes semblent varier avec leur niveau de contamination.

Pour conclure, nos résultats indiquent que les outils géochimiques s'avèrent pertinents pour l'étude des problématiques viticoles, et notamment pour améliorer la compréhension des mécanismes impliqués dans le transfert élémentaire dans le continuum sol-vigne-vin.

Mots-Clefs : *sol ; effet terroir ; transfert élémentaire ; isotopes du cuivre ; solution du sol ; nutrition des plantes*

Acknowledgements

Before this thesis really begins I would like to take time to thank all the people who have been involved in this work and made my time in Toulouse the great time it was.

At first I would like to thank the two who have accompanied this work from the beginning to the end : Eva Schreck and Priscia Oliva. I still remember both of them telling me not to put too much hope in getting funding for a PhD here, how Eva was rather sick when she presented the subject etc... And then it just happened.

Eva was a great 'directrice'. She was always there to defend my interests, using her contacts to open doors, and reminding me to take breaks at some point. She was always ready to help with whatever needed to be done. Besides she was the one who kept the focus on getting things to an end : 'Le mieux est l'ennemi du bien'. Priscia was there even earlier from the moment arrived in Toulouse as an ERASMUS student. I am still very grateful for the role she played, alongside with Séverine Alibert, the computer science team and the other UPSSITECH - lectures from the GET, in my decision to quit engineering and do more fundamental science. That has been a great decision up to now. During this thesis I got to work a lot more with her (often at rather unlikely times of the day) and I enjoyed a lot discussing mechanisms to least detail, sharing perfectionism, digging holes and marveling at the beauty of soils. I learned a lot from her in the past years, not only about soils but also about her vision of sciences. It took me quite some time to realize that approaches of Eva and Priscia can be quite different and there were moments when I preferred to work with one or the other, but I always thought of them as a brilliant and complementary team. Thank you so much for everything!

Great thanks as well to Jérôme Viers my third supervisor, who certainly was a lot less present but still he was there when help was needed with the mysteries of isotopes and their measurements.

Next I would like to thank the scientists external to the GET that took me for a journey in their respective sciences. There is Christophe Laplanche who introduced me to the world of statistics and coding, I was always amazed by his clean coding style, readable even for a beginner. Sophie Sobanska who took me for a ride into the world of orbitals, spins and spectroscopy. Nicolas Saurin and Olivier Geoffroy who introduced me to their respective domain in wine growing and making. And Laurence Denaix who got me involved with more agronomic aspects of soil science. Thanks a lot to you all – on most occasions I would have loved to learn even more but time is sometimes a limiting factor.

Also I would like to thank in advance Catherine Keller, Philippe Hinsinger and Dominik Weiss who accepted to examine this work without knowing much about what to expect. Thanks as well Philippe Behra who was external supervisor of the progress of this work.

Besides those scientist there were also the Master students William, Vincent, Valentin and Adrien who actually contributed a lot of hands on work and data to this thesis and who I enjoyed supervising and spending time with. Thanks a lot !

The next people to thank are Filippo and Paola, the owners of the vineyard in Soave where a large part of the field work for this thesis took place. After initial skepticism the two gave us a warm welcome every time we came to Castelcerino. We felt like part of the family, being invited to dine in the old castles kitchen on each trip. Besides their hospitality they gave us access to everything we needed in the field and even partly delivered their secrets of wine making – and their wine is really good ! Good wine was necessary, giving me arguments to get a team together for three days of hard work ! Gracie mille !

Gracie mille also to Giuseppe, who did most of the translation and communication for our work in Italy and finally embarked on a field trip to Soave. The missions to Soave were often the most intense moments during this thesis. Starting from organizing a trip for harvest time, Filippo calling a couple of days earlier, getting a team together in the “rentrée” period and finally driving more than 10 h to Castelcerino. Over three years I have done this trip with different colleagues and I would like to thank all of them. Still I want to name two who have done this trip multiple times with me. There is Pierre who had the idea for the subject, connections to Italy and the STRONG conviction that soil or even more geological setting is responsible for the taste of wine. Being a contagious orator he got people from the GET to the Filippi vineyard even before this thesis started. Stéphane who did the physical work for me in Soave but also in St. Mont and other places. Trips with those two were always accompanied by deep, philosophical and controversial discussions, which significantly shortened the long drives. Thanks a lot !

Back at the GET many people contributed to this work and helped me in one way or the other. Thanks Christine who took care of me in all issues related with teaching and modeling. The geophysics crew with José and Muriel. And then all the people with whom I spent time actually wearing the white lab coat : Fred, Carol, Jérôme, Manu, Aurélie, Ludo, Stéphanie, Jo, Michel, Cyril. But also Celine and Dominique at the LCA, Philippe Telouk in Lyon and Sylive, Cecile and Thierry in Bordeaux. Thanks you so much for your help !

But there is no work without play. Starting from the laboratory with all the PhD students and other ‘non-permanents’ than made being at the lab a pleasant experience, from lunchbreaks to occasional volley- and basketball matches, too late-night or weekend labwork – thanks a lot. In the last month there were Mathieu, Léandre and Fiorella the same situation as I was and I want to particularly thank them for being there (on weekends), discussions and help with all sorts of issues.

For me doing this PhD thesis was tightly linked to life in Toulouse. As my time in Toulouse likely comes to an end soon I want to thank at least Sam, Patrick and Emma who became close friends and really made my time outside the lab. Also I would like to thank the whole rest of the Toulozen crew, with whom I rode the roller-coaster of sharing much more than a house for the past years. It has been a great time!

Even in the south of France I had my bit of German ‘Heimat’ with me. With Mieke who was there and made me feel understood in all ups and downs and I could never imagine this past three years without her. But also Jan, Johanna and Marilen who were always there to get my head straight again and cooked dinner for more than once in the last months. Also thanks to Michel who was further away but always somehow there.

Last but clearly not least I want to thank my parents who have supported me in every possible way not only here but for the last 30 years. Thanks a lot for everything.

Now before we go back to sciences I want to thank the people that got me hooked up with physical science in the first place : Hans Joachim Ratsch who was disappointed that I was more interested in bicycles than in pharmacy at high school, Claudia Pauck who introduced me to copper and alcoholic drinks and Gerd Könemann who would now just smile and say : “A specialist is someone who knows more and more about less and less until he knows everything about nothing”. I think his influence is still visible in this thesis.

Again thanks to all of you, I had a great time in Toulouse and at the GET, and am curious for the next adventure.

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Chapter 1

Introduction

1.1 Foreword: The link between soil and wine

Even before their role in plant nutrition was known, distinctive qualities of wines were attributed to their respective vineyard soils. Nowadays, especially in southern Europe wine growers put a lot of emphasis on their soil. This is reflected in the appellation of origin schemes most popular in France, Italy and Spain. These schemes certify that geographical setting and production techniques are ‘typical’ for a region. This led to the common naming of wines by their origin- think of Champaign, Chablis or St. Emilion wines. These appellations of traditional European wine growing areas (“the old world”) is in opposition to the common naming in the English speaking wine production areas (“the new world”) where a higher importance would be accorded to the biology of wine growing. In the new world wines would commonly be called by the grape variety – a pinot noir, a gewürztraminer, etc. However grape variety in Europe is commonly included in appellation criteria as well. Yet these criteria go a lot further than variety and often contain constraints on geographic, geologic and pedologic properties, leaving room for extensive discussions on the importance of a certain soil type for a certain wine. One of the ideas behind the introduction of appellation laws was the prevention of fraud. Fraud is still an issue in the wine business and a clear link between vineyard soils and wines is yet to be established.

Furthermore, renowned vineyard soils have sustained grapevines for a long time. As grapevine is sensitive to diseases, treatments have been used for at least 150 years. Inorganic pesticides as the “Bouillie Bordelaise” are so commonplace that questions are being asked about their fate. This is especially true as some regions report problems replanting their vineyards and the concerns of an impact on viticulture is growing. Total content of Cu appears to be a poor indicator of toxicity towards grapevine, and mechanisms of Cu mobility appear to differ depending on physico-chemical conditions

in soils. In the European Union efforts are being made to ban Cu treatment but have up to this point led nowhere due the irreplaceability of Cu treatment especially in organic viticulture. From an ecotoxicologic point of view transport of Cu in aquatic ecosystems is of major interest as Cu develops its highest toxicity towards aquatic microorganisms.

Source tracing and analysis of mixing process through elemental analysis and isotopic techniques are classical tasks routinely performed in geochemistry. Traditionally geochemical tools are mostly used to trace rock forming components or mixing of different water sources. Here these techniques will be applied to viticultural systems. Furthermore, there is an emerging use of non-traditional stable isotopes such as Cu. Even though this tool is still in development, discussions are still ongoing on whether source signature or biogeochemical transformation controls Cu isotope ratios in natural samples. Regardless, the tool appears to be useful in viticultural settings: If source signature is controlling isotope ratios, the provenience of Cu could be traced otherwise the biogeochemical mechanisms of Cu retention and cycling could be determined.

In this global context, this PhD work aims to approach the large question of mobility of elements in soils, in particular Cu, and their availability to plants. It focuses specific questions for viticultural practices by using traditional and innovative geochemical tools as elemental ratios, isotopic techniques and EPR (electron paramagnetic resonance) analysis.

1.2 Current viticultural problems in relation with soil geochemistry

1.2.1 Mineral nutrition and wine taste

Soils are considered of utmost importance in viticulture. Most visibly the nature of soils is used in marketing of wines to justify a special taste from a certain wine region. “Blue schist” from Moselle, “Red soils” from South Africa or “Volcanic soils” from Italy are emblematic of wine regions. Most marketing efforts at least imply a certain impact of soil quality on taste and since the year 2000 explicit descriptors of “minerality” in wine are more and more commonly used (Maltman, 2013). However, the differentiation between soil and rock is not always clear in these descriptions. In popular wine literature taste descriptions like ‘packed with minerals’, a ‘flinty’ or ‘chalky’ taste or a ‘granitic smell’ are easily found (Figure 1.1) however there is no equivalence in scientific tasting schemes (Maltman, 2013). Some confusion in the discussion certainly comes from the differences in use of the word “mineral”. In geosciences, minerals are the building blocks of rocks. They are crystalline, chemically homogenous compounds.

By contrast, in plant biology this term “minerals” often refers to inorganic ions taken up by plants as nutrients (Marschner and Marschner, 2012).



Figure 1.1 – Advertisement for the Soave appellation with suggestive images using active volcanos, grapevines and white wine to represent the link between a “volcanic soil” and a “mineral” wine (image from *Essere Soave*, 3/2015).

Nevertheless in France soil type is often one of the criteria applied to be allowed to sell wine under a certain AOC (appellation d’origine contrôlée) label (Kuhnholz-Lordat, 1963). Even though the impact of environmental factors like water availability, temperature and sunshine are extensively studied and proven, these refer to physical properties of soils, such as depth, pore size distribution reflectance etc. in conjunction with climate (Bramley et al., 2011; Gonzalez-Barreiro et al., 2015; Styger et al., 2011; Van Leeuwen et al., 2009; van Leeuwen et al., 2004). Evidence of chemical soil properties or the presence of certain minerals or bedrock influencing wine quality is scarce. However a growing body of scientific literature reports differences in wine quality most likely due to chemical properties of soil (Benciolini et al., 2006; Costantini et al., 2012; Imre et al., 2012; Mackenzie and Christy, 2005).

It is evident that the influence of minerals contained in a soil do not directly give a ‘mineral taste’. Firstly, minerals are not directly absorbed by plants but rather ions, their dissolvable constituents (Marschner and Marschner, 2012). Secondly, taste thresholds of ions are high compared to organic compounds so it is unlikely that mineral composition of wine is detected (Epke and Lawless, 2007; Sipos et al., 2012). Thirdly, mineral

nutrition does not pass directly from soil to plant but is subject to an active regulation mechanism of uptake and translocation so that healthy plants often have rather similar elemental compositions in important organs even if they are grown on different soils (Marschner and Marschner, 2012). This homeostatic control has its limits most evidently manifested in toxic effects of overabundance of, for example, metals or lack of nutrients (Figure 1.2).



Figure 1.2 – *Photograph of swollen yellow grapevine roots due to Cu toxicity on the left. Photograph of chlorotic leaves due to Mg deficiency to the right (from vignevin-sudouest.com).*

A possible influence of soil chemistry on wine needs to pass through an influence of mineral nutrition on the synthesis of organic compounds responsible for wine flavour (Maltman, 2013). Besides its role in plant functioning elemental composition of grapes furthermore serves as a source of mineral nutrients for organisms of fermentation (Navasques, 2005). Even though main nutrients as NO_3 or K might be added at this stage, too high concentrations of metals such as Cu are reported to disturb the fermentation process and change yeast strains present in grapes and musts (Brysch-Herzberg and Seidel, 2015; Milanovic et al., 2013; Ribéreau-Gayon et al., 2012). To better constrain the possible influences of soil chemistry on wine it is important to understand the mobility of different elements from different soil types to grapevine plants.

1.2.2 Use of elemental composition for origin tracing

Besides considerations of plant nutrition and its impact on wine taste, the elemental composition of plant tissues and wines recently got much attention in scientific literature for use in origin tracing and fraud control (Almeida and Vasconcelos, 2001; Coetzee et al., 2014; Day et al., 1995; Greenough et al., 2005; Kwan et al., 1979). As geographic origin is the cause for high price differences in wine, it is interesting to be able to verify what is written on the bottle label. Using elemental contents of wines it appears to be

possible to differentiate wines grown on a limited number of different soils (Almeida and Vasconcelos, 2001; Coetzee et al., 2014; Day et al., 1995; Greenough et al., 2005; Kwan et al., 1979). In most of those studies, nutrients are included in analytical dataset but the greatest discrimination power often comes from non-essential elements such as Co, Rb, Sr and Ba. But some nutritive elements such as Mn and Mg are also frequently used to determine product origin. However most studies on origin tracing only empirically link elemental composition to a certain geographic zone and few (Coetzee et al., 2014).

From studies on other plants it is known that non-nutritive trace elements appear to be less tightly regulated than nutrients and their concentration in plant tissues thus is more dependent on environmental gradients. For example in their study across a gradient of varying geologic material in 4 tree species, Chiarenzelli et al. (2001) found that relative standard deviation of Fe and Ni concentrations in leaves were low (16 and 21 %, respectively) whereas those of Co and Cd varied much (151 and 148 %, respectively) independently of plant species. Even though older studies often dealt only with few chemically similar elements, it became clear in research on radioactive elements that uptake between chemically similar elements is coupled (Ehlken and Kirchner, 2002). For example, radioactive Sr and Cs enter plants due to their chemical similarities to Ca and K (Ehlken and Kirchner, 2002). It was shown that the same transporters are involved in K and Cs uptake (Qi et al., 2008), implying that the plant elemental content of Rb and Sr mostly depend on the Ca/Sr and K/Rb ratio in the source of plant nutrition (Shaw, 1993). Furthermore it was reported that plant tissue content of many elements changes significantly after modification of pH of the same soil even some plant nutrients as Mg, K and Mn are significantly influenced by pH changes (Tyler and Olsson, 2001). To be able to generalize the use of elemental contents as origin tracers and to understand the functioning and limitations of the technique there is a need to understand what soil properties actually induce different elemental profiles in wine.

1.2.3 Viticulture and Cu toxicity

Concerns are growing on the effect of Cu on viticultural practice. Cu based pesticides have been used for around 150 years to protect grapevine plants from downy mildew (Richardson, 2000; Viala and Ferrouillat, 1887). However Cu does not kill the germs but only inhibits sporulation so that a constant film of Cu needs to be present on grapevine leaves that is at least partly washed off to the soil in rain events (Richardson, 2000). This leads to repeated Cu application throughout the year and accumulation of Cu in vineyard soils (Chaignon et al., 2003; Flores-Vélez et al., 1996). Today's EU regulation allows spaying of up to 6 kg ha yr⁻¹ but application was more excessive in

the past (RCE No. 889/2008, Viala and Ferrouillat, 1887). We found literature advising up to $60 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Viala and Ferrouillat, 1887). However Cu is still the only permitted pesticides in organic agriculture efficient against downy mildew due to its ‘natural’ origin (RCE No. 889/2008). Indeed there have been efforts to prohibit use of Cu pesticides on EU level but they were abandoned due to the economic importance and lack of alternatives. However in Germany and Switzerland Cu use in organic agriculture is limited to $3 \text{ kg ha}^{-1} \text{ yr}^{-1}$ whereas in Denmark and the Netherland the use of Cu has been completely banned.

Cu is highly toxic to aquatic organisms as algae, so that a traditionally important issue is the mobility of Cu from soils to the hydrosphere (Flemming and Trevors, 1989). However a large part of Cu treatments remains in vineyard soils (Komárek et al., 2010). Also in soils microbial life is most impacted by Cu contamination, microbial communities differ between contaminated and non-contaminated soils and some studies find lower respiration rates in more contaminated soils (Celardin and Chatenoux, 2003; Dell’Amico et al., 2008; Hiroki et al., 1985). Higher plants and especially grapevine have been considered tolerant to Cu contamination for a long time evidence for negative impact of Cu concentration in vineyard soils on viticulture is growing (Anatole-Monnier, 2014; Cambrollé et al., 2015; Juang et al., 2014). Toxic effects to grapevine include decrease in root growth, inhibition of nutrient uptake (especially P, Mg, Fe), reduction in photosynthesis (Toselli et al., 2009) and an increase of vulnerability towards diseases (Anatole-Monnier, 2014). Furthermore an influence of Cu contamination on factors relevant for wine making such as soluble sugar and chlorophyll contents of grapevine leaves has been reported (Romeu-Moreno and Mas, 1999). These effects are observed even though Cu is considered of low mobility in soils. Especially in carbonate-rich soils displaying high pH values, Cu is of low mobility and thus accumulates in soils, mainly in topsoil horizons (Duplay et al., 2014; Komárek et al., 2010; Pietrzak and McPhail, 2004; Ponizovsky et al., 2007). Usually an association of Cu with soil organic matter is observed (Boudesocque et al., 2007; Flogeac et al., 2004; Strawn and Baker, 2008, 2008) but some studies report an association with Fe-(oxy)-hydroxydes (Sayen and Guillon, 2010). The latter would be in line with model predictions (Bradl, 2004). Also soil carbonates appear to play a role in Cu retention (Komárek et al., 2009; Ponizovsky et al., 2007). Furthermore an aging effect is observed making older Cu less mobile than Cu from recent applications (Arias-Estevéz et al., 2007; Ma et al., 2006; Sayen et al., 2009). As Cu retention and mobility in soils is complex there is an increasing demand for further understanding of both the mobility and bioavailability of Cu in vineyard soils.

1.3 What drives elemental cycling in the soil – plant system?

1.3.1 The plant demand: Plant mineral nutrition

Besides major building blocks of plant tissues such as H, C, N, and O, 14 other elements (B, Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Mo) are taken up as nutrients by the roots from the soil (Marschner and Marschner, 2012). Most of these nutrients are present in rather small quantities but occupy important functions within the plants. For example redox active metals as Fe, Cu, Zn or Mo are the main functional groups in enzymes responsible for electron transfer (Marschner and Marschner, 2012). Their content in plant tissue is often tightly regulated as their redox activity can also harm plant tissues when over abundant (Marschner and Marschner, 2012).

These elements are taken up in their ionic form or complexed to low molecular weight substances from the soil (Marschner and Marschner, 2012). To be taken up into the root cells solutes have to pass through pores in the cell wall in a passive mass diffusion process (Marschner and Marschner, 2012). However as pore size is around 5 nm, there is some restriction on the movement of larger molecules such as humic or fulvic acids (Marschner and Marschner, 2012). Cell walls furthermore have a surface charge dependent on the environmental pH and as such can adsorb cations (Marschner and Marschner, 2012). To be considered as ‘absorbed’, solutes need to further cross the cell membrane, for ions there is the possibility of entering through ion channels due to an electrochemical gradient between cell and solution or by active transport proteins directly requiring energy for the transport (Marschner and Marschner, 2012). Through this system solute import is decoupled from water transport for most elements (Marschner and Marschner, 2012; Zhao et al., 2010). Active ion transporters are not always selective for one element but rather for a group of ions and can involve chemical transformation of the solute as reduction or oxidation (Korshunova et al., 1999). The most studied transporter probably is the IRT1 transporter which transports Mn, Fe, Co and Zn but can also be inhibited by Cu (Korshunova et al., 1999). Within the plant ions are then distributed through xylem and phloem vessels to different tissues (Marschner and Marschner, 2012).

As this process mainly transports small solutes, the speciation of mineral nutrients and their mobility in the soil solution are of crucial importance for plant uptake. To satisfy their needs plant can deploy various chemical means to adjust the solubility of nutrients (Hinsinger, 1998). Especially in the close vicinity of roots called the rhizosphere, modification of physico-chemical properties of the soil solution are observed.

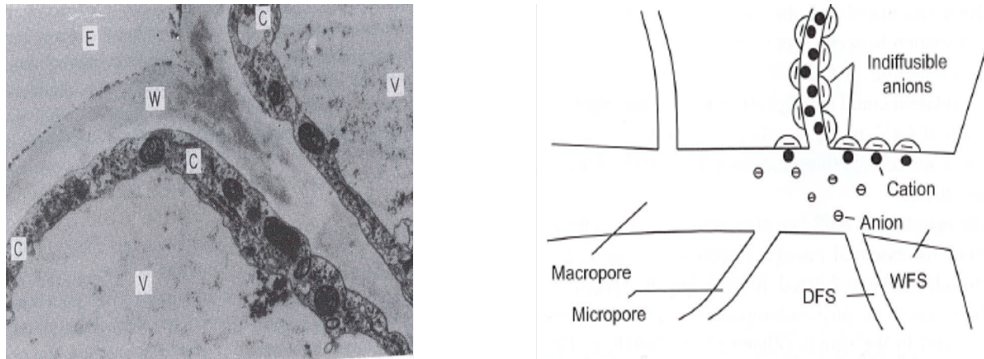


Figure 1.3 – *On the right: Microscopic image of roots. With the external solution (E), the cell walls (W) and the inside of cells with the cytosol (c) and the vacuole (V). On the left: Schematic sketch of pores in cell walls allowing diffusion and adsorption of ions (both from Marschner and Marschner, 2012)*

This reaches from displacement of chemical equilibria (Hinsinger, 1998) due to uptake of large amounts of for example K, adjustment of pH to either increase or decrease solubility of a certain nutrient (Bravin et al., 2012; Hinsinger et al., 2003) and exudation of molecules complexing specific insoluble nutrients as Fe or changing the redox potential (Hinsinger, 1998; Kraemer, 2004). Thus plants are able to modify essential parameters controlling solubility and speciation of elements. Furthermore symbiotic interactions with microorganisms play an important role in mineral nutrition of plants but this is a topic in itself and exceeds the scope of this thesis (Aerts and Chapin, 1999; Landeweert et al., 2001; Marschner and Marschner, 2012).

1.3.2 Elemental dynamics in soil – interaction between solid and liquid phases

Soils are living systems at the intersection of lithospheric, hydrologic atmospheric and biologic influences (Blume et al., 2016). They are made up of a solid, a liquid and a gaseous phases (Blume et al., 2016). Source of inorganic compounds is either physical or chemical rock degradation. Fresh mineral matter is either in place (bedrock) or transported to the soil by the action of rivers, slope or the atmosphere (Chadwick et al., 1999; Jenny, 1994). These mineral constitutes are the basis of the soil which are chemical degraded under the action of water and biological activity (Jenny, 1994). Weathering processes release ions to the liquid phase (Jenny, 1994). Depending on their position in different minerals and their solubility, elements can be more or less easily released to the liquid phase (Carroll, 2012). For example Si is a constituent of many minerals occurring in soils but most of them are only scarcely soluble (Carroll, 2012). In most cases fresh rock is available in the deeper layers of the soil column and more and more weathered and thus immobile material constitutes the upper layers (Blume et al., 2016; Jenny, 1994).

The liquid phase of soil is in permanent contact with mineral and organic surfaces and thus surface adsorption of elements plays an important role for their actual presence in the liquid phase (Koretsky, 2000). Most surface active constituents are particles of small volume and thus high volume to surface ratios that have been formed during soil development. Some have a constant surface charge as clay minerals (Carroll, 2012), but for other constituents surface charge depends on the pH as natural organic matter or Fe-(oxy-)hydroxydes that can act as adsorption sites when they get deprotonated at higher pH (Blume et al., 2016). The rates of dissolution and surface adsorption highly depend on physico-chemical properties in the soil as pH, Eh and ionic strength but also on available ligands to keep a certain ion in solution (Carroll, 2012; Kabata-Pendias, 2004; Velde and Meunier, 2008). So that the mobility of natural occurring elements mainly depends on (1) their abundance in primary material, (2) the solubility minerals at given physico-chemical conditions, (3) its affinity to surface adsorption under given physico-chemical conditions and (4) the availability of ligands that keep the ion in solution, say their speciation in solution. Note that not one most important factor can be identified physico-chemical conditions can make one compound highly mobile even at low abundance in mineral matter but also surface adsorption can almost totally immobilize a soluble constituent. Plants not only influence the elemental mobility and chemical equilibria in the close circumference of their roots. But also they reintroduce elements from deeper layers in the soil on the top of the soil column through leaf fall and dead tissues (Jobbagy and Jackson, 2001; Lucas et al. 2006, Lucas, 2001). Plants are thus a major player in elemental mobility also within soils.

To this chemical mobility in soils, a physical mobility can be added if a given compound migrates with a particulate phase (Blume et al., 2016; Jenny, 1994). As most surface active constituents of soils are rather small adsorbed elements can migrate through pores with those particles (Blume et al., 2016; Jenny, 1994). Also the dissolution of a solid adsorbent leads to the mobilization of immobilized compounds at their surface as for example liberation of As through reductive dissolution of oxy-hydroxydes of Fe (Nickson et al., 2000).

Finally, in agricultural ecosystems the elemental cycling is further modified by human activity. Some elements are take of the system with the harvest, others are introduced at the top of the soil column by fertilization, use of pesticides or other agricultural practices. Then exogenic compounds could be largely introduce to this dynamic system and can thus modify equilibria in place.

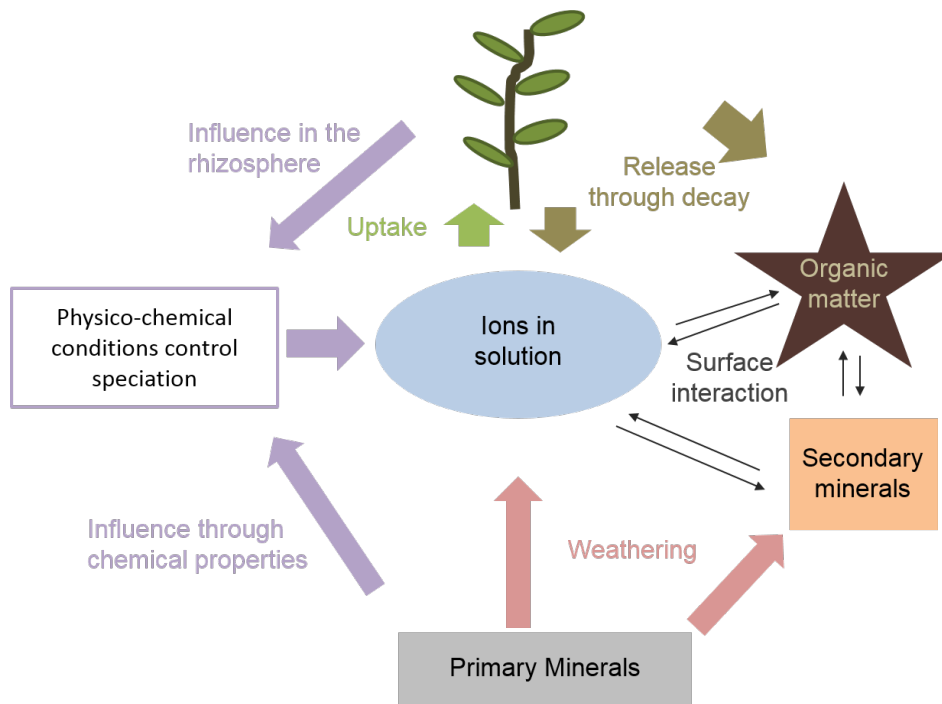


Figure 1.4 – Schematic sketch illustrating interplay of major mechanisms controlling elemental mobility in soils.

1.4 Potentials of a geoscientific approach in viticulture

In order to identify influences of soil type on wine quality one needs at first to establish differences in soil functioning. In soil sciences, soil functioning is typically described in more or less homogenous horizontal layers called horizons of a soil, from the bedrock to edge of the atmosphere (Blume et al., 2016; Jenny, 1994). This type of description appears to be more adapted to viticultural issues as traditional agricultural techniques only working on the first 20 cm of a soil column as grapevine is a deep rooting plant with rooting depth observed up to 6 m (Benciolini et al., 2006; Branas and Vergnes, 1957; Doll, 1954; Seguin, 1972). Also a clear distinction between soil and bedrock is made in soil science (Jenny, 1994). These distinctions are often not being made in viticulture and often blurs arguments as descriptions confuse properties of bedrock and soil (Maltman, 2013). So systematic pedological description is necessary for a sound discussion of the subject.

1.4.1 The use of traditional geochemical techniques

Furthermore geochemical methods routinely use Inductively Coupled Plasma Mass Spectrometry (ICP-MS) based analysis giving access to concentrations of many main and trace elements in different compartments. This data can then be used in multivari-

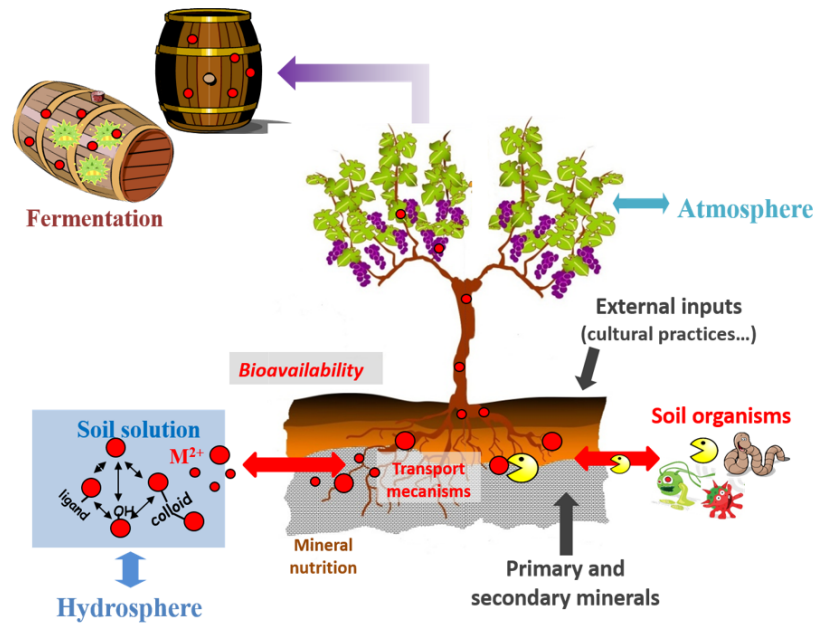


Figure 1.5 – Schematic display of factors influencing elemental transfer between soil and grapevine: Primary minerals from the bedrock are weathered within the soil, supplying soluble elements, other elements are amended by agricultural practice. Microorganisms and physicochemical conditions in the soil solution control speciation of elements and thus their availability to the plant. Transport and uptake mechanism within the plant regulate the transfer to grapes and ultimately wine.

ate statistical approaches to find specific patterns for a category. These techniques have been commonly used to differentiate empirically wines from a small number of regions (Coetzee et al., 2014; Martin et al., 2012; Mercurio et al., 2014). A more traditional geochemical technique exploits ratios of chemically similar elements for source tracing. For example Ca/Sr ratios are commonly used to trace Ca nutrition in forest ecosystems (Blum et al., 2012, 2012; Schmitt et al., 2017; Wiegand et al., 2005). Also the K cycling in ecosystems can be readily traced by K/Rb ratios (Chaudhuri et al., 2007; Likens et al., 1994; Peltola et al., 2008). Elemental ratios are also at the origin of mass transfer calculation in soils developed to trace mass losses due to chemical weathering but readily applicable also to gains of matter (Brimhall et al., 1988). Furthermore stable isotopic ratios of the same element are common tracers used in geochemistry. A common tracer of origin are radiogenic Sr isotope ratios, the isotopic ratios mostly depends on isotopic ratios of the source of strontium but does not significantly fractionate during geochemical or biological transitions of Sr (Blum et al., 2000; Capo et al., 1998; Horn et al., 1993; Pett-Ridge et al., 2009).

Geochemistry thus provides a variety of methods that can be readily used for origin tracing. This is not only interesting for the determination of geographical origin of a wine but also to identify soil pools contributing to plant nutrition.

1.4.2 Use of non traditional stable isotopes in agricultural settings

Stable isotope ratio analysis especially of light elements is by now a common tool in many scientific disciplines. Isotope ratios can give additional information on sources of the element (as isotope ratios of different sources may differ) or on reaction pathways as heavy and light isotopes react slightly different. Cu-isotope ratios at natural abundances are typically measured on a Multiple Collector – Inductively Coupled Plasma - Mass Spectrometry (MC-ICP-MS). The measurement methods are straight forward and most working groups rely on the methods published by Maréchal et al. (1999). However as relatively small differences are measured in the order of the tenth per mil variance between ^{63}Cu to ^{65}Cu ratio, measurement requires high purity of the sample and correction of the internal machine fractionation (Maréchal et al., 1999). While mass bias correction by either Ni or Zn spikes is routinely performed, the sample purification, especially for environmental samples where Cu is only a trace element, remains critical (Chapman et al., 2006; Ehrlich et al., 2004; Maréchal et al., 1999). This means that the Cu separation protocol was adapted by almost every working group to their specific matrices (Archer and Vance, 2004; Babcsanyi, 2015; Bigalke, 2010; Borrok et al., 2007; Larner et al., 2011; Liu et al., 2014; Maréchal et al., 1999).

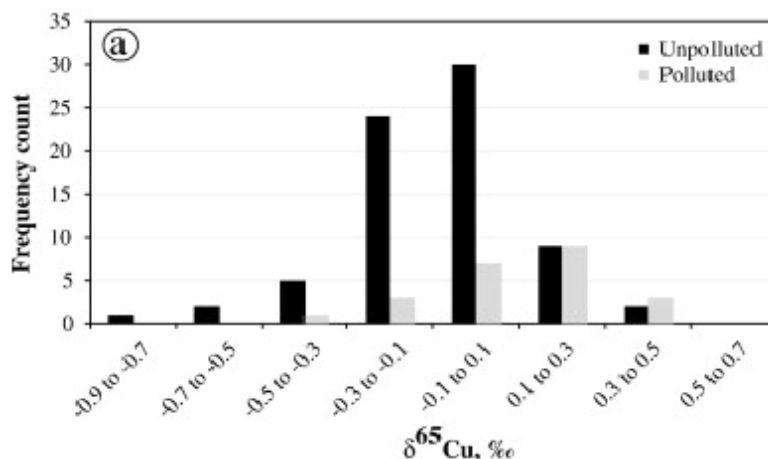
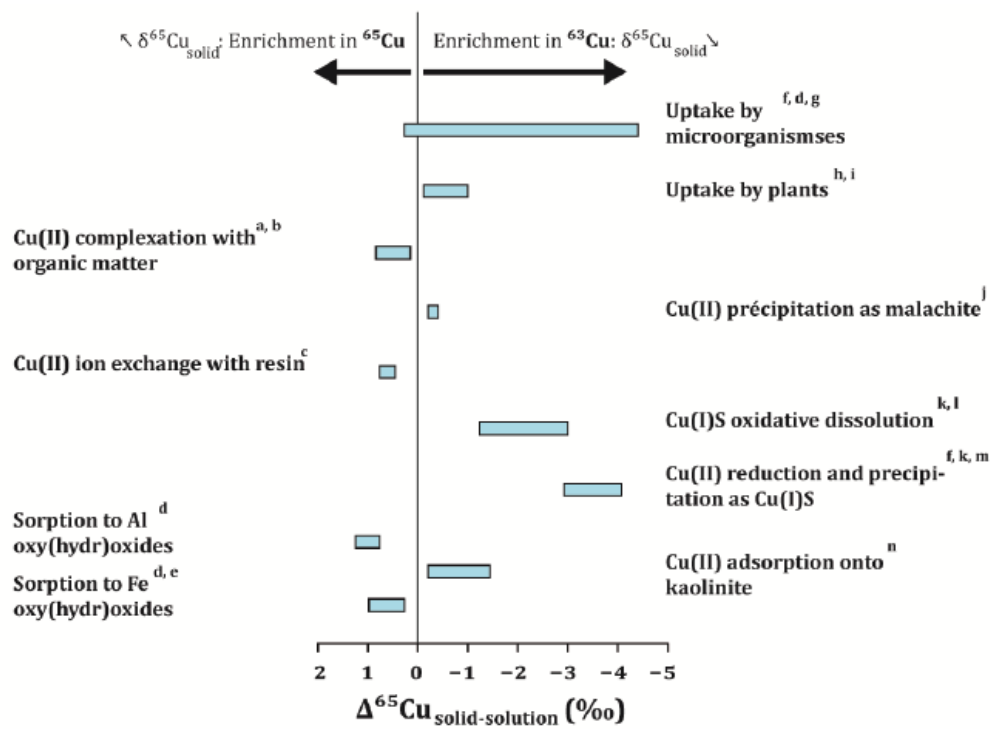


Figure 1.6 – Histogram of Cu isotope ratios measured in French agricultural topsoils contaminated by Cu (black) and uncontaminated (grey) (Fekiacova et al., 2015).

Cu isotope analysis on environmental samples was first performed by Maréchal et al. (1999), followed by initial studies on plants and soils some 10 years later (Bigalke, 2010; Bigalke et al., 2010b, 2011; Weinstein et al., 2011). Even though metal contaminated soils have been in the focus of Cu isotope studies from the beginning to conclusion has been reach on weather source signature or biogeochemical mechanisms control the Cu isotope ratios within a soil column (Babcsányi et al., 2016; Bigalke et

al., 2010a). However a study on a large number of French topsoils shows a tendency for heavier isotope ratios in polluted soils underlining the potential of the method in polluted environments (Fekiacova et al., 2015). Also in plants the discussions on Cu isotope fractionation is still ongoing. Even though light fractionation during uptake is commonly admitted fractionation patters of root to shoot transport remain unclear. Variable results from different growth settings suggest that fractionation of Cu isotopes depends on environmental conditions (Jouvin et al., 2012; Li et al., 2016; Ryan et al., 2013; Weinstein et al., 2011).

By now fractionation patters under experimental conditions have been reported in literature (Figure 1.7). Cu isotope analysis is thus an interesting tool to investigate Cu mobility in vineyard environments besides not being an out of the box solution yet.



a: Ryan et al., 2014, b: Bigalke et al., 2010, c: Maréchal et Albarède, 2002, d: Pokrovsky et al., 2008, e: Balistrieri et al, 2008, f: Zhu et al., 2002, g: Navarrete et al., 2011, h: Jouvin et al., 2012, i: Ryan et al., 2013, j: Maréchal et Sheppard, 2002, k: Mathur et al., 2005, l: Kimball et al., 2009, m: Ehrlich et al., 2004, n: Li et al., 2015

Figure 1.7 – Cu isotope fractionation during various reactions reported in literature. Graphic taken from (Babcsanyi, 2015).

1.5 Aim and scope

This thesis aims to contribute to the larger question of elemental mobility in soils and their availability to plants by applying geochemical tools to specific viticultural problems. The underlying hypothesis is that pedogenetic mechanisms (mineral weathering, and formation of secondary minerals, interaction with organic matter etc....) and physico-chemical factors influence biochemical and physiological functioning of the grapevine plant. In a viticultural setting this hypothesis means questioning the role of soil chemistry in the “terroir effect”. Besides natural influences vineyard soils are subject to human inputs (fertilizers, pesticides etc...) like all cultivated soils. The use of Cu-based pesticides is particularly widespread in vineyard ecosystems and a special focus of this work is on the mobility of Cu in vineyard environments.

In this thesis classical geochemical tracers as elemental ratios and mass balances are used, alongside with biochemical markers and emerging tools such as stable Cu-isotope ratio analysis and electron paramagnetic resonance (EPR). The method development necessary to be able to measure Cu isotope ratios in vineyard settings is described in Appendix C.

In **Chapter 3** right after this introduction, the development of the idea of soil chemistry on wine quality is addressed through a historic review. This chapter aims to link popular perception of wine growing and wine taste and scientific questions surrounding it.

Chapter 4 describes a study of elemental composition of more than 200 western European wines. The influences of environmental parameters such as soil type and meteorological conditions on the elemental composition of wines are investigated. *Is the ‘terroir effect’ a statistical reality? Is it possible to link soil and wine using geochemical tracers?*

In the **fifth chapter** a comparative study between two vineyard plots on different soil types (calcaric *vs.* vertic) in Soave (Italy) is presented. On the scale of two catenas, soil forming processes and geochemical functioning of soils is investigated. Furthermore elemental and biochemical markers in grapevine plants are measured. *Which soil properties influence the elemental transfer to grapevine plants? Does elemental composition of grapevine plants depend on soil geochemistry?*

The **sixth chapter** focuses on the transport of Cu in the soils presented in **Chapter 5**. The mechanisms of retention and transport of Cu-based pesticides are investigated through mass balance calculations, stable Cu-isotope ratios and EPR-measurements.

Do Cu-isotope ratios in soils reflect the isotope ratios of pesticides? Or do they reflect physico-chemical mechanisms of Cu mobility?

Finally the **Chapter 7** examines Cu transfer between soil, soil solution and grapevine plant in a greenhouse experiment. Elemental concentrations and Cu-isotopic ratios are measured in soils, soil solutions and plants as well as indicators of metal stress in plants. *Can we gain insights on the mechanisms of Cu mobility through the analysis of Cu isotope ratios? How does the presence of Cu in vineyard soils influence plant health and elemental cycling in the grapevine environment? Does the presence of Cu influence the isotopic composition of the plant?*

This thesis uses different approaches (statistics, field observations, greenhouse experiment) and different observation scales to extend knowledge in two areas. At first we want to extend the definition of the "terroir effect" through an experimental geochemical approach. Secondly the potential of stable Cu isotope ratios for the understanding of mechanisms of Cu cycling in vineyard environments is investigated.

Chapitre 2

Introduction en français

2.1 Préface : Du sol au vin

Avant même que soit étudiée et comprise la nutrition minérale des plantes, la qualité des vins était déjà mise en relation avec leur « sol d'origine ». Aujourd'hui, l'importance donnée au sol comme paramètre du « terroir » est particulièrement grande dans le sud de l'Europe, comme illustré par les appellations d'origine couramment utilisées en France, en Italie et en Espagne. Ces appellations certifient une origine géographique et une façon de produire typique d'une région. La systématique des appellations a été incorporée dans la dénomination populaire des vins. On boit par exemple « un Chablis », « du Champagne » ou « un St. Emilion ». Dans les régions viticoles anglophones, au contraire, les vins sont nommés par leur cépage. On boit du « pinot noir » ou du « gewürztraminer »... Cela dit, les cépages sont également inclus dans les cahiers des charges des appellations européennes. Une des idées derrière l'introduction des critères des appellations était la protection des vins contre la contrefaçon. Mais jusqu'à aujourd'hui le lien direct entre sol et vin reste à établir selon des arguments scientifiques.

Comme les grands domaines viticoles existent depuis plusieurs siècles et que la vigne est une plante vulnérable face aux maladies, les sols viticoles ont connu des traitements depuis quasiment 150 ans. L'utilisation des pesticides cupriques étant très répandue, de nombreuses questions se posent sur leur devenir dans les sols. La question est particulièrement sensible depuis que dans certaines grandes régions viticoles soient rapportés des problèmes lors des replantations. Les teneurs totales en Cu dans les sols ne permettent pas de faire des prédictions satisfaisantes quant à la toxicité du Cu et sa mobilité dans les sols. Toxicité et mobilité varient, entre autres, avec les conditions physico-chimiques rencontrées dans les sols. L'importance économique de l'utilisation des fongicides cupriques, notamment en agriculture biologique, est telle que les tentatives d'interdiction par l'union européenne de l'utilisation de ces pesticides ont échoué.

D'un point de vue écotoxicologique c'est le transport du Cu depuis les sols vers les écosystèmes aquatiques qui est le plus problématique car Cu est fortement toxique envers les microorganismes aquatiques.

L'utilisation des teneurs élémentaires et des rapports isotopiques des éléments comme outils de traçage de sources ou de mécanismes est une approche classique en géochimie. Traditionnellement, ces traceurs sont utilisés dans des contextes de formation de roches ou des mélanges des eaux. Dans cette thèse, ces approches sont appliquées au sein de systèmes agronomiques. L'utilisation croissante des isotopes dits non-traditionnels en géochimie, dont le Cu, offre des perspectives intéressantes en milieu viticole même si l'état des connaissances sur ces systèmes isotopiques n'est pas encore au niveau des systèmes des isotopes légers et que les causes des fractionnements observés sont encore en discussion. Si les signatures isotopiques des sources du Cu sont suffisamment différentes, les rapports isotopiques peuvent être envisagés comme traceurs de l'origine du Cu ; si les mécanismes biogéochimiques au sein du sol fractionnent les isotopes du Cu, les rapports isotopiques pourraient permettre de tracer les transformations chimiques qui influencent le cycle du Cu dans les sols viticoles.

Dans ce contexte, le travail présenté dans cette thèse vise à alimenter plus largement la connaissance sur la mobilité des éléments, et en particulier Cu, dans le continuum sol-plante. L'utilisation conjointe des outils géochimiques traditionnels et innovants en contextes viticoles constitue un des enjeux de ce travail.

2.2 Problèmes actuels de la viticulture en lien avec la géochimie des sols

2.2.1 La nutrition minérale et le gout du vin

Les sols ont une importance particulière en viticulture, le plus visible étant leur utilisation à des fins de « marketing » pour justifier le gout particulier de vins provenant d'une certaine région. Les schistes bleus de la Moselle, les sols rouges d'Afrique du sud ou les sols volcaniques d'Italie sont l'image même de leur région. Au regard de ces images, le rôle du sol dans le goût du vin semble implicite. A partir des années 2000, la notion de « goût minéral » devient de plus en plus courante dans la littérature populaire du vin (Maltman, 2013). Mais en viticulture populaire, la différenciation entre sol et roche n'est pas toujours exacte. Des descriptions de vins avec un goût « minéral » (Figure 2.1), comme un « gout de craie » ou une « odeur de granite » se retrouvent facilement dans les description de vins (Maltman, 2013). Dans cette discussion une confusion est apportée par l'utilisation du mot « minéral » : En géosciences, il s'agit des entités

chimiquement homogènes qui constituent les roches ; par contre en biologie, c'est ainsi que l'on nomme les ions nutritifs indispensables aux organismes vivants (Marschner and Marschner, 2012).



Figure 2.1 – *Image publicitaire pour les vins « volcaniques » italiens qui suggère un lien entre origine géologique du sol et le goût du vin (image de Essere Soave, 3/2015).*

Néanmoins, en France, les propriétés pédologiques (ex. teneur en calcaire actif des sols) font souvent partie des critères à remplir pour vendre un vin sous une certaine appellation (Kuhnholz-Lordat, 1963). Les influences des différents facteurs environnementaux comme la disponibilité en eau et les conditions météorologiques ont beaucoup été étudiées et démontrées. Pourtant il s'agit majoritairement de facteurs physiques du sol comme la profondeur, la taille des pores et leur réflectance (Bramley et al., 2011 ; C. Van Leeuwen et al., 2009 ; Gonzalez-Barreiro et al., 2015 ; Styger et al., 2011 ; van Leeuwen et al., 2004). Il n'existe pas d'évidence directe de l'influence des propriétés chimiques des sols sur la qualité des vins. Cependant, de plus en plus d'études s'intéressent à cette problématique et suggèrent un lien entre « chimie » du sol et vins (Benciolini et al., 2006 ; Costantini et al., 2012 ; Imre et al., 2012 ; Mackenzie and Christy, 2005).

Il est évident que les minéraux contenus dans le sol ne donnent pas directement un goût « minéral » au vin. Tout d'abord parce que les solides minéraux ne sont pas directement absorbés par la plante mais plutôt les constituants solubles, les ions (Marschner and Marschner, 2012). De plus, les teneurs qui seraient nécessaires pour pouvoir ressentir le « goût » des ions sont extrêmement élevées par rapport aux constituants

organiques des vins (Epke and Lawless, 2007 ; Sipos et al., 2012). Enfin l'absorption des ions est un mécanisme très régulé par les plantes et il n'est pas rare de trouver des concentrations élémentaires semblables dans les tissus de plantes cultivées sur différents sols (Marschner and Marschner, 2012). Néanmoins ce contrôle n'est pas infini et la nutrition minérale peut avoir un impact important sur le fonctionnement de la plante, par exemple par le biais d'une toxicité (métallique,..) ou d'une carence en éléments minéraux essentiels (Figure 2.2).



Figure 2.2 – *A gauche : Racines de vigne jaunies et gonflées à cause de la toxicité de Cu. A droite : Feuilles chlorotiques à cause d'une carence en Mg (image de vignevin-sudouest.com).*

Dans ce contexte, l'influence de la chimie du sol sur le vin peut être envisagée au travers de la synthèse des molécules organiques en lien avec la nutrition des plantes (Maltman, 2013). Au-delà de leur rôle dans le fonctionnement biochimique de la plante, les éléments minéraux dans les raisins servent aussi de source de nutrition minérale pour les levures (Navascues, 2005). Même si des nutriments principaux comme le NO_3 ou le K sont ajoutés par le viticulteur lors de la fermentation, des teneurs trop élevées en métaux peuvent perturber la fermentation (Brysch-Herzberg and Seidel, 2015 ; Milanovic et al., 2013 ; Ribéreau-Gayon et al., 2012). C'est pourquoi, pour mieux comprendre les influences possibles du sol sur le vin il est important de mieux contraindre les transferts élémentaires entre le sol et la plante.

2.2.2 Utilisation de la composition élémentaire du vin comme outils de traçage de l'origine du vin

Au-delà de l'influence du sol sur le goût du vin, le profil élémentaire des vins peut aussi être utilisé comme outil de traçabilité de leurs origines géographiques (Almeida and Vasconcelos, 2001 ; Coetzee et al., 2014 ; Day et al., 1995 ; Greenough et al., 2005 ; Kwan et al., 1979). Dans la mesure où l'origine d'un vin fait partie des arguments de

vente, sa traçabilité géographique est importante. Dans la littérature, de nombreux auteurs rapportent qu'il est possible de différencier la provenance des vins (sur la base d'un nombre d'origines restreint) par leurs profils élémentaires (Almeida and Vasconcelos, 2001 ; Coetzee et al., 2014 ; Day et al., 1995 ; Greenough et al., 2005 ; Kwan et al., 1979). En général, les différences rencontrées dans les profils élémentaires des vins de régions différentes ont été attribuées à une différence dans la « chimie » des sols. Les éléments les plus utilisés dans la mise en place de ces classifications d'identification d'origine sont des éléments en traces qui ne sont pas connus pour être des micronutriments essentiels comme Co, Rb, Sr ou Ba. Mais dans certains cas, les vins semblent se différencier aussi par leurs profils en Mn ou Mg qui sont des nutriments essentiels.

Les éléments traces qui ne sont pas considérés comme des micronutriments sont moins strictement régulés par les plantes lors de l'absorption. Par exemple, Chiarenzelli et al. (2001), dans une étude s'intéressant à différents domaines géologiques et à différentes espèces de plantes, reporte que l'écart-type des teneurs en Fe et Ni dans les végétaux est de 16 et 21 %, respectivement alors que les teneurs en Co et Cd ont des écart-types de 151 et 148 %. Traditionnellement, les études portant sur l'absorption des éléments par les plantes ont considéré un nombre restreint d'éléments. Pourtant, il existe des évidences d'interactions entre éléments lors de l'absorption par les plantes. Par exemple, les études de l'absorption des éléments radioactifs montrent que Sr et Cs sont absorbés par la plante du fait de leur propriétés physico-chimiques proches de Ca ou K (Ehlken and Kirchner, 2002). De plus, il a été démontré que les mêmes transporteurs sont responsables de l'absorption de K et de Cs (Qi et al., 2008). Par conséquent, les quantités absorbées de ces éléments en trace par les plantes dépendent des rapports Ca/Sr ou K/Rb des sources de la nutrition minérale (Shaw, 1993). Il a aussi été démontré que la teneur en certains éléments dans les tissus végétaux dépend fortement du pH de la solution de sol, même pour des nutriments majeurs tels que Mg, K et Mn (Tyler and Olsson, 2001).

Afin de pouvoir généraliser et formaliser l'utilisation du traçage de l'origine des vins à l'aide de leur composition élémentaire, il est désormais important de comprendre quelles sont les propriétés du sol et les mécanismes dans le continuum sol/plante/vin responsables de ces différences dans les profils élémentaires observés dans les vins.

2.2.3 Viticulture et toxicité du cuivre

Les pesticides cupriques et en particulier la fameuse « bouillie bordelaise » ont été utilisés depuis 150 ans en viticulture (Richardson, 2000 ; Viala and Ferrouillat, 1887). L'ion cuprique inhibe la sporulation du mildiou et pour que le traitement soit efficace

il est nécessaire de traiter les vignes après chaque pluie afin de garder un film de Cu sur les feuilles (Richardson, 2000). Ainsi, cette pratique implique plusieurs traitements par an et est à ce jour responsable d'une accumulation importante de Cu dans les sols viticoles (Chaignon et al., 2003; Flores-Vélez et al., 1996). Les réglementations européennes permettent depuis 2008 l'application de pesticide cuprique jusqu'à 6 kg ha yr⁻¹ de Cu mais les quantités qui ont été appliquées sur les parcelles viticoles étaient plus importantes dans le passé. En effet, dans la littérature, des auteurs conseillaient des applications jusqu'à 60 kg ha⁻¹ yr⁻¹ (Viala and Ferrouillat, 1887). En agriculture biologique le Cu reste le seul pesticide autorisé contre le mildiou à cause de son origine « naturelle » et son utilisation traditionnelle. Même si ces initiatives ont échoué, certains pays de l'union européenne limitent l'utilisation du Cu en deçà des limites de la réglementation européenne. En Allemagne et en Suisse, l'utilisation du Cu en agriculture biologique est limitée à 3 kg ha⁻¹ yr⁻¹ et est complètement interdite au Danemark et au Pays Bas.

Le Cu est reconnu comme étant particulièrement toxique envers les microorganismes aquatiques (Flemming and Trevors, 1989). Ainsi, le transport de Cu depuis les sols contaminés vers les milieux aquatiques est une question cruciale. Il a été montré que la majeure partie du Cu issue des traitements reste immobilisée dans les sols (Komárek et al., 2010). C'est pourquoi, dans les sols c'est la vie microbienne qui semble le plus impactée. En effet, des communautés bactériennes différentes ont été observées en sols contaminés et non contaminés et la respiration du sol est moins importante dans les sols contaminés (Celardin and Chatenoux, 2003; Dell'Amico et al., 2008; Hiroki et al., 1985).

Les plantes et en particulier la vigne étaient généralement considérées comme résistantes vis-à-vis de la toxicité de Cu mais de plus en plus d'études récentes démontrent des effets négatifs des contaminations en Cu des sols sur les plantes (Anatole-Monnier, 2014; Cambrollé et al., 2015; Juang et al., 2014). Ces effets liés à la toxicité du Cu sur les plantes vont de l'inhibition de la croissance racinaire et la diminution de l'absorption des nutriments (particulièrement P, Mg, Fe), à la réduction de l'activité photosynthétique (Toselli et al., 2009) et une vulnérabilité accrue envers les maladies (Anatole-Monnier, 2014). Aussi des effets sur des paramètres importants pour la production de vins ont été observés comme une baisse en sucres solubles et une augmentation de la teneur en chlorophylle liée à une toxicité du Cu (Romeu-Moreno and Mas, 1999).

Ces effets ont été observés alors que Cu est considéré comme immobile dans les sols. En particulier, en environnement carbonaté, l'immobilité du Cu entraîne son accumulation dans les horizons de surface (Duplay et al., 2014; Komárek et al., 2010; Pietrzak

and McPhail, 2004; Ponizovsky et al., 2007). La plupart du temps, l'association du Cu avec la matière organique du sol est observé (Boudeocque et al., 2007; Flogeac et al., 2004; Strawn and Baker, 2008, 2008) mais certaines études ont montré l'existence de l'association du Cu aux oxy-hydroxydes de Fe, ce qui est en accord avec les résultats de modélisations (Sayen and Guillon, 2010; Bradl, 2004). Les carbonates semblent jouer aussi un rôle important dans la rétention du Cu dans les sols (Komárek et al., 2009; Ponizovsky et al., 2007). Certaines études ont démontré qu'il existe un effet de « vieillissement du Cu » dans les sols responsable d'une mobilité plus faible du Cu avec le temps (Arias-Estevez et al., 2007; Ma et al., 2006; Sayen et al., 2009).

2.3 Quels sont les moteurs des transferts élémentaires dans le système sol – plante ?

2.3.1 La demande biologique : La nutrition minérale des plantes

Au-delà des constituants majeurs des tissus vivants (H, C, N et O), les plantes ont besoin de 14 autres nutriments essentiels (B, Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Mo) absorbés par leur racines (Marschner and Marschner, 2012). La plupart de ces éléments sont présents en petites quantités dans les tissus de la plante mais ils occupent des fonctions importantes. Par exemple les métaux Fe, Cu, Zn et Mo sont utilisés dans des groupement fonctionnels des enzymes responsables des transferts des électrons (Marschner and Marschner, 2012). Leurs teneurs dans les tissus de la plante sont extrêmement régulées car l'activité redox peut aussi endommager les tissus de la plante (Marschner and Marschner, 2012).

Tous ces éléments sont absorbés sous forme ionique ou complexés à des composés de faible masse moléculaire (Marschner and Marschner, 2012). Afin d'être absorbés, les ions doivent d'abord diffuser à travers les pores dans la paroi cellulaire (Marschner and Marschner, 2012). La taille de ces pores, autour de 5 nm, exclue des ions complexés à des molécules relativement grandes comme des acides humiques et fulviques (Marschner and Marschner, 2012). De plus, les parois cellulaires ont une charge de surface dépendante du pH impliquant une plus grande adsorption des cations pour des pH basiques (Marschner and Marschner, 2012). Pour entrer à l'intérieur des cellules végétales, les ions doivent traverser la membrane cellulaire. Pour les cations, il est possible de passer passivement à travers les canaux cellulaires sous l'effet du gradient électrochimique existant entre l'intérieur et l'extérieur de la cellule (Marschner and Marschner, 2012). Mais il existe aussi des mécanismes de transport actifs, consommateurs d'énergie, qui ciblent le transport de certains ions en opposition avec les gradients chimiques (Marschner and Marschner, 2012). Ces transporteurs peuvent impliquer une transformation

chimique comme l'oxydation ou la réduction de l'ion en question (Korshunova et al., 1999). Le transporteur le plus étudié est probablement le IRT1 responsable du transport du Fe mais qui est connu aussi comme le transporteur de Mn, Co et Zn. L'activité de IRT1 peut être inhibée en présence de Cu (Korshunova et al., 1999). A l'intérieur de la plante, les ions sont ensuite distribués via les circulations dans le xylème et le phloème (Marschner and Marschner, 2012).

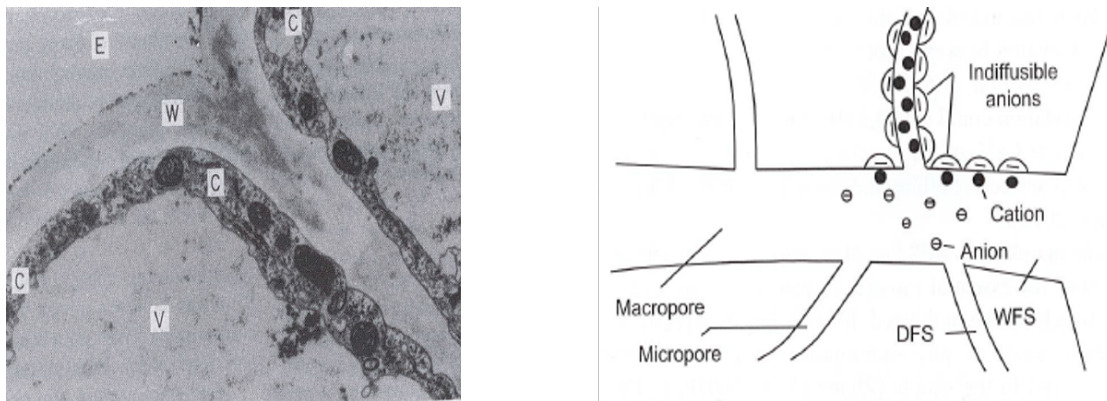


Image au microscope de cellules racinaires de maïs avec la solution externe (E), la paroi cellulaire (W) et l'intérieur des cellules avec le cytosol (C) et la vacuole (V). A droite sont schématisés les pores dans les parois cellulaires permettant la diffusion et l'adsorption des cations (Images de Marschner and Marschner, 2012).

Comme tous ces processus transportent des éléments provenant de la solution du sol, la spéciation des éléments est un paramètre primordial pour leur absorption par les plantes. Afin de satisfaire leurs besoins nutritifs les plantes peuvent modifier les conditions physico-chimiques autour des leurs racines (Hinsinger, 1998). Ces modifications sont particulièrement importantes à proximité des racines dans la rhizosphère. Les modifications peuvent aller du simple déplacement des équilibres chimiques (Hinsinger, 1998), à cause par exemple de l'absorption de quantités importantes de K, à la modification du pH pour augmenter ou diminuer la solubilité ds certains éléments (Bravin et al., 2012 ; Hinsinger et al., 2003) ou à l'exsudation de molécules organiques permettant de complexer ou d'influer sur l'état redox des nutriments peu solubles comme Fe (Hinsinger, 1998 ; Kraemer, 2004). Des symbioses microbiennes sont aussi impliquées dans la nutrition des plantes mais cela est un domaine scientifique à part entière qui dépasse les limites de cette thèse (Aerts and Chapin, 1999 ; Landeweert et al., 2001 ; Marschner and Marschner, 2012).

2.3.2 Transferts élémentaires dans les sols – interactions entre phases solides et liquides

Les sols sont des systèmes dynamiques à l'interface entre la lithosphère, l'hydrosphère, l'atmosphère et la biosphère (Blume et al., 2016). Ils sont composés de phases solides, liquides et gazeuses (Blume et al., 2016). Les constituants minéraux du sol sont issus de la dégradation physique et chimique des matériaux géologiques à la surface de la terre (Viers and Oliva, 2017; Chadwick et al., 1999; Jenny, 1994). Ces matériaux parentaux dont sont issus les sols peuvent être en place (roche mère) ou transportés via les rivières, le long des pentes ou par voie éolienne (Chadwick et al., 1999; Jenny, 1994). Ces matériaux géologiques sont la base de la formation des sols et sont altérés sous l'effet conjoint de l'activité biologique et des eaux météoriques (Jenny, 1994). L'altération des minéraux libère des ions en solution (Jenny, 1994). En fonction de la position des éléments au sein des réseaux cristallins, de la solubilité intrinsèque des minéraux et des conditions physico-chimiques du milieu d'altération, les ions sont plus ou moins facilement libérés en solution (Carroll, 2012). Par exemple le Si qui est un constituant majeur des minéraux silicatés est relativement peu présent dans les solutions d'altération (Carroll, 2012). Dans la plupart des cas, il existe un gradient d'altération entre les roches « fraîches » en profondeur plus altérables et les matériaux déjà altérés en surface, enrichis en minéraux résiduels plutôt inertes vis-à-vis de l'altération et en minéraux secondaires issus de ces processus d'altération minérale (Blume et al., 2016; Jenny, 1994).

La phase liquide du sol, ou solution du sol (Figure 2.3) est toujours en contact avec les surfaces des composés organiques et minéraux du sol. Les équilibres physico-chimiques entre les solides et la solution, en particulier les mécanismes d'absorption/désorption jouent un rôle important dans la chimie des éléments en solution (Koretsky, 2000). Les particules ayant les plus grandes surfaces réactives sont les phases de petites dimensions, généralement les minéraux secondaires formés lors du développement du sol. Certaines phases, comme les argiles ont des charges de surface permanentes; pour d'autres phases la charge de surface dépend du pH comme dans le cas de la matière organique ou les oxy-hydroxydes de fer (Blume et al., 2016). Les équilibres d'adsorption/désorption ainsi que les cinétiques d'altération sont fonction des conditions physico-chimiques comme le pH, le Eh, la force ionique ou la présence de ligands en solution (Carroll, 2012; Kabata-Pendias, 2004; Velde and Meunier, 2008).

Cela signifie que la mobilité des éléments dans le sol dépend de plusieurs facteurs (1) de leur présence dans les minéraux primaires, (2) de la solubilité des minéraux sous des conditions physico-chimiques données, (3) des équilibres d'adsorption/désorption sous des conditions physico-chimiques données, (4) de la spéciation des éléments dans la so-

lution. Il n'y a pas de facteur qui domine, par exemple, les conditions physico-chimiques peuvent entraîner une concentration très importante d'un composé en solution alors que sa présence dans la matière solide est très faible. Les plantes influencent la mobilité des éléments non seulement à travers la modification des conditions physico chimiques mais aussi par le phénomène de recyclage à l'échelle de la zone critique. En effet l'absorption des éléments en profondeur par le système racinaire des plantes permet de « remonter » les nutriments qui sont ensuite déposés en surface lors du dépôt de litière (e.g., Jobbagy and Jackson, 2001 ; Lucas et al. 2006, Lucas, 2001). Les plantes jouent un rôle fondamental dans les cycles biogéochimiques de certains éléments dans les sols.

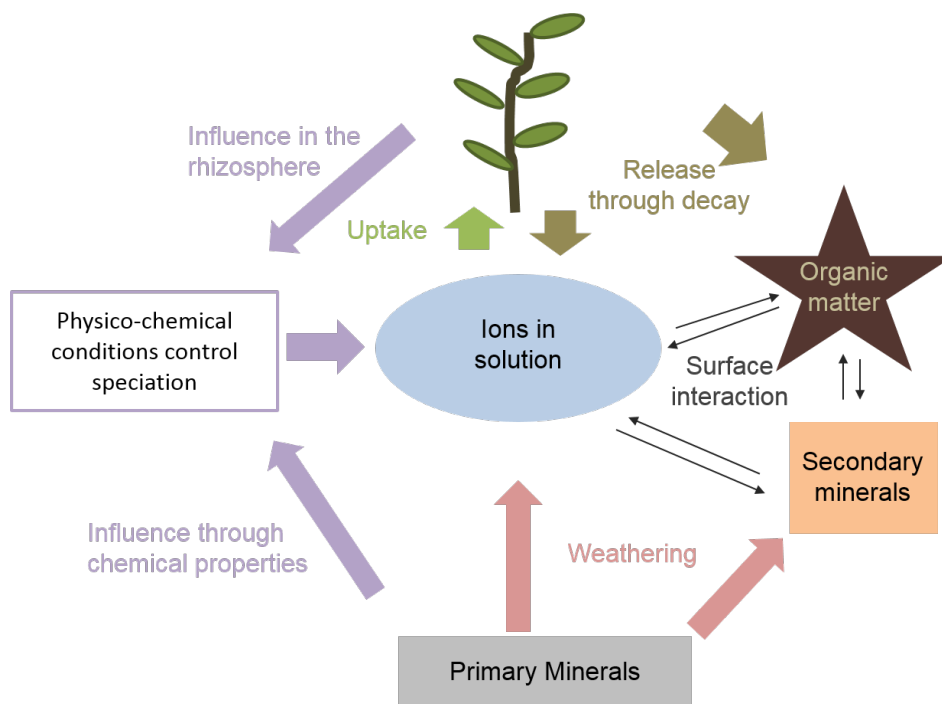


Figure 2.3 – Schéma de principe illustrant les facteurs majeurs qui influencent la mobilité des ions dans la solution du sol.

La mobilité physique se rajoute à la mobilité chimique des éléments dans les sols du fait des migrations verticales et latérales de particules fines à travers la porosité (e.g., Blume et al., 2016 ; Jenny, 1994, Kusonwiriawong et al. 2017). Dans les systèmes agricoles, les cycles biogéochimiques des éléments sont aussi influencés par les récoltes ou par les ajouts d'engrais et de pesticides. Les substances qui sont ainsi ajoutées dans ces systèmes dynamiques peuvent modifier considérablement les équilibres en place.

2.4 Le potentiel des géosciences en viticulture

Afin d'identifier les différences dans la qualité du vin qui peuvent être liées à la nature des sols, il est d'abord nécessaire de caractériser le fonctionnement des sols. En

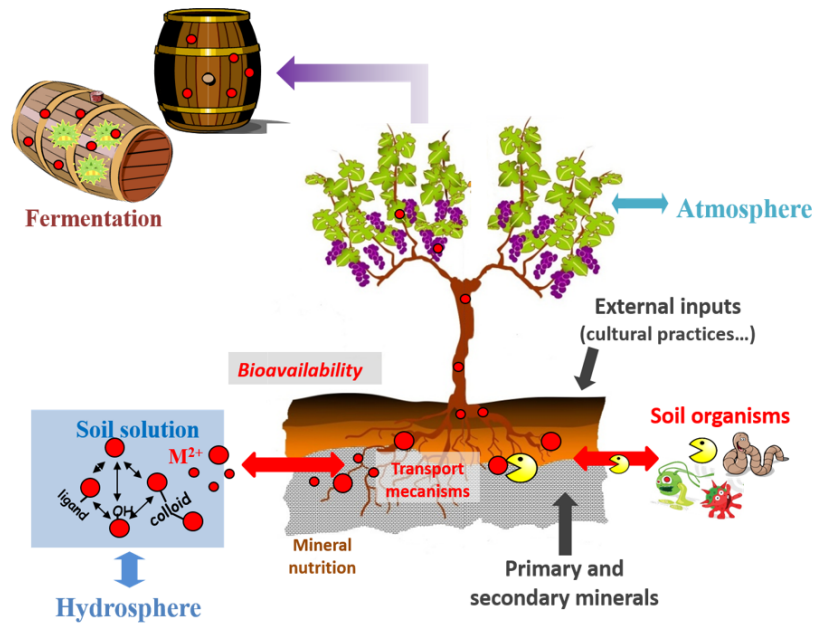


Figure 2.4 – Schéma illustrant le transfert élémentaire entre sol et vin. Les minéraux primaires sont altérés dans les sols, libérant des ions dans la solution. D'autres éléments sont rajoutés par les pratiques viticoles. Les microorganismes et les conditions physico-chimiques contrôlent la spéciation en solution et par conséquent la disponibilité des éléments pour la plante. Les mécanismes d'absorption et de transport régulent le transfert des éléments dans les différents tissus de la plante.

pédologie, la caractérisation des sols se fait par une description en horizons, couches « horizontales » plus ou moins homogènes qui s'individualisent de la roche mère jusqu'à l'atmosphère (Blume et al., 2016 ; Jenny, 1994). Comme la vigne est une plante à enracinement profond ce type de description semble plus adapté à la viticulture que la description des premiers 20 cm communément utilisée en agronomie (Benciolini et al., 2006 ; Branans and Vergnes, 1957 ; Doll, 1954 ; Seguin, 1972). De plus, en sciences du sol, une distinction est faite entre sol et matériel parental ; cette considération est souvent absente dans les discussions autour du vin (Maltman, 2013).

2.4.1 Utilisation des outils géochimiques traditionnels

Les méthodes géochimiques utilisent la spectrométrie de masse en routine pour la détermination des teneurs en éléments majeurs et traces dans différentes matrices. Par cette technique des données sur une multitude d'éléments peuvent être acquises simultanément et peuvent ensuite être traitées par une approche statistique (statistiques multivariées). C'est ce qui a déjà été entrepris pour distinguer la provenance de vins à partir d'un nombre d'origines limités (Coetzee et al., 2014 ; Martin et al., 2012 ; Mercurio et al., 2014). Plus traditionnellement, les rapports entre éléments sont utilisés en géochimie pour le traçage de source. Par exemple le couple Ca/Sr a été utilisé afin de

déterminer les source de Ca dans un écosystème forestier (Blum et al., 2012, 2012; Schmitt et al., 2017; Wiegand et al., 2005). De la même manière, les rapports K/Rb ont prouvé leur utilité pour le traçages de la nutrition par les plantes et les pertes des écosystèmes forestiers (Chaudhuri et al., 2007; Likens et al., 1994; Peltola et al., 2008). Les rapports entre éléments sont également à la base des calculs de transferts de masses lors de l'altération chimique (Brimhall et al., 1988). Ces calculs permettent de déterminer la part d'un élément qui est exporté d'un sol en référence à un élément immobile. Cette technique est aussi utilisée pour calculer les apports d'éléments extérieurs par rapport aux teneurs initiales dans la roche mère (Brimhall et al., 1988). Les rapports isotopiques du Sr constituent un autre type de traceur communément utilisé en géochimie. Les rapports des isotopes radiogéniques du Sr ne sont pratiquement pas fractionnés lors des réactions chimiques de basse température et font ainsi d'excellents traceurs d'origine dans une multitude de situations (Blum et al., 2000; Capo et al., 1998; Horn et al., 1993; Pett-Ridge et al., 2009).

La géochimie nous fournit alors une variété d'outils qui peuvent être facilement utilisés en environnement viticole. Ces outils semblent adaptés pour l'étude des sources des nutriments des plantes et la détermination de l'origine des vins.

2.4.2 Utilisation des isotopes non-traditionnels en milieu agromonique

Les rapports des isotopes stables des éléments légers sont devenus des outils standards dans une multitude de domaines. Les rapports isotopiques peuvent ajouter une information supplémentaire aux données de concentrations si les mécanismes de fractionnement sont connus. Généralement, les rapports isotopiques peuvent être utilisés soit pour tracer les sources d'un élément dans un compartiment, soit pour acquérir des informations sur les mécanismes chimiques mis en œuvre lors du transfert de ces éléments. Ceci est possible parce que les isotopes réagissent différemment en fonction de leur masse pour une grande majorité de réactions. Les rapports isotopiques du Cu sont communément mesurés à l'aide d'un ICP-MS à multi-collection. La mesure est relativement simple et la plupart des équipes qui travaillent sur les isotopes stables du Cu utilisent le protocole proposé par Maréchal et al. (1999). Mais comme les différences observées sont relativement petites (de l'ordre du dixième de pour mille des rapports ^{65}Cu sur ^{63}Cu), la mesure nécessite une grande « pureté » de l'échantillon et une correction du fractionnement induit par la machine par une mesure simultanée du Zn ou du Ni (Chapman et al., 2006; Ehrlich et al., 2004; Maréchal et al., 1999). Cela implique la séparation du Cu des autres éléments de la matrice de l'échantillon, en particulier lorsque le Cu n'est qu'un élément trace dans les matrices environnementales

(Chapman et al., 2006 ; Ehrlich et al., 2004 ; Maréchal et al., 1999). La purification est l'étape critique dans la détermination des rapports isotopiques du Cu et le protocole de purification nécessite une adaptation aux matrices étudiées (Archer and Vance, 2004 ; Babcsanyi, 2015 ; Bigalke, 2010 ; Borrok et al., 2007 ; Larner et al., 2011 ; Liu et al., 2014 ; Maréchal et al., 1999).

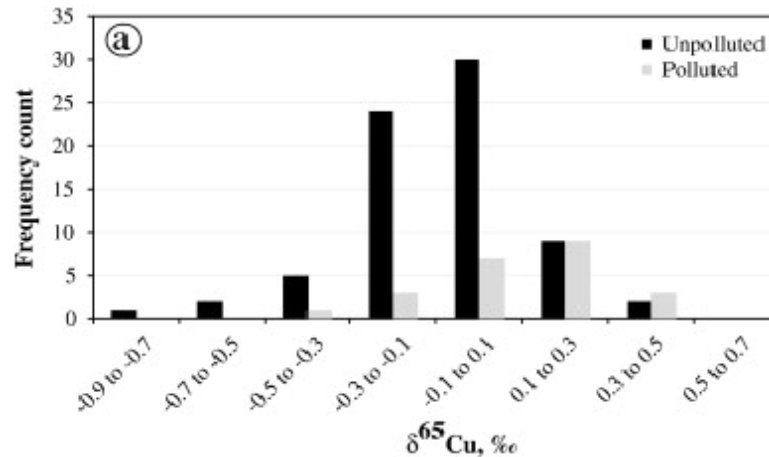


Figure 2.5 – Histogramme des rapports isotopiques mesurés dans des sols agricoles pollués par du Cu (noir) et non-pollués (gris) en France (Fekiacova et al., 2015).

Les premières études des rapports isotopiques dans des échantillons environnementaux ont été conduites par Maréchal et al. (1999). Les premières analyses dédiées aux sols et aux plantes ont été réalisées environ 10 ans plus tard (Bigalke, 2010 ; Bigalke et al., 2010b, 2011 ; Weinstein et al., 2011). Même si les sols contaminés en Cu ont été ciblés dès le début des études utilisant l'isotopie de Cu, il n'est toujours pas établi si les rapports isotopiques mesurés sont contrôlés par la signature des sources du Cu ou par le fractionnement lors des mécanismes de transfert (Babcsányi et al., 2016 ; Bigalke et al., 2010a). Néanmoins des tendances générales vers des rapports isotopiques élevés dans les sols sont visibles dans une étude sur les sols agricoles en France (Fekiacova et al., 2015).

Dans les plantes, la discussion sur les mécanismes entraînant un fractionnement isotopique des isotopes du Cu est encore largement débattue. Il est généralement admis que l'absorption par les plantes enrichit les tissus végétaux en isotopes légers mais différentes observations existent sur le fractionnement du Cu à l'intérieur de la plante, suggérant une dépendance des conditions environnementales (Jouvin et al., 2012 ; Li et al., 2016 ; Ryan et al., 2013 ; Weinstein et al., 2011).

Néanmoins, le fractionnement entraîné par une multitude de réactions en conditions expérimentales a été reporté dans la littérature (absorption par des plantes/microor-

ganismes, complexation par de la matière organique, précipitation sous forme de malachite, réduction et oxydation, adsorption sur des minéraux secondaires) (Balistrieri et al., 2008 ; Bigalke et al., 2010c ; Ehrlich et al., 2004 ; Jouvin et al., 2012 ; Kimball et al., 2009 ; Li et al., 2015 ; Maréchal and Albarède, 2002 ; Marechal and Sheppard, 2002 ; Mathur et al., 2005 ; Navarrete et al., 2011 ; Pokrovsky et al., 2008 ; Ryan et al., 2014, 2013 ; Zhu et al., 2002).

Il s'avère au regard de cela que les rapports isotopiques du Cu sont un outil en développement intéressant pour les problématiques en milieu viticole.

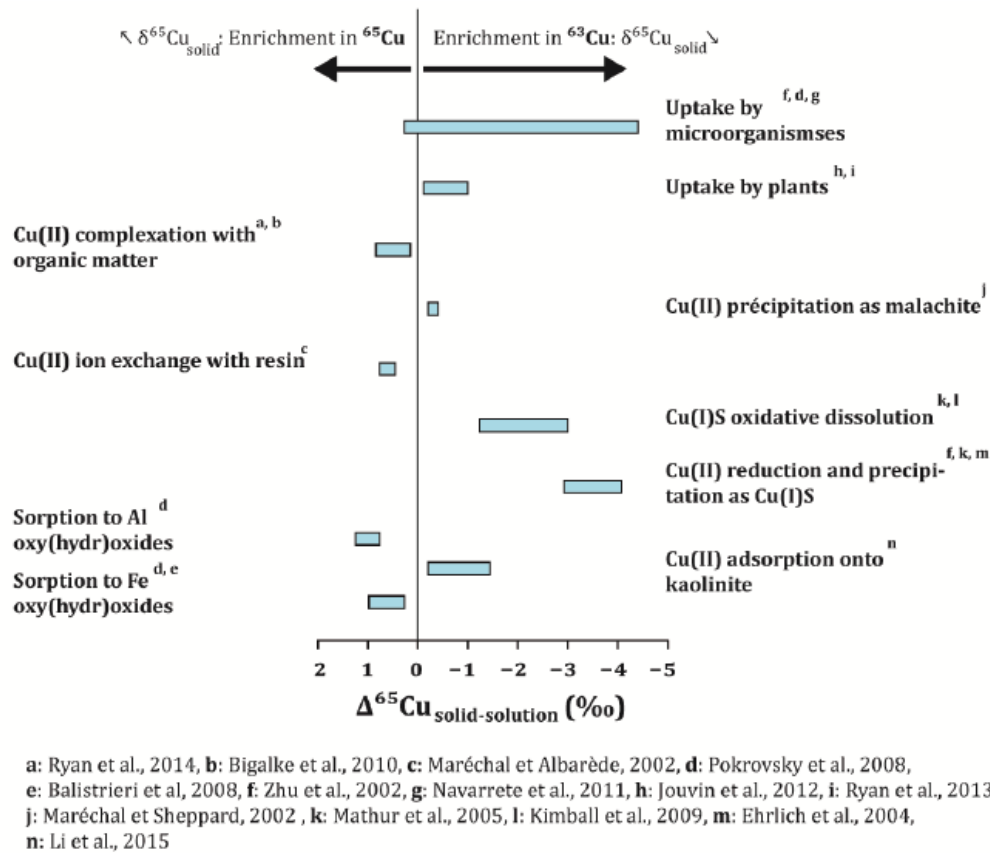


Figure 2.6 – *Fractionnements des isotopes de Cu lors de différentes réactions chimiques reportées dans la littérature. La Figure est tirée de (Babcsanyi, 2015).*

2.5 Objectifs et portée scientifique

Cette thèse vise à contribuer à la question scientifique fondamentale de la mobilité des éléments dans le continuum sol-plante, au travers de l'utilisation d'outils géochimiques en contexte viticole. L'hypothèse ayant initiée ce travail est que les mécanismes de pédogénèse (altération minérale et formation des phases secondaires, dégradation et incorporation de la matière organique) et les facteurs physico-chimiques et biologiques

(facteurs pédogénétiques, contaminations, . . .) dans les sols, entraînent des différences dans le fonctionnement biochimique et physiologique des plantes. En contexte viticole, cette hypothèse revient à questionner le rôle du compartiment sol dans « l'effet terroir ». Les sols viticoles, comme tous les sols cultivés, ne sont pas simplement soumis aux facteurs naturels mais aussi à l'activité humaine via l'apport d'intrants (pesticides, engrais, . . .). Les apports de pesticides cupriques étant particulièrement importants en viticulture, il est impossible de ne pas les considérer dans les travaux sur les signatures élémentaires dans le continuum sol-vigne.

Pour aborder ces aspects, nous avons choisi de combiner une approche géochimique classique (rapports élémentaires, bilans de masses) à l'utilisation de marqueurs biochimiques et au développement de l'outil « isotopes stables du Cu », dans le but d'améliorer les connaissances relatives aux mécanismes impliqués dans les transferts élémentaires au sein du continuum sol-plante en milieu viticole. Le travail méthodologique d'amélioration des protocoles de séparation des isotopes du cuivre qui a été nécessaire pour mener à bien cette étude est développé en annexe C dans le manuscrit.

Le **chapitre 3** après cette introduction traite de la composante sol de « l'effet terroir » au travers d'un résumé historique et bibliographique destiné à mettre en perspective les aspects scientifiques et la perception commune de la question de l'influence du sol sur la vigne et le vin.

Le **quatrième chapitre** examine, via un travail mené sur plus de 200 vins provenant de toute l'europe de l'ouest, les conditions environnementales (nature du sol et facteurs climatiques) permettant la ségrégation des vins, à partir de leur profil élémentaire. *Le rôle de la composante « sol » sur la signature élémentaire des vins est-elle une réalité statistique ? Si oui existent-il des traceurs géochimiques permettant de relier sols et vins ?*

Le **cinquième chapitre** présente une étude pédo-bio-géochimique comparative de deux types de sol viticoles (sols calcaires versus sols vertiques) issus de deux parcelles juxtaposées à Soave en Italie. Cette étude recentrée à l'échelle de la toposéquence de sols permet d'investiguer le rôle des processus pédogénétiques ainsi que des paramètres bio-géochimiques associés sur la signature biochimique et chimique des plantes. *Quels paramètres descripteurs des sols sont pertinents dans le cadre de l'étude des transferts élémentaires sol/plante ? Des propriétés des différents sols induisent t-elles des différences sur la composition de la plante ?*

Le **sixième chapitre** se focalise sur le cuivre dans les deux types de sols du chapitre précédent (toposéquences de sols à Soave, Italie). Il s'agit de quantifier et comprendre les mécanismes de rétention/transfert du Cu dans ces sols afin d'évaluer les probables implications des apports cupriques sur le continuum sol-plante. Pour cela des calculs de bilans élémentaires associés à l'outil isotopique et à l'utilisation de la RPE ont été mis en oeuvre. *Les apports anthropiques de cuivre doivent-ils être considérés dans l'étude des transferts élémentaires dans le sol et entre le sol et la plante en milieu viticole ? Les signatures isotopiques du Cu dans les sols reflètent-elles la signature des pesticides ? Reflètent-elles des mécanismes physico-chimiques se déroulant dans les sols ?*

Enfin, le **septième chapitre** s'intéresse au transfert du Cu et des nutriments entre différents types de sol et les plantes dans le cadre d'une expérimentation en conditions contrôlées, sous serre. Les compositions chimiques et les rapports isotopiques du Cu des sols, solutions de sol et plantes sont étudiées ainsi que les traceurs biochimiques de stress métalliques. *Les isotopes du Cu permettent-ils de caractériser les mécanismes de transferts du Cu dans le continuum sol – solution du sol – plante ? Dans quelle(s) mesure(s) la présence de cuivre dans les sols viticoles influence-t-elle la nutrition/santé de la plante ? Sa composition chimique et isotopique en Cu ?*

Grâce à la mise en œuvre de différentes approches (statistique, terrain, mésocosme), menées à différentes échelles et combinant de multiples techniques, cette thèse souhaite in fine répondre à deux problématiques que sont la pertinence de l'approche géochimique dans la compréhension de « l'effet terroir » en viticulture, mais aussi celle de l'utilisation des isotopes du cuivre pour comprendre les mécanismes impliqués dans les transferts élémentaires sol-plantes du cuivre.

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Chapter 3

From soil to wine: A historical perspective

3.1 The concept of terroir in viticulture

The concept of terroir is of uttermost importance in viticulture ; It relates environmental factors such as climate, geology, soil type, cultivar and regional production techniques to wine quality (Van Leeuwen and Seguin, 2006). Hence, it is frequently used to justify differences in wine quality and thus price. Terroir and references to the wine growing environment are often used in advertising and in Europe barely any bottle of wine is sold without some reference to environmental conditions the plants were grown in. Even though the influence of climate, biologic material and regional winemaking techniques on wine quality are widely accepted and scientifically demonstrated, there is an ongoing discussion about the effect of soil and geology (Bramley et al., 2011; Van Leeuwen et al., 2009; Gonzalez-Barreiro et al., 2015; Maltman, 2008, 2013; Styger et al., 2011). In scientific literature, influence of geology has often been reduced to its role in geomorphology and thus microclimate of winegrowing (Bramley et al., 2011; Maltman, 2008). Similarly soils have been shown to influence wine quality through their role in water availability to vines (van Leeuwen et al., 2009). Nevertheless since 2000 references to bedrock type and "minerality" of wines are increasing in popular wine literature (Maltman, 2013). A mineral taste of wine is not clearly defined and may refer to anything from a perception of high mineral content to a taste of rock as in a "chalky taste" or "smell of granite" (Maltman, 2013). Even though this might be an exaggeration of a metaphorical taste descriptor, it has been shown that soil pH influences important winemaking parameters (Bates et al., 2002; Maltman, 2013). Furthermore mineral nutrition of plants depends largely on soil biogeochemistry as it regulates the bioavailability of essential nutrients (Marschner and Marschner, 2012) so that there is a possibility for the influence of soil-biogeochemistry on wine quality. In the following paragraph we will look at the historic evolution of that idea.

3.2 Emergence of the concept of mineral nutrition of plants and the beginning of wine classification

The quality of soil has been a sales argument for wines for a long time. Visiting the chateau Haut Brion in 1677, the philosopher John Locke is quoted as saying the following:

Vine de Pontac, so much esteemed in England, grown on a rising open to the West, in a white sand mixed with little gravel, which one would think bear nothing; but there is so much particularity in the soil, that at Mr. Pontac's near Bordeaux the merchants assured me that the wine growing in the very next vineyards, where there was only a ditch between, and the soil, to appearance, perfectly the same, was by no means so good.

John Locke in Van Leeuwen and Seguin, 2006

It is impressive that the difference in quality is assigned to the soil, yet there is no apparent difference with the adjacent wine growing plots. What might have been a sales gimmick at the time, reflects the idea that there must be something in the soil that makes plant live but it was not quite clear what it was. Different concepts have been proposed emphasizing the importance of various constituents:

Clays are abundant in soil most appropriate to plant growth. Calcareous earth, sand, gravel and riversides and are only there to reduce the compacity of clayey soils, absorb water and make it possible to root. But clays are the only soil constituent that enters the plant.

Baumé, 1770

However up to the mid 19th century the humus – theory was the most widely accepted explanation of plant growth (Russel, 1952). It was believed that plants live on humus derived extracts containing water soluble C, H, O and N (van der Ploeg et al., 1999). Based on this they built their tissues and were thought able by an intrinsic vital force (*vis vitalis*) to generate other constituents such as Si and K (Russel, 1952).

The idea of a mineral nutrition taken up by plants from the soil, that in turn is necessary for plant growth was introduced when de Saussure (1804) published works on gas exchange by plants and the absorption of salts and N by roots. The next step towards modern understanding of plant nutrition were the works of Sprengel (1826, 1828). He stated that salts are not always equally abundant in soil and that fertilization with salts less abundant will increase crop yields whereas salts already sufficiently present will not increase plant growth (the so called "principal of the minimum"). He considers some 20 elements beneficial for the plans including N, P, K, S, Mg and Ca. Both authors received relatively few attention for their works in the beginning.

Still in 1838, Boussignault states that analysis of arable soil did not provide any evidence towards a marked influence of their [chemistry] on plant growth (cited in Grandeau, 1889). Mineral nutrition of plants and the "principal of the minimum" only got recognized after the publication of Liebig's "Organic Chemistry in its Applications to Agriculture and Physiology" in 1840 (Liebig, 1840). Its popularity was probably due to Liebig's international name as a chemist (his book got published in more than 20 editions by 1848 in Germany, England, France, the USA, Italy, Denmark, Holland, Poland and Russia) as well as timing in the mid-19th century when depleted soils were a big issue both in Europe and the United States (van der Ploeg et al. 1999).

In 1855, Bordeaux wines were classified for the world exposition in Paris. Classification criteria were based on sales price as an indicator of wine quality; however environmental parameters did not play a role for this classification (Markham, 1998; Van Leeuwen and Seguin, 2006). Meanwhile, Victor Rendu stated in his "ampélographie française" that grapevine can adapt to any terrain given that water does not stagnate. He went on to state that in France there are many renowned wines growing on very different soil e.g. bedrocks: chalk, schists, granites, limestone and sand (Rendu, 1854). However he questioned the influence of soil type on the wine quality and asked if there is a best soil for quality wine making (Rendu, 1854). To our knowledge this was the first time that winegrowing and geology were systematically matched.

3.3 The great crisis

Before these thoughts were further explored, different challenges were waiting for European viticulture. In 1845, odium was for the first time observed in France and in 1863 phylloxera (Dion, 2011; Galet, 1977). Phylloxera is not strictly speaking a disease but a parasitic insect. Even though an efficient pesticide was found for odium (sulfur sprays), phylloxera was harder to control (Viala and Ferrouillat, 1887). In contrast to former diseases, phylloxera not only affected the annual yield but destroyed the plant, and led to the replantation of European wine regions with European cultivars grafted on American rootstocks (Dion, 2011). This replantation completely reshaped the geography of wine growing in Europe.

By 1878 downy mildew was introduced to Europe probably with phylloxera resistant root stocks from America (Viennot-Bourgin and Heim, 1949). Mildew was not as easy to fight as odium either and different treatments as liming and sulfur applications were explored (Viala and Ferrouillat, 1887). Lime treatments, mainly in use in Italy, were thought to need to cover all green parts of the plants and needed to be washed of at har-

vest by sulfuric or tannic acid (Viala and Ferrouillat, 1887). Sulfur treatments involved letting native sulfur oxidize on the soil underneath the plants to have a constant SO_2 flow around the leaves, or burning sulfur sticks underneath the wine plant. Also foliar application Zn and Fe sulfates were experimented but their effect was largely inferior to Cu salts. By this time Cu was already known to have fungicidal properties. Prevost discovered in 1807 that Cu ions would inhibit sporulation of fungi and Welsh journalists had reported that fields close to Cu smelters were not hit by potato blight (Viala and Ferrouillat, 1887). So that means of treatment were at hand when it became clear that mildew could be treated with Cu, however it got its emblematic name as “Bouillie Bordelaise” through the marketing of Millardet (Viala and Ferrouillat, 1887).

The fact that Cu would not kill mildew but only inhibit sporulation meant that treatment was preventive and needed to be applied before the infection of the plants (Viala and Ferrouillat, 1887). As symptoms of downy mildew develop only several days after inoculation, caution needs to be taken to choose the first date of treatment. Aim of the treatment was to keep a constant film of Cu on the leaves so that every water droplet would contain enough Cu to inhibit mildew sporulation, implying retreatment after heavy rain and a well-adjusted solubility of the treatment to be partly dissolved in water but not instantly washed of (Viala and Ferrouillat, 1887). These properties were judged to best be met by “bouillie bordelaise” so that it became the common place treatment in vineyards. Viala and Ferrouillat recommended a preparation of 8 kg $\text{CuSO}_4 \cdot 5(\text{H}_2\text{O})$ in 100 L of water mixed with 15 kg of lime suspended in 30 L of water. This preparation translates in a solution containing around 22 g L^{-1} of Cu. They further suggest a treatment five to six times per year (also after harvest) with 150 to 500 L ha^{-1} (less in the first treatment in spring). This gives 3.3 to 11 kg of copper per hectare and treatment. And hence a Cu input between 16.5 and 66 $\text{kg}_{\text{Cu}} \text{ha}^{-1} \text{a}^{-1}$ (Viala and Ferrouillat, 1887). Leading to the high Cu contents observed in vineyard soils until today (Chaignon et al., 2003; Mirlean et al., 2007). In the 1920s and 30s, the “Bordeaux mixture” was replaced by less phytotoxic basic Cu-sulfate, cuprous oxide, copper oxychloride and later on organic fungicides (Richardson, 2000). However Cu pesticides are still in use today as they are less prone to provoke resistance in germs and are up to the date the only permitted pesticides to fight downy mildew in organic viticulture (RCE No. 889/2008; Richardson, 2000).

3.4 French viticulture after the great diseases

The moment when the great diseases came technically under control fell together with other major technical advances. On the one hand railways were installed and wine could now easily be transported from one region to another (Pomerol, 1984; Van Leeuwen

and Seguin, 2006). This was especially new for the inland vineyards and largely increased competition between wine regions. If formerly every region produced its own wines now wine quality came into focus and many small vineyards producing low quality wines were not replanted (Pomerol, 1984). Also grapes from low quality and high yield wine regions could now be transported and blended with wines from higher quality regions (Barnea, 2017).

On the other hand, fertilization was now better understood so that Grandeau (1889) promised a great future to the educated farmer suggesting the possibility to overcome shortcomings of their soils. In the same year Vermorel and Michaut (1889) published a textbook on grapevine fertilization. They stated that grapevine can be found on very different soil types in France and rarely any other plant adapts as well to different environmental conditions. Nevertheless they reported soil analysis on the great French vineyards, Cote d'Or, Chablis, Bordeaux, Midi, recognizing that for example in the Cote d'Or when soil type changes due to superposition of geological layers a different wine is produced. Furthermore stated that the best wines are produced on carbonated soils and on the opposite side wine growing on granitic bedrock rarely produced distinguished wine. They remark as well that poor soils gave good wine in general, which is why many wine experts especially in Italy and Spain were opposed to fertilization. They suggested carrying out more field trials for several years in order to fix the right treatment for a specific plot. They insisted that even though nutrient exportation through wine is low, these nutrients need to be replaced in the soil. Finally they gave general rules for fertilization of vineyards on different soil types. They remark that even though there have been problems with pests throughout the century wine remains the best paying crop of their time.

In this context of increased competition, emerging fraud with wine denominations and scientific advances, various social movements emerged to call for protection of their regional wine production. The forms of demands and protests were different, however the French state intervened in the beginning of the 20th century with laws protecting the appellation of wines. The delimitation of zones allowed to carry a certain appellation was not always evident and largely disputed (and changed) throughout the 20th century (Jacquet, 2010; Vincent and Jacquet, 2012). In these disputes, geology was introduced to defend claims of delimitations (Vincent and Jacquet, 2012). However, if the geological borders did not match social demands they could be rather flexibly interpreted or deleted as a whole (Vincent and Jacquet, 2012). Nevertheless, geology and soil type became important criteria for vineyard zonation by the INAO. For soils, the fertility plays a role as well as the calcareous or silicious character of the soil (Kuhnholz-Lordat, 1963). The common place practice of limitation that was adopted is to identify the best

wines of one region ‘noyau d’élite’ and attribute the appellation to adjacent terrains having similar environmental properties (Kuhnholz-Lordat, 1963).

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Chapter 4

The influence of soil chemistry and meteorological conditions on the elemental profiles of West European wines:

A new tool for the identification of geological wine provenience

4.1 Abstract

Elemental profiles of wines have successfully been used to distinguish their geographical provenience around the world. Yet the causes of differences of elemental signatures between wines remain unclear.

In this study, contents of Ba, Ca, Mg, Mn and Sr were determined in 215 wines from several West European winegrowing areas. The methodology consisted of an easy-to-perform analysis by ICP-OES. Major environmental and winemaking parameters (soil type, rainfall, temperature and wine color) were used to explain variations within the dataset.

The combined effect of winemaking process and soil type explained 28.5 % of total variance. Results show that carbonate occurrence in soils clearly influence wine elemental composition. The effect of climatic conditions explained 24.1 % of variance and could be interpreted as intensity of drought stress. These findings provide insights to mechanisms underlying elemental fingerprinting and make it possible to predict which wine growing regions are easily distinguishable.

Keywords: *Soil; Wine; Terroir; Origin tracing; Elemental analysis, Environmental parameters*

4.2 Introduction

The influence of soil on wine taste is controversially discussed even in geologic literature (Maltman, 2013). The essence of this discussion is the French notion of ‘terroir’ that can be described as an interactive ecosystem, in a given place, including topography, climate, soil, and vine characteristics as rootstock and cultivar (Froni et al., 2017). Human factors such as viticulture techniques and landscape environment can also be included, (van Leeuwen et al., 2004) making each wine as a unique combination of all these parameters (Frost et al., 2015). Even though the effect of climate, topography, biological material and production techniques are well studied the influence of soil chemistry remains unclear (Bramley et al., 2011; C. van Leeuwen et al., 2009; Gonzalez-Barreiro et al., 2015; Styger et al., 2011; van Leeuwen et al., 2004). Mackenzie and Christy (2005) reported that a relationship could be established between geological bedrock, soil chemistry and the related wine products (Mackenzie and Christy, 2005). Recently, due to fraud of wine products over the world, interest in tracing wine origin has increased, as quality and authenticity issues often depend on local geographical factors (soil properties, available water amount and composition, vine variety or environmental conditions), as highlighted by Rodrigues et al. (2011).

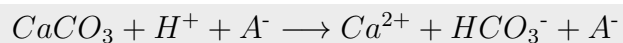
In fact, elemental compositions of wines have successfully been used to determine their geographical origin, as reported by more than twenty studies in scientific literature around the world: three in South Africa (Coetzee et al., 2014, 2005; van der Linde et al., 2010), three in north America (Greenough et al., 2005; Taylor et al., 2003, 2002), three in south America (Bentlin et al., 2011; Di Paola-Naranjo et al., 2011; Fabani et al., 2010), two in Australia and New Zealand (Angus et al., 2006; Martin et al., 2012), four in eastern Europe (Kaunova et al., 2013; Kment et al., 2005; Koreňovská and Suhaj, 2005; Šelih et al., 2014) and also four in the classical wine growing countries of the old world (Almeida and Vasconcelos, 2003; Galgano et al., 2008; J. Baxter et al., 1997; Jurado et al., 2012). One study compared wines from Europe and North America (Kwan et al., 1979). All cited studies cover a small number of wine growing regions and assign wines to these areas using their elemental contents on an empirical basis. To date, there is no study of elemental contents on a global scale, nor is there a unique combination of elements allowing regional classification of wines. Still some elements are recurrent in those 20 studies (excluding Kment et al. 2005 and Almeida et al. 2003 for not reducing element set and using just one of the Taylor publications as they use essentially the same elements). Rubidium (Rb) is used in 14/20 studies, strontium (Sr) in 12/20 studies, manganese (Mn) in 11/20 studies, barium (Ba) in 10/20 studies, followed by cobalt (Co) and magnesium (Mg) used in 6/20 cases. Multiple elements start to be used an equal number of times. Three of those most used elements are alkaline

earth elements (Ba, Mg and Sr). For two of the elements (Ba and Mn) Tyler and Olson (2001) reported that their content in plant tissue was highly dependent on the addition of lime and thus variation of soil pH.

The differences encountered in elemental content analysis between vineyard regions are usually assigned to differences in geology, pedology or climatic conditions but most of the studies do not include them in data analysis. Only Greenough et al. (2005), links trace element composition to degree days, whereas others (Galgano et al., 2008; Taylor et al., 2002) state that there was no effect of vintage. Three studies give information on soil type or geology of the covered regions. (Coetzee et al., 2005; Taylor et al., 2002; van der Linde et al., 2010) Few studies tried to investigate the link between soil and wine composition on an interregional scale. All of them empirically link total or extracted element contents of soil to wine composition (Almeida and Vasconcelos, 2003; Day et al., 1995; Geana et al., 2013; Jurado et al., 2012; Kment et al., 2005; Taylor et al., 2003; van der Linde et al., 2010). However, to our knowledge, no study investigating the influence of macroscopic soil conditions widely used in agronomy (such as lime content or soil pH) has been reported, even though these parameters are known to influence major wine growing properties such as photosynthetic activity, leaf surface, yield and root-to-shoot biomass ratios (Bates et al., 2002; Bavaresco and Poni, 2003).

Grapevine, as all higher plants needs to take up at least 16 essential nutrients from the soil solution (Mengel et al., 2001) using active or passive pathways (White, 2012). For example, plants have developed different mechanisms to either enhance or block absorption due to lack or surplus of elements (George et al., 2012; Hinsinger, 2001). Thus most mineral nutrients are present in a fixed range in healthy plant tissue which can be different from soil mobility, due to a process called homeostasis (Mengel et al., 2001). Also non-nutritive elements can be present in plants, depending on environmental factors. For example the presence of Sr is directly linked to the uptake of Ca, a necessary nutrient (Blum et al., 2000, 2012).

In soil sciences, it is widely accepted that total element content in soil is a poor predictor of their phytoavailability (Kabata-Pendias, 2004). However the bioavailability of elements largely depends on their speciation in the soil solution and solubility of soil minerals, both depending on physico-chemical soil properties such as pH and redox potential (George et al., 2012; Hinsinger, 2001; Sauvé et al., 2000). The presence of Ca- and Mg-carbonates is an important factor as they are highly soluble. In fact, dissolution of these carbonates consumes protons from the soil solution resulting in a pH_c7 in carbonated soils (van Breemen et al., 1983). The equation beneath shows the reaction of calcite dissolution for a generic acid A.



Furthermore, soil solutions of soils rich in carbonate phases are characterized by their high Ca^{2+} content. The Ca^{2+} cations contribute to the flocculation of clay minerals and thus to soil structuration (Haynes and Naidu, 1998). Liming experiments showed that Mg and Ca contents in biomass increased with rising pH, whereas contents of Ba, Mn and Sr declined and the effect on Rb was less pronounced (Tyler and Olsson, 2001).

The presence of water as a transport vector and weathering agent is crucial on the earth surface dissolving ions necessary for plant nutrition (Gislason et al., 1996; Oliva et al., 2003; Tipper et al., 2006). Besides, sufficient water supply is essential to plant functioning. It has also been reported that climatic factors play a crucial role in element availability to plants including nutrients such as Ca, Fe, Mg, Mo and S (Sardans et al., 2012, 2008). Available water, temperature and sunshine condition transpiration rate and thus soil-solution throughput of plants (Collatz et al., 1991; Sack and Holbrook, 2006). An accumulation of non-nutritive elements in plants with high transpiration rates (Couder et al., 2015; Greenough et al., 2005) has been observed, allowing a climatic effect on elemental concentration of plants. Other than from grapes, the elemental content of wines can be also influenced by the winemaking process (Hopfer et al., 2015).

Even though physiological and pedological processes are complex, region specific elemental patterns can be found in wine, especially by using elements often used in above mentioned fingerprinting schemes that are ubiquitous in soils and of relative high mobility. Magnesium (Mg) is contained in dolomite or ferromagnesian minerals which are commonly encountered in volcanic rocks (Rothwell, 1989). In the plant, Mg is phloem mobile and is involved in photosynthesis process (Hawkesford et al., 2012; Rogers et al., 2006). Calcium (Ca) is a major constituent of limestone. In the plant, Ca is transported through the xylem fascicles (Rogers et al., 2006; White, 2003, 2001) and is important for cell wall stability (Hawkesford et al., 2012; P. J. White, 2003). The chemical behaviors of Ba and Sr are largely similar to that of Ca in the environment, and have formerly been used to trace Ca sources in forest ecosystems (Blum et al., 2000, 2012; Poszwa et al., 2000). The ionic radius increases from Ca over Sr to Ba. The greater difference in ionic radius between Ca and Ba than between Ca and Sr leads to a higher Ca/Ba fractionation in plant uptake (J. Blum et al., 2000), but more importantly to the relative absence of Ba in carbonates and making those possible tracers of carbonate contribution to plant nutrition. An estimate using data from Horn and Adams (1966) show that Ba/Ca ratios in silicate rocks are 5 to 100 fold larger than in carbonates. Finally, Mn is an essential nutriment for redox processes and a common

cofactor for enzymes in the plant. Its phytoavailability highly depends on pH (Burnell, 1988; George et al., 2012; Tyler and Olsson, 2001). It occurs in soils mainly in form of oxides (George et al., 2012), highly soluble at low pH and low redox potential, but is on the other hand not a major constituent of material in wine processing material as is Fe (Kabata-Pendias, 2004).

Moreover, the nutritive elements in grapevines are located in different tissues. Thus differences in elemental composition of wines can be induced by differences in production techniques for example between red and white wine. In white wines, juices are fermented without contact to the pressing residues of grapes, whereas red wines are macerated with grape residues and fermented in their presence, allowing a potentially greater extraction time of elements from the grape (Ribéreau-Gayon et al., 2012). For rosé and vin gris wines, maceration is either short or absent and fermentation takes place without contact with grape residues (Ribéreau-Gayon et al., 2012). Another major difference between red wines and white, rosé and gris wines is the malolactic fermentation step conducted in virtually all red wines but few white, rosé and gris wines (Ribéreau-Gayon et al., 2012). Thus the wine color, an indicator of the winemaking process, needs to be tested for its influence on elemental composition.

This study aims to contribute to the understanding of mechanisms by which environmental factors influence the elemental compositions of wines. Contents of the five chosen elements (Ba, Ca, Mg, Mn, and Sr) were analyzed in 215 wines from France and adjacent winegrowing regions in Western Europe (Germany, Italy, Spain) by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES). Multivariate statistical methods were used to explore the contribution of various parameters (wine color as a marker of winemaking process, ‘calcareous’ or ‘non-calcareous’ soil conditions, and meteorological conditions) to the elemental composition of wines. Even if the effect of other parameters during the winemaking processes is expected to introduce some scatter in the present study, it should not affect overall results as the same soil and meteorological conditions from several regions and countries were examined conjointly. Thus the novelty of this work comes also from the produced dataset: it is essentially different from former studies using a high number of wines from only a small number of regions. The multiplicity of wine provenience in our study allows analyzing global effects on wine elemental composition in order to provide a rapid, innovative and relevant tool to discriminate wines, at the global scale, depending on soil chemistry and meteorological conditions.

4.3 Materials and Methods

4.3.1 Wines collection and storage

We collected 215 wine samples through private consumption and research stocks at the Geosciences Environment Toulouse Laboratory and from the French Agronomic Institute INRA (Institut National de la Recherche Agronomique) in Gruissan and Bordeaux. The collected wines contained 128 red wines, 77 white wines, 11 rosé wines and 2 vin gris (wines.txt, Supplementary Information).

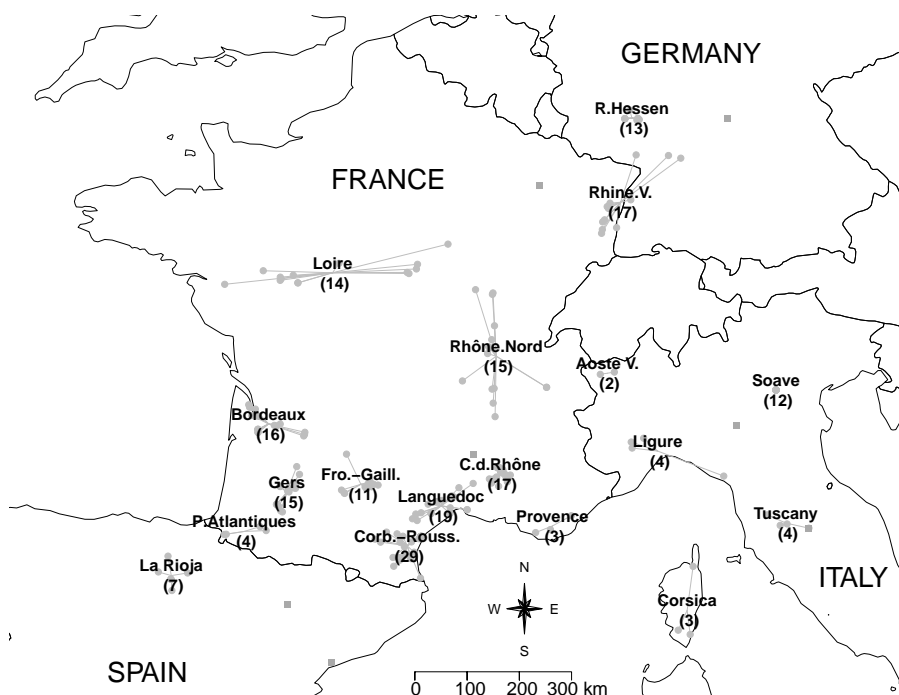


Figure 4.1 – Map of the geographical origin of wines measured and their attribution to major winegrowing areas. Regions with only one wine sample are marked by a square, otherwise region names and number of wines are given and grey dots show provenience of wine samples. Abbreviations stand for Aoste.V – Aoste Valley, C.d.Rhône - Côtes du Rhône, Corb.-Rouss. – Corbières et Roussillon, Fro.-Gaill. – Fronton and Gaillac, P.Atlantiques – Pyrénées Atlantiques, R.Hessen for Rheinhessen and Rhine.V – Rhine Valley.

Small samples were stocked in 30 mL PP vials until ICP-OES analysis, no longer than 3 months. With different measurement sessions, occurring in several dates throughout these 3 months, we confirmed that ageing, oxidation of alcohol and deposition did not influence the measured element contents more than analytic uncertainty. Average (\pm SD) recovery of same wine samples measured in a new dilution after 3 month of

storage were Ba 94.5 ± 5.5 %, Ca 98.5 ± 4.2 %, Mg 95.6 ± 4.0 %, Mn 102.9 ± 5.7 %, Sr 103.2 ± 9.6 %. Wine provenience and the number of samples for each wine-growing area were reported in the Figure 4.1. Wines were finally classified in a dataset according to different parameters (Appendix A - Wine Data).

4.3.2 Soil type determination

Information on soil lime content was directly determined on the field for samples from within the working group ($n=23$). For others, soil data was derived from pedologic maps of the wine region ($n=33$). Main sources of soil maps were the French INRA series (Arnal, 1984; Bégon, 2013; Bonfils, 1993; Bonneau, 1978; Bornand et al., 1977; Bouteyre and Duclos, 1994; Chrétien, 1975; J Chrétien, 1996; Jean Chrétien, 1996; Séguy, 1975; Servant, 1970; Wilbert et al., 2013) and the geological surveys of German Länder (Landesamt für Geologie und Bergbau Rheinland-Pfalz, 2017; Regierungspräsidium Freiburg, Landesamt für Geologie, Rohstoffe und Bergbau, 2017). For these two sources of soil information, wines were assigned to a ‘calcareous’ soil when there was effervescence in the organo-mineral horizon, a lime content given or when soil pH was higher than 7. If these conditions were not met, wines were assigned to the ‘non-calcareous’ soil group.

Otherwise product description of winegrowers was used ($n=96$). The presence of lime was accepted if indications were stated on the bottle, appellation description or promotion web site, such as ‘chalky-clay soil’, ‘limestone’, or similar. If soil or bedrock was described as ‘non-calcareous’, ‘gneissic’, ‘granitic’ or ‘schistous’, wine samples were assigned to the ‘non-calcareous’ group. One exception was the Maury area where some wine growers explicitly stated carbonate-silicate schists as the bedrock and thus soils were assigned to the calcareous soil group. Soil data which was derived from vineyard description (serving mainly for marketing purposes) is expected to contain errors. The final dataset thus contains 51 wines grown on non-calcareous soils and 101 wines on calcareous soils, leaving some wines ($n=63$) without soil information.

4.3.3 Climatic conditions

The coordinates of the actual wine growing spot was either known ($n=33$), or found from the address of the winery ($n=53$) or of the village of provenience ($n=46$). Weather data for Germany was taken from publicly available stations of the “Deutscher Wetterdienst” (DWD) and the weather survey of the Geisenheim University. The majority of French weather data was supplied by the AgroClim Unit in Avignon that manages the

agroclimatic network of INRA. Further French weather data came from data published by the NOAA. Spanish weather data came from the AMET. All the above-mentioned data is available on the websites of the respective organisms. The only Italian weather data used was measured by our working group in the Soave region. The closest available weather station with data available for the year of production was chosen. If the closet available weather station farther than 50 km away no weather data was assigned. Monthly rainfall and average temperature were recorded from Mars to September for the year of harvest for 132 wines.

4.3.4 Chemical content analysis

The first objective was to develop a rapid, efficient and easy to perform method to determine the contents of Ba, Ca, Mg, Mn, and Sr in various wines without any previous preparation step (except dilution) of samples or mineralization process. We aim to analyze wines directly by ICP-OES.

Table 4.1 – *Details of ICP-OES settings and dilutions used for the measurement of Ca, Mg, Mn Sr and Ba in red, rosé and white wine samples.*

Parameter	Settings	
	For Ca, Mg, Mn, Sr	For Ba
Dilution	x 10	x 3.5
Operating Power	1200 W	1200 W
Nebulization Pressure	1.98 bar	1.98 bar
Nebulizer Flow	0.82 L min ⁻¹	0.70 L min ⁻¹
Nebulizer Type	PEEK Mira Mist	PEEK Mira Mist
Rinsing Time	60 s	60 s
Stabilisation Time	20 s	20 s
Integration Time	4 s	4 s
Wavelength	Ba 455.403 nm, Ca 317.933 nm, Mg 279.079 nm, Mn 257.610 nm, Sr 407.771 nm	

In order to calibrate the direct ICP-OES measurements, a first calibration experiment was performed. Ten wine samples (red and white) were mineralized in three steps. First, 1 mL of suprapure H₂O₂ (30 % v/v) was added to wine samples (10 mL) and left to react 2 h to avoid explosion through ethanol-HNO₃ reaction (when HNO₃ is added just after). Subsequently, 5 mL of double sub-boiled HNO₃ solution was slowly added

and the mixture was heated to 120 ° C in a closed (Savillex) digestion vessel for at least 4 h. Then samples were evaporated to dryness at 90 °C. The second digestion step used 4 mL of double sub-boiled HCl (10 mol L⁻¹), 2 mL double sub-boiled HNO₃ (14.5 mol L⁻¹) and 1 mL ultrapure HF (22.4 mol L⁻¹ from Merck). These acids were added to the digestion vessel and heated to 120 °C for at least 4 h. Then the samples were again evaporated to dryness and a last digestion step using 5 mL double sub-boiled HNO₃ was performed at 120°C for at least 4 h. After evaporation to dryness, samples were dissolved in 20 mL HNO₃ 20% (v/v), diluted ten times and spiked with in house In/Re standard. Measurements were performed on an Agilent 7500ce Q-ICP-MS. During measurements, SLRS-5 standards were run to assure result quality. Recovery was within a $\pm 10\%$ range of certified values for the five considered elements.

After this first test, wine elemental contents were then determined by ICP-OES, using ICP-MS measurements to calibrate the direct measurement method. The 10 wines previously studied by ICP-MS analysis were run as standards in ICP-OES batches to assure measurement quality. A $\pm 10\%$ deviation from ICP-MS measurements for each element was accepted. For ICP-OES measurements, wines were diluted in ultrapure water (18.2 M Ω): ten times for Ca, Mg, Mn and Sr measurements and 3.5 times for Ba analysis. Analyses were carried out on an Ultima Expert by Horiba. Standards were prepared in ultrapure water containing 1.2 % (v/v) and 4 % (v/v) Et-OH. One drop (about 30 mg) of double subboiled HNO₃ was added to 100 mL of Ba standards to assure solution stability. Machine settings are denoted in Table 4.1. Different methods have already been published for analyzing elemental contents in wine by ICP-OES but there still seem to be no out-of-the-box solution as parameters need to be adapted to the material used as red wines in particular have a strong impact on plasma stability.

4.3.5 Statistical treatments and data interpretations

Data analysis was carried out using R software version 3.2.5 (R Core Team, 2016). Elemental contents in wines were log-transformed prior to statistical analyses. The script used to perform data analysis is provided in Appendix B. Linear Discriminant Analysis (LDA) was used to explore the relationship between elemental contents in wines with wine color and soil type (calcareous/non-calcareous). Three LDAs were performed, a first one using wine color only, a second one using soil type only, and a third one using both factors. These LDAs were performed using the portion of the data containing both soil type and meteorological data (n=91), in view of the analyses which are presented in the next paragraph. The classification ability of the 3 LDAs was evaluated by cross-validating using remaining available samples (n=124 for the first LDA and n=61 for the second and third LDA since 63 wines were left without soil type data).

Finally, the relative contribution of the various predictors (wine color, soil type, and meteorological variables) to the variability of elemental contents in wines as well as the relation between meteorological variables and elemental content were measured by conducting a Redundancy Discriminant Analysis (RDA). RDA is the extension of multiple linear regression to multiple explanatory variables, in our case elemental contents in wines (5 variables) were expressed as a function of wine color (0 for red; 1 otherwise), soil type (0 for non-calcareous; 1 for calcareous), and meteorological variables (14 variables). We conducted a partial RDA in order to measure the effect of meteorological variables given that the effect of wine color and soil type were already accounted for. The remaining covariance structure of elemental contents in wines was investigated by conducting a Principal Component Analysis (PCA) on RDA residuals.

4.4 Results

4.4.1 Effect of wine color on elemental composition

In a first approach, red wines appear to have higher Mg concentrations than white wines, rosé wines and vin gris. In LDA analysis using all five elements rosé and vin gris wines are not separated from white wines. If the LDA is performed using two groups (i.e. red vs white/rosé/gris) 92.3 % of the 128 wines containing soil and meteorological data are rightly assigned. Cross validation using wines without soil and meteorological data rightly classified 84.7 % of the wines.

Table 4.2 – *Contribution of different elements to LDA analysis separating wine color, soil type and the combination of wine color and soil type.*

	LDA	Ca	Mg	Mn	Sr	Ba
Wine Color	LD1	6.87	-12.51	-0.50	-0.47	-0.23
Soil Type	LD1	-0.18	-3.81	-1.55	0.71	-3.45
Wine Color	LD1	-5.28	12.03	1.24	0.00	2.08
+ Soil Type	LD2	5.35	-4.31	0.96	-0.82	2.92

Factors contributing to the discrimination axis (Table 4.2) show that the influence of Mg and Ca is at least 10 fold higher than the influence of other elements. Figure 4.2 shows the results of a LDA between red wines and white, rosé and vin gris wines using only Mg and Ca concentrations of wines. The decision criterion is $[Mg]^2/[Ca] < 100$, than a wine is classified as white/rosé/gris and if $[Mg]^2/[Ca] \geq 100$ the wine is classified as red. This criterion rightly classifies 89.8 % of the 215 wines.

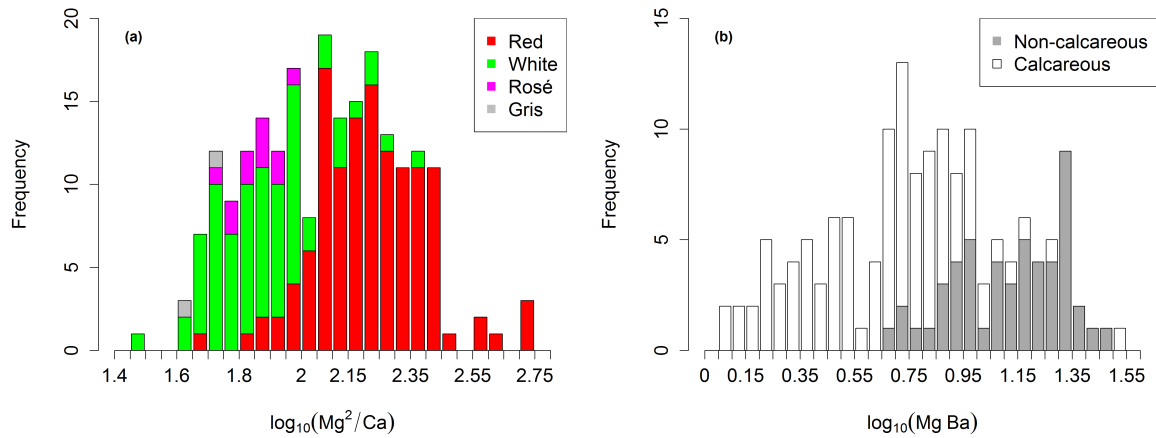


Figure 4.2 – (a) LDA analysis explaining wine color (red=red wine, green=white wine, violet=rosé wine and grey=vin gris) based on Mg and Ca compositions of the wine. (b) LDA analysis explaining soil geochemistry (as ‘calcareous’ or ‘non-calcareous’) based on Mg and Ba contents in wine.

4.4.2 Effect of soil geochemistry (calcareous vs non-calcareous) on elemental composition of wines

The wine elemental content also enabled identification of the soil type factor ‘calcareous’ containing the values ‘yes’ and ‘no’ (Figure 4.2). Principal factors allowing separation were content values of Ba, Mg and Mn (Table 4.2). The influence of Ca concentration on the identification of soil properties was the lowest, about 20 fold less than Mg, the most influential element. Using all five elements 89.0 % of the wines containing soil and meteorological data were rightly classified and 85.2 % of the cross validation dataset. Again the criterion of classification could be reduced to two elements. Figure 4.2 shows a LDA rightly classifying 84.2 % of the 152 wine containing soil data. The decision criterion is $[Mg] \cdot [Ba] < 10$, then the wine is assigned to the calcareous soil group. Otherwise, if $[Mg] \cdot [Ba] \geq 10$ the wine is classified with a non-calcareous soil.

4.4.3 Soil-Color interaction

As the effects of wine color and soil type were determined on the same dataset, it is possible that some degree of interaction exists. Figure 4.3 shows a LDA analysis using four classes: ‘red+calcareous’, ‘red+non-calcareous’, ‘non-red+calcareous’ and ‘non-red+non-calcareous’. Even though the four groups overlap on the edges and some clear outliers are visible, the centers of each group are well separated. The influences of color and soil type are mostly orthogonal. RDA analysis (Figure 4.4) shows that the combined effect of soil type and color accounted for 28.5 % of the variance in the dataset.

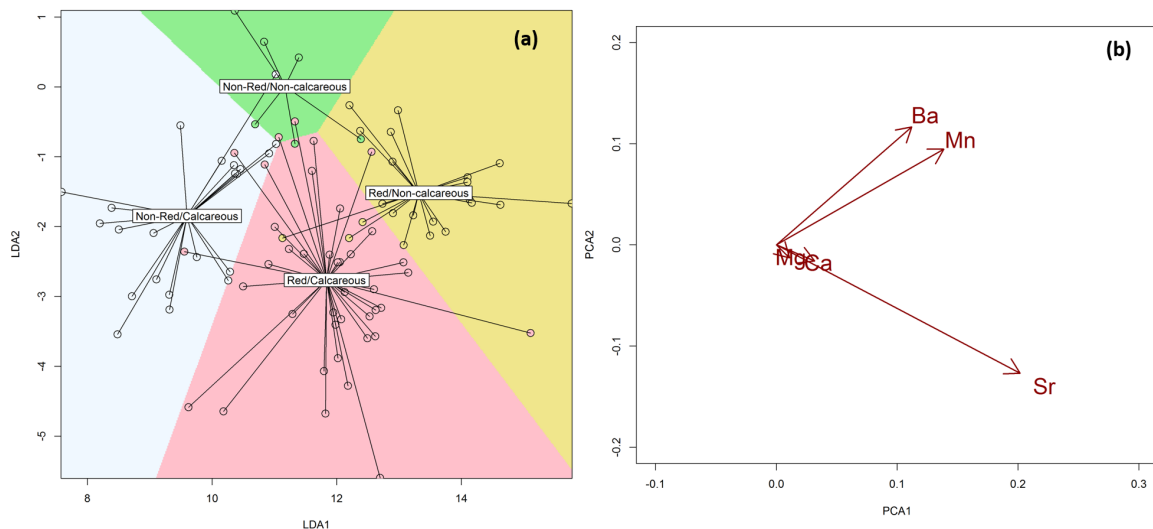


Figure 4.3 – (a) LDA analysis explaining soil geochemistry (as ‘calcareous’ or ‘non-calcareous’ soil) and wine color (as red or white/rosé/grey) based on wine elemental composition (from Ba, Ca, Mg, Mn and Sr contents). Background color indicates the area in which wines are assigned group and point markers are colored in the color of real group affiliation. (b) PCA analysis performed on the residues of two subsequent RDA analysis of (1) color and soil and (2) metrological data.

4.4.4 Effect of climatic parameters on elemental composition of wines

RDA analysis was performed on the residuals of the RDA determining the effect of color and soil type on elemental compositions (Figure 4.4), and showed that meteorological factors explain 23.4 % of the remaining variance. The principal axis in the RDA can be summed up as temperature minus rainfall in the summer month. Correlation of Ca and Mg concentration with this axis was weak as their variance was largely exploited for color and soil type classification. The concentration of Sr was correlated with higher temperature and Ba and Mn with higher rainfall during summer. The same RDA performed on the dataset without subtracting the effect of color and soil type explained 24.1 % of total variance.

4.4.5 Remaining variance

After subtracting the effect of wine color, soil and meteorological factors, 54.8 % of the total variance in the dataset remains unexplained. In a PCA performed on the residues of the RDA analysis described in section 3.4 two nearly orthogonal contributions are visible (Figure 4.3): Ba and Mn point in one direction whereas Sr points in another. Contribution of Mg and Ca is low as their variance was largely exploited in color and soil type analysis.

4.5 Discussion

4.5.1 Influence of the wine color as indicator of winemaking process on the elemental profiles of wines

The comparison of magnesium contents in red wines with the content in white/rosé/gris wines suggest a slow extraction of Mg from the grape residues during maceration and fermentation. In literature, Mg is reported to accumulate in the seeds which are present during red wine fermentation (Rogers et al., 2006). Mg was already mentioned to be part of the elements allowing to separate white from red wines, (Martin et al., 2012; Rodrigues et al., 2011) even though it did not appear in other classifications of wine color (Coetzee et al., 2005; J. Baxter et al., 1997; van der Linde et al., 2010). The Ca content in red wines tends to be lower in red wines than in white/rosé and gris wines especially compared with Mg as the separation axis can be resumed as $[Mg]^2/[Ca]$. This is probably due to malolactic fermentation of red wines less common in white wines (Ribéreau-Gayon et al., 2012). Malolactic fermentation consumes malic acid in the wine, a major inhibitor of precipitation of Ca-tartrate (Mckinnon et al., 1995). This second fermentation thus enables Ca removal from the wine.

4.5.2 Influence of environmental factors on the elemental profiles of wine

The presence of lime influences major properties of soil chemistry. As a highly soluble mineral, the dissolution of $CaCO_3$ controls soil pH by consuming protons to form HCO_3^- (George et al., 2012; van Breemen et al., 1983). Thus, it determines the solubility of mineral nutrients as well as promotes competition of Ca ions with other bivalent nutrient ions (White, 2012). Furthermore, Ca is a powerful flocculating agent for clay minerals and organic matter, and thus also influences soil structure (Haynes and Naidu, 1998). Calcareous soils were historically thought to produce better wines (Vermorel and Michaut, 1889). By contrast, the absence of lime usually translates to higher mobility of most metals including Al and Mn which can be toxic to plants as grapevine (George et al., 2012). For this reason, most non-calcareous vineyard soils have a long history of liming (Vermorel and Michaut, 1889). Nevertheless this study allows to determine the pedological provenience of wines based only on their combination of cation contents. Elements measured (with exception of Mg) are not considered as mobile in the phloem and as such transported by the xylem fascicles. As they are transported via the water supply system, a greater influence by environmental factors as transpiration rate or concentration in soil solution can be expected.

In the LDA analysis, higher concentrations in Ba, Mg and Mn are associated with non-calcareous soils. The influence of Ca and Sr is small to absent but higher concentrations Sr rather point towards calcareous soils. Considering the greater mobility of Ca in calcareous soils, this points towards an active regulation of Ca content by the plant. Barium and Mn contents in plant tissues are known to be highly decreased (by a factor 6 and 4, respectively) by the addition of lime and subsequent pH changes from 5.2 to 7.8 (Tyler and Olsson, 2001). For Mn, this effect is due to increased solubility of Mn-oxides at acid pH levels up to toxicity to plants (George et al., 2012). The case of Ba is less clear; in carbonated environments carbonates are the primary source of Ca nutrition for plants in contrast to silicate minerals in non-calcareous soils. Barium is virtually absent in carbonates thus Ba uptake may be influenced by its content in the calcium source and associated Ca/Ba ratios in soil solutions. Higher Mg contents in wines on non-calcareous soils could be due to competitive absorption between Ca and Mg, (White, 2012) but naturally Mg is also more abundant in silicate rocks, with respect to Ca, than in carbonates.

In any case, soil pH and Ca-homeostasis mechanisms seem to condition the differences in elemental composition between wines grown on calcareous vs. non-calcareous soils. The link between soil chemistry and wine elemental composition shows for the first time that there is an influence of parameters such as soil pH that reportedly influence grapevine quality (Bates et al., 2002; Bavaresco and Poni, 2003) and the elemental composition of a wine.

However, the observed effect of calcareous soils is somewhat surprising as most non-calcareous soils have a long history of liming. As soil liming is conducted on the surface, one possible explanation is that grapevines take up at least a part of their mineral nutrition in deeper soil horizons or directly through biological weathering of the bedrock. Thus, a clear effect of soil type on wine geochemistry was stated, even though our dataset on soil type measures is rough. Separation performance is expected to be higher if soil data is more accurate. Finally, combining the effects of wine color and soil type shows that both factors are close to be orthogonal, suggesting that their influence from dataset is independent of each other.

Environmental factors include the climatic influence on elemental composition of wines. After subtracting the former mentioned influences of maceration process and soil chemistry on the wine elemental composition, weather conditions explain 23.4 % of the remaining variance. Higher concentrations in Ba and Mn were associated with higher rainfall during summer months. And higher Sr contents were associated with higher temperatures. The main axis of the RDA analysis opposed this to factors, thus the main

climatic effect could be interpreted as drought stress which increases Sr concentrations and lowers Ba and Mn concentrations. The correlation of Mn and Ba concentration with higher precipitation levels especially in the summer month could either be due to a higher mobility of these elements in wet soils with low redox potential or to a stronger acidification of soils receiving more rain (Kabata-Pendias, 2004; Magalhães et al., 2012; van Breemen et al., 1983).

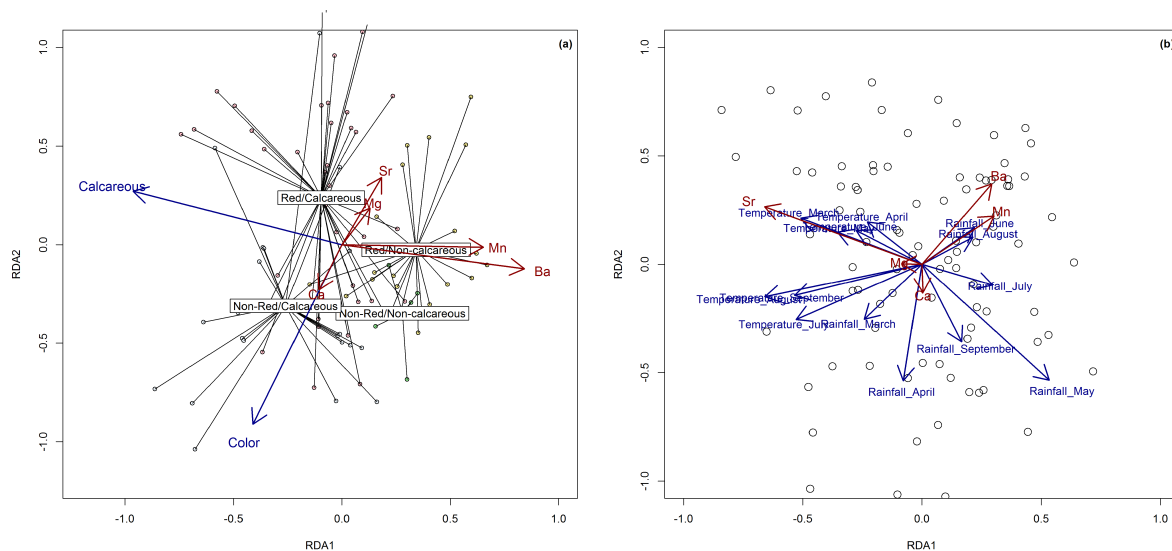


Figure 4.4 – (a) Triplot of a partial RDA using soil type and wine color as response variables and elemental concentrations of Mg, Ca, Mn, Sr and Ba as explanatory variables. (b) Triplot of a partial RDA using meteorological parameters as response variables and elemental concentrations as explanatory variables, after subtracting the effect of soil and color.

4.5.3 Causes of leftover variance

With the three factors examined above (wine color, soil type and climate), 45.2 % of the total variance in the dataset could be explained. A PCA performed on the residues (Figure 4.3) shows an axis influenced by Sr concentration and another by Ba and Mn, conjointly. Strontium content in wines, as one of the most used parameters in fingerprinting schemes was not explained to a big extent by the environmental factors used in this study. As strontium chemistry is very similar to that of Calcium, the ratio of these two elements should depend on their signature in the substratum. It turns out that Sr content in limestones varied between 250 mg kg⁻¹ in Valaginen age to 1650 mg kg⁻¹ in the early Miocene with important fluctuations over the past 140 million years (Renard, 1985). On calcareous soils, the Sr/Ca variation should thus differentiate limestone formation of different ages and geologic circumstances, and indicate the actual geological formation providing Ca nutrition. This implies that Sr content is a good tool to discriminate various vine growing regions where Sr/Ca ratios differ in geological

material. But on a global scale, it is likely to encounter same ratios in different vineyard regions.

The unexplained variation of Ba and Mn might be due to soil pH as a more detailed measure of soil chemistry than the presence of carbonates, whereas they could also be influenced by redox potential and thus soil water status (Kabata-Pendias, 2004; Magalhães et al., 2012). Some production procedures have also been shown to influence elemental composition of wines (Hopfer et al., 2015). But as our dataset is composed of wines from 183 different wineries, no systematic effect can be assumed. As well some influence due to root stock and cultivar species are expected (Angus et al., 2006; Martin et al., 2012). As many wines in our dataset are assemblies and as we work on a large spatial distribution, the analysis of the cultivar effect remains non-significant. Moreover, data on rootstock was unfortunately not available in most cases.

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Chapter 5

The role of soil in the terroir effect: A geochemical perspective

5.1 Abstract

The effect of soil on wine mineral composition and taste is controversially discussed in viticulture. Mineral nutrition of the grapevine is one possibility for an influence of soil chemistry on winemakeywording. However effects of soil chemistry are difficult to isolate from other physical and biological factors.

In this chapter two winegrowing plots in the Soave region lying side by side on contrasted soil types are investigated. Firstly, we examine environmental and geochemical factors that influenced soil formation and characterize soil chemistry and mineralogy. In a next step, we investigate the influence of soil type on the elemental composition (Mg, Al, P, S, K, Ca, Mn, Fe, Cu, Zn, Sr, Mo and Ba), sugar contents and fatty acid ratios of grapevine plants. Radiogenic Sr isotope ratios are used to identify the influence of different bedrocks.

Even though the morphology of soils is different, chemical characteristics are similar between both vineyard plots. Nevertheless Sr isotope ratios show influence of different bedrocks on their genesis. Also the composition of grapevine plants is similar between both plots even though there is a tendency for higher elemental contents on more calcareous soil. Yet Sr-isotope ratios differ between plant samples from the two plots. Finally, neither sugar contents nor fatty acid composition in the plants are significantly different from one soil type to another mainly due to high variability between plant samples.

Keywords: *vineyard soil; terroir effect; soil formation; geochemical and biochemical tracer*

5.2 Introduction

Soil properties are often indicated on wine bottles and are part of appellation schemes (Kuhnholz-Lordat, 1963; Maltman, 2013). Often an influence on wine quality is inferred (Maltman, 2013). Even though the influence of climate, biologic material and regional winemaking techniques on wine quality are widely accepted and scientifically demonstrated, there is an ongoing discussion about the effect of soil and geology (Bramley et al., 2011; Van Leeuwen et al., 2009; Gonzalez-Barreiro et al., 2015; Maltman, 2008, 2013; Styger et al., 2011).

One way of possible influence of soil chemistry on wine quality is plant nutrition and translocation of elements through the plant (Maltman, 2013). Some 17 elements are essential nutrients for plant functioning and are taken up from the soil (Marschner and Marschner, 2012). However almost the whole periodic table of elements can be found in plants. Element concentration vary systematically between different wines (Almeida and Vasconcelos, 2001; Coetzee et al., 2014; Day et al., 1995; Greenough et al., 2005; Kwan et al., 1979). In the precedent chapter studying at a large scale the use of elemental contents in wines as markers of soil characteristics, it was shown that some of those elemental contents in wines vary with carbonate contents in soils. Carbonate content thus appears to be one possible influence on plant functioning. It is known that in carbonated environments certain nutrients are scarcely available (especially P and Fe) and that plants have to deploy specific mechanisms satisfy their needs (Marschner and Marschner, 2012; Strom, 1997).

Even though differences in elemental contents likely do not have a proper taste, it is possible that smaller variations in element availability may influence the synthesis of compounds essential for wine taste such as aroma precursors, organic acids or sugars (Epke and Lawless, 2007; Sipos et al., 2012). For example the K/Ca equilibrium is important for acidity in wine but K was also found important in polyphenol synthesis (Brunetto et al., 2015; Daudt and Fogaça, 2008). Furthermore many elements act as nutrients for fermenting yeasts or catalyzers for synthesis of aromatic compounds (Pohl, 2007). For example variations in metal ion contents have been show to influence grape properties as sugar and essential amino acid content in grapes, musts and wines (Pereira, 1988).

Mobility and phytoavailability of elements generally depend not only on the soil content but is controlled by various soil properties such as pH, Eh, CEC or organic matter content (Kabata-Pendias, 2004; Tyler and Olsson, 2001). Thus thorough pedologic investigation is needed to identify soil properties controlling plant nutrition. Elemental

contents of grapevine organs and their link with soil properties have been subject to several studies with some identifying a direct link between soil concentrations or extractions whereas other do not (Angel Amoros et al., 2013; Cugnetto et al., 2014; Likar et al., 2015; Mercurio et al., 2014; Vazquez Vazquez et al., 2016). Only few studies have investigated grapevine nutrition on contrasted soils (Mackenzie and Christy, 2005; Peuke, 2000). In these studies, differences in elemental contents and winemaking parameters as sugar or acid contents have been identified (Mackenzie and Christy, 2005; Peuke, 2000). However vineyards investigated in these studies lie several kilometers apart thus meteorological conditions likely varied and no information is given on slope or exposition. The next step would thus be to isolate soil factors from meteorological factors that have been demonstrated to influence elemental compositions of grapevine (Boselli et al., 1998).

Besides elemental techniques, isotope ratios, and especially Sr isotopes, are used to determine geographical origin of wines (Horn et al., 1993). Radiogenic Sr isotope ratios between ^{87}Sr and ^{86}Sr have been used to trace sources in geology and soil sciences for many years, as stable isotope fractionation of Sr isotopes is negligible compared to variance in source signature even in biological systems (Blum et al., 2000; Capo et al., 1998). Sr isotope ratios are thus a valuable trace of the source of Ca nutrition as chemical behaviors of the two elements is extremely similar (Blum et al., 2000; Poszwa et al., 2000; Schmitt et al., 2017). This is especially interesting as carbonates are involved in the mineral nutrition of grapevine plants.

In the present chapter, two adjacent plots in the Soave area (Northern Italy, Veneto region) were studied in order to determine in real field conditions the role of soil composition in the “terroir effect”. More precisely, we aim to determine if the chemical nature of soil can be transferred through the grapevine plant. In a first approach, the 2 studied plots only differ by their soil properties, each one influenced by its respective bedrock (carbonates vs. basalts). Both parcels are cultivated in the same way with the same grapevine cultivar, under same climatic conditions and sun exposure, allowing then their use to the influence of different rocks and soil types on the mineral nutrition of grapevine.

After a pedological and geophysical characterization of each plot, soil formation (morphologic processes leading to the formation of different soil types in these two adjacent parcels) as well as mineralogical and geochemical differences were investigated. In a next step elemental contents, sugar concentrations and fatty acid ratios were measured in grapevine leaves to establish a potential link between soil properties, plant elemental composition and biochemical properties, under the same meteorological and agronomic

conditions. The ultimate goal is to identify geochemical tools (elemental contents and Sr isotopes ratio) allowing a better understanding of the role of soil geochemistry in the plant nutrition mechanisms and in the large notion of the “terroir effect”.

5.3 Materials and Methods

5.3.1 Geologic Setting

The Soave vineyard is located in the southern foothills of the Alps, in Northern Italy, in the Veneto region. This winegrowing area comprises different types of soils that have previously been mapped (Benciolini et al., 2006). Two main soil types have developed on contrasted parent material: Vertic cambisols develop on basalts and volcanic tuff mainly in the Alpone valley and calcaric cambisols have developed on limestone in the western parts of the appellation area (Benciolini et al., 2006). Volcanic rocks come from tertiary volcanic activity during the alpine orogenesis with the closest eruptive centers in Alpone valley and north of Cazzano di Tramigna village (De Vecchi et al., 1976). Limestones are from paleogenic and lower Miocene but siliclastic marine sediments and marls are also found in the area as some parts of the Lessini shelf became emerged in early Oligocene whilst others remained on the slope of Thetys ocean (Bassi and Nebelsick, 2010; De Vecchi et al., 1976).

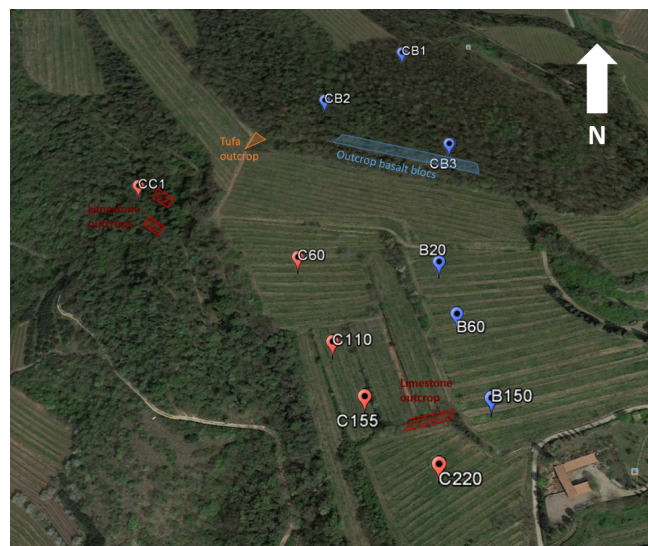


Figure 5.1 – Aerial image of the experimental site in the Soave vineyard including the two catenae: C for calcareous (point C60 to C220) and B for basaltic (point B20 to B150), different control soils (CC1, CB2 and CB3) and rock outcrops (colored areas). Aerial image taken from google earth.

5.3.2 Study site

The study was performed in the Cantina Filippi domain, in the Castelcerino vine-growing area, from the Soave appellation (45.464911 N, 11.236905 E). Geologically, the studied site lies between different bedrocks. Basalt outcrops are mainly found to the north– east, tufa to the north and west, and limestones in the south and west of the vineyard (Figure 5.1). The plots are cultivated with the typical Garganega cultivar in organic viticulture by the same vine grower. Treatment, pruning and harvesting intervals differ only by days and treatment amounts are the same on both plots. Both plots are in viticulture for 65 years. Plant material is still the first generation planted thus no deep ploughing occurred since the beginning of cultivation. Vineyard management has been organic for the last 12 years and grapevine plants are irrigated in case of severe droughts.

5.3.3 Geophysical survey

The studied vineyard was mapped using a kinetic differential GPS and an elevation map grid was calculated by kriging between 47968 data points using Surfer 13 software package to propose a vector map indicating slope direction (Figure 5.9). Meteorological condition on the studied site were followed by a Davis Vantage Pro 2 weather station at the B150 sampling spot. The station was measuring precipitation, temperature and humidity in intervals of 2 h. To have an impression of spatial heterogeneity of the field electrical resistivity measurements were carried out using a Terrameter 4000. Electrodes put in with 1 m spacing in slope direction and measurement was carried out using a Wenner type electrode setting. Inversion models were created using RES2DINV software package version 3.59.

5.3.4 Pedological study

Pedological characterization were mainly performed on the field during a dedicated campaign in October 2013. In order to evaluate the potential role of slope in soil pedogenesis, soil profiles were studied along catenas within the two studied parcels (Figure 5.1). Pedological investigations in depth were made by the use of an Edelman soil corer down to the depth of 120cm maximum. Structure, texture and pedological features (e.g., mottling, coatings, secondary phases precipitations, root and pores distributions) were described directly on the field (i.e., at field moisture). Colors were determined using a Munsell color chart in the year 2000 revised version. The occurrence of carbonate phases (effervescence method) was established using 1 M HCl.

5.3.5 Sampling of rocks, soils and plants

Rock samples of limestone, tufa and basalt were collected from the respective outcrops marked in Figure 5.1. Limestone outcrops are situated within the C catena, basalt outcrops are uphill of the B catena and tufa outcrops uphill from the C Soil sampling was performed following the two catenas identified in Figure 5.1 "B" (basalt) catena for soils over basaltic bedrock (in blue) and "C" (carbonate) catena for soils over calcareous bedrock (in red). Numbers are distances from the gravel road on top of the parcels. Soils were sampled using an Edelman auger and morphologic horizons were determined in the field according to soil structure, texture, color and effervescence to hydrochloric acid. Soil identification was then performed according to the World Reference Base (WRB) classification (2015). Leaves and grapes were sampled as composites from 5 plants surrounding the soil sampling site. Only tissues that appeared healthy was sampled in chest height (about 150 cm) using a ceramic knife. Leaves were washed three times in ultrapure water and thereafter frozen and freeze dried. Grapes were sampled including the pedicel and rinsed with ultrapure water and subsequently freeze dried. Plant samples were ground under liquid nitrogen using an agate mortar.

5.3.6 Soil physico-chemical properties

Soil samples were air-dried for several days under a laminar flow hood and sieved to 2 mm. Soil pH was measured on 1 g in ultrapure water (18.2 M Ω) following ISO 11464 protocol. For measurement of the cation exchange capacity (CEC), 1 g of soil was shaken in 20 mL of a 0.017 mol L⁻¹ cobalthexamine solution during 1 h, solutions were subsequently centrifuged and supernatant filtered at 0.22 μ m. Then cobalthexamine loss from solution was determined by absorbance loss at 475 nm with a Varian Cary 50 photospectrometer. Total Organic Carbon and Total Inorganic Carbon (respectively TOC and TIC) were calculated after subsequent measures of raw and calcined samples on a Horiba EMIA – 320V CS automate. For granulometric analysis, 2 g of soil samples were suspended in distilled water and sieved to 500 μ m. The fine fraction was subsequently treated with 10 mL hydrogen peroxide to remove organic matter. Treatment was repeated until no foam or gas formation was observed when adding H₂O₂. Finally samples were treated with an acetic acid/ammonium acetate buffer at pH \approx 4.5 to eliminate carbonates phases. Treatment was then repeated until no effervescence was visible anymore. Finally samples were washed in ultrapure water and grain size distribution was measured on a Horiba LA – 950 laser granulometer.

5.3.7 Mineralogy of rock and soil samples

Rock samples were washed with ultrapure water. Approximately 200 g of sieved soil samples (>2 mm fraction i.e., fine earth) and rock samples were crushed using a planetary mill made from agate. X-Ray Diffraction (XRD) spectra were acquired directly using an INEL diffractometer equipped with a CPS 120 detector at 40 kV and 25mA applied to a Co anticathode ($\lambda K\alpha = 0.179$ nm; $\lambda K\beta = 0.162$ nm) for crushed soil samples and a Bruker D8 advance at 40 kV and 40 mA applied to a Cu anticathode ($\lambda K\alpha = 0.154$ nm; $\lambda K\beta = 0.139$ nm) for crushed rock samples. The crystallized compounds were identified through the comparison with the COD 2013 database. Clay type was identified on oriented thin sections using the >2 μ m fraction of decarbonated soil samples. Orientated thin sections were measured as natural sample, heated to 550°C for two hours and treated with ethylene glycol. Diffraction spectra were then measured using the Bruker D8 device with the setting described above.

5.3.8 Elemental contents in rock, soil and plant samples

For total elemental content analyses, 100 mg of each crushed soil or rock sample were digested in a CEM MARS 5 microwave oven using ultrapure acids (9 mL HNO₃ : 2 mL HCl : 3 mL HF). For each digestion run, an experimental blank and a standard (SRM 2709a or BCR-2) were included. Ground plant samples (200 mg) were digested in a CEM MARS 5 microwave oven suprapure acids (10 mL HNO₃ : 3 mL HCl : 0.2 mL HF). For each digestion run, an experimental blank and a standard (SRM 1515) were included.

After acid digestion, element contents were measured with an Agilent 7500ce ICP-MS at GET Toulouse and leaves with an iCAPQ Thermo Scientific at Hydrosiences Montpellier using an In-Re internal standard to correct data for instrumental drift and plasma fluctuation. Ca, Mn and Fe contents in soil samples were measured using an ICP-OES Horiba Ultima 2. Element concentrations were expressed in dry weight (mg kg⁻¹, DW). Quality and measurement traceability were ensured by measuring replicates of SLRS-5 river water standard.

5.3.9 Sr isotope analysis

From above described sample digests, aliquots were taken to contain 300 ng of Sr. They were redissolved in 0.5 mL 2 M HNO₃ and added to a column containing 150 μ l of Sr-Specific resin. Matrix was eluted using 0.4 mL 2 M HNO₃ followed by 1.5 mL 7 M HNO₃ and another 0.3 mL of 2 M HNO₃. Strontium was then recovered in 1 mL of 0.05 M HNO₃. All acids used for purification were double subboiled prior dilution.

Samples were evaporated to dryness and deposited on W-filaments. Measurement was carried out on a Thermo Triton. NBS 987 standards were run to verify precision of the method and Sr values are denoted as ratio between ^{86}Sr and ^{87}Sr :

$$^{87}\text{Sr}/^{86}\text{Sr} = \frac{^{87}\text{Sr}}{^{86}\text{Sr}}$$

5.3.10 Sugar content

Grape sugar content was measured on the field using a Hanna Instruments 96801 refractometer on at least 15 berries. Berries were sampled on harvest day in September 2015 at shoulder height from different plants surrounding the soil sample spots.

5.3.11 Fatty acid ratios

The ratio of saturated and unsaturated fatty acids containing an 18 carbon chain were proposed as indicator of metal contamination, named Omega-3 (Le Guédard et al., 2012). In case of metal contamination the relative content of unsaturated fatty acids decline due to oxidative stress (Le Guédard et al., 2012). Ratios between saturated and unsaturated fatty acids were measured in leave samples taken at harvest time in 2013 and 2015. In 2013 one sample was take per sampling sight in 2015 three. A sample consists of around 1 cm^2 taken from leaves at the soil sampling spot and directly put into a methanol solution for conservation. Samples were then sent to LEB Bordeaux for GC- MS analysis as described in Le Guédard et al. (2012).

5.4 Results

5.4.1 Field morpho-pedologic description and soil identification

Studied soil profiles from the C catena feature grumous to polyhedral soil structure on top of the soil profile (0-10 cm). Structure evolve to polyhedral in structural horizons, whereas the deepest soil horizons display single grained structure for C220 and powder like structure in C155. Only C60 display a more massive structure in subsoil horizons. Texture is loamy clay on topsoils and getting coarser towards the bottom. Soil colors are mostly 10YR 4/3 but the lowest horizon of C220 display 10 YR 5/3 colors.

All studied soil profiles from the C catena present an increase in carbonate content as judged to their reaction with 1 M HCl. The increase in carbonate phase content with depth is due to two main processes: primary carbonate dissolution in surface horizons

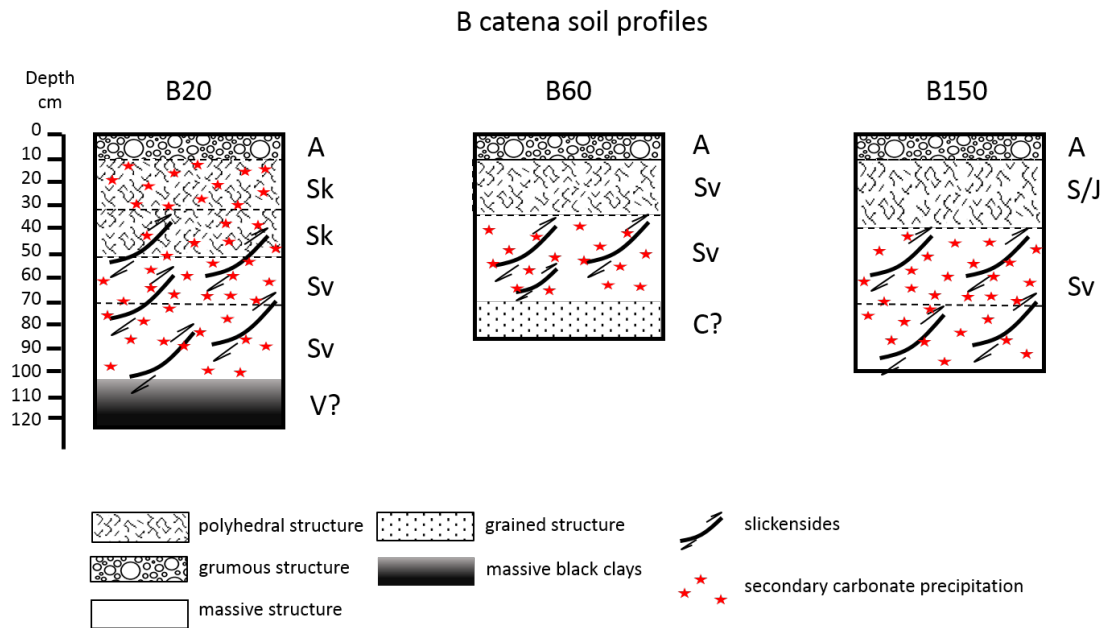


Figure 5.2 – Schematic sketches of soil profiles of the B catena including field observations.

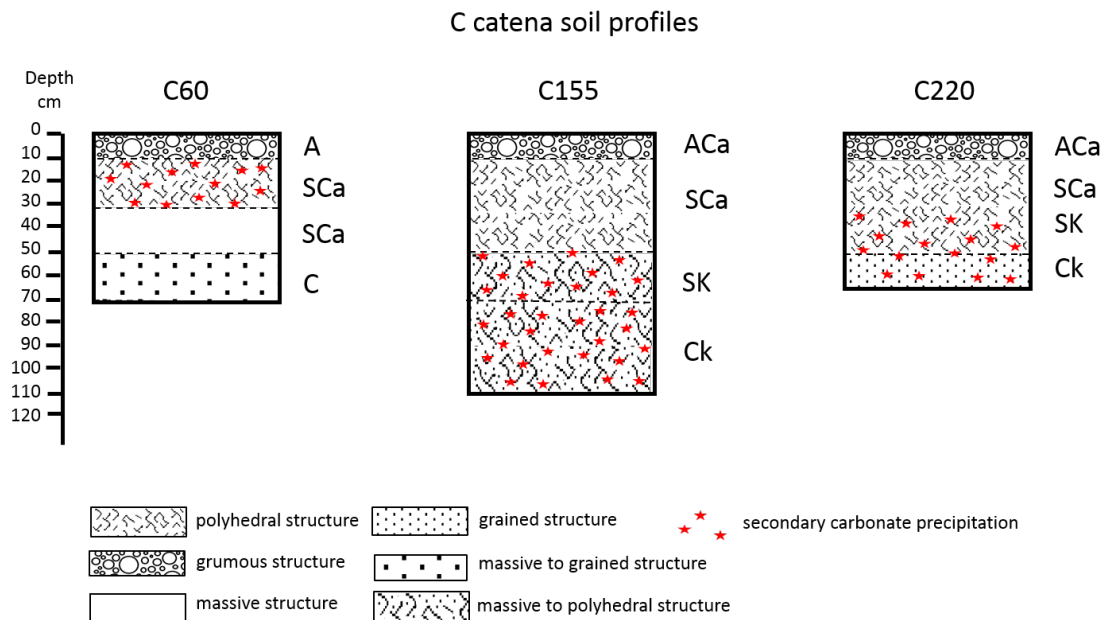


Figure 5.3 – Schematic sketches of soil profiles of the C catena including field observations.

and secondary carbonate formation in subsoil. The soils from the C catena were identified as calcaric cambisols (WRB soil classification), as described in Figure 5.3. Soil profiles from the B catena show grumous to polyhedral structure within the first 10 cm. Deepest horizons investigated generally display massive soil structure with vertic properties. However the deepest horizon of B60 features a single grained structure and the two deepest horizons of B150 have powder like to polyhedral structure. Intermediate horizons show massive to polyhedral structure. Texture reaches from loamy clay in topsoil horizons to clay in vertic horizons. Again the bottom layer of B60 differs from the rest of the studied soil profiles being clayey-loamy-sand and thus much coarser. Colors are darker than calcaric soils (mainly 10YR 3/2 and 10 YR 2/1). Only B60 bottom layer is brighter with 10YR5/6. The occurrence of slickensides was established for most of the subsoil horizons after 30/40 cm depth. However, those structures resulting from shrinking and swelling properties of clays are not considered as currently active because of a quasi-constant humidity all year long (climate with no dry season and irrigation) so that vertic properties are poorly expressed at the soil profile scale. Secondary precipitate of carbonate phases was observed at various depth following the soil profile (see figure 5.2). Colluvic material was only established for B150. B20 and B60 were identified as vertic cambisols whereas B150 was identified as a vertic colluvic cambisol (WRB soil classification).

5.4.2 Mineralogical and physico-chemical properties of soils

Mineralogical and physico-chemical characteristics of soil samples are summarized in Table 5.1. The mineralogy of parental rocks and soils is expressed as XRD spectra in Figure 5.4 and 5.5. The XRD spectra of limestones taken from the outcrops between the parcels exclusively show calcite peaks. The tufa samples also contain the peaks typical for calcite, but also exhibit peaks from clay minerals identified as smectites (Figure 5.5). Only the basalt samples have more complex mineralogy (Figure 5.4) containing albite, titanite, chabazite and diopside. In most soils of the C catena calcite remains the main mineral. Only in the topmost horizon of C60 quartz is the major phase (examples of diffraction spectra are shown in Figure 5.5).

In all soils peaks belonging to smectite type mineral are detected (Figure 5.6). Furthermore albite and titanite peaks are present in C60 and C155. Diffraction patterns for soils of the B catena show the same minerals phases than basalt rock, except chabazite. Furthermore smectite is detected in all of those soils and some horizons show calcite. In the lowest horizon of B60, calcite is more abundant than the two other minerals. In B60 50-70 cm, quartz is found to be the most abundant mineral.

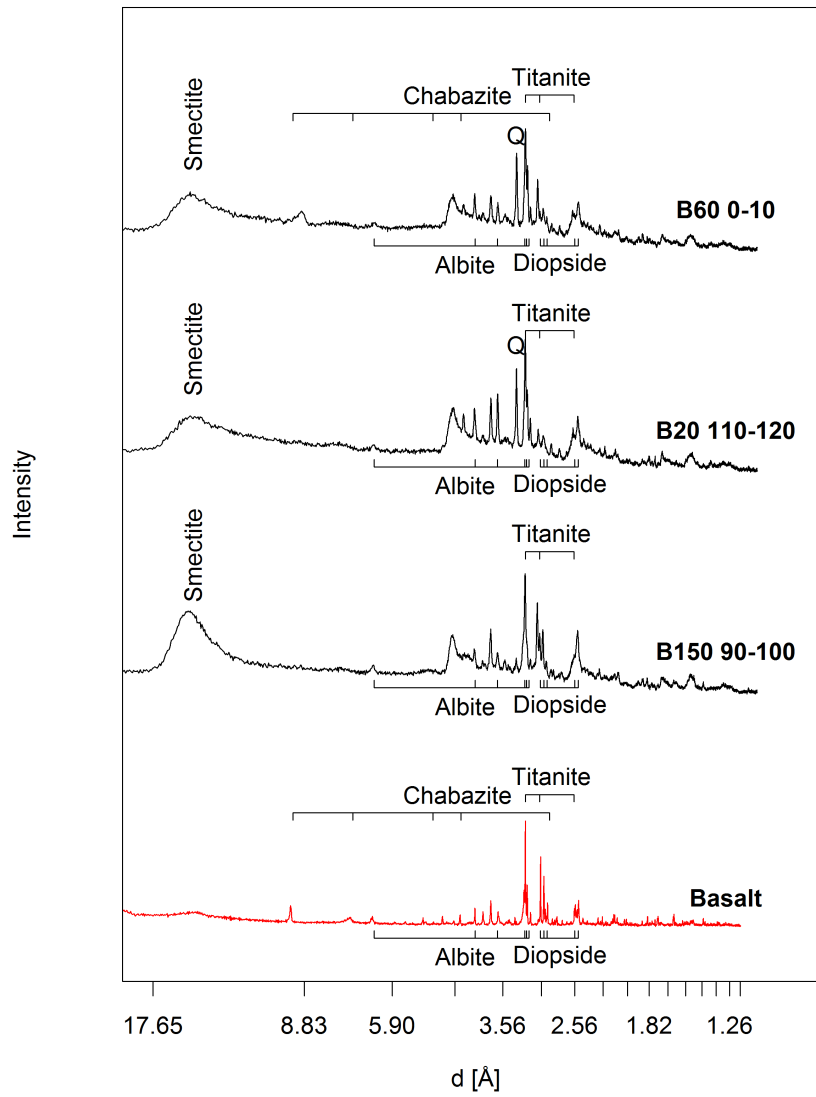


Figure 5.4 – X-Ray Diffraction spectra of basalt rocks and B catena soil horizons. Peaks for main mineral phases are indicated, for clarity only main peaks are indicated for silicates.

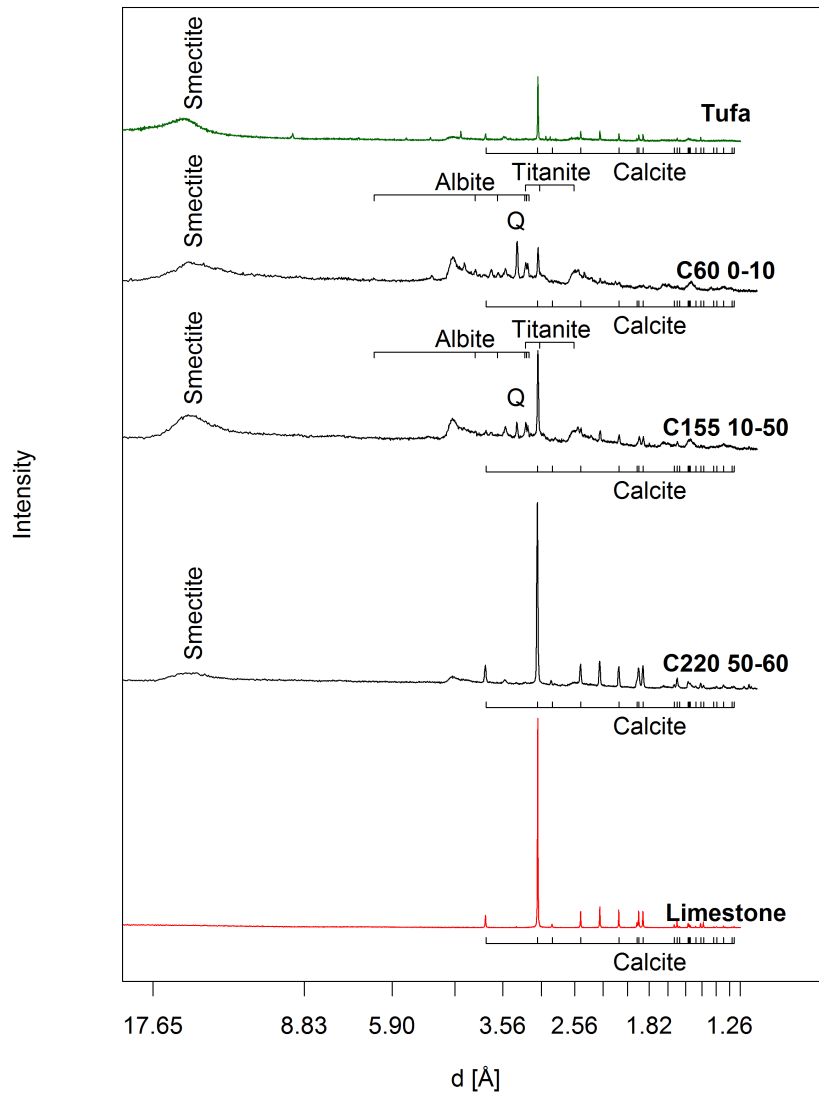


Figure 5.5 – X-Ray Diffraction spectra of carbonated rocks and *C catena* soil horizons. Peaks for main mineral phases are indicated, for clarity only main peaks are indicated for silicates.

Table 5.1 – Measurements of mineralogical and physico-chemical properties of the Soave soil horizons.

Soil ID and depths (cm)	Horizon (Référentiel Pédologique)	Main mineralogical Phases	pH (H20)	CEC	TIC	TOC	Clay	Silt	Sand	cmol kg ⁻¹		
										wt. %	wt. %	wt. %
B20												
0–10	A	calc., felds., smec.	7.5	72.1	1.8	4.3	29	67	3			
10–30	SK	calc., felds., smec.	7.7	66.1	2.2	1.3	39	60	2			
50–70	SV	felds., smec.	7.8	71.9	0.5	0.7	25	71	6			
70–120	S/V	felds., smec.	7.7	75.2	0.1	0.8	33	64	2			
B60												
0–10	A	felds., smec.	7.2	82.9	0.5	7.1	38	62	1			
10–30	SV	felds., smec.	7.9	-	0.4	1.3	42	57	1			
30–60	SV	felds., smec.	7.8	77.7	0.3	0.9	34	66	5			
60–70	SV	qtz., felds., smec.	7.8	78.3	0.3	0.6	36	61	3			
70–90	CS	calc., felds., smec.	7.9	82.8	1.6	0.1	16	66	19			
B150												
0–10	A	felds., smec.	7.3	80.2	0.2	4.0	55	45	1			
10–40	S/J	felds., smec.	-	-	0.3	-	50	50	1			
40–50	SV	felds., smec.	7.6	78.9	0.1	0.9	52	48	1			
50–70	SV	felds., smec.	-	-	0.1	-	55	45	1			
70–90	SV	felds., smec.	7.8	70.0	0.2	1.3	50	46	2			
90–100	SV	felds., smec.	7.8	72.2	0.6	0.1	55	45	1			
C60												
0–10	A	qtz., calc., felds.	7.2	80.4	0.5	5.0	63	37	1			
10–30	SCa	qtz., calc., felds.	7.7	77.9	0.6	1.3	32	65	5			
30–50	SCa	qtz., calc., felds.	7.6	84.5	0.8	0.9	31	69	1			
50–70	CS	calc., qtz., felds.	7.8	79.6	1.9	0.1	40	60	5			
C155												
0–10	A	calc., felds., smec.	7.3	83.0	1.4	2.0	32	63	6			
10–50	SCa	calc., felds., smec.	7.6	79.5	1.5	0.9	32	61	8			
50–70	SKCa	calc., felds., smec.	7.8	81	2.1	0.2	35	57	11			
70–100	CKs	calc., felds., smec.	7.9	82.6	1.9	0.1	10	82	7			
C220												
0–10	A(Ca)	calc., felds., smec.	7.2	66.0	3.7	4.9	25	66	8			
20–50	SCa(K)	calc., felds., smec.	7.9	55.1	4.9	0.5	29	53	19			
50–60	CKs	calc., felds., smec.	8.1	50.8	5.6	0.2	-	-	-			

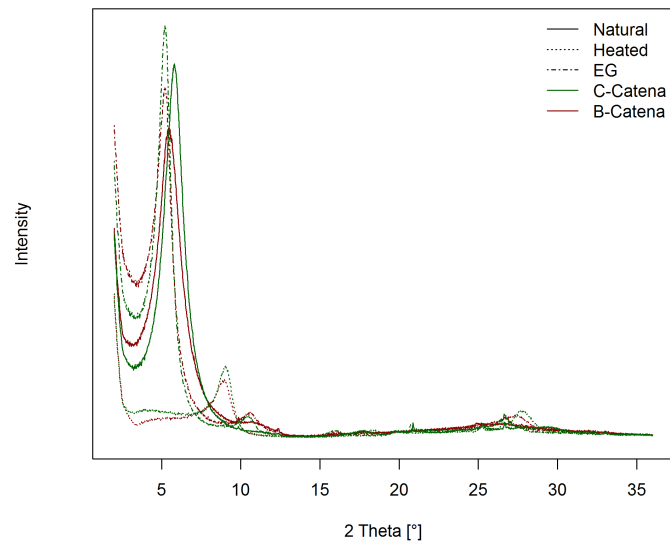


Figure 5.6 – Orientated thin section X-ray diffraction spectra on the clay fraction ($2\mu\text{m}$) of Soave soils. Samples were measured in natural state heated and after ethylene glycol (EG) treatment.

All studied soil horizons have pH values higher than 7. The lowest pH values within a soil profile are systematically found in topsoils and reach from 7.2 in C60 and C220 to 7.5 in B20. The pH values of subsoils vary between 7.6 in B150 40-50 cm, C60 30-50 cm and C155 10-50 cm. The highest pH value is 8.1 measured in the deepest horizon of C220. Cation exchange capacity (denoted CEC) is around 80 cmol kg^{-1} in most horizons.

The deepest horizons of B150 have slightly lower CEC with values of 70.0 and $72.2 \text{ cmol kg}^{-1}$. The soil C220 has systematically the lowest CEC with values between $66.0 \text{ cmol kg}^{-1}$ in the topsoil and $55.8 \text{ cmol kg}^{-1}$ in the lowest soil horizon. The soil C220 is also the soil with the highest inorganic carbon contents reaching from 3.7% in the topsoil to 5.6% in the lowest soil horizon. Inorganic carbon contents in the different soil profiles are shown in Figure 5.7. Inorganic carbon contents of around 2% (wt) are measured in the deepest horizon of C60 and the two deepest horizons of C155 alongside with the two topmost horizons of B20. Values between 1.4 and 1.6% are measured in the two upper horizons of C155 and the lowest horizon of B60. All other horizons have inorganic carbon contents of less than 1% . Organic carbon contents decline in all soil columns with depth. Organic carbon contents in topsoils reach from 7.1% in B60 0-10 cm to 2.0% in C155. Organic carbon systematically declines to $< 0.1\%$ in lowest soil layers only B20 110-120 cm and C220 50-60 cm contain measurable amounts of organic carbon (0.8 and 0.2% respectively).

Granulometric analyses after carbonate removal show that most of the soil horizons have similar granulometric composition characteristic of silty clay to silt clay loam textures (Figure 5.8): between 30 and 40 % of clay and very few sand (mainly $\leq 10\%$). The soil B150 has systematically higher clay contents of around 50 % as well as the topsoil of C60 which can be classified as having a heavy clay texture. Soils from the C catena have slightly higher sand contents than soils from the B catena. The highest sand contents of 11 to 19 % are found in B60 70-90 cm, C155 50-70 cm and C220 20-50 cm.

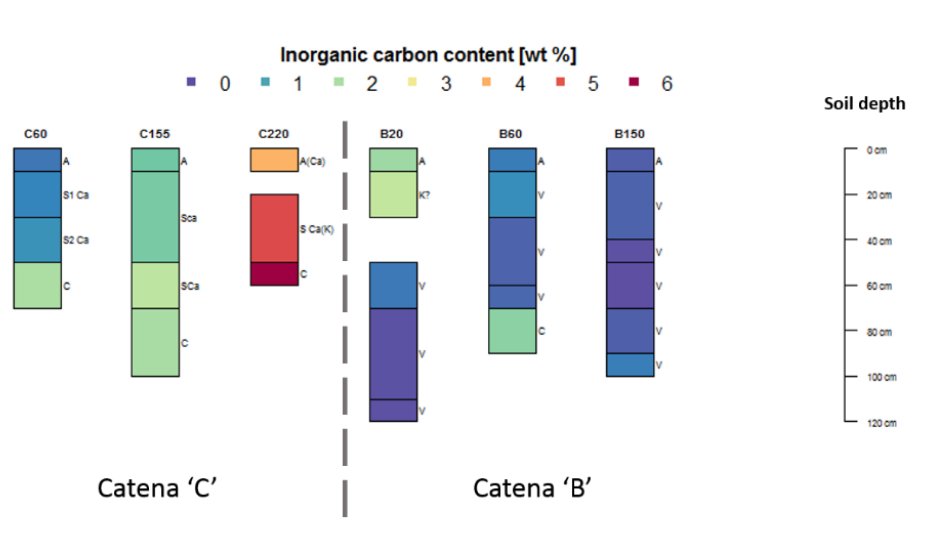


Figure 5.7 – Total inorganic carbon content in soil columns by pedological horizon.

5.4.3 Topography

Elevation of the vineyard plots is the highest in north east and the lowest in the south west (Figure 5.9). The slope direction is generally south on the 'B' catena and around C60 slightly southwest. Only around the C155 sampling point, the terrain is orientated west. Magnitude of slopes is generally around 10° . It is lower around 5° only around C220 sampling point. Uphill from B60 sampling point there is a stretch in the 'B' plot extending east to west with a width of about 20 m with no apparent slope direction indicated by various arrow directions on the stretch. The highest slopes are found in the sections separating the plots of the 'C' and 'B'.

5.4.4 Geophysical survey

Results from the geophysical survey are shown in Figures 5.10 and 5.11. Resistivity values are low in both catenas. This suggests the presence of conductive material in both plots down 5 meters. This might be water (also pore water in rock) or clay minerals. More resistive material is only found in the middle of the slope corresponding to a slight

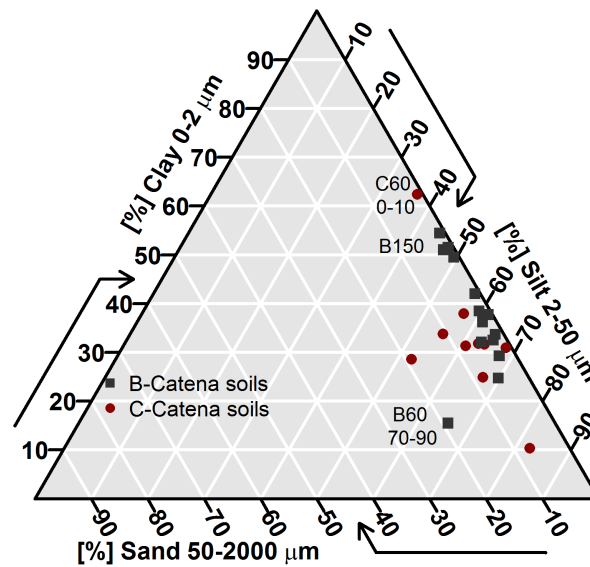


Figure 5.8 – Textural triangle of the studied soil horizons. Particle size distribution was measured after acid treatment to eliminate carbonates.

hill in topography next to the B60 sampling spot. In the C catena geophysical surveys are more complicated to conduct due to walls installed to inhibit erosion. Even though values are also very low we observe greater heterogeneity than in the B catena. However more resistive material is found in about 6 meters depth around C155 sampling spot and upslope of C220 sampling spot. The measured values suggest the presence of solid rock but basalt and limestone cannot be differentiated.

5.4.5 Elemental contents and Sr isotope ratios in rocks, soils and plants

Elemental concentrations of major elements Mg, K, Ca and Mn in basaltic rock (44.2, 9.9, 55.3 and 1.25 g kg⁻¹ respectively) are within the range of values found by other studies in the area (De Vecchi et al., 1976). In our study we find between 1.5 and 2 times higher contents of Fe, Al and P in basalt than reported in literature (De Vecchi et al., 1976). Concentration of all measured elements except Ca (394 g kg⁻¹) are relatively low in limestone. Especially the content of transition metals Mn (0.67 g kg⁻¹), Fe (3.32 g kg⁻¹), Cu (2.57 mg kg⁻¹) and Zn (3.81 mg kg⁻¹) is low compared to basalt rock (1.25 and 81.9 g kg⁻¹ respectively for Mn and Fe, and 49.1 and 120.4 mg kg⁻¹ for Cu and Zn). The only element more concentrated in limestone than in any other rock besides Ca is Mo (0.25 mg kg⁻¹) which is with more abundant in limestone than in tufa



Figure 5.9 – Map of elevation and slope direction of the studied vineyard plots interpolated from differential kinetic GPS measurements.

(0.17 mg kg^{-1}) but still most concentrated in basalts (1.10 mg kg^{-1}). Sr isotope ratios are also the highest in limestone with $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.7070. The tufa rock concentrations of elements are intermediate between basalt and limestone for most elements. Ba, Cu, P and Al have almost identical concentrations in tufa and basalt, whereas all other elements are more concentrated in the basalt rock. However Sr isotope ratios of tufa rock are with 0.7067 close to limestone. In basaltic rocks, the most abundant measured element is Fe in contrast to tufa and limestone where Ca is far more abundant. Sr isotope ratios of basaltic rock is 0.7032.

In soil profiles, Ca and Fe are the most abundant elements in the order of hundreds of grams per kg bulk soil. Some of the elements show distinct variations with depth. This is the case for Cu and S which are much more concentrated on the top of soil columns. The content and migration of Cu in the Soave soils will be discussed in chapter 6. However also K, Zn and to some extent P show increasing concentrations on the top of soil profiles. For Ca the trend is inversed with higher concentrations in the bottom horizons of the soils, especially on catena C. Other elements are more or less equally distributed through the soil profile. For Mg, there is a depletion in the top soil horizons of B150 and C60. There are few systematic differences in elemental content between catena B and catena C. The Mo content tends to be slightly higher in soils of the B catena than in the C catena, and Sr isotope ratios are higher in soils of the C catena than in those of the B catena. However there is a trend that C220 soil horizons are depleted in Mg, Al, K, Mn, Fe and Ba with respect to all other soils and more concentrated in Ca.

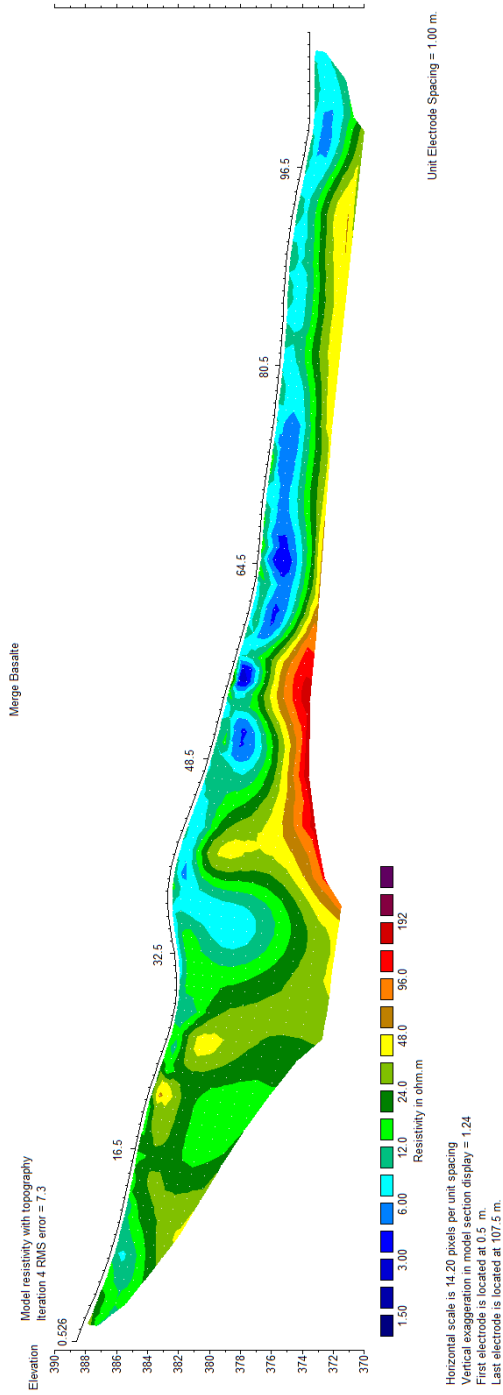


Figure 5.10 – Resistivity profile of the B catena starting from B20 sampling spot.

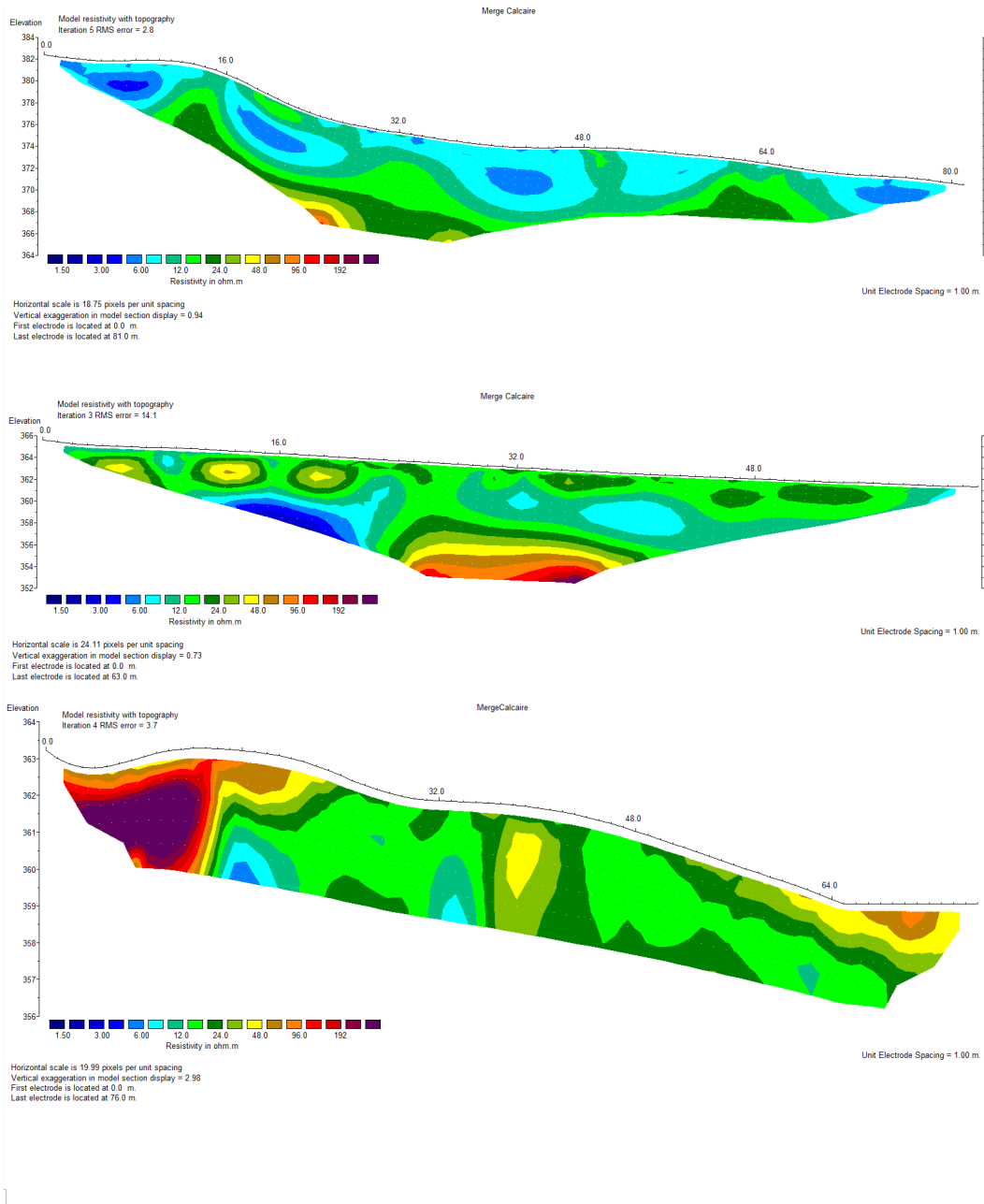


Figure 5.11 – Different parts of the electrical survey of the C catena. From the upslope plot around C60 (a), the middle plot around C155 (b) and the downslope plot C220 (c)

Table 5.2 – Total concentration of selected elements and $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in Soave soils and rocks.

ID	Type	Depth	Mg g/kg	Al g/kg	P g/kg	S mg/kg	K g/kg	Ca g/kg	Mn g/kg	Fe g/kg	Cu mg/kg	Zn mg/kg	Sr mg/kg	Mo mg/kg	Ba mg/kg	$^{87}\text{Sr}/^{86}\text{Sr}$ 2SD	
B20	Bulksoil	0-10	19.36	56.28	2.85	679	7.10	86.35	1.00	62.08	356.6	162	257	1.02	282	0.7056	0.00011
B20	Bulksoil	10-30	20.37	56.12	2.24	317	5.90	107.34	1.02	66.83	209.6	138	295	1.03	299	-	-
B20	Bulksoil	50-70	12.52	36.96	1.75	-	4.05	40.82	1.08	75.40	91.00	125	256	0.94	257	0.7047	0.00010
B20	Bulksoil	110-120	10.9	57.53	1.03	76	4.23	23.34	1.35	84.36	63.6	118	179	0.57	258	0.7051	0.00008
B60	Bulksoil	0-10	15.83	49.21	3.36	965	7.17	42.37	1.18	66.74	563.7	196	221	1.36	306	0.7054	0.00011
B60	Bulksoil	10-30	17.71	61.77	1.78	-	4.12	39.62	1.16	72.27	135.9	115	193	1.06	265	-	-
B60	Bulksoil	30-60	11.70	54.29	1.57	177	4.45	32.68	1.33	77.28	79.9	112	188	0.90	301	0.7057	0.00013
B60	Bulksoil	60-70	15.55	51.80	1.79	-	6.25	31.78	1.15	82.37	56.7	112	176	2.69	342	-	-
B60	Bulksoil	70-90	33.04	50.89	4.17	-	2.39	95.64	0.97	77.08	40.3	101	406	1.17	373	0.7053	0.00013
B150	Bulksoil	0-10	7.93	36.57	3.34	547	8.76	31.16	1.08	71.77	277	163	250	1.40	343	0.7043	0.00011
B150	Bulksoil	40-50	8.62	46.07	2.42	155	6.09	27.82	1.27	83.35	72.8	140	217	1.25	296	0.7043	0.00011
B150	Bulksoil	70-90	18.22	61.35	2.88	-	7.48	45.06	1.20	93.37	61.0	139	420	1.34	394	-	-
B150	Bulksoil	90-100	17.95	70.39	3.05	-	7.92	52.64	1.32	98.88	59.1	133	441	1.46	424	0.7037	0.00009
C60	Bulksoil	0-10	9.94	47.98	3.29	707	9.02	35.16	1.13	72.18	351.9	177	137	0.91	394	0.7067	0.00009
C60	Bulksoil	10-30	5.39	39.17	2.64	255	6.82	32.46	0.95	60.33	186.5	131	128	0.70	344	0.7066	0.00011
C60	Bulksoil	30-50	14.54	56.04	2.44	-	6.11	44.11	1.08	71.93	160.5	134	152	0.82	413	-	-
C60	Bulksoil	50-70	23.01	55.10	3.25	-	4.65	92.30	1.07	74.01	83.90	119	178	0.60	386	0.7064	0.00008
C155	Bulksoil	0-10	23.08	57.24	3.55	400	6.92	71.22	0.96	70.68	164.7	132	152	0.68	497	0.7061	0.00011
C155	Bulksoil	10-50	22.70	53.08	2.92	254	4.61	73.03	0.92	68.88	124.9	119	153	0.69	475	0.7061	0.00009
C155	Bulksoil	50-70	23.25	52.57	3.03	-	4.05	89.64	0.99	73.49	111.2	123	171	0.70	504	-	-
C155	Bulksoil	70-100	27.50	60.79	3.57	-	2.77	92.84	0.92	73.07	37.3	113	147	0.48	563	-	-
C220	Bulksoil	0-10	12.87	33.72	3.17	784	5.21	135.10	0.70	42.68	261.9	156.5	186.5	0.90	286	0.7069	0.00010
C220	Bulksoil	20-50	14.23	40.23	2.24	240	2.80	194.42	0.66	46.29	69.8	82.9	216.6	0.98	300	0.7068	0.00010
C220	Bulksoil	50-60	11.31	36.49	1.98	168	1.37	232.26	0.42	35.76	31.4	65.9	195.7	0.69	137	0.7071	0.00010
Basalt	Rock		44.22	55.91	2.96	-	9.87	55.34	1.25	81.86	49.12	120.36	1516	1.10	930	0.7032	0.00001
Tufa	Rock		17.28	46.15	2.72	-	2.16	105.42	0.83	52.95	44.02	67.53	173	0.17	903	0.7067	0.00009
Limestone	Rock		3.07	0.86	0.33	-	0.10	394.17	0.67	3.32	2.57	3.81	169	0.25	10	0.7070	0.00002

5.4.6 Elemental contents and Sr isotope ratios in leaves

Elemental contents in leaves of both catenas are of the same order of magnitude (Table 5.2). Values measured are around average contents reported in literature Mg, P, Mn and Zn (Marschner and Marschner, 2012). Cu and Ca contents are elevated with 74 mg kg⁻¹ compared to 6 mg kg⁻¹ reported in literature and 32 compared to 5 g kg⁻¹ in literature. K and Fe value are relatively low with 7.2 g kg⁻¹ compared to 10 g kg⁻¹ in literature and 43.7 compared to 100 mg kg⁻¹ in literature.

However, there is a tendency for higher concentration in leaves from the C catena all measured elements but Sr (Figure 5.16). Those differences are not significant due to variability of concentrations between sampling spots. Isotope ratios of Sr are higher in leaves from the C catena than in those from the B catena.

5.4.7 Sugar contents

Sugar contents in grapes are shown in Figure 5.12. Mean values are between 18.4 and 21.6 °Brix. However sugar content of grapes surrounding the same sampling spot were variable especially in the two downslope spots of catena B. Variability within one spot is higher than differences between different sampling spots.

5.4.8 Omega-3 biomarker

The relative content of fatty acids in leaves was higher in 2013 than in 2015 in all plants (Figure 5.13). In values were higher in the spots C110 and C155. Compared to other sampling points. However this difference was not measured in 2015. All samples have similar median values. Only the leaves at C220 show lower values than the rest of leaves from their respective catena. B150 values are elevated compared to the B catena. However variance in samples taken from B150 is large.

5.5 Discussion

5.5.1 Influence of geology versus pedological processes on soils properties in the Soave appellation

The soils of the two catenas are clearly different following a pedological approach. Color, structure and inorganic carbon content show distinct features following the soil type. There is a tendency for more massive structures in the B catena and more poly-

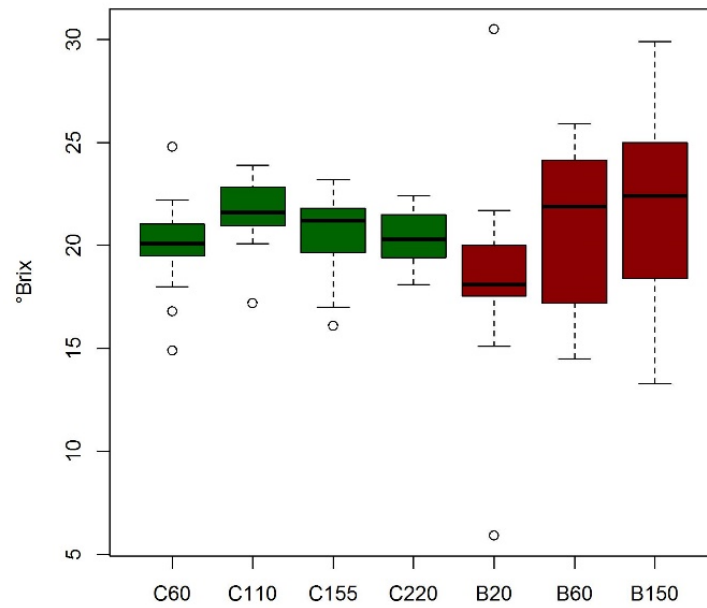


Figure 5.12 – Sugar content in grapes harvested in 2015 at the different soil sampling points.

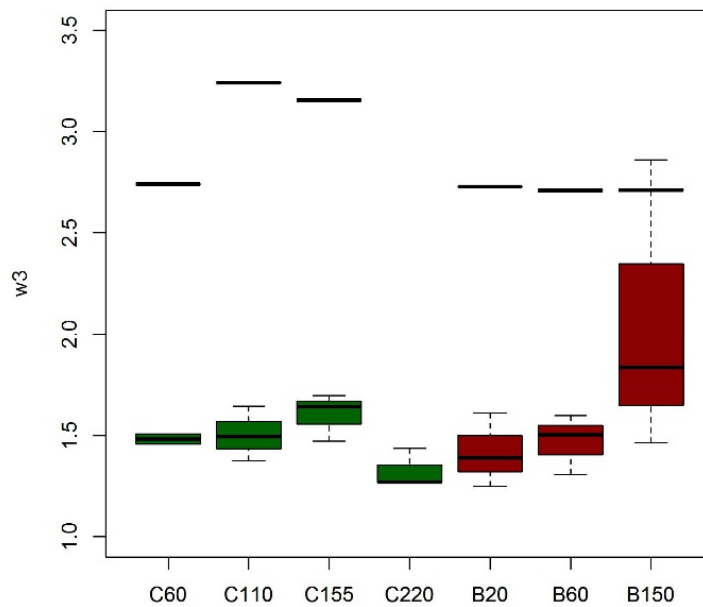


Figure 5.13 – Omega-3 biomarker values in leaves from the Soave vineyard. Boxes represent three samples taken in 2015 and black lines single samples from 2013.

Table 5.3 – Elemental composition and Sr isotopic ratios of Soave leaves.

ID	Type	Mg g kg ⁻¹	Al mg kg ⁻¹	P g kg ⁻¹	S g kg ⁻¹	K g kg ⁻¹	Ca g kg ⁻¹	Mn mg kg ⁻¹	Fe mg kg ⁻¹	Cu mg kg ⁻¹	Zn mg kg ⁻¹	Sr mg kg ⁻¹	Mo mg kg ⁻¹	Ba mg kg ⁻¹	⁸⁷ Sr/ ⁸⁶ Sr	2SD
B20	Leaf	2.81	24.4	2.24	1.63	6.85	29.7	35.7	33.0	99.3	18.4	49.9	0.118	8.79	0.7065	0.00008
B60	Leaf	2.95	25.3	2.53	1.41	7.05	31.7	23.0	34.4	70.5	24.1	66.3	0.036	8.34	0.7061	0.00012
B150	Leaf	4.24	27.1	2.39	1.44	4.67	32.1	51.9	44.3	43.6	23.2	54.2	0.042	18.41	0.7062	0.00010
C60	Leaf	3.15	29.9	2.14	1.78	8.40	26.9	49.6	64.2	125.4	25.6	43.1	0.271	36.14	0.7072	0.00009
C155	Leaf	5.49	25.3	4.08	1.67	7.32	37.7	58.8	40.6	33.9	35.2	48.7	0.166	22.65	0.7068	0.00012
C220	Leaf	2.74	40.7	2.77	1.60	9.04	32.6	47.9	45.7	74.3	28.8	38.6	0.114	20.42	0.7070	0.00013

hedral to particulate structure in the C catena. Furthermore soils of the C catena are lighter in color and tend to have higher inorganic carbon contents and all show traces to large amount of calcite in XRD spectra. By contrast, soils from the B catena clearly have inherited minerals from basalt and evidence vertic properties. Furthermore soils from the C catena tend to have higher sand contents even after elimination of carbonates. However taking a more detailed look also similarities emerge. There are several carbonated horizons in the upslope soils of the B catena and similar CEC values in all soils, only C220 has a bit lower values than the other soils but still very high. pH values are ≈ 7 in all horizons. This is attributable to a control by carbonates phases on soil pH in all soils. Also in their chemical composition the soils are surprisingly similar (Figure 5.14). The only apparent difference is higher Ca contents in carbonated horizons whereas contents in metals as Al, Mn and Fe are relatively high in all soils samples. The C220 soil contains more Ca and metals than the other soil profiles. Apparently all soils are formed under mixed influence of the three bedrocks present, making them different in some properties but very similar in others.

In the C catena a distinct increase in Ca contents with depth as well as the increase in relative importance of the calcite peaks in XRD spectra indicates that carbonate weathering is one of the soil forming processes involved in soils (figure 5.5). However high metal and smectite contents as well as an increase in Al/Fe ratios in soils compared to limestone indicate that weathering of that rock is not the only soil forming process. Weathering of the tufa rock would possibly explain these observations, and is supported by the $^{87}\text{Sr}/^{86}\text{Sr}$ values close to tufa rock and limestone in the soils of the C catena (Figure 5.15). Besides soils of the C catena mainly in more upslope positions contain trace of feldspars and titanite typically inherited from basaltic rock (Figure 5.5). The presence of minerals inherited from basalt indicate that at least basalt influences soils from the C catena.

Soil forming processes along the B catena are also complex. Finer texture in soils in downslope (B150) indicate that erosive transport (colluvic processes) occurs. The presence of carbonated horizons together with non-carbonated horizons in upslope soils (B20 and B60) indicates an influence of carbonated rock through erosion processes (particulate transport) or secondary carbonate precipitation implying the circulation of water charged with carbonates. The presence of quartz also corroborate an erosive contribution from sedimentary rocks. Only Cu contents decrease with depth within the soil profiles, this is obviously due to fungicide treatment. Orientated thin sections XRD indicate no difference between smectites types from the B and the C catena (Figure 5.6). This suggests that all the smectites encountered formed under the same conditions possibly through basalt weathering.

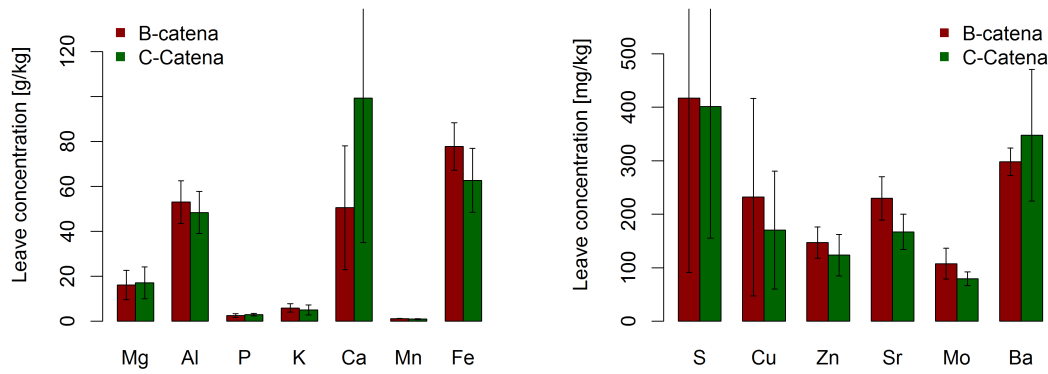


Figure 5.14 – Mean elemental contents of all measured horizons in the Soave soil profiles. Error bars are standard deviation of different horizons in one soil column and Mo concentration has been multiplied by 100 to cover visible surface.

As at least some contribution from tufa rock weathering is expected in catena C, this suggests that smectites contained in tufa rocks might be formed from past basalt weathering as still occurring in the area. The presence of tufa rocks fits into a greater geological context with variations in land and ocean levels that left behind a variation of calcareous and siliclastic rocks in the Lessini Mountains (Bassi and Nebelsick, 2010; De Vecchi et al., 1976). Tufa rocks seem to have considerable impact on the soil formation in the area. A mixing diagram (Figure 5.15) was drawn using contents of Ca and Sr and Sr isotope ratios of the three rocks and soil horizons to illustrate the mixed influences. It shows clear tendencies, even though soil horizons lie outside of the mixing triangle as decrease in Ca/Sr ratios is expected during weathering (e.g. Pett-Ridge et al., 2009). The C220 horizons lie on the mixing line between limestone and tufa with decreasing Ca/Sr ratios towards the surface. The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are unchanged from limestone bedrock indicating that weathering of limestone is the major soil forming process. Also for the two topmost horizons of C60 the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are in the range of values measured in tufa and limestone, their shift towards lower Ca/Sr values indicates that weathering of one or both of these rocks played a role in their formation. For the other horizons $^{87}\text{Sr}/^{86}\text{Sr}$ values differ from all bedrocks indicating a mixed influence. The deeper horizons of C60 lie together with the C155 horizons and rather close to the $^{87}\text{Sr}/^{86}\text{Sr}$ values of tufa and limestone, indicating a major influence of those rocks. If they were derived mainly from tufa bedrock weathering played a minor role in their formation as Ca/Sr values are not far from those of tufa rocks. If the main source material was limestone weathering was even stronger than in C220 horizons. A basalt influence is also a possibility to explain those signatures. It would be coherent with the presence of mineral phases from basalt such as titanite in the soils from the C catena.

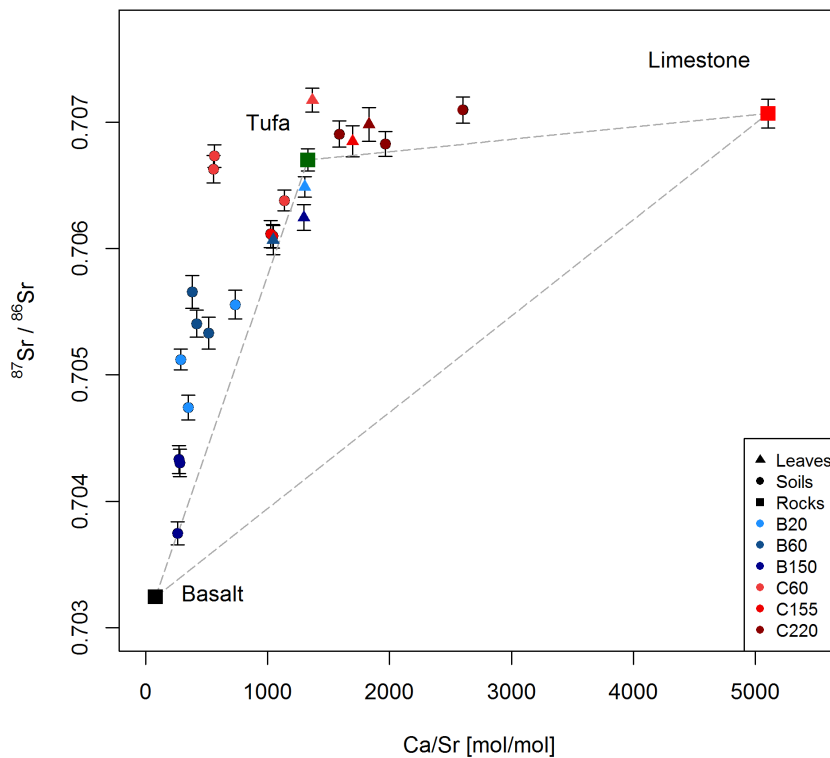


Figure 5.15 – *Mixing diagram using Sr isotope ratios and the Ca/Sr ratio of different source rocks, soils and leaves.*

$^{87}\text{Sr}/^{86}\text{Sr}$ values of all B catena horizons align between the level of basalt and a pole with lower Ca/Sr ratio and higher ^{87}Sr signature than tufa and limestone. We suggest that this pole correspond to secondary carbonate formation in basaltic soil. B150 soils are the closest to the basalt pole whereas B20 and B60 were more strongly influenced by carbonated rocks and secondary carbonates. This illustrates the complex interplay between at least three parental sources and pedogenetic processes (weathering, secondary phase precipitation) in the area. Erosive transfer, pedogenesis and a complicated bedrock geometry make the vineyard zones less clearly distinct in their biogeochemical properties as would have been stated from macroscopic pedodological parameters.

5.5.2 Influence of soil geochemistry on plant elemental content: a relevant marker of terroir effect?

The complex pedogenesis of the Soave soils has to a large extend homogenized elemental contents of bulk soils even though structure and color are clearly different in

field observations. A dominant influence of one bedrock's on soil chemistry is only visible for few elements. One remaining difference is the Ca content as marker of the degree of carbonate content. The increase of Ca contents with depth in the 'C' catena reveals a weathering gradient within the soil column, underlining the fact that those soils have not been deeply ploughed recently. Even more the differences observed for Ca and inorganic carbon contents within the different soils and their respective horizons indicate different contents of carbonates. The presence of carbonates is an important control on pH and thus the mobility of elements (Kabata-Pendias, 2004). The decrease in Cu and S concentrations in all soils is due to progressive migration of those elements frequently used in plant protection especially in organic viticulture. The gradients of nutrients K, Zn and P stem more likely from an effect the uptake from depth by the plants and deposition through litter fall (Jobbagy and Jackson, 2001). The fact that Mo is more concentrated in soils from the 'B' catena is easily explained by its higher abundance in the basaltic bedrock.

On the mixing diagram (Figure 5.15) leaves from different catenas have different $^{87}\text{Sr}/^{86}\text{Sr}$ values. Leaves from the B catena all have significant lower values than tufa and limestone rock indicating an influence of basalts on their mineral nutrition. However samples cannot be assigned to their respective soil of origin. Plants from C catena all have isotope ratios in the range of tufa or limestone. Leaves from C155 and C220 also have higher Ca/Sr ratios than leaves from the B catena. This shows that even in a context where plants are influenced to different degrees by the same rock formation their respective geological origin can be traced using alkaline earth elements. Elemental contents of other elements are not conclusive for the separation of two terroirs. However there is a trend for higher elemental concentration in the C catena for all measured elements but Sr. Also elevated Ca and low Fe values compared to literature are typical for plants grown in carbonated environments and thus potentially be used to trace influence of carbonates (Marschner and Marschner, 2012). Cu contents in leaves seem to have a trend to higher concentrations in upslope positions. This is interesting as all plants were sprayed equally and soils did not show this trend even though their Cu contents were elevated due to use of Cu-based pesticides. Cu contamination has been shown to alter plant properties important for wine making such as lower contents of soluble sugars and higher chlorophyll and carotenoid contents in plant tissues (Romeu-Moreno and Mas, 1999). Cu transfer in vineyards will be discussed in the following chapters.

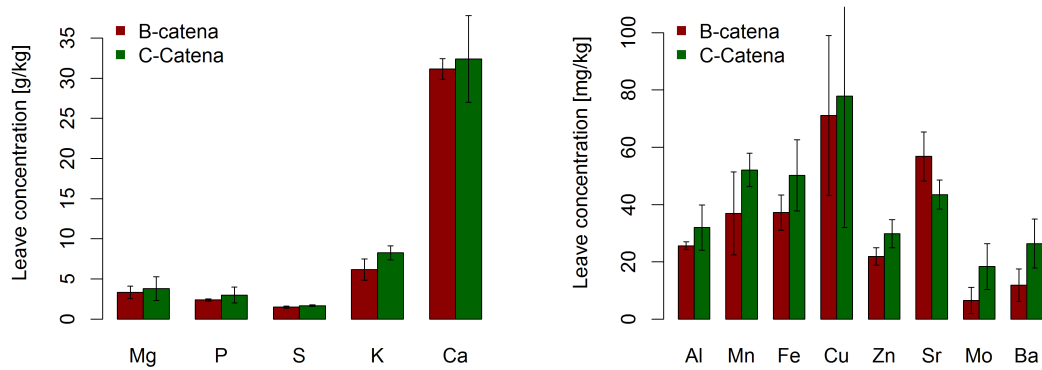


Figure 5.16 – Mean elemental contents of leaves from each sampling spot. Error bars are standard deviation and Mo concentration has been multiplied by 100 to cover visible surface.

5.5.3 Role of the biochemical markers in the "terroir effect"

For both sugar content and Omega-3 biomarker, no systematic differences between soil types were detected. For sugar contents the variance within a sample spot was large compared to the variance between different spots indicating that vegetative effects were stronger than possible effects of soil. For Omega-3 biomarker strong differences were observed between the two years of sampling. This means that other parameters than metal stress induced by soil content influence on the biomarker. Possibly diseases, drought or the action of ozone. However differences between soil types observed in 2013 were not reproduced in 2015.

5.6 Conclusion

This field study was performed on two vineyard plots in the Soave vineyard. In field observations the two examined soils showed clear morphologic differences. Complex formation processes and the mixing of different bedrocks have resulted in rather homogeneous chemical soil composition, suggesting that soils that are morphologically different can still have rather similar chemical functioning.

Similar soil chemistry is reflected in similar elemental concentrations in grapevine leaves. However there appears to be a trend for higher elemental concentration in plants grown on more calcareous soil. Yet there are no significant differences in plant nutrition between the two plots. It is still possible to tell apart leaves by their $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios and to some extent ratios of Ca and Sr, underlining the role of carbonates in plant nutrition stated in **Chapter 4**. Looking at sugar and fatty acid composition, no

significant differences were observed between the two soil types. All plant parameters that were examined from mineral to sugar contents or fatty acid ratio were subject to important variations between sampling spots that did not depend on soil type. Thus the biological variability is larger than the influence of soil in the studied vineyard. Finally, the distribution of Cu in soils and plants showed remarkable patterns that will be more thoroughly examined in the following chapters.

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Chapter 6

The fate of Cu pesticides in
vineyard soils:

A case study using $\delta^{65}\text{Cu}$ isotope
ratios and EPR analysis

6.1 Abstract

Copper (Cu) based pesticides are still widely used in viticulture and are the only pesticides permitted in organic viticulture. Due to its extensive long term use, Cu accumulates in vineyard soils and ecotoxicological implications are growing.

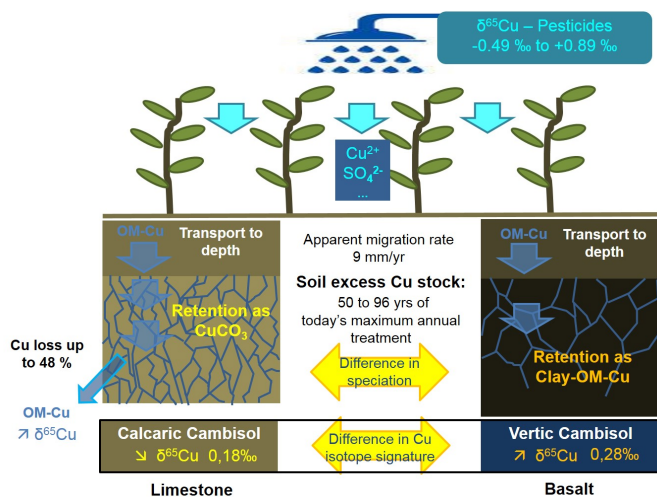


Figure 6.1 – *Graphical Abstract - Summary of major mechanisms controlling Cu transport in the Soave vineyard*

In this study, the cycling of Cu based pesticides was investigated in vineyard environments using copper mass balance, Electron Paramagnetic Resonance (EPR) spectroscopy and Cu isotope analyses. Soils (i.e. Vertic Cambisol and Calcaric Cambisol) from the Soave vineyard (Italy) were studied. Kinetic extractions were performed on soil samples using $\text{Na}_3\text{-citrate}$ to assess Cu bioavailable fraction. Results show that isotope ratios of pesticides depend on Cu speciation and their manufacturing date, covering a large range of isotope ratios $\delta^{65}\text{Cu}$ (from $-0.49 \pm 0.05 \text{‰}$ to $0.89 \pm 0.01 \text{‰}$) making it difficult to trace sources of Cu in soils. Mass balance calculations based on Ti as invariant element permitted to put in evidence large excess Cu stocks in both studied soils. Excess Cu is transported to depth with approximately the same apparent rate (0.0092 m yr^{-1}) in both soils, faster than formerly reported in literature. A substantial amount of Cu was missing from calcaric cambisols (6 to 48 %) when compared to vertic cambisols, implying a relative loss of Cu such soils via the soil solution. In bulk soils, there are slight but significant differences in mean Cu isotope ratios depending on soil type ($\delta^{65}\text{Cu}$ from 0.28 vs 0.18 ‰ in vertic and calcaric soils respectively), illustrating the loss of dissolved, heavy Cu from carbonated soils. EPR analysis confirms a difference in Cu speciation between vertic and carbonate-rich soils, indicating an influence of carbonates on Cu retention besides the role of Cu-organic matter interactions. Kinetic extractions showed that the bioavailable fraction displays isotopically heavier Cu isotopes signature than bulk soil, whatever the soil type.

This chapter was published in Chemical Geology (doi: 10.1016/j.chemgeo.2017.11.032).

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Results show that isotope ratios of pesticides depend on Cu speciation and their manufacturing date, covering a large range of isotope ratios $\delta^{65}\text{Cu}$ (from $-0.49 \pm 0.05 \text{‰}$ to $0.89 \pm 0.01 \text{‰}$) making it difficult to trace sources of Cu in soils. Mass balance calculations based on Ti as invariant element permitted to put in evidence large excess Cu stocks in both studied soils. Excess Cu is transported to depth with approximately the same apparent rate (0.0092 m yr^{-1}) in both soils, faster than formerly reported in literature. A substantial amount of Cu was missing from calcaric cambisols (6 to 48 %) when compared to vertic cambisols, implying a relative loss of Cu such soils via the soil solution. In bulk soils, there are slight but significant differences in mean Cu isotope ratios depending on soil type ($\delta^{65}\text{Cu}$ from 0.28 vs 0.18 ‰ in vertic and calcaric soils respectively), illustrating the loss of dissolved, heavy Cu from carbonated soils. EPR analysis confirms a difference in Cu speciation between vertic and carbonate-rich soils, indicating an influence of carbonates on Cu retention besides the role of Cu-organic matter interactions. Kinetic extractions showed that the bioavailable fraction displays isotopically heavier Cu isotopes signature than bulk soil, whatever the soil type.

6.2 Introduction

Copper (Cu) has been used as a fungicide in European vineyards since the appearance of downy mildew in the late 19th century (Richardson, 2000). In conventional viticulture, those pesticides have partly been replaced by synthetic agents but remain the only permitted choice in organic farming (RCE No. 889/2008). Even though its application is foliar, Cu from fungicides eventually reaches the soil after being washed off or following leaf fall (Flores-Vélez et al., 1996; Pérez-Rodríguez et al., 2016). Especially in carbonate-rich soils displaying high pH values, Cu is of low mobility and thus accumulates in soils, especially in topsoil horizons (Duplay et al., 2014).

Exportation from soils is low (i.e., estimated to be around 1% of applied Cu in the following rain event) and is mainly linked to soil erosion (Babcsányi et al., 2014; El Azzi et al., 2013; Ribolzi et al., 2002). European soil Cu background median was estimated to be around 14 mg kg⁻¹ in topsoils (Lado et al., 2008). Concentrations of Cu in vineyard soils can reach 1200-1500 mg kg⁻¹ in European vineyards (Chaignon et al., 2003; Flores-Vélez et al., 1996) and maximum amounts increase up to 3200 mg kg⁻¹ in vineyards under tropical climate conditions (Mirlean et al., 2007). Even though ecological implications of contaminations have been in the focus of research for a while (see Komárek et al., 2010 for a review), Cu-contaminated soils have long time been considered of no harm to grapevine itself. However, recent evidence for negative effects of Cu contaminated soil on grapevine have been reported (Anatole-Monnier, 2014; Cambrollé et al., 2015; Juang et al., 2014). Toxic effects to grapevine include decrease in root growth, inhibition of nutrient uptake (especially P, Mg, Fe), reduction in photosynthesis (Toselli et al., 2009) and an increase of vulnerability towards diseases (Anatole-Monnier, 2014).

Few is known about mechanisms controlling Cu bioavailability, uptake and transfer in vineyards and results are sometimes contradictory. On one hand, speciation of Cu in vineyard soils has generally been studied through chemical extractions (Chaignon et al., 2003; Lejon et al., 2008; Pietrzak and McPhail, 2004) that divide total soil copper into bioavailable (mainly fraction bound to organic matter) and not bioavailable fractions. These findings are supported by studies of Cu speciation using Extended X-Ray Absorption Fine Structure (EXAFS) (Boudesocque et al., 2007; Strawn and Baker, 2009, 2008) or Synchrotron X-Ray Fluorescence (S-XRF) (Jacobson et al., 2007) in topsoil layers. On the other hand, there is evidence for surface precipitation of copper as carbonate species especially in experimental settings with alkaline pH (Ponizovsky et al., 2007). Other experimental approaches underlined the importance of soil and pesticide type for speciation of retained Cu (Komárek et al., 2009). In a modelling approach, a

high affinity of Cu for Mn and Fe oxides in soils is predicted but experimental evidence is scarce (Bradl, 2004). A EXAFS study by Sayen and Guillon (2010), suggests preferential binding of Cu with FeOOH phases associated organic matter in soils. As highest Cu concentrations are found in topsoils (Besnard et al., 2001; Duplay et al., 2014; Flores-Vélez et al., 1996), investigations of deeper soil horizons are rare, even though some studies have reported a transfer of Cu to depth (Chopin et al., 2008; Mirlean et al., 2007; Pietrzak and McPhail, 2004). Transfer rate to depth was quantified as 0.0066 m yr^{-1} in a contaminated soil next to a smelter (Bigalke et al., 2010a). An aging effect has been also observed, responsible for the increase of the soil Cu retention capacity and the decrease of Cu bioavailability due to Cu (co)precipitation (Arias-Estevez et al., 2007; Ma et al., 2006; Sayen et al., 2009). Thus, mechanisms involved in Cu retention seem complex and are not fully understood.

Cu isotope analyses have been successfully used to trace Cu based pesticides behavior at a catchment scale (Babcsányi et al., 2014; El Azzi et al., 2013) as well as to identify the different sources contribution of Cu in rivers (Petit et al., 2013, 2008; Vance et al., 2008). Fractionation patterns of reactions likely to occur in soils are readily found in literature: sorption on Al and Fe oxy-hydroxides (Balistrieri et al., 2008; Pokrovsky et al., 2008), adsorption on clay minerals (Li et al., 2015), complexation with organic matter (Bigalke et al., 2010b; Ryan et al., 2014) and carbonate precipitation (Marechal and Sheppard, 2002). The largest fractionation of Cu isotopes is observed for redox processes (Ehrlich et al., 2004; Markl et al., 2006). These processes are also observed at the soil plant interface (Jouvin et al., 2012; Ryan et al., 2013) but in soils the Cu fractionation due to redox processes was less conclusive (Kusonwiriawong et al., 2017). To analyze Cu isotope ratios in soils, the classic separation procedure (Maréchal et al., 1999) had to be modified to suit on complex soil matrices (Bigalke et al., 2011, 2010a, 2010c), as Cu elution varies depending on matrix types (Chapman et al., 2006). More recently, studies have underlined the potential use of Cu isotopes analysis in the context of polluted agricultural soils (Babcsányi et al., 2016; Fekiacova et al., 2015). It was suggested that polluted soils have higher $\delta^{65}\text{Cu}$ ratios than unpolluted soils (Fekiacova et al., 2015) and that Cu isotope signatures differ between different granulometric fractions of vineyard soils (Babcsányi et al., 2016). However disaccord remains on the effects of transport mechanisms on isotopic Cu signature especially in deeper soils: It was suggested that transport mechanism are responsible for distinct isotopic signatures (Bigalke et al., 2010a) whereas another study found that natural isotopic variations are masked by the pollutant source signature (Babcsányi et al., 2016). As these studies were conducted in different soil types (i.e., differing physico-chemical conditions). So that further study is needed to determine the influence of transport mechanisms and source signature in Cu isotope ratios in soils. Other studies analyzed Cu isotope ratios

to determine the isotope signature of different Cu pools in soils leached by sequential extractions, showing a strong isotope fractionation of highly soluble soil pools due to speciation changes (El Azzi et al., 2013; Kusunwiriawong et al., 2016).

Knowledge of Cu speciation in different compartments, from Cu sources to soil horizons, is thus essential to better understand both Cu retention mechanisms and Cu isotope fractionation patterns. As Cu^{2+} is a paramagnetic element with an electron spin $S=1/2$, Electron Paramagnetic Resonance (EPR) spectroscopy can be a useful technique to get information on the Cu binding environment. As stated above some disagreement exists between EXAFS studies and other speciation approaches so that EPR analysis can provide new insights on Cu speciation in soils. Relevant EPR spectra have been recorded in literature underlining the potential of this technique for vineyard soil characterization: clay adsorbed Cu (Yang et al., 2015), Cu bound to humic and fulvic acid (Guimarães et al., 2001; Jezierski et al., 2000, 1998), dissolved Cu^{2+} (Tikhonov et al., 2006) and Cu adsorbed to cell surfaces (de Carvalho et al., 2003). Finally there are also examples of EPR spectra of single vineyard soil samples showing Cu complexation with organic matter (Flogeac et al., 2004) and soil solutions tracing evolution of Cu speciation after sewage sludge application (Cheshire et al., 1994).

In the present study, a combined approach using mass balances, kinetic citrate extractions, isotopic geochemistry and EPR spectroscopy is taken to investigate behaviour of Cu in a vineyard soils. For this purpose, two adjacent parcels of grapevine displaying soils with contrasted properties (i.e. calcaric and vertic) were studied. Both parcels are planted with Garganega grape variety and are conducted in organic viticulture (Cu application $\simeq 2.5 \text{ kg ha}^{-1} \text{ a}^{-1}$). Copper mobility was investigated in soils via kinetic citrate extractions, as well as total Cu contents, Cu isotope ratios and EPR analyses in different compartments: a panel of Cu based-pesticides and soil horizons.

The aim of this study was (a) to investigate differences in vertical Cu transfer in different soil types, (b) contribute to the development of Cu isotope techniques for the investigation of Cu polluted environments and (c) to propose mechanisms of Cu transport/immobilization in different soil types.

6.3 Materials and Methods

6.3.1 Field experiment

The study was conducted in the Soave wine region in northern Italy, located in the piedmont of the Alps. In this zone, soils develop over two distinct bedrock types:

basaltic rocks to the north-east and limestone to the south west. Geologically, the study site lies at the interface between the two bedrocks. Two adjacent plots featuring distinct soil cover types were selected: one plot with Cambisols Calcaric on limestone bedrock (i.e., “calcaric”) and one plot with vertic cambisols on basalt parental rock (i.e., “vertic”). The two plots are cultivated with Garganega cultivar in organic viticulture by the same vine grower. Treatment, pruning and harvesting intervals differ only by days and treatment amounts are the same on both plots.

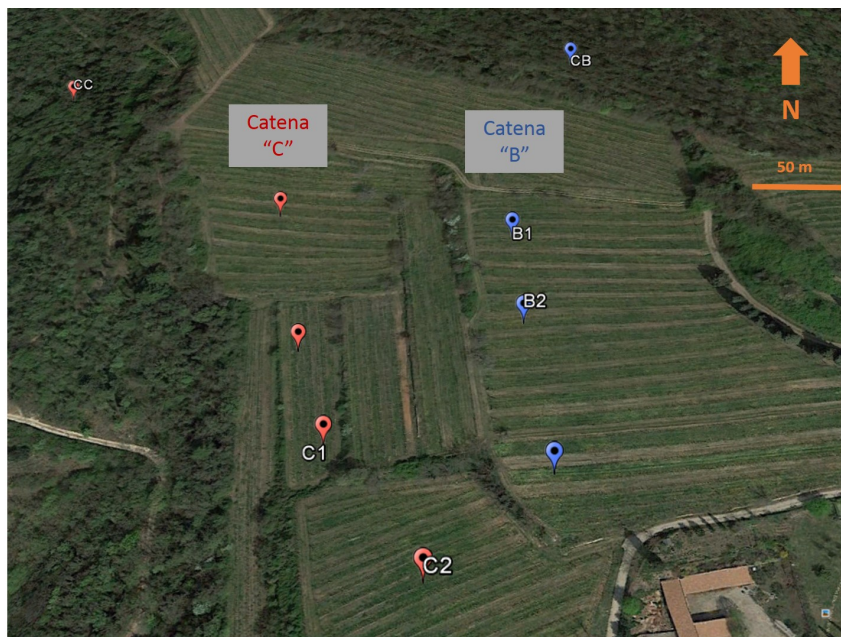


Figure 6.2 – *Sampling points along the two soil catenas: catena C for soils over calcareous bedrock to the left and catena B for soils over basalt rock to the right). Control soils are situated CC and CB in the adjacent forests (aerial picture from Google Maps (2017)).*

Both plots are in viticulture for 65 years. Plant material is still the first generation planted thus no deep ploughing occurred since the beginning of cultivation. Vineyard management has been organic for the last 12 years. Pesticides used were of three types: two types of CuSO_4 bearing pesticides and one type of $\text{Cu}(\text{OH})_2$ pesticide applied as rapid intervention after heavy rain.

6.3.2 Soil and fungicides sampling and characterization

Soil sampling was performed following two catenas (Figure 6.2): “B” (basalt) catena for soils over basaltic bedrock and “C” (carbonate) catena for soils over calcareous bedrock. Control soils for estimation of geochemical background Cu were taken in the adjacent forests north and west of the studied catenas. In control soils CC and BC

only deepest horizons are given. For this study, two soil profiles for each catena were investigated (i.e., B1, B2, C1 and C2), reflecting at best the different soil types. For all soils, sampling was performed following horizons identified morphologically on site and sampled separately. The two calcareic cambisols feature grumous soil structure on top of the soil column going to polyhedral structure in cambic horizons, whereas the lowest soil horizons display single grained structure for C2 and powderlike structure in C1. Texture classes were silt loam in deepest horizons and C2 0-10. All other horizons of the C catena were silty clay loam. Soil colors are mostly 10 YR 4/3 only the lowest horizon of C2 is 10 YR 5/3 (determined on field using a Munsell color chart in the year 2000 revised version). The vertic cambisols feature grumous to polyhedral structure in the topsoils whereas vertic horizons show massive to polyhedral structure. Only the deepest horizon of B2 features a single grained structure. Texture class of the B catena was mostly silty clay loam with B2 10-30 classed slightly finer as silty-clay and B2 70-90 being coarser than the other horizons, classed as silt loam. Colors are darker than calcareic soils 10 YR 3/2 and 10 YR 2/1. Only B2 bottom layer is brighter with 10 YR 5/6. Hydraulic conductivity at saturation was estimated by infiltration tests at constant hydraulic head (Porchet and Laferre, 1935) in 45 cm deep standardized boreholes.

A set of Cu fungicides as CuSO_4 and $\text{Cu}(\text{OH})_2$ containing samples dating back to the 1950s to modern pesticides were studied to account for the variety of Cu-based fungicides used in viticulture since late 19th century. The Cu-based fungicides used were for one part purchased in 2015 in Castorama, France from main producers (commercial names are not divulged but could be given on demand), and others were obtained from the stocks of different wine growers in France and in Italy. Old pesticides come from different private stocks. They were dated by the year of inscription to the trade catalogue of the product and the closure date of the factory (which gave about 5 year ranges).

Soil pH was measured on 1 g of soil (sieved at 2 mm) in ultrapure water (18.2 M Ω) following ISO 11464 protocol. Total Organic Carbon and Total Inorganic Carbon (respectively TOC and TIC) were calculated after subsequent measures of raw and calcined samples on a Horiba EMIA – 320V CS automate. X-Ray Diffraction (XRD) spectra were acquired directly from soil and pesticide powder after crushing with an agate mortar using an INEL diffractometer equipped with a CPS 120 detector at 40 kV and 25 mA applied to a Co anticathode ($\lambda K\alpha=0.179$ nm; $\lambda K\beta=0.162$ nm) for soil samples and a Bruker D2 phaser at 30 kV and 10 mA applied to a Cu anticathode ($\lambda K\alpha=0.154$ nm; $\lambda K\beta=0.139$ nm) for pesticide samples. The crystallized compounds were identified through the comparison with the COD 2013 database. For Cu content analyses, 100 mg of each ground soil sample were digested in a CEM MARS 5 microwave oven using

ultrapure acids (9 mL HNO₃ : 2 mL HCl : 3 mL HF). For each digestion run, an experimental blank and a standard (SRM 2709a or BCR-2, recovery values reported in Table 6.3) were included. Pesticides were dissolved in ultrapure water, when necessary the solution was acidified to dissolve the whole of the sample.

6.3.3 Total element contents

Element contents were measured with an Agilent 7500ce ICP-MS using an In-Re internal standard to correct data for instrumental drift and plasma fluctuation. Element concentrations were expressed in dry weight (mg kg⁻¹, DW) and only Ti and Cu contents will be provided for the present study. Quality and measurement traceability were ensured by measuring replicates of SLRS-5 river water standard. Mean Cu recovery was 17.6 ± 0.9 µg L⁻¹ for a certified value of 17.4 ± 1.3 µg L⁻¹. Relative standard deviations (RSD %) of each measurement were < 5 %. All reference material had satisfying recoveries noted in Table 6.3.

6.3.4 Mass balance calculations

Copper mass balances were calculated using Ti as an invariant element. The same calculations were tested for Zr as invariant element, even though numbers changed slightly overall conclusion stayed the same so that only Ti calculations are presented here. Calculations were carried out using the deepest horizons of the control soil as reference. τ_{Ti} values are computed according to Brimhall et al. (1988) in the following way:

$$\tau_{Ti} = \left[\frac{\left(\frac{c_{Cu}}{c_{Ti}} \right)_{Sample}}{\left(\frac{c_{Cu}}{c_{Ti}} \right)_{Reference}} \right] - 1$$

Excess copper is calculated using Cu/Ti ratios of the deep horizons of control soils for the respective catena. Mass per surface is calculated using a bulk density of 0.81 t m⁻³ for topsoils and 1.18 t m⁻³ for subsoils of toposequence 'B'. The values for toposequence 'C' were 0.94 and 1.20 t m⁻³ for surface soil and subsoil respectively. Bulk densities were determined for soils C1 and B2. For each topsoil and subsoil 100 cm³ core of intact soil were sampled weighed before and after oven drying at 105°C for 48h.

6.3.5 Kinetic citrate extractions

Since the kinetic extraction study by Bermond et al. (1998) using EDTA, it is widely accepted that kinetic extractions can be used to determine operationally labile and stable pools of metals in soil samples. Na₃-citrate extractions (Labanowski et al., 2008)

were performed on two selected soil samples (vertic horizon B2 30-60 and carbonate-rich horizon C2 50-60) using 0.1 molar tri-sodiumcitrate solution (citric acid trisodium salt dehydrate, ACS reagent, Acros Organics) in triplicates. Only the most contrasted soil samples of the study were selected after pedological characterization for these kinetic extraction experiments. 3 g of powdered soil samples were shaken for 0.5, 1, 3 and 6 h in 30 mL of citrate solution to investigate citrate-soil reaction kinetics. All sample were processed in triplicates.

For each reaction time, soil-citrate suspensions were centrifuged at 8000 g. The supernatant was filtered at 0.22 μm , diluted in 2 % (v/v) HNO_3 and analyzed by ICP-MS within 24h. Copper concentrations in experimental blanks were 105 ± 75 ng Cu for at least 7 μg Cu in samples. One subsample per triplicate was transferred to a teflon vessel for Cu-isotope measurements, evaporated to dryness and digested twice using HNO_3 and aqua regia (both 120°C for 12h). To check consistency of isotope ratios between triplicates a second subsample of one time step per soil was analyzed. Their respective Cu isotopes ratios are within the reported 2SD range of the first measured samples.

Table 6.1 – *Modifications of the Cu separation procedure*

	Fungicide samples	Soil samples	
		First step	Second step
Resin Volume	2 mL	2.5 mL	2 mL
Conditioning	6 mL 7 M HCl, 0.01% H_2O_2	6 mL 7 M HCl, 0.01% H_2O_2	6 mL 7 M HCl, 0.01% H_2O_2
Sample Loading	1 mL 7 M HCl, 0.01% H_2O_2	1 mL 7 M HCl, 0.01% H_2O_2	1 mL 7 M HCl, 0.01% H_2O_2
Matrix elution	8 mL 7 M HCl, 0.01% H_2O_2	9 mL 7 M HCl, 0.01% H_2O_2	9 mL 7 M HCl, 0.01% H_2O_2
Cu elution	24 mL 7 M HCl, 0.01% H_2O_2	15 mL 4 M HCl, 0.01% H_2O_2	23 mL 7 M HCl, 0.01% H_2O_2

6.3.6 Sample purification and Cu isotopes measurement

For isotope analyses, aliquots of digested samples containing 500 ng of Cu were purified using anionic AG MP-1 resin. Separations were carried out at least twice per soil sample using a protocol adapted from Maréchal et al., (1999), details on method development are given in Appendix C. For soil and BCR-2 samples, sample elution was modified as described in the Table 6.1. A first separation step was performed on 2.5 mL of AG MP-1 resin, loading the sample with 1 mL of 7 M HCl and 0.01 % H_2O_2 .

Matrix was then eluted by 9 mL of the same acid, followed by Cu elution using 15 mL 4 M HCl containing 0.01 % H₂O₂. Residues were re-dissolved in 1 mL 7 M HCl containing 0.01 % H₂O₂ and charged to a Biorad column containing 2 mL of AG MP-1 resin. Matrix was eluted by 9 mL of 7 M HCl (0.01 % H₂O₂) and then Cu was eluted using 23 mL of the same acid. For fungicide samples, separation was performed using 2 mL of of AG MP-1 resin and collecting Cu from the 8th mL of elution onto the 32nd. No second purification step was necessary as isotopic ratios measured were identical for simple and double purification.

Recovery was checked to be $100 \pm 5\%$ and BCR-2 standards were run to assure result quality. Measured isotopic ratios of BCR-2 are reported in Table 6.3, reference material was digested twice and three different purification runs were performed. Our measured values ($0.21 \pm 0.03 \text{ ‰}$) are in line with values formerly published $0.20 \pm 0.10 \text{ ‰}$ (Babcsányi et al., 2014) and $0.22 \pm 0.05 \text{ ‰}$ (Bigalke et al., 2010b). Purified solutions were spiked with IRMM Zn standard for measurement on a MC-ICP-MS (Nu Plasma 500, Nu Instruments) and a MC-ICP-MS (Neptune plus, Thermo Finnigan) respectively at ENS Lyon and GET Toulouse. Exponential laws were used to correct for mass bias using 66/64, 68/64 and 68/66 Zn isotope ratios. Cu isotope ratios are expressed in ‰ relative to NIST 976 Cu standard :

$$\delta^{65}\text{Cu} = \left(\frac{(^{65}\text{Cu}/^{63}\text{Cu})_{\text{Sample}}}{(^{65}\text{Cu}/^{63}\text{Cu})_{\text{NIST946}}} - 1 \right) * 1000$$

6.3.7 Carbonate removal procedure

To remove carbonates the soil horizons C2 50-60 cm and B2 30-60 cm, 5 ml of 0.1 molar HCl was added to 1 g of ground soil sample and shaken for 2 h. Samples were centrifuged at 3000 g and supernatant was removed. Procedure was replicated until no effervescence was visible when acid was added. Subsequently samples were oven dried at 60 °C and ground in an agate mortar.

6.3.8 Electron Paramagnetic Resonance (EPR) spectroscopy

CW X-band EPR measurements were performed using a Bruker ELEXSYS E500 spectrometer at room temperature. About 60 mg of powdered soil samples were weighted, modulation amplitude and microwave power were respectively set to 2 G and 2 mW. The g-factor, which is a constant of proportionality, was determined in order to gain information on Cu properties. When there is a hyperfine structure, it is possible to determine the hyperfine constant A which corresponds to the distance between two consecutive stripes. The accuracy of the determination of EPR parameters was ± 0.005 and ± 0.0005 for g values of metal ions and radicals, respectively. The

accuracy of the estimation of the hyperfine splitting constant was $\pm 10^{-4} \text{ cm}^{-1}$. Cu speciation was determined through the comparison of parallel component values deduced from our experimental spectra and literature values. EPR spectra were recorded for all horizons of B1, B2, C1 and C2 soil samples and decarbonated fractions from C2 20-50 and B2 30-60.

6.3.9 Thermodynamic Modelling

In absence of soil solution samples we performed thermodynamic calculations on citrate extracts as model of the mobile Cu fraction. The models aim to predict if Cu carbonates are oversaturated at soil pH levels and if these phases can be mobilized by citrate extractions. Saturation indices of different Cu bearing phases were modelled using Minteq software package version 3.1. Input data was the final (6h) concentration of Cu in citrate extracts of the calcareous soil extract put in contact with an infinite amount of solid calcite. In a first step 0.3 mol L^{-1} of Na^+ and 0.1 mol L^{-1} of citrate³⁻ were added to the solution. Then saturation indices were modeled for pH values between 6 and 9.75. In a second step citrate and sodium ions were removed from the input data and saturation indices for the same pH values were modeled. Modeled saturation indexes are shown in Figure 6.5 and Figure 6.5.

6.4 Results

6.4.1 Copper content and mineralogical properties of fungicides

Copper content in fungicides ranges between 17 and 27 % (wt), as summarized in Table 6.2. Most CuSO_4 - rich pesticides have a Cu content ranging from 17 to 27 % and the two $\text{Cu}(\text{OH})_2$ -rich samples show concentrations of 24 and 27 %. XRD spectra summarized in Table 6.2 indicate that, in historic pesticides, various Cu bearing phases exist (e.g., atacamite and malachite). Traces of brochantite are also found. In all recent CuSO_4 pesticides, Ca bearing mineralogical phases are the most abundant (data not shown), mostly gypsum/bassanite or calcite. The main Cu bearing mineral is brochantite for recent CuSO_4 pesticides with exception of the pesticide "E" where atacamite is the main Cu bearing phase. In Cu-hydroxyde labeled pesticides spertiniite is the main Cu bearing phase. Historic CuSO_4 pesticides contain less organic carbon ($3.5 - 6.7 \text{ g kg}^{-1}$) than recent CuSO_4 pesticides ($18.3 - 75.7 \text{ g kg}^{-1}$). $\text{Cu}(\text{OH})_2$ pesticides are the richest in inorganic carbon content ($91.5 - 106.2 \text{ g kg}^{-1}$).

Table 6.2 – *Mineralogical, chemical and isotopic data of Cu pesticides. The table is ordered by Cu isotopic ratio. Chemical formulas of mineral phases are atacamite ($Cu_2Cl(OH)_3$), malachite ($Cu_2CO_3(OH)_2$), brochantite ($Cu_4SO_4(OH)_6$), sinnerite ($Cu_6As_4S_9$), spangolite ($Cu_6Al(SO_4)(OH)_{12}Cl.3(H_2O)$) and spertiniite ($Cu(OH)_2$).*

ID	Cu bearing minerals	Cu wt %	Ca wt %	TOC g kg ⁻¹	$\delta^{65}Cu$ ‰	2SD ‰
Cu-Sulfates						
A*	atacamite, malachite, brochantite	18.1	0.18	6.7	-0.49	0.05
B*	atacamite, malachite, brochantite	16.6	0.17	3.5	-0.10	0.01
C	brochantite	18.6	30.95	62.5	0.01	0.08
D	brochantite	19.1	30.19	18.3	0.22	0.03
E	atacamite, brochantite, malachite	20.0	9.68	36.4	0.22	0.01
F	brochantite, sinnerite	18.6	35.68	46.7	0.23	0.03
G	brochantite,spangolite	26.5	9.12	25.1	0.31	0.03
H	brochantite	20.6	39.76	24.0	0.31	0.01
Cu-Hydroxydes						
I	spertiniite	24.2	0.28	106.2	0.81	0.01
J	spertiniite	27.3	20.64	91.5	0.89	0.09

*Pesticides dating from the 1950s

6.4.2 Physico-chemical properties of soils and Cu lability

Main soil properties are reported in Table 6.3. Soils on calcareous bedrock are slightly shallower (i.e. between 60 and 100 cm deep) than soils developed over basalt (i.e., between 90 and 120 cm deep). Soil pH levels are slightly alkaline to alkaline in both catenas (7.17 to 8.14). Physico-chemical measurements confirm field observation as calcareous cambisols from the C catena are the richest in inorganic carbon (TIC), related to the presence of calcite. The highest values of TIC are measured in the deepest horizon of C2 (i.e., 5.6%) and the lowest values were measured in the deep vertic horizon of B1 (i.e., 0.1%). Surface horizons from B1 and deepest horizon from B2 exhibit non negligible amounts of TIC in relation with the occurrence of calcite in those horizons. XRD investigations corroborate TIC analysis as calcite is the major mineralogical phase in soils from the C catena. The occurrence of carbonate phases is minor for the B catena soils. In vertic cambisols, major mineralogical phases are silicate minerals (i.e. feldspar, smectite and quartz).

Soil hydraulic conductivity is lower in vertic soils (2×10^{-6} to 10^{-5} m s⁻¹) than in calcareous ones (3 to 7×10^{-5} m s⁻¹). The lowest hydraulic conductivity is measured where the infiltration test depth includes the vertic horizon. Organic carbon contents

logically decline with depth to reach values $< 0.8\%$. TOC contents range between 2 and 7 % in topsoils and there is no obvious link between organic carbon content and soil type.

The highest Cu contents are found in topsoils of vertic cambisols (i.e. 326 and 564 mg kg⁻¹ for B1 and B2, respectively). Copper contents decline with depth in all soil profiles. In carbonate-rich soils, Cu content in surface samples is lower (164 and 262 mg kg⁻¹ for C1 and C2 respectively) and decline more progressively with depth. In the deepest horizons, soil Cu ranges between 31 and 64 mg kg⁻¹ for C2 and B1. In control soils Cu contents in deepest soil horizons is 32 and 55 mg kg⁻¹ for calcaric and vertic soils respectively.

Kinetic of citrate extraction experiments performed on vertic and calcaric horizons (Figure 6.6) show a rapid leaching of Cu in the first 30 minutes. In the C2 50-60, the equilibrium is reached after one hour leaching 2.1 mg_{Cu}kg_{soil}⁻¹ corresponding to 6.8 % of total Cu content. For the vertic horizon (B2 30-60), we also observe a rapid extraction ($\simeq 6.4$ mg kg⁻¹ h⁻¹) during the first 30 min. This first rapid extraction is followed by a small but significant increase in extracted Cu up to 6 h. However the amount extracted between 1 h and 6 h represents only a 10th of the amount extracted in the first hour. After 6 h extraction time 3.6 mg_{Cu}kg_{soil}⁻¹ are leached corresponding to 4.5 % of total Cu content.

6.4.3 Mass balance calculations

Table 6.3 summarizes τ – values (τ_{Ti}) and excess copper calculations based on mass balances. Negative τ – values correspond to a loss of Cu with respect to the reference sample. Highest τ – values are situated in the topsoil horizons of B2 and C2 showing a 10 - fold and 8.5 - fold increase in Cu/Ti ratios with respect to the reference soil horizons. These are also the horizons with highest organic carbon contents of the respective catena. τ – values decrease with depth in all soil samples. The decrease is rather progressive in B1 and C1. Note that C2 10-20 cm horizon was not measured. The deepest horizons of soils B2 and C1 are the only samples showing negative τ – values of -0.35 for B2 70-90 cm and -0.18 for C1 70-100 cm respectively. Negative τ – values correspond to a loss of Cu with respect to the reference sample CC 75-95 cm and CB 70-110 cm.

The excess Cu calculations show that around 500 kg excess Cu per hectare is stocked in the vertic soils B1 and B2. The excess Cu stock is not limited to surface horizons and is present down to horizons reaching 70 cm of depth in B1 and C1, 60 cm depth in B2 and the entire C2 soil profile (i.e. down to 60 cm of depth). If we divide 60 cm by 65

Table 6.3 – Physico-chemical properties of soil samples

Soil ID and depth cm	Horizon (WRB)	Mineralogical Phases	pH (H ₂ O)	TIC wt %	TOC wt %	Ti wt %	Cu mg kg ⁻¹	$\delta^{65}\text{Cu}$ ‰	2SD ‰	Ti^i	Excess Cu kg ha ⁻¹	g-factors	K m s ⁻¹
B1													
0-10	Mollie	calc., felds.,smec.	7.5	1.8	4.3	1.12	326	0.28	0.06	6.48	251	2.101	1 * 10 ⁻⁵
10-30	Calcic	calc., felds.,smec.	7.7	2.2	1.3	1.16	210	0.21	0.05	3.26	189	2.101	
50-70	Vertic	felds.,smec.	7.8	0.5	0.7	1.33	91	0.25	0.07	0.61	41	2.101	
70-120	Vertic	felds.,smec.	7.7	0.1	0.8	1.54	64	0.31	0.04	-0.03	0	2.120	
							sum:				481		2 * 10 ⁻⁶
B2													
0-10	Mollie	felds., smec.	7.2	0.5	7.1	1.18	564	0.37	0.05	10.22	417	2.105	
10-30	Vertic	felds.,smec.	7.9	0.4	1.3	1.26	165			2.09	132	2.102	
30-60	Vertic	felds.,smec.	7.8	0.3	0.9	1.36	80	0.31	0.02	0.39	26	2.095	
60-70	Vertic	qtz., felds.,smec.	7.8	0.3	0.6	1.32	57	0.26	0.01	0.01	1	2.056	
70-90	Cambic (Ca)	calc., felds.,smec.	7.9	1.6	0.1	1.46	40	0.12	0.04	-0.35	0		
							sum:				576		3 * 10 ⁻⁵
C1													
0-10	Mollie	calc., felds. smec.	7.3	1.4	2	1.47	164	0.22	0.06	2.99	115	2.064	
10-50	Cambic (Ca)	calc., felds. smec.	7.6	1.5	0.9	1.40	124	0.14	0.08	2.19	103	2.060	
50-70	Cambic (Ca)	calc., felds. smec.	7.8	2.1	0.2	1.48	111	0.16	0.02	1.68	84	2.066	
70-100	Cambic (Ca)	calc., felds. smec.	7.9	1.9	0.1	1.64	37	0.16	0.05	-0.19	0	2.059	
							sum:				302		7 * 10 ⁻⁵
B2													
0-10	Mollie	calc., felds. smec.	7.2	3.7	4.9	0.98	262	0.21	0.04	8.56	219	2.078	
20-50	Cambic (Ca)	calc., felds. smec.	7.9	4.9	0.5	1.04	70	0.17	0.02	1.39	49	2.086	
50-60	Cambic (Ca)	calc., felds. smec.	8.1	5.6	0.2	0.94	31	0.19	0.01	0.19	6	2.082	
							sum:				274		
Control Soils													
CC 75-95	Cambic(Ca)	calc.,smec. qtz.	8.0	3.6	0.6	1.12	32						
CB 70-110	Vertic	felds.,smec. calc.	8.0	0.7	0.4	1.28	55						
Reference Materials													
BCR2 (n=3)							1.38 ± 0.09			17 ± 1		0.21	0.03
Certified													
SRM 2709a (n=4)							1.35 ± 0.03			19 ± 2			
							0.36 ± 0.05			30.6 ± 3.7			
							0.34 ± 0.07			33.9 ± 0.5			

Notes: calc. = calcite, felds. = feldspar, smec. = smectite; dominant mineral phase in bold. Uncertainties for reference materials are given as 1 SD. Excess Cu is calculated using the Cu/Ti ratio of reference horizons CC 75-90 for the C catena and CB for the B catena to calculate geologic Cu concentration in each horizon, considering Ti invariant. Conversion to mass per surface is done using bulk soil densities measured in the field.

years of viticultural activity as a conservative estimate of Cu transport we can estimate an apparent average transport rate of 0.0092 m yr^{-1} . However the sum of excess Cu in C1 and C2 with 302 and 274 kg ha^{-1} is lower than in the vertic soils. Again note that in C2 the 10-20 cm horizon was not measured.

6.4.4 Isotope analyses

Cu isotope ratios of fungicides (given in Table 6.2 and summarized in Figure 6.3) cover a large range of isotope signatures from $-0.49 \pm 0.05 \text{ ‰}$ (historic CuSO_4 pesticide) to $0.89 \pm 0.01 \text{ ‰}$ ($\text{Cu}(\text{OH})_2$ pesticide). The Cu isotope signatures of recent CuSO_4 pesticides range from 0.01 ± 0.08 to $0.31 \pm 0.01 \text{ ‰}$. For the pesticide "I", the Cu isotope signature measured is close to the value reported in literature (0.91 ‰ , Babcsányi et al., 2016) the other pesticides measured here were not reported anywhere else. Moreover, the observation that Cu isotope signatures are heavy in $\text{Cu}(\text{OH})_2$ pesticides compared to CuSO_4 pesticide is also supported by Babcsányi et al. (2016). However, the third study that provides Cu pesticide isotope signature of just one pesticide found negative (-0.34 ‰) Cu isotope ratio for a CuSO_4 pesticide (El Azzi et al., 2013).

Soil isotope ratios are shown in Table 6.3. Mean values vary slightly but significantly between soil types though 2SD interval overlap (one sided Wilcoxon Rank Sum test $p=0.013$). If we discard the B60 70-90 horizon for having obviously calcareous properties, the p -value decreases to <0.002 with respective mean isotope ratios of 0.18 ‰ for calcareous cambisols and 0.28 ‰ for vertic soils.

B2 surface horizon contains the heaviest measured Cu signature for soil samples in the present study (i.e., $0.37 \pm 0.05 \text{ ‰}$), this is also the horizon containing the most Cu and organic carbon. The lowest isotope ratio is found in the deepest horizon of B2 ($0.12 \pm 0.04 \text{ ‰}$). That horizon contains a considerable amount of inorganic carbon (1.6 %), and calcite is the most abundant mineral. All measured soil Cu isotope ratios in the range of pesticide Cu isotope ratios. Isotope ratios of citrate extracts are positive and constant with time (Figure 6.6). Extraction solutions $\delta^{65}\text{Cu}$ values are higher than those of the respective bulk soils and, despite important uncertainties for some samples. Mean values over time are 0.99 ± 0.08 and $0.8 \pm 0.08 \text{ ‰}$ for respectively vertic and calcareous soils.

6.4.5 EPR analyses

For all samples large broad-band EPR spectra were observed and their g -values reported in Table 6.3. Spectra of the soils B2 and C2 are shown in Figure 6.4. The broad-band EPR spectrum is attributed to an average spectrum due to both Cu^{2+} and

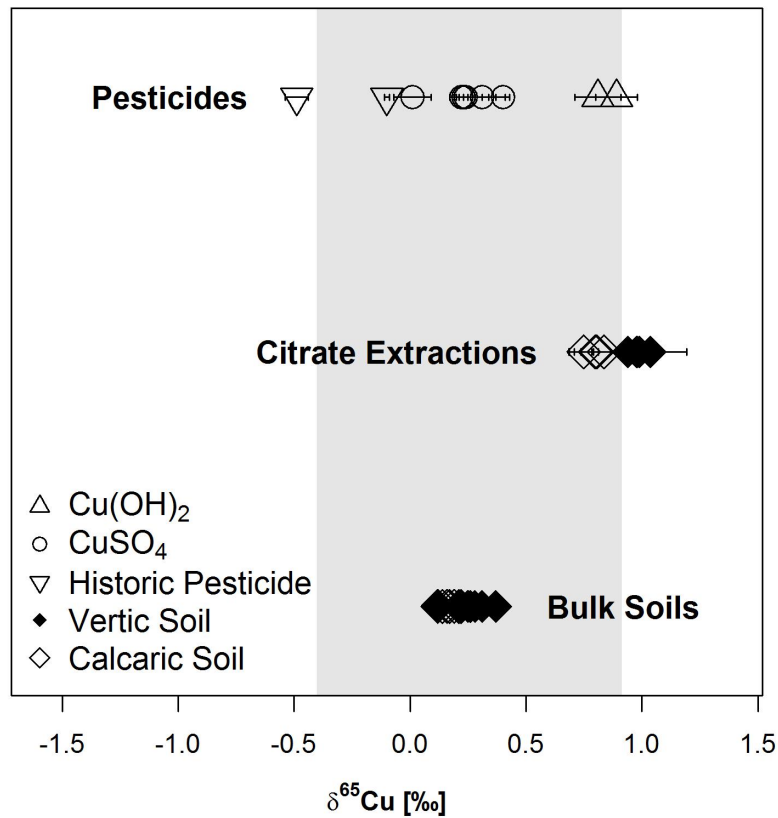


Figure 6.3 – $\delta^{65}\text{Cu}$ isotope ratios in pesticides, soils extracts and bulk soils. The area covered in grey represents isotope values of uncontaminated soils found in literature (Fekiacova et al., 2015; Vance et al., 2016)

Mn^{2+} contributions which are found in all samples. The g-values measured varied between 2.056 and 2.120. For soils of the B catena (i.e., over basalt), values are around 2.100. The deepest horizon of B2 has particularly low g values (2.056) similar to those of carbonate-rich soils that range between 2.059 and 2.086. In carbonate-rich horizons, EPR spectra show six characteristic equally-spaced peaks due to the hyperfine interaction of high spin ($S=5/2$) state of Mn with its nuclear spin ($I=5/2$). In vertic soils, this hyperfine structure is observed only in horizons containing a significant amount of carbonates (i.e., B1 0-10, B1 10-30, B2 70-90). A shift of g-values from 2.086 to 2.104 together with disappearance of the Mn hyperfine structure occurred in the C2 20-50 when decarbonation procedure was applied to the sample before measurement (Figure 6.4). No shift was observed for the B60 30-60 horizon after carbonate removal. This clearly confirmed the presence of the Mn and Cu-carbonated phases in C2 20-50 and their relative absence in B60 30-60. As the g value shifts from a typical value for carbonated soils to a value typical for vertic soils, this implies that carbonated species are at the origin of the difference in EPR spectra but speciation in other pools is similar in both soil types.

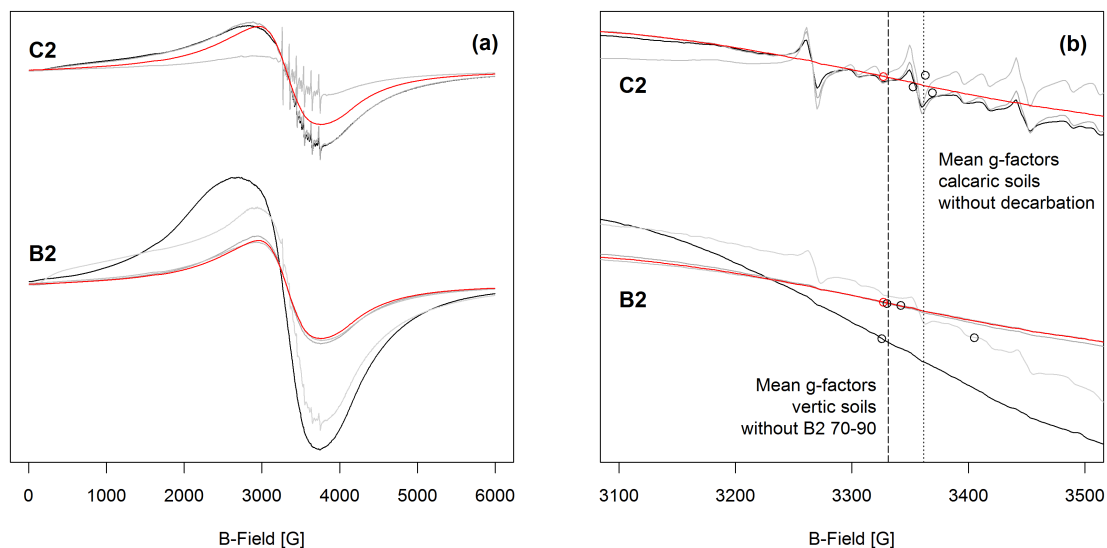


Figure 6.4 – (a) EPR spectra of soils C2 and B2. Spectra go from black to grey with depth and the red line is a decarbonated sample of the respective soil. (b) Zoom on inflection points of C2 (top) and B2 (bottom), vertical lines indicate mean position of inflection points not including decarbonated horizon for C2 and the lowest horizon (B2 70-90)

For C1, C2 and B1 topsoil horizons, four equally spaced EPR peaks related to the hyperfine coupling of Cu^{2+} electron spin ($S = 1/2$) with its nuclear spin ($I = 3/2$) were observed. A zoom on the hyperfine interaction of B1 is shown in Figure 6.4, typical values of $A_{//} = 150 \cdot 10^{-4} \text{ cm}^{-1}$ and $g_{//} = 2.38$ were calculated for all hyperfine couplings observed. Such values of $g_{//}$ and $A_{//}$ are consistent with a four-oxygen coordinated organic complexes such as Cu(II) ligated with carbonyl oxygen (Peisach and Blumberg, 1974).

EPR spectra of soils (Figure 6.4 show few variations with depth: only amplitude lowers as Cu contents declines with depth. In carbonate-rich soils, we observe a shift of peak position when decarbonation procedure is applied to the sample before measurement. In carbonate-rich soils, EPR spectra show six characteristic peaks of Mn(II) due to the interaction of high spin ($S=5/2$) state of Mn with its nuclear spin ($I=5/2$), increasing with depth. In vertic soils, those peaks are only present in horizons containing a significant amount of carbonates (i.e., B1 0-10, B1 10-30, B2 70-90).

6.4.6 Thermodynamic modelling

Modelled saturation indices show that, in presence of the added amount of $\text{Na}_3\text{-citrate}$, none of the considered Cu phases is oversaturated even at the relatively high pH values measured in the extracts. The main Cu species in solution at the pH value

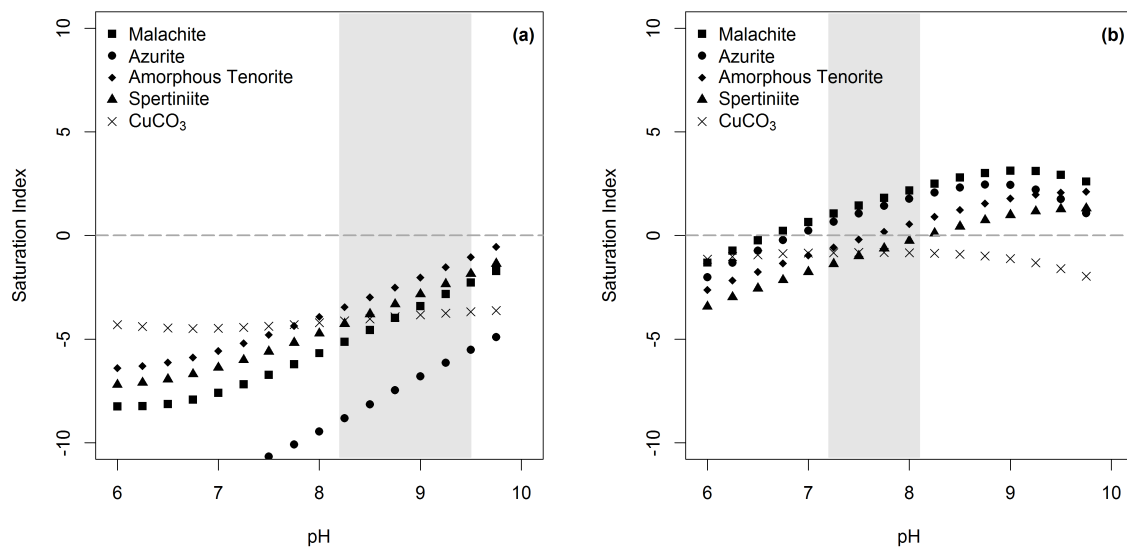


Figure 6.5 – (a) Modelled saturation indices for Cu-bearing minerals in a solution containing the final amount of Cu extracted from calcareous soils in contact with calcite for various pH values. Saturation indices are modelled in presences of $0.1 \text{ mol L}^{-1} \text{ Na}_3\text{-citrate}$. (b) Saturation indices are modelled without citrate

measured after 6 h extraction time in calcareous soil (pH=9.5) are Cu-citrate₂⁴⁻ (87.0 %) and Cu-citrate⁻ (12.6 %). If the extractant is removed from the model we observe a supersaturation of the Cu-carbonates Malachite and Azurite for the whole range of pH values observed in the soils and supersaturation of amorphous tenorite starting from pH 7.75.

6.5 Discussion

6.5.1 Isotope analysis as tracer of Cu origin in vineyard soils

Large differences from -3.03 to $+5.74$ ‰ have been observed for Cu isotope ratios in Cu ores (Maréchal et al., 1999; Markl et al., 2006). Even though there are tendencies for different minerals to scatter around different mean isotopic ratios, isotope ratios for main ores malachite and chalcopyrite overlap between -1 and 1 ‰ (Markl et al., 2006). This overlap falls right in the range of values measured in this study, however the variations we observe in isotope ratios of Cu pesticides seem to be rather linked to production techniques than source signature, reflecting both production date and Cu speciation. Production date most likely plays a key role because of a change in raw material from primary Cu to recycled Cu in the 1970s, bringing along change in production techniques (Richardson, 2000). This is reflected by a variety of Cu bearing phases in older pesticides, such as atacamite and malachite with traces of brochantite. In recent CuSO_4 pesticides, brochantite is clearly the dominating Cu bearing mineral. Only one recent pesticide contains atacamite. Older pesticides show the lowest isotope ratios (i.e., -0.49 and -0.1 ‰). In recent pesticides, isotope ratios are distinct between isotopically heavier $\text{Cu}(\text{OH})_2$ (i.e. 0.81 and 0.89 ‰) and lighter CuSO_4 (i.e. 0.01 to 0.31 ‰) conditioned pesticides (Table 6.2).

XRD spectra confirm the speciation difference between CuSO_4 and $\text{Cu}(\text{OH})_2$ pesticides. XRD data also show that added compounds vary between different pesticides: old pesticides and $\text{Cu}(\text{OH})_2$ conditioned pesticides contain mainly Na-sulfates (i.e., thenardite), whereas recent copper sulfates mainly contain Ca bearing sulfates or carbonates (i.e., gypsum, bassanite and calcite). TOC values reflect a shift towards organic additives in more recent pesticides. Differences in Cu isotope signatures between the different producers exist but are not as large as those related to speciation and manufacturing date. The isotope ratios reported here between -0.49 to 0.31 ‰ largely extend the range of values reported in literature for CuSO_4 pesticides between -0.35 and -0.06 ‰ (Babcsányi et al., 2016; El Azzi et al., 2013). This means that isotopic ratios of CuSO_4 pesticides overlap with isotopic ratios of cuprous oxide (-0.21 ‰), cupric hydroxychloride (0.11 and 0.22 ‰) as well as the Cu hydroxydes (0.22 and 0.91 ‰) based pesticides reported in literature (Babcsányi et al., 2016).

As variations of Cu isotope ratios between pesticides are significant and recover the differences in isotope ratios observed in most soils (Figure 6.3), $\delta^{65}\text{Cu}$ ratios can hardly be used for source tracing of Cu in agricultural environments, above all if the pesticide mix is unknown. This case was already reported in literature where Cu contaminated vineyard soils had the same Cu isotope ratios as a deep horizon of a control soil (Babcsányi

et al., 2016). Indeed, $\delta^{65}\text{Cu}$ ratios of unpolluted soils in literature range from -0.40 to 0.92 ‰ depending on soil type (Bigalke et al., 2010b, 2010a, 2011; Fekiacova et al., 2015; Vance et al., 2016), highlighting that added Cu might just have the same isotope ratios as the original soil – making source tracing impossible.

6.5.2 Labile and stable Cu pools in soil

In the studied soils at least two main processes are expected to control Cu mobility: (1) Cu sorption to organic matter as frequently identified as main Cu species in polluted soils and (2) Cu-carbonate precipitation on mineral surfaces (Boudesocque et al., 2007; Chaignon et al., 2003; Komárek et al., 2009; Lejon et al., 2008; Pietrzak and McPhail, 2004; Ponizovsky et al., 2007; Strawn and Baker, 2009). As Cu has a high affinity for citrate ($\log K \text{ Cu-citrate}^- = 7.6$ and $\log K \text{ Cu-citrate}_2^{4-} = 8.9$), Cu carbonates should be extracted from the soils. This is confirmed by thermodynamic modelling showing that even at high pH values Cu carbonates are undersaturated in the last step of our extraction procedure (Figure 6.5). For Cu-NOM complexes stability constants reported in literature between $\log K$ 3.7 and 10.5, so that a large part of organic matter sorbed Cu should be extracted by citrate but not necessarily all (Tipping and Hurley, 1992). Furthermore it was reported that adsorption of humic acids to clay surfaces enhances their Cu adsorption capacity (Arias et al., 2002). As shown in Figure 6.6, extractable Cu in both carbonate-rich and vertic horizons are dominated by rapidly extractible, labile Cu. After its extraction, little to no Cu is leached by the extractant from both soils.

However, the percentage of total Cu extracted by citrate is higher in the carbonated horizon (6.8 ‰) than in the vertic horizon (4.5 ‰). Isotope ratios stay within the 2 standard deviation (σ) interval over time in both soil types are thus considered constant. This is in line with labile Cu controlling extractable Cu in both soils. Lower isotope ratios in extracts from the carbonate-rich soil indicate a greater contribution of a lighter pool of Cu to the labile Cu fraction. This would be consistent with the extraction of Cu from precipitated Cu-carbonates. Furthermore Cu binding to carbonates in calcareous soils is supported by the observed shift in EPR spectra (Figure 6.5).

Isotope ratios in extracts of the vertic horizon are also constant over time, indicating that extracted Cu is dominated by the rapidly extractible labile fraction. However higher isotope ratios than in the calcareous horizon indicates a contribution of a heavier Cu pool to the mobile Cu. It would be expected that desorption from organic matter surfaces is a rather rapid reaction extracting most of the adsorbed Cu within the first 30 min even though there is a tendency that more strongly bound Cu is slower to be

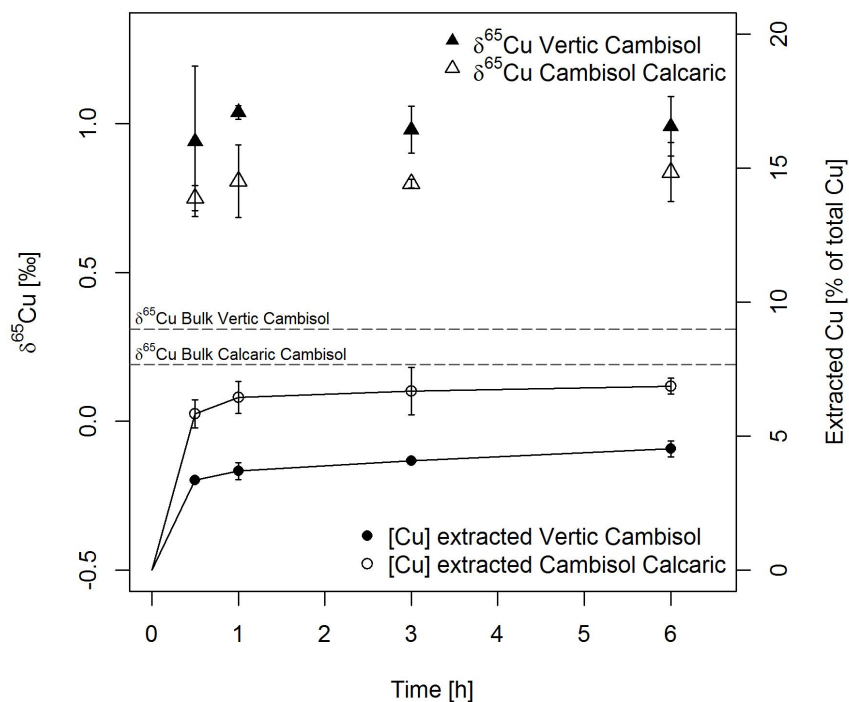


Figure 6.6 – $\delta^{65}\text{Cu}$ isotope values of kinetic extractions over time and the corresponding percentages of total Cu extracted. Error bars show 2SD range.

desorbed (Rate et al., 1993). An enrichment in ^{65}Cu would be expected in Cu adsorbed to organic matter (Bigalke et al., 2010c; Ryan et al., 2014). Other pools contributing to the labile Cu pool could be clay sorbed Cu, however this pool would be expected to have lighter isotope ratios than organic bound Cu (Babcsányi et al., 2016; Li et al., 2015). Even though we cannot identify the actual speciation of extractible Cu similar extraction kinetics indicate that processes of liberation are also similar. Isotopic values indicate a bigger contribution of Cu-carbonates to the mobile Cu in calcareous soils whereas in vertic soils organic matter adsorbed Cu plays a relatively larger role. It is important to note that there is a relatively greater mobile Cu pool in calcareous soil than in vertic soil even though more exogenous Cu is contained in the vertic soil. This indicates that amended Cu is more strongly immobilized in the vertic soil.

The equation $\Delta^{65}\text{Cu}_{\text{ligand-free}} = 0.0364 * \log(K) - 0.1459$ derived from Donnan dialysis (Ryan et al., 2014) relates Cu isotope fractionation due to an organic ligand to its stability constant of the Cu complex. This equation can be used to estimate the isotope effect due to the extractant, giving a fractionation $\Delta^{65}\text{Cu}_{\text{citrate-free}} \approx +0.2 \text{ ‰}$. We can estimate the isotopic signature of the extracted Cu pool by subtracting this extractant induced fractionation and the bulk isotope ratios of the respective horizon from the average isotope ratios of extractions. The extracted Cu pool is probably different from

mobile Cu in the soil solution but represents a potentially phytoavailable pool as organic complexants as citrate are exuded by plants to facilitate metal uptake especially in carbonated soils (Strom, 1997). The Cu-isotope fractionation between soil total and extractable Cu ($\Delta^{65}\text{Cu}_{\text{mobile} - \text{bulk soil}}$) is around +0.4 ‰, regardless of the soil. This value suggests that heavy Cu is more mobile than light Cu and that a heavy Cu pool is available to plants.

This observation is consistent with Cu isotope analysis from river studies showing that dissolved phase is considerably enriched in heavy Cu with respect to the particulate phase due to complexation with organic matter (Babcsányi et al., 2014; Petit et al., 2013; Vance et al., 2008). Hence our findings suggest that organic matter complexed Cu contributes to the mobile Cu fraction also in the deep horizon of the calcareous cambisols. Note that extracted Cu amounts to only 7 % of total Cu of the carbonated horizon and 4.5 % of the vertic horizon suggesting that this mobile Cu pool associated to organic matter is by no means the main speciation of Cu in those soils. This finding also shows that a higher percentage of Cu is rapidly extracted from the carbonated soil even though the vertic horizon contains more total Cu, excess Cu and more organic matter.

6.5.3 Copper mass balance of soils

High τ_{Ti} – values in topsoils and their decline with depth indicate that Cu is accumulated from the top. Especially in the vertic soils one would expect some loss of Cu due to weathering (Vance et al., 2016) but in fact we state an accumulation. This is not surprising for the topmost horizons as Cu is regularly sprayed as a fungicide on the plots, but the amount of transport to depth is higher than expected (Bigalke et al., 2010a; Duplay et al., 2014). The fact that excess Cu reaches down to 60 cm depth in all soils means that apparent transport rates in the studied soils are around 0.0092 m yr⁻¹ and thus 50 % higher than the apparent transport rate of 0.0066 m yr⁻¹ reported in literature (Bigalke et al., 2010a) even though the soil pH values are higher in our study.

These estimated rates are mean transport rates over the time of Cu application, the actual rate might vary with meteorological condition and soil depth, furthermore it might depend the quantity of Cu applied. Nevertheless these observations show that Cu can migrate in carbonated environments. In B2 where the vertic layer is located close to the surface depth τ_{Ti} – values are higher in the surface layers than in B1 and decline more abruptly with depth, indicating a bloc of depth transport in this soil. This is consistent with the finding that Cu is only slowly released in vertic horizons. In B2

70-90 cm horizon the τ_{Ti} – value is negative in the deepest soil horizons and coincide with lower g-values and higher inorganic carbon contents, reflecting an influence of carbonates. Thus the low τ_{Ti} – values might be due to a mixed influence between basaltic and calcareous rock material.

Sums of excess Cu are somewhat lower in B1 (481 kg ha⁻¹) than in B2 (576 kg ha⁻¹) but note that in B1 the 30-50 cm horizon was not measured. A conservative estimated would be to assume the same amount of excess Cu as in the underlying 50-70 cm horizon. This estimation of total excess Cu would increase the sum to 522 kg ha⁻¹, showing that those two soils contain a similar amount of excess Cu. A maximum estimate for total C2 excess Cu would be to attribute the excess Cu of the 0-10 cm horizon to the 10-20 cm horizon, as in all other soils the second horizon contain less excess Cu than the surface horizon. This gives a maximum estimate of 493 kg ha⁻¹ for C2. The actual amount of amended Cu might be higher due to losses from the soil through erosion making the calculated number a low estimate of an unknown total (Babcsányi et al., 2016; Ribolzi et al., 2002). The total of excess Cu in the ‘B’ catena amounts to a theoretical 87 – 96 years of nowadays maximum allowed Cu treatment (RCE No. 889/2008). Keeping in mind that the vineyard has been exploited for only 65 years and that treatments of the preceding years was only 2.5 kg ha⁻¹, it becomes clear that amounts applied in the early years must have been higher. We found literature that recommended Cu doses of up to 60 kg ha⁻¹ a⁻¹ (Viala and Ferrouillat, 1887) in vineyards. As treatment is the same for the two plots we expect at least 522 kg ha⁻¹ of Cu to be applied to each soil. Total excess Cu of both calcaric cambisols is lower than this amount even taking a maximum estimate for C2. In comparison with vertic soils Cu loss from carbonated soil can reach up to an estimated 48 %. This is much more than found by Babcsányi et al. (2016) and contradicts the thesis of relative immobility of Cu in carbonated environments (Duplay et al., 2014). If we compare the ratio of extracted Cu after 6 h (i.e. 6.8 % for C and 4.5 % for B) the mobility of Cu is higher in the “C” catena by a factor 1.5 than in the “B” catena. This value is in the same order of magnitude as the ratio of total excess Cu in the two soil where all horizons were measured (B1 and C1, ratio 1.9).

6.5.4 Mechanisms of Cu transfer and retention in soils

Geographically erosion should play a limited role in this study as grass cover is maintained around the year and ranks are planted perpendicular to the steepest slope. In the “C” catena low walls were built to form terraces minimizing the vineyard slope and in the field no erosion figures were found. Depth transfer might be particulate as Cu has been reported to accumulate in smallest particle size fractions (Babcsányi et al., 2016) but lighter isotope ratios in calcareous soils suggest that heavy Cu is lost

from those soils. As shown in section 6.5.2 extractable Cu is heavy but also in river and pore water studies heavy Cu is associated with the dissolved phase likely due to complexation with dissolved organic matter (Babcsányi et al., 2014; Kusonwiriawong et al., 2017; Petit et al., 2013; Vance et al., 2008). This implies that Cu loss from carbonated soils is mainly dissolved Cu. However we cannot exclude a contribution of organic colloids as those are reported to have essentially the same isotope ratio as dissolved Cu in organic matter complexes (Ilina et al., 2013). Lower isotope ratios in carbonated soils are also in line with precipitation of Cu-carbonates as a mechanisms of Cu-retention (Marechal and Sheppard, 2002; Ponizovsky et al., 2007). The EPR-study confirms this hypothesis: low g-factors in carbonated soils and carbonated horizons of vertic soils suggest this values to be associated to Cu-carbonates. This is supported by the g-factor shift to values of the vertic of the decarbonated horizon of catena “C”. The thermodynamic models shows that Cu-carbonates are oversaturated in our soils in absence of an organic ligand. However if an organic ligand as citrate with high affinity to Cu is added, Cu-carbonates become undersaturated (Figure 4). This is a possible process for remobilization of Cu carbonates in the studied soils as for example humic acid can have as high stability constants for Cu complexes as citrate (Tipping and Hurley, 1992). Thus Cu seems to migrate with organic matter via the soil solution in the calcaric cambisols some of which precipitates in form of Cu carbonates in lower soil horizons whereas the remaining heavy Cu fraction complexed with organic matter reaches the hydrosphere. This hypothesis is supported by isotopic differences and g-factor values. The organic matter might come from decay of plant material or agricultural amendments. Cu reprecipitation in lower soil horizons could be due to mineralization of the transporting organic matter.

RPE measurements suggest a difference in Cu speciation in the two soil types which implies that retention mechanism are different. In the vertic soils Cu migration is blocked by the vertic layer as illustrated by abruptly dropping τ_{Ti} – values and low extraction percentages. This might be due to adsorption of organic matter to clay surfaces. Thus organic matter bound Cu that migrates in calcareous soils would be retained in vertic horizons. Furthermore adsorption to clay surfaces increases Cu binding capacity of organic matter, that would explain the lower extractability of Cu in vertic horizons (Arias et al., 2002). A direct adsorption of Cu on clay surfaces is contradicted by the fact that clay adsorbed Cu tends to be isotopically light (Babcsányi et al., 2016; Li et al., 2015). Cu mobility might be initially similar between both soil types but that heavy organic matter bound Cu that is lost in calcaric cambisols is sorbed to vertic layers of the vertic soils. This is also coherent with heavier isotope ratios in vertic soils.

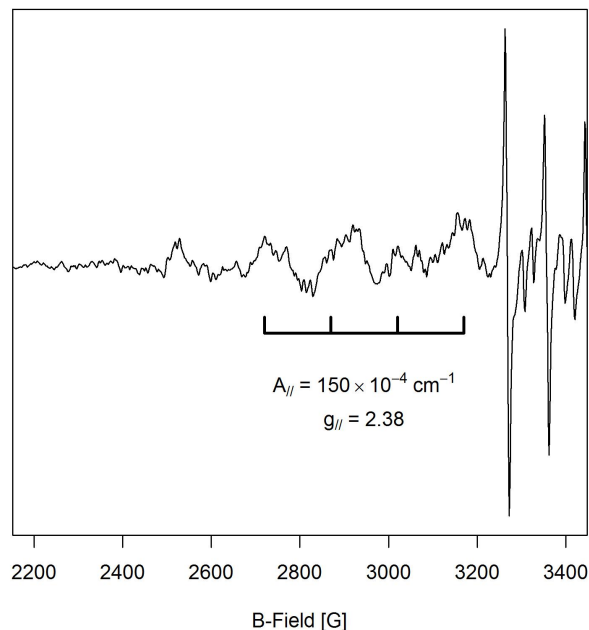


Figure 6.7 – Zoom on hyperfine interaction of Cu in the B1 0-10 EPR spectrum after subtracting a curve approximation of 15th order.

Hyperfine interaction (Figure 6.7) in EPR spectra of topsoil shows values ($A_{//} = 150 \times 10^{-4} \text{ cm}^{-1}$ and $g_{//} = 2.38$), these values are in the range of Cu – organic matter complexes (Valko et al., 1999) and soil particles (Flogeac et al., 2004) in literature. Similar values of $A_{//} = 139 \times 10^{-4} \text{ cm}^{-1}$ and $g_{//} = 2.34$ were formerly reported in literature for Cu-humic acid complexes (Davies et al., 1997). This is in line with a large body of literature showing that Cu is complexed with organic matter in topsoils (Boudesocque et al., 2007; Chaignon et al., 2003; Jacobson et al., 2007; Lejon et al., 2008; Pietrzak and McPhail, 2004; Strawn and Baker, 2008, 2009) as well as with the transport mechanisms proposed above.

6.6 Conclusion

In this case study we show that significant vertical transfer of Cu can occur even in calcareous soils. Apparent transfer rates can be as high as 0.0092 m yr^{-1} in the order of magnitude of acid soils. Isotope analysis show that isotopically heavy Cu is lost from calcareous soils. This is consistent with extraction results from kinetic extractions indicating that mobile Cu is heavy compared to bulk soils. Thermodynamic modelling shows that Cu carbonates can be remobilized by sufficiently strong organic ligands. It is suggested that Cu migrates in the dissolved phase complexed to organic matter in both soil types. Whereas Cu-organic matter complexes are retained by clay rich layers in vertisols, a part of the Cu is lost to the hydrosphere in carbonated soils. EPR

analysis show that in calcareous soils Cu carbonates are one of the species involved in Cu retention. Finally it is shown that large variations in Cu isotope signature occurs in different Cu based pesticides.

6.7 Acknowledgements

The authors would like to thank the French INSU national program EC2CO (BIO-EFFECT) for its financial help in the TERVIT program. We want to also express our gratitude to Filippo Filippi, the Italian vine grower and vine marker for providing us technical information, allowing us to collect samples in his vineyard and for his kindly and warm welcome in Castelcerino. Finally, we want to acknowledge the RENARD Platform for its help in EPR experimental set-up and data treatment.

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Chapter 7

Study of soil solution and Cu isotope fractionation for a better understanding of the rhizospheric mechanisms involved in the grapevine mineral nutrition in a greenhouse experiment

7.1 Abstract

After 150 years of pesticide use Cu, has become an ubiquitous pollutant in vineyard environments. Increasingly toxic effects on grapevine are reported. However Cu mobility in soils is complex and depends on many factors. Here we investigate Cu transfer between soil, soil solution and grapevine in a greenhouse experiment. Therefore *vitis vinifera* cv. Tannat on a 101.14 rootstock are planted on 6 different vineyard soils. Soil solution concentration and Cu isotopic ratios are followed over a growth period of 16 weeks. Subsequently plants are destructively harvested and also elemental contents and Cu-isotope ratios are measured. Cu mobility depends on the release of organic matter to the soil solution rather than total content of bulk soil. However solution contents of organic matter and various elements vary over time. Isotope fractionation between bulk soil and solution depends on soil type and only rarely change over time. Cu contamination appears to reduce plant production but is not the only factor involved. Plants accumulate Cu in the roots with increasing solution Cu content. Leaves do not show similar Cu contents independent of root or solution Cu content. The more Cu is contained in the root the more root Cu is isotopically heavy. The inverse trend is observed in the leaves the more Cu is contained in leaves the lighter is the leave Cu. Our results suggest that organic matter type and its binding in the soil are important factors for Cu mobility. Plants appear to modify the release of Cu to the soil solution over time. Finally isotope fractionation within plants seems to trace different mechanisms involved in Cu homestasis depending on contamination level.

7.2 Introduction

Copper is a redox active element that is both an essential nutrient for plants but in excess also a potentially toxic pollutant (Marschner and Marschner, 2012). In viticulture Cu based pesticides have been in use for more than 150 years and are still the only permitted treatment against downy mildew in organic viticulture. The long term treatment of vineyard soils led to increased Cu contents especially in topsoils (e.g. Chaignon et al., 2003; Duplay et al., 2014; Pietrzak and McPhail, 2004). Grapevine plants were longtime believed to tolerate Cu but recently reports of Cu toxicity are increasing (Anatole-Monnier, 2014; Toselli et al., 2009). Also when other cultures are grown on former vineyard soils issues of Cu toxicity are common place (Chaignon et al., 2003). However the mobility and toxic effects of Cu in soils does not exclusively depend on its total content but also on physico-chemical conditions and its speciation possibly controlling phytoavailability (Kabata-Pendias, 2004). Mobility of Cu in soil is reported to be controlled by different soil factors such as organic matter (e.g. Komárek et al., 2010; Ren et al., 2015; Sauvé et al., 1998), clay minerals (Babcsányi et al., 2016), carbonates (Ponizovsky et al., 2007) or Fe-oxyhydroxydes (Bradl, 2004; Sayen and Guillon, 2010). Furthermore Cu in soils is affected by an aging effect making older Cu pools less bioavailable than freshly introduced Cu (Arias-Estevéz et al., 2007; Ma et al., 2006; Sayen et al., 2009). This makes Cu mobility complicated to predict especially as different mechanisms can counteract each other as shown in **Chapter 6**.

Cu mobility in soils is commonly assessed using either single step or sequential extractions (e.g. Chaignon et al., 2003; El Azzi et al., 2013). However it was argued that these techniques only give a flashlight of bioavailability not taking into account the kinetics of restoration of a mobile pool nor the quantity that can be renewed by the soil stock (Zhang et al., 2001). Furthermore plants modify physico-chemical conditions in the rhizosphere in order to satisfy their needs in nutrient uptake but also to avoid toxicity (Bravin et al., 2009, 2012; Hinsinger, 2001; Hinsinger et al., 2003; Marschner and Marschner, 2012). One would thus expect changes in bioavailability of elements over time, especially in soils under perennial plants with relatively high biomass.

Also, toxicity of Cu towards plants has been reported to depend on various soil properties but not mainly on total concentration (Ruyters et al., 2013). Higher toxic effects in grapevine plants were observed if sand was mixed to contaminated soils (Toselli et al., 2009). High Ca availability was reported to increase Cu toxicity whereas Na and K showed no effect and Mg decreased toxicity (Juang et al., 2014; Lock et al., 2007). To be taken up by plants elements need to be in ionic form or complexed to low molecular weight organic substances and be diffused through pores of the cell wall (Marschner

and Marschner, 2012). Concentration in soil solution appears to be the best precursor for this process even though elements present in larger complexes might not pass cell wall pores and representative sampling of soil solution is still matter of discussion (Coutelot et al., 2014; Marschner and Marschner, 2012; Reynolds et al., 2004). Furthermore a measurement of soil solution over time gives the possibility to look at the evolution of certain elements as plants might enhance or lower their solubility but also soil stocks might be depleted by plant uptake (Hinsinger, 1998; Zhang et al., 2001).

As copper has two stable isotopes, ^{65}Cu and ^{63}Cu variations in their relative occurrence can be used to further constrain sources and mechanisms of Cu transfers. Due to their difference in mass, both isotopes react slightly different during chemical reactions and variations in the ratio between heavy and light Cu are observed. Experimental values for reactions controlling Cu mobility in soils are available in literature: the adsorption on (oxy-)hydroxides favors heavy Cu isotopes (Balistrieri et al., 2008; Clayton et al., 2005; Pokrovsky et al., 2008), as well as the complexation with organic ligands (Bigalke et al., 2010a; Ryan et al., 2014). In contrast the adsorption of Cu on clay minerals was evaluated to favor light Cu isotopes (Babcsányi et al., 2016; Li et al., 2015). In real soil settings mobile Cu from polluted soils was evaluated as heavy through extraction procedures (Li et al., 2016). A light isotope enrichment of the soil with respect to pore water in an agricultural but not strongly polluted soil in France suggests that heavy copper is more mobile also in close to natural settings (Kusonwiriawong et al., 2017). This is consistent with global values of French soils where unpolluted soils are depleted in heavy Cu isotopes and data from Scottish and Hawaiian soils showing that soil layers weathered under oxic conditions are depleted in heavy Cu (Fekiacova et al., 2015; Vance et al., 2016). These observations underline the potential of Cu isotope ratios as possible tracers of biogeochemical mechanisms in soils.

Also Cu isotope analysis has been a powerful tool to identify pathways of Cu uptake and translocation in plants. The founding study of copper isotopes in plants investigated leaves, stems and soil from Virginia wild rye, lentil seeds germinated or not and hairy-leaved sedge (Weinstein et al., 2011). This study concluded that (a) lentil germs only grown on nutrients present in the seeds accumulated light Cu in the shoots and heavy Cu in the seeds, and (b) that isotope ratios in leaves depend on height (the higher the leave the lighter the Cu) and duration of growth period (Weinstein et al., 2011). The following studies of Cu isotopes in plants were carried out in hydroponic growth (Jouvin et al., 2012; Ryan et al., 2013). Jouvin and coworkers report for tomato that root isotopic ratios are always lighter than nutritive solution, in their study a sufficient amount of Cu was supplied in form of organic complexes at $\text{pH} \approx 6$. They assign this light fractionation to a preferential passive uptake of free Cu^{2+} expected to be lighter than complexed

Cu and to a reduction step necessary for specific uptake (Ehrlich et al., 2004; Zhu et al., 2002). For root to shoot transfer, as translocation process, they reported that for some modalities leaves were lighter than roots after 42 days of growth whereas others were in the same range (Jouvin et al., 2012). The light isotope fractionation during uptake was confirmed by a study of Cu isotopes in tomatoes (Ryan et al., 2013). However this study found that tomato leaves are enriched in heavy isotopes with respect to the roots. This observation was attributed to a superior technique used to desorb Cu from the root apoplast (Ryan et al., 2013). However, in their study a higher Cu supply was given and uptake mechanisms might be concentration specific (Ryan et al., 2013). Moreover Cu was supplied in an acid solution and main species was free Cu^{2+} , so that open questions remain on the influence of speciation and pollution on Cu isotope fractioning in plants. The most recent study on Cu isotopes in plants investigated Cu fractionation in Cu tolerant strategy I plant *Elsholtzia splendens* in moderately contaminated soils under different treatments. Isotope fractionation between roots and mildly polluted soil was confirmed to favor light isotopes. Their untreated data showed light to neutral isotope fractionation between roots and leaves for pot grown plants and clearly light isotope fractionation for young leaves in field grown plants, but they estimated root adsorbed Cu to claim that leaves are actually heavy with respect to roots (Li et al., 2016). In conclusion the discussion on Cu isotope fractionation during uptake and translocation in plants is still ongoing.

From this literature review it becomes clear that Cu transfer in vineyard soils is complex in both soils and plants. Most studies evaluating Cu influence on plants have been reduced complexity through hydroponic growth. In this study we approach real world condition one step further by introducing various soil types to identify main factors and mechanisms controlling Cu mobility in soils and its availability to plants. Namely we measure Cu concentrations in soils, soil solutions, roots and leaves. We couple these measurements with measurements of physico-chemical conditions in the soil solution and concentrations of plant nutrients to evaluate other impacts on plant functioning than copper stress. Soil solutions are sampled in two weeks intervals to observe the influence of plant growth on its compositions. Furthermore we use stable Cu isotopic ratios to shed light on transport mechanisms involved and use fatty acid ratios to estimate metal stress on plants.

7.3 Materials and Methods

7.3.1 Experimental Setting

Six different vineyard soils showing different total Cu content were sampled for this study. Vineyard soils came from «Bordeaux» (denoted CO, HBN and OB; n=3) and St. Mont (denoted STM; n=1) regions in France and Soave in Italy (denoted CI and VI; n=2) (Anatole-Monnier, 2014, **Chapter 5**). About 100 kg of each soil were sampled from the first 20 cm. All soils, except OB, have been under viticulture for more than 50 years. The OB soil was in viticulture for only 4 years before sampling.

Pedological, agricultural, mineralogical and physico-chemical properties were previously determined for each studied soil and reported in Table 7.1. These soils differed in various properties: mineralogy, viticultural practice (organic or conventional) and physico-chemical parameters (as reported in Table 7.1), and also in their Cu treatment history.

Table 7.1 – *Pedological, mineralogical and physico-chemical properties of vineyard soils in the greenhouse experiment.*

Soil ID	Region	Culture	Soil type	Mineral phases	pH	CEC	TIC	SOC	Cu
						cmol kg ⁻¹	% wt	% wt	mg kg ⁻¹
STM	St. Mont	Con.	Stagnosol	Quartz	6.6	5.5	0.1	0.1	10
VI	Soave	Org.	V. Cambisol	Fels., Smec., Qtz.	7.7	59.8	0.4	2.5	229
CI	Soave	Org.	C. Cambisol	Calc., Fels., Smec.	7.8	49.9	5.1	2.1	214
OB	Bordeaux	Con.	Fluvisol	Quartz	7.6	3.8	0.1	0.5	3
HBN	Bordeaux	Con.	Fluvisol	Quartz	7.4	7.7	0.1	1.3	251
CO	Bordeaux	Con.	Fluvisol	Quartz	7.2	5.2	0.1	0.6	115

Notes: *Con.* = Conventional Agriculture, *Org.* = Organic Agriculture; *V. Cambisol* = Vertic Cambisol, *C. Cambisol* = Calcaric Cambisol; *calc.*=calcite, *felds.*= feldspar, *smec.*= smectite

Soils were sieved to 2 mm, and plants potted in 5 L pots with 5 replicates per soil. In each pot, one grafted grapevine (*Vitis vinifera* cv. Tannat grafted on rootstock *V. riparia* x *V. rupestris* cv. 101.14) was planted per pot. A microporous cup (RHIZON MOM 10 cm, Rhizosphere Research Products) was inserted in each pot. After planting, pots were saturated with water and placed on heated ground until bud break (5 d). Then pots were placed in a greenhouse at the ISPA laboratory (INRA Institute, Bordeaux) under artificial lightning miming 12 h of daily sunshine (Figure 7.1). Plants were regularly watered using demineralized water to obtain a soil moisture corresponding to 80 % of the water holding capacity of each soil.



Figure 7.1 – *Experimental setting in the greenhouse experiment.*

7.3.2 Sampling of soil solution and plant tissues

Beginning from one week after potting, soil solutions were sampled using RHIZON samplers every two weeks. Total Organic Carbon and Total Inorganic Carbon (respectively TOC and TIC), NO_3^- , SO_4^{2-} and Cl^- concentrations as well as pH were directly measured in soil solutions. Leftover solutions were acidified with ultrapure nitric acid to 2 % (v/v) and later analyzed for elemental contents.

Plants were cultivated for 16 weeks before three healthy plants per soil modality were destructively harvested. At harvest, leaves were cut using ceramic knives. They were separated in two samples: the first called “young leaves” and included the first 5 healthy leaves of every branch counting from the top, and the second one leftover leaves starting from the sixth that were put together with brown leaves and leaves showing signs of chlorosis. Elemental and isotope analyses were performed only on the first ‘young leaves’ sample whereas all leaves were considered for biomass weight. Field grown leaf samples were collected from the two catenas in the Soave vineyard introduced in **Chapter 5**. Samples were collected at approximately 150 cm height above ground from five plants surrounding one soil sampling spot of the catena at harvest time 2015. Note that rootstock (420a) and cultivar (*Garganega*) of the field samples differ from pot grown plants.

Roots were extracted from soil and then washed under flowing demineralized water. When no more soil particles were visible, the roots were cut from the trunk and put into sample bags. Root and leaf samples were then washed three times. After a first step using demineralized water, the next two washing steps were performed in ultrapure water (18.2 M Ω). All samples were frozen and freeze-dried. Once dried, the biomass was expressed for each plant tissue in dry weight (g, DW), and leaf and root samples were ground to powder using a planetary mill with Zr-containers and balls. Soils were air-dried and powdered in a planetary agate mill by SIEBTECHNIK.

7.3.3 Digestion and total elemental contents determination

Total elemental contents were determined in soil and plant samples after an acidic mineralization step. For elemental analysis, 100 mg of each ground soil sample was digested in a CEM MARS 5 microwave oven using ultrapure acids (9 mL HNO₃ : 2 mL HCl : 3 mL HF). For each digestion run, an experimental blank and a standard were run. Standard for soil digestions was the BCR-2 basalt standard with Cu recovery of 96 ± 10 % (n=3) and for plant samples the apple leaves standard (SRM1515) with Cu recovery of 92 ± 5 %.

Plant samples were digested on hotplates in three steps. For each plant sample, 200 mg of powder were weight into Savillex Teflon vessels. Then 1 mL of ultrapure H₂O₂ was added and left to react for 2 h. Subsequently 5 mL of double subboiled HNO₃ were added in 1 mL steps to each vessel and left to react overnight. Then the vessels were heated to 120 °C for at least 4 h and evaporated to dryness at 90 °C. Once dried, 4 mL of double subboiled HCl and 2 mL of double subboiled HNO₃ were added along with 1 mL of suprapure HF. Again vessels were heated to 120 °C for at least 4 h and evaporated to dryness at 90 °C. A final digestion step was performed using 5 mL HNO₃ and the same heating protocol as before. All digested samples were then redissolved in 20 % (v/v) HNO₃ and diluted ten times in ultrapure water (18.2 MΩ) before analysis.

Total element contents were measured in soil and plant mineralized samples with an Inductively Coupled Plasma Mass Spectrometry (Agilent 7500ce ICP-MS). Soil solutions were directly analyzed by ICP-MS, after tenfold dilution and acidification with ultrapure nitric acid to 2 % (v/v). Quality and measurement traceability were ensured by measuring replicates of SLRS-5 river water standard (n=6). Relative standard deviations (RSD %) of each measurement were ≤ 5 %. Recovery of SLRS5 standard in alkaline earth elements were 113 ± 4 % for Ca and 97 ± 6 % for Mg. Transition metal recovery from SLRS5 was 96 ± 4 % for Fe and 100 ± 5 % for Cu. Recovery of K was 94 ± 5 % and recovery of As was 89 ± 4 %.

7.3.4 Isotope analyses of different matrices

To reach 500 ng necessary for isotope measurements, solutions of STM and VI and the late sampling points of OB were pooled. For VI sample, the recovered soil solution was pooled for all replicates at the 4, 8 and 12 weeks' time steps. For STM soils, all recovered soil solutions of the 4 weeks' time step were pooled and for a second sample all recovered solutions from 12, 14 and 16 weeks' time step. However solution yields in 14 and 16 time step were relatively low so that the second STM sample was marked after 12 weeks (as reported in Figure 3). To obtain the 12 weeks sample of the shown

OB replicate, 12, 14 and 16 weeks' time step were pooled for this one replicate. Aliquots of soil solutions were digested in three steps as described above for plant samples.

For isotope analyses, aliquots of digested samples containing 500 ng of Cu were purified using anionic AG MP-1 resin. Method development is described in detail in Appendix C. Separations were carried out twice per soil sample using a protocol adapted from Maréchal et al. (1999), previously described in **Chapter 6**. For soil and BCR-2 samples as well as STM and OB root and all leaf samples, purification was carried out as described for soil samples in **Chapter 6**. VI, CI, HBN and CO roots were purified using the second soil purification step described in **Chapter 6**. For STM, VI, CI and OB soil solutions purification using the second soil purification step were carried out twice, whereas for HBN and CO samples a simple purification step was sufficient to yield same results as double purification.

Recovery of purification protocol was checked to be 100 ± 5 % and BCR-2 standards were run to assure result quality. Measured isotopic ratios of BCR-2 are reported in Table 7.4, reference material was digested three times and four different purification runs were performed allowing 10 measurement runs. Our measured values (0.26 ± 0.09 ‰) are slightly heavier than values formerly published 0.20 ± 0.10 ‰ (Babcsányi et al., 2014) and 0.22 ± 0.05 ‰ (Bigalke et al., 2010b) but within the 2 SD range of those results. Purified solutions were spiked with IRMM Zn standard for measurement on a MC-ICP-MS (Nu Plasma 500, Nu Instruments) and a MC-ICP-MS (Neptune plus, Thermo Finnigan) respectively at ENS Lyon and GET Toulouse. Exponential laws were used to correct for mass bias using 66/64, 68/64 and 68/66 Zn isotope ratios. Cu isotope ratios are expressed in ‰ relative to NIST 976 Cu standard (eq.1).

$$\delta^{65}\text{Cu} = \left(\frac{(^{65}\text{Cu}/^{63}\text{Cu})_{\text{Sample}}}{(^{65}\text{Cu}/^{63}\text{Cu})_{\text{NIST946}}} - 1 \right) * 1000$$

7.3.5 Thermodynamic Modelling

Speciation of Cu in the different soil solutions was modeled using Minteq software package version 3.1. Data used for modelling were mean concentrations of all replicates of cation analysis using the 8, 12 and 14 weeks' time steps per modality. Elements entered to the model were the major cations: Na, Mg, K, Ca; the metals: Al, Mn, Fe, Co, Ni, Cu, Zn and Pb; and the anions : P, As, Mo. This data was combined to mean anionic analysis of NO_3^- , SO_4^{2-} and Cl^- and median DOC, DIC and pH values of all samples available per modality. Organic matter contents were entered using a Gaussian model and a Nica-Donnan model using default parameters (in witch 80 % of humic

substances are considered as fulvic acid and ratio DOM to DOC is 1.65). Base on this data, solution speciation of Cu and saturation of malachite and Fe-oxyhydroxydes were modeled.

7.3.6 Omega-3 biomarker

The ratio of saturated and unsaturated fatty acids containing 18 carbon chain were proposed as indicator of metal contamination called Omega-3 biomarker (Le Guédard et al., 2012). In case of metal contamination, the relative content of unsaturated fatty acids declines due to oxidative stress (Le Guédard et al., 2012). Ratios between triple unsaturated fatty acids with 18 carbon atoms to other fatty acids with 18 carbon atoms were measured in leave samples: a small piece of the leaf was cut directly from the greenhouse plants, just before harvest. A sample consists of around 1 cm² taken from leaves at the soil sampling spot and directly put into a methanol and H₂SO₄ solution for conservation. Samples were then send to LEB Bordeaux for GC-MS analysis as described in Le Guédard et al. (2012).

7.4 Results

7.4.1 Plant biomass

Total leaf biomass differed significantly between different modalities (Figure 7.3). The highest leave biomass was recovered from CI and VI grown plants with 32 to 40 g dry mass and 31 to 37 g dry mass, respectively. HBN soils yielded intermediate biomasses between 26 and 30 g dry mass. Lower leave biomasses were recovered from STM and OB soils between 21 and 25 g. The lowest leave biomass was harvested from CO soils with 11 and 14 g for the two lighter replicates, however one replicate yielded 24 g of leaf biomass. Root biomass varied less than leaf biomass with again largest amounts harvested in CI and VI with 14 to 21 g dry weight followed by CO and HBN grown plant with 11 to 17 g root biomass. The least biomass was recovered from OB and STM soils with 10 to 12 g.

7.4.2 Omega-3 biomarker in plant leaves

Highest mean biomarker values were detected plants grown on STM and HBN soil (1.27 ± 0.17 and 1.27 ± 0.33 , respectively). Lowest values were measured VI (0.55 ± 0.17) and CI (0.55 ± 0.09) grown plants. Plants grown on OB and CO soils had intermediary values with 1.09 ± 0.44 on OB soils and 1.10 ± 0.26 on CO soils.

7.4.3 Elemental contents in soil solution

Main nutrient contents (P and NO_3^-) in soil solutions are shown in Figure 7.3. Variations over time were large for nitrate concentrations in solution (Figure 7.3), but still there is a trend for higher concentrations in CI, OB and HBN soils and lower concentrations in STM soil. VI and CO soils show somewhat intermediate NO_3^- concentrations. Phosphorus contents were higher in OB, HBN and CO soil solutions from Bordeaux area than in STM, CI and VI soil solutions. VI and STM P concentrations ranged between 0.5 and 1.2 mg L^{-1} in soil solution. P concentration in CI soil was a little lower: between 0.4 and 0.9 mg L^{-1} . However some variations over time and between replicates occurred. Phosphorus concentration in OB and CO soils was much higher, between 3.4 and 7.7 mg L^{-1} . P concentrations in HBN were intermediate with 1.6 to 4.7 mg L^{-1} . K concentrations also higher in soil solutions from Bordeaux soil. Highest concentrations are found in CO soils with up to 86 mg L^{-1} . Lowest values in Bordeaux soil solutions are from OB. Values are around 20 mg L^{-1} in samples taken at the end of the experience. K concentrations in CI soil solutions are in the same concentration range between 20 and 30 mg L^{-1} . Soil solution samples from STM and VI soils contain between 10 and 20 mg L^{-1} of K. Also Mg concentrations are also higher in solutions from Bordeaux soils (Figure 7.4). However concentrations fluctuate over time. Lowest values are often found in the end of the experience. Mg concentrations in Bordeaux soil solutions are between 6 and 86 mg L^{-1} . In CI soil solutions Mg contents are between 13 and 24 mg L^{-1} and thus somewhat higher than in VI and STM modalities with 3 to 12 mg L^{-1} . Ca solution contents are highest in the CI modality with 238 to 318 mg L^{-1} , followed by the other Italian soil VI containing between 128 and 142 mg L^{-1} Ca (Figure 7.4). Bordeaux soils and STM show about the same Ca solution content between 20 and 76 mg L^{-1} .

7.4.4 Cu concentrations in soils and soil solutions

Cu contents in soils are reported in Table 7.4. Soils contained between 3 and 251 mg kg^{-1} of Cu. These values range from lower than average European background concentration (about 14 mg kg^{-1}) to contamination typical for vineyard soils (Chaignon et al., 2003; Lado et al., 2008). The lowest concentrations were found in OB and STM with respectively 3 and 10 mg kg^{-1} , followed by 115 mg kg^{-1} in the CO soil. The highest Cu concentrations were measured in CI, VI and HBN soils (214, 229, 251 mg kg^{-1} , respectively).

For soil solutions, results are reported in (Figure 7.4 and highly variable Cu contents were measured. Mean Cu concentrations in solutions are also noted in Table 7.4 and time variations are discussed later. The lowest mean concentrations of Cu were mea-

sured in solutions taken from STM soils ($13 \mu\text{g L}^{-1}$), followed by CI and VI with 37 and $76 \mu\text{L}^{-1}$. The highest mean Cu concentrations were measured in soils from Bordeaux with 358, 890 and $2509 \mu\text{g L}^{-1}$ on average.

Contents of As are highest in Bordeaux soils between around 20 to $250 \mu\text{g L}^{-1}$ (Figure 7.4). Highest values are found in CO modality whereas lowest values of Bordeaux soils are found in OB modality. STM soil solutions contain between 2 and $9 \mu\text{g L}^{-1}$ and CI and VI soil solutions contain around $2 \mu\text{g L}^{-1}$ of As.

7.4.5 Evolution of elemental concentrations in soil solution over time

In Figure 7.2, concentrations of the transition metals Cu and Fe, the major nutrients P, Mg, K and Ca as well as As in soil solutions are shown over time for one replicate in each soil type. The concentrations are normalized to the first considered soil solution sample 4 weeks after plantation to be able to compare evolutions in different modalities. Measured values of these samples are shown in Table 7.2, values are commented for each soil modality.

STM soil. Soil solution of STM soils become slightly more alkaline over time, with the initial pH value of 6.4 going up to 7. DOC contents of STM solutions appear to increase slightly from 18.5 mg L^{-1} to about 30.9 mg L^{-1} . The Ca concentration is constant around 40 mg L^{-1} until the 10 weeks sample to then slightly decline to 29 mg L^{-1} . P concentrations fluctuate around 1 mg L^{-1} to slightly decline in the last two steps. Mg and K concentrations also decline over time from 11 to 2 mg L^{-1} and 20 to 10 mg L^{-1} respectively. Cu concentration declines from 12.3 to $7.2 \mu\text{g L}^{-1}$ between 4 and 6 weeks to then slowly reincrease to about $10 \mu\text{g L}^{-1}$. Fe and As have lower concentration than in the initial samples after 6 and 8 weeks but steeply increase in the 10 weeks' sample to fall back values lower than in the initial in the next sample. Values of As and Fe remain relatively stable at about 80 % of the initial values until the end of the experience.

VI soil. In VI soil solutions, pH and DOC values are relatively constant with values around pH 8 and 5 mg L^{-1} DOC. Concentrations Mg, P, K, Ca and As also remain constant around the initial value over time. Only in the 14 weeks sample there is an increase of about 40 % in Ca and Mg. Cu concentrations decrease slightly from $38.2 \mu\text{g L}^{-1}$ to $22.6 \mu\text{g L}^{-1}$ after 12 weeks. Iron concentrations are constant over time with values around stable values around $43 \mu\text{g L}^{-1}$. Only in the ten weeks sample value quadruplet to $168.7 \mu\text{g L}^{-1}$. As concentrations in solution scatter around $2 \mu\text{g L}^{-1}$.

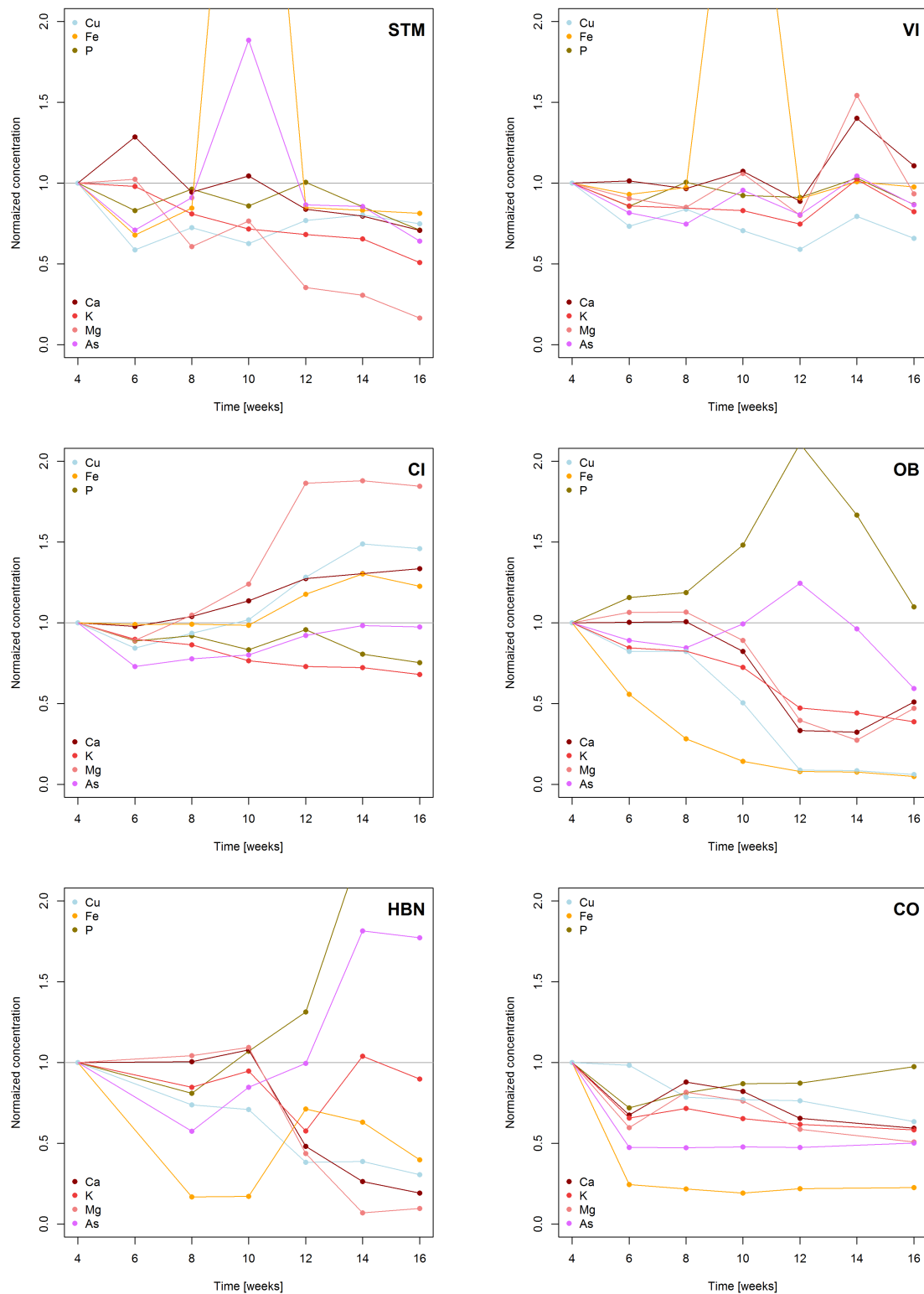


Figure 7.2 – Evolution over time of concentrations of metals and metalloids (Cu, Ni, Fe, As), man nutrients (P and K) and alkaline earth elements (Mg and Ca) in the soil solutions of the different investigated vineyard soils (STM, VI, CI, OB, HBN and CO)

CI soil. For the CI soil, an alkalization of the soil solution is visible with pH values increasing from 7.5 to 8.0. Also DOC content increases over time from around 77 mg L⁻¹ to 118.5 mg L⁻¹. In the CI soil solution also Ca and Mg concentrations increase over time. The increase is much higher for Mg which increases from 13 to 24 mg L⁻¹, whereas Ca contents increase from 238 to 318 mg L⁻¹. Cu and Fe concentrations follow a similar trend with constant values until the 10 weeks sample but then increase as well up to about 25 % more than the initial value. Cu concentrations at first decline slightly from 99.7 µg L⁻¹ to 84.2 µg L⁻¹ but then reincrease steadily until the end of the experiment reaching 145.4 µg L⁻¹. As concentrations vary slightly between 2.5 and 18. µg L⁻¹ during the experiment. P and K contents slightly decline over time. P concentrations are between 0.79 mg L⁻¹ and 0.60 mg L⁻¹ and K concentrations between 31 and 21 mg L⁻¹.

OB soil. In the OB solutions, pH values also increase during the experiment from 7.7 in the beginning to 8.2 at the end. An inverse trend is observed for DOC content with values around 110 mg L⁻¹ until 10 week of experiment and a sharp drop to about half the values in later samples. P concentrations start with 3.89 mg L⁻¹ much higher than in all former samples. P concentration increase over time to reach more than double the initial values (8.21 mg L⁻¹) after twelve weeks to then fall back close to initial contents (4.28 mg L⁻¹) at the end of the experiment. As concentrations are also much higher than in former soil and mostly follow the P evolution showing a peak after twelve weeks (29.0 µg L⁻¹) and a clear decrease in the last samples (22.4 and 13.9 µg L⁻¹). Transition metal concentrations continuously decline from the first sample. The decline of Fe is the steepest starting from 1287 µg L⁻¹ and decreasing by a factor almost 20 to 65.2 µg L⁻¹ in the last sample. Cu follows this trend but the decline is more progressive from 283.8 to 17.6 µg L⁻¹. Ca and Mg concentration remain constant until 8 weeks' time and then decline sharply to about third of their initial concentration of the initial value (63 mg L⁻¹ for Ca and 18 mg L⁻¹ for Mg) in the sample after 12 weeks. Their concentrations remain more or less constant until the end of the experiment. K contents also decline over time from 54 to 21 mg L⁻¹ but the decline is more progressive than for Ca and Mg.

HBN soil. In HBN soil solution, no clear trend is visible for pH or DOC contents. DOC values fluctuate from 31.9 mg L⁻¹ after 8 weeks to 131.5 mg L⁻¹ after 10 weeks back to 34.1 mg L⁻¹ after twelve weeks. Fe concentrations are highest in the first sample with 368.4 µg L⁻¹ in the next two samples values drop to around 60 µg L⁻¹ to reincrease to 262.4 µg L⁻¹ after 12 weeks followed by a steady decline to 146.9 µg L⁻¹ in the final sample. Cu, Ca and Mg concentrations decline over time. Cu concentrations decline to about two thirds of the initial values after 8 weeks' time (i.e. 1376.9 to 1015.8 µg L⁻¹

for Cu). Cu concentrations then decrease steadily from $1376.9 \mu\text{g L}^{-1}$ to reach $423.2 \mu\text{g L}^{-1}$ in end of the experience. Ca and Mg values remain constant until 10 weeks' time (around 65 mg L^{-1} and 60 mg L^{-1} , respectively) and then drop sharply decline to reach 12 mg L^{-1} and 6 mg L^{-1} in the final sample. K concentrations fluctuate around 70 mg L^{-1} a significantly lower value of 42 mg L^{-1} after 12 weeks' time. The only elements with increasing concentrations at the end of the experience are P and As. Both elements are less concentrated in the 8 weeks solutions (20 % less for P and 40 % less for As) to then increase until 14 weeks' time. P concentrations more than double from initial 1.98 mg L^{-1} to final 4.32 mg L^{-1} . As concentrations start from initial $67.9 \mu\text{g L}^{-1}$ than go down to $39.1 \mu\text{g L}^{-1}$ after 8 weeks to reach $120.4 \mu\text{g L}^{-1}$ in the final sample.

CO soil. Solution of CO soil is the only one showing a trend for acidification over time starting from pH 8.2 and reaching 7.6 after 12 weeks' time. Organic carbon contents measured scatter but show a trend for lower values at the end of the experiment. All element contents but Cu decline between the 4 and 6 weeks sample to remain about constant until the end of the experiment. Fe concentration show the strongest decrease from 409 to $101 \mu\text{g L}^{-1}$. Mg and Ca declines are relatively small with 67 to 40 mg L^{-1} and 71 to 48 mg L^{-1} respectively. Cu contents also decline but the decline is more progressive with concentrations between 1595.2 and $1011.3 \mu\text{g L}^{-1}$.

7.4.6 Cu isotopic ratios in soils and soil solutions

Mean isotopic ratios are displayed in Table 7.4. Soil isotopic ratios are between $0.02 \pm 0.09 \text{ ‰}$ in CO soils and $0.33 \pm 0.01 \text{ ‰}$ in VI. Intermediate values are found in CI, OB and HBN with 0.21 ± 0.04 , 0.27 ± 0.24 and $0.21 \pm 0.04 \text{ ‰}$, respectively. Cu isotope ratios of uncontaminated Cu soil was $0.17 \pm 0.11 \text{ ‰}$. Mean soil solution values are also highest in VI with $0.69 \pm 0.46 \text{ ‰}$, followed by CI and STM with 0.58 ± 0.11 and $0.56 \pm 0.14 \text{ ‰}$, respectively. The lightest mean isotopic ratios in soil solutions are found in soils from Bordeaux: $0.21 \pm 0.10 \text{ ‰}$ in OB, $0.12 \pm 0.09 \text{ ‰}$ in HBN and $0.02 \pm 0.20 \text{ ‰}$ in CO. The evolution over time of Cu isotopic ratios in soil solutions with respect to soil isotope ratios of Cu is reported in Figure 7.7. For STM and VI, these values are pooled over all replicates. Isotope ratios in STM, VI and CI soil solutions are clearly heavier than bulk soils (around $+0.4 \text{ ‰}$). The observed values are close to the value reported in **Chapter 6** for citrate extractions. However for VI, isotope ratios are significantly higher : $0.63 \pm 0.03 \text{ ‰}$ in the first sample and then decline over time until $0.21 \pm 0.10 \text{ ‰}$. Also CO and HBN isotope ratios are heavier in the soil solution sample taken after 4 weeks' time ($0.15 \pm 0.09 \text{ ‰}$ and $0.29 \pm 0.09 \text{ ‰}$ respectively) and then decline in the same way around bulk soil values. OB samples slightly vary over time but stay close to bulk soil isotope ratios.

Table 7.2 – *Evolution of elemental concentrations in soil solutions*

Soil	Time	pH	TOC	TIC	P	Mg	K	Ca	Fe	Cu	As
			mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	µg L ⁻¹	µg L ⁻¹	µg L ⁻¹
STM	4	6.41	-	-	0.98	11	20	41	60	12.3	4.4
STM	6	6.38	18.5	2.9	0.81	12	19	53	41	7.2	3.1
STM	8	-	-	-	0.94	7	16	39	51	8.9	4.0
STM	10	6.92	57.1	7.4	0.84	9	14	43	293	7.7	8.3
STM	12	6.44	32.5	4.4	0.98	4	13	34	51	9.5	3.8
STM	14	6.97	30.9	4.5	0.83	3	13	33	50	9.9	3.8
STM	16	-	-	-	0.69	2	10	29	49	9.2	2.8
VI	4	7.91	-	-	1.03	7	16	128	44	38.2	2.5
VI	6	8.01	-	-	0.89	7	14	130	41	28.0	2.1
VI	8	-	50.9	4.2	1.04	6	14	124	43	32.1	1.9
VI	10	8.01	58.3	7.7	0.95	8	13	137	169	27.0	2.4
VI	12	7.98	48.0	6.5	0.94	6	12	114	40	22.6	2.0
VI	14	7.86	42.2	8.5	1.07	11	17	179	45	30.4	2.7
VI	16	-	-	-	0.9	7	13	142	43	25.2	2.2
CI	4	7.51	-	-	0.79	13	31	238	49	99.7	2.5
CI	6	7.83	76.8	15.5	0.70	12	28	233	49	84.2	1.8
CI	8	-	96.3	20.3	0.73	14	27	248	49	93.3	1.9
CI	10	7.54	98.5	7.7	0.66	16	24	271	48	101.5	2.0
CI	12	7.76	107.8	14.5	0.76	25	23	304	58	127.9	2.3
CI	14	8.01	118.5	10.1	0.64	25	23	311	64	148.4	2.4
CI	16	-	-	-	0.60	24	21	318	60	145.4	2.4
OB	4	7.72	-	-	3.89	46	54	63	1287	283.8	23.3
OB	6	7.79	118.7	8.2	4.50	49	45	63	718	233.7	20.8
OB	8	-	104.3	15.2	4.62	49	44	63	364	233.8	19.7
OB	10	7.87	112.8	12.5	5.77	41	39	52	185	143.5	23.2
OB	12	8.16	55.9	10.2	8.21	18	25	21	104	25.5	29.0
OB	14	8.15	46.0	11.5	6.49	13	24	20	100	24.3	22.4
OB	16	-	-	-	4.28	22	21	32	65	17.6	13.9
HBN	4	7.66	-	-	1.98	57	72	65	368	1376.9	67.9
HBN	8	-	31.9	39.2	1.60	59	61	65	62	1015.8	39.1
HBN	10	7.60	131.5	8.0	2.12	62	68	70	64	976.5	57.6
HBN	12	7.74	34.1	32.1	2.60	25	42	31	262	528.6	67.6
HBN	14	-	-	-	4.65	4	75	17	233	535.4	123.3
HBN	16	-	-	-	4.32	6	65	12	147	423.2	120.4
CO	4	8.19	-	-	4.73	67	86	71	409	1595.2	244.0
CO	6	7.65	83.2	13.6	3.40	40	56	48	101	1568.0	115.8
CO	8	-	105.3	4.0	3.85	55	61	62	89	1252.8	115.7
CO	10	-	33.9	67.8	4.12	51	56	58	79	1229.7	116.8
CO	12	7.62	60.1	7	4.13	39	53	46	90	1219.2	115.7
CO	16	-	-	-	4.61	34	50	42	93	1011.3	122.4

7.4.7 Cu speciation modelling in soil solution

In the Gaussian DOM model, concentrations of different Cu species calculated follow the same increasing trend from STM to CO as total concentrations. Only organic matter bound Cu is more abundant in STM than in VI. However in all samples except STM CuCO_3 is a major species in soil solution. In CI and VI soils from Italy, CuCO_3 in solution is even more abundant than organic matter bound Cu. Note that in all solutions except STM are oversaturated in Cu-carbonated species. Saturation indexes in VI and CI are 0.32 and 0.61, respectively. In Bordeaux soils, oversaturation is stronger with saturation indices for OB 1.86, HBN 2.42 and CO 3.33. Also cupric Fe species, Fe oxy-hydroxydes and carbonates are oversaturated in all solution but as Fe(II) to Fe(III) ratios values were not measured, these phases cannot be accurately modelled.

Table 7.3 – *Modelled Cu speciation in mean soil solution using Nica - Donnan and Gaussian DOM models.*

		STM	VI	CI	OB	HBN	CO
GAUSSIAN							
Cu^{2+}	%	8.5	4.8	5.8	3.0	2.7	3.8
Cu-DOM	%	88.9	21.6	28.3	58.5	54.8	62.4
CuCO_3	%	0.9	61.8	56.9	29.1	36.1	23.4
CuOH^+	%	0.5	8.8	6.8	5.9	3.9	5.7
NICA-DONNAN							
Cu^{2+}	%	0	0	0	-	0.01	0.04
Cu-FA	%	61.1	70.9	70.6	-	73.9	75.4
Cu-HA	%	38.8	29	29.4	-	25.9	24.1
CuCO_3	%	0	0	0	-	0.15	0.28
CuOH^+	%	0	0	0	-	0.02	0.07

For the Nica-Donnan model, essentially all Cu in solution was organic matter bound. Only in HBN and CO modalities small amounts of other Cu species 0.2 % and 0.4 %, in both mainly CuCO_3 are found. Cu carbonated species are undersaturated in all modalities but approach equilibrium in HBN and CO with saturation indices of -4.8 and -2.3 for Azurite and -2.3 and -0.5 for Malachite respectively. Ferrhydrite, Goethite and Hematite are all highly undersaturated with saturation indices between -14.0 and -32.9 in all modalities.

7.4.8 Cu contents and isotopic ratios in plant tissues

Cu contents in roots and shoots were reported in Table 7.4. The lowest mean Cu concentrations in roots are found in STM and OB grown plants with 30 and 25 mg kg⁻¹, respectively. In the Italian soils, CI and VI mean Cu concentrations of 199 and 81 mg kg⁻¹ are measured. The highest concentrations in roots are found in CO and HBN soils with 579 and 768 mg kg⁻¹, respectively.

Isotopic ratios of Cu in roots are constant within 2SD range around 0.3 ‰ in all samples. As isotopic ratios measured in soil solutions vary, we observe significant fractionation between solution and roots (Figure 7.10). There is a trend for light isotope enrichment in roots grown in less Cu concentrated soil solutions and heavy isotope fractionation in more polluted soil solutions.

In leaves, the lowest Cu concentrations are measured in OB and STM soils with 5.0 and 5.1 mg kg⁻¹. OB is the only modality where Cu is more concentrated in leaves than in roots. Cu concentrations in HBN grown leaves (6.1 mg kg⁻¹) are lower than in VI and CI grown leaves (7.4 and 6.7 mg kg⁻¹ respectively). The highest Cu concentrations are measured in CO grown leaves with 10.9 mg kg⁻¹ on average. However, in literature average values for healthy plant tissues are reported to be around 6 mg kg⁻¹ and most modalities are close to this values only OB and STM show somewhat lower values whereas CO nearly contains nearly double as much Cu (Marschner and Marschner, 2012). Isotopic ratios in leaves lie between 0.24 ± 0.01 and 0.20 ± 0.07 ‰ in OB and STM grown leaves respectively, and between -0.20 ± 0.17 and -0.14 ± 0.25 ‰ in HBN and OB, respectively. There is a trend towards lighter isotope discrimination in root to leaf transfer in samples with high root Cu content whereas highest isotope ratios are found in samples with low root Cu content (Figure 7.10). Especially in OB and STM samples where root Cu is lower than leaf Cu. Isotope ratios measured in the field are with -0.9 to -1.7 ‰ much lower than in the greenhouse trial, even though CI and VI soils were taken from the Soave area (Figure 7.11).

Table 7.4 – Mean Cu contents and Cu isotope ratios in soils, soil solutions, roots and leaves from the greenhouse experiment for each modality.

		Type	STM	VI	CI	OB	HBN	CO
SOC	% wt	Soil	0.1	2.5	2.1	0.5	1.3	0.6
Cu	mg kg ⁻¹	Soil	10	229	214	3	251	115
$\delta^{65}\text{Cu}$	‰	Soil	0.17	0.33	0.21	0.27	0.12	0.02
2SD	‰	Soil	0.11	0.01	0.04	0.24	0.07	0.09
pH		Solution	6.4	7.9	7.7	8	7.8	7.9
TOC	mg L ⁻¹	Solution	43	41	62	102	76	97
Cu	$\mu\text{g L}^{-1}$	Solution	13	37	76	358	890	2509
$\delta^{65}\text{Cu}$	‰	Solution	0.56	0.69	0.58	0.21	0.12	0.02
2SD	‰	Solution	0.14	0.46	0.11	0.1	0.09	0.20
Cu	mg kg ⁻¹	Roots	30	81	199	25	768	579
$\delta^{65}\text{Cu}$	‰	Roots	0.25	0.3	0.37	0.26	0.3	0.24
2SD	‰	Roots	0.04	0.05	0.03	0.08	0.20	0.18
Cu	mg kg ⁻¹	Leaves	5.1	7.4	6.7	5.0	6.1	10.9
$\delta^{65}\text{Cu}$	‰	Leaves	0.20	0.00	-0.08	0.24	-0.20	-0.14
2SD	‰	Leaves	0.07	0.16	0.30	0.01	0.17	0.25
$\Delta^{65}\text{Cu}$	‰	Solution-Soil	0.4	0.36	0.37	-0.06	-0.09	0.00
2SD	‰	Solution-Soil	0.17	0.46	0.12	0.26	0.10	0.22
$\Delta^{65}\text{Cu}$	‰	Root-Solution	-0.31	-0.39	-0.21	0.05	0.18	0.23
2SD	‰	Root-Solution	0.14	0.47	0.12	0.13	0.22	0.27
$\Delta^{65}\text{Cu}$	‰	Leaves-Roots	-0.36	-0.69	-0.66	0.03	-0.32	-0.15
2SD	‰	Leaves-Roots	0.08	0.17	0.31	0.08	0.26	0.31
BCR-2	‰	(n=10)	0.26	± 0.09				

7.5 Discussion

7.5.1 Mineral nutrition and plant growth

Biomass production. Plants on the Italian soils (CI and VI) have the highest biomass yields of leaves and roots (Figure 7.3). For the other modalities there appears to be no direct link between root and leaf biomass. As plants were grown in a greenhouse experiment, certain parameters as temperature and light were the same for all plants. Water holding properties might differ between soils but as water supply was kept at water holding capacity for all soils no drought stress should occur independent of soil properties. In this context, factors that may explain variations in biomass are nutrient supply and toxicity of contaminants. Dry matter yield decrease has generally been accepted as the standard measure for comparisons of toxicity (Reichman, 2002). As roots are in contact with the soil solution, the elemental concentration in soil solution gives an estimate of potential bioavailability in different soils. However physico-chemical differences in the immediate space surrounding the roots (rhizosphere) may change the concentrations locally so that actual bioavailability can differ (Hinsinger, 2001, 1998; Marschner and Marschner, 2012).

Nutrient supply. For N nutrition, we only have data available on NO_3^- concentration in solution (Figure 7.3). However N might also be taken up as NH_4^+ . Also NO_3^- concentration shows the largest variations over time for the major nutrients shown due to microbial activity and organic matter degradation controlling its supply (Marschner and Marschner, 2012). The samples with the highest leaf biomasses (CI and VI) do not have the highest NO_3^- medians in solution. In the same manner STM has the lowest NO_3^- concentration in solution but intermediate leaf biomass. HBN has the highest NO_3^- concentration median but not the highest overall biomass production. However it has the highest leaf biomass of Bordeaux soils. CO is the soil with the lowest leaf biomass and the Bordeaux soil showing the lowest NO_3^- solution concentration. N nutrition thus seems to be one factor influencing biomass production but not the single controlling factor in our setting.

For the nutrients P and K, the Bordeaux soils show significantly higher solution concentrations than the other soils (Figure 7.3). The P concentration in solution is significantly lower in HBN than in other Bordeaux soils still HBN is the soil with highest biomass production of Bordeaux soils, so that P supply does not appear to be a major control on biomass production. This observation is further supported by low P concentrations in VI and especially CI soils with the highest plant biomass production. Also K supply seems to have limited effect on biomass production as CO with the lowest leaf biomass is the one with the highest K concentration in solution and VI with the lowest K concentrations is one of the soils with the highest biomass production.

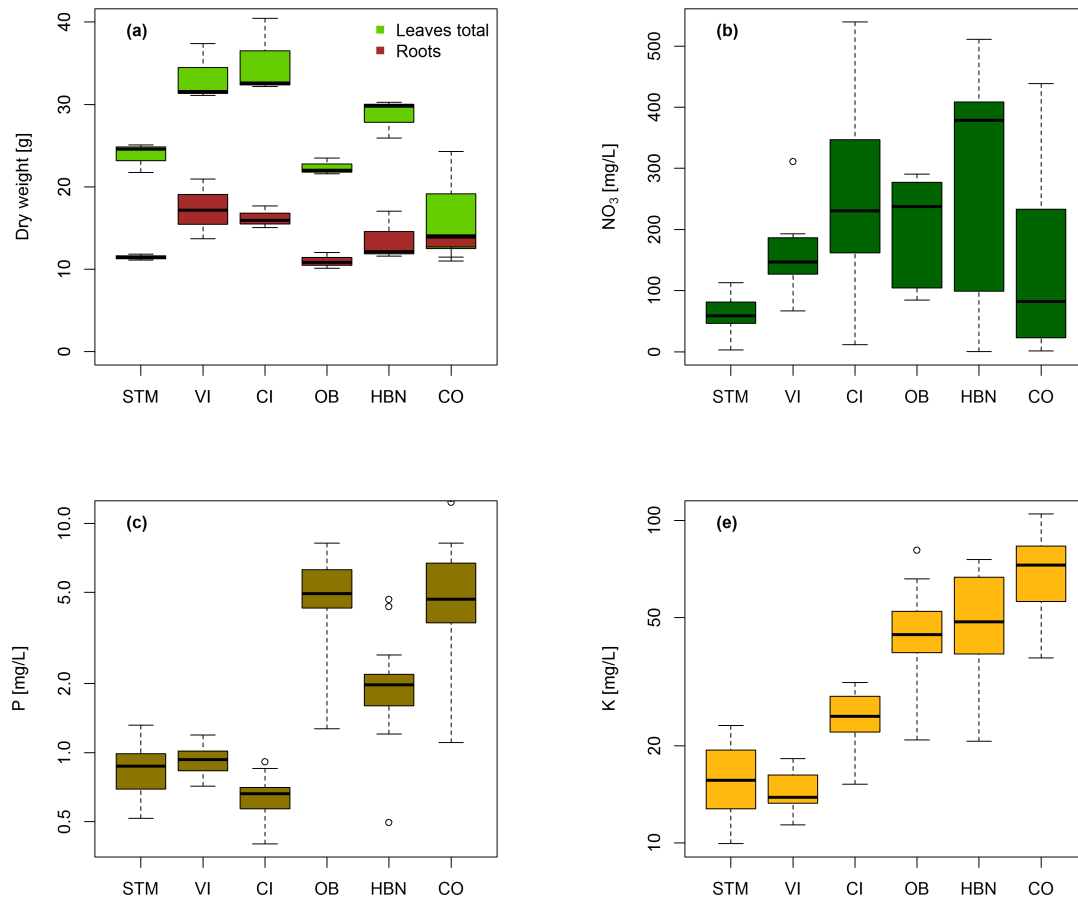


Figure 7.3 – Boxplots of plant biomasses (a), nitrate concentration in soil solution (b), phosphorus content in soil solution (c) and potassium (d) concentration in soil solutions for all measured samples in the different soil modalities. Note that P and K plots are in log scale.

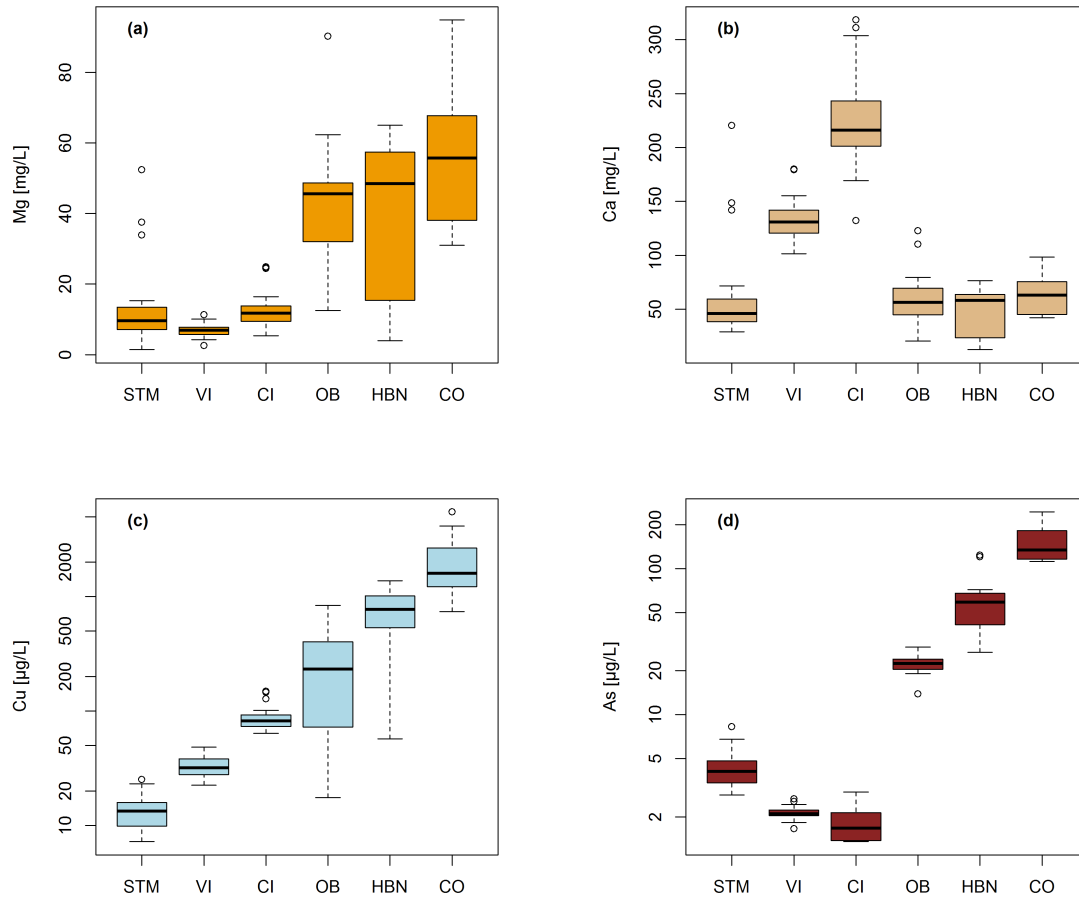


Figure 7.4 – *Boxplots of Magnesium (a), Calcium (b), Copper (c) and Arsenic (d) concentrations in soil solutions for all measured samples in the different soil modalities. Note that Cu and As plots are in log scale.*

Contaminant influence. For Cu contents in solutions, we observe large differences in solution concentrations between around $10 \mu\text{g L}^{-1}$ and around $5000 \mu\text{g L}^{-1}$ (Figure 7.4). The solution concentrations of Cu increase in the order STM ; VI ; CI ; OB ; HBN ; CO and interquartile ranges do not overlap. However extreme values measured in OB overlap with both, the soils VI and CI with on average lower solution concentrations and the next higher samples HBN and CO due to a strong variations in Cu concentrations over time. As CO the soil with the highest Cu concentration in solution has the lowest biomass production but not the lowest nutrient supply there appears that contamination could influence the biomass production (Cambrollé et al., 2015; Juang et al., 2014; Reichman, 2002). However STM with the lowest Cu concentration in solution does not have the highest biomass production and leaf biomass of the second most contaminated soil HBN is more important than STM leaf biomass (Figures 7.3 and 7.4). Also biomass production of HBN is higher than OB even though Cu concentration in solution is higher in HBN than in OB, and OB nutrient supply is comparable to that

of HBN (Figure 7.3). Cu concentration in solution thus appears to have some effect on biomass production but it is not the single controlling factor.

Solution concentration of As is much higher in Bordeaux soils than in STM, CI and VI (Figure 7.4). Within Bordeaux soils the As concentrations are increasing in the same order than Cu and thus As might have an influence on biomass production in the same as Cu (Cambrollé et al., 2015; Nagajyoti et al., 2010). The lowest concentration of As are measured in VI and CI soils corresponding to samples with the highest biomass production. STM modality with lower biomass production does have slightly higher As contents in solution.

Thus, no single factor in plant nutrition appears to control biomass production in the experiment but an interplay between nutrient supply and contamination appears to influence plant growth. However, there is a trend for higher leaf biomass in leaves containing less Mg (Figure 7.5). This does not appear to be a deficiency problem as the highest biomass is found in leaves with the lowest Mg contents and to our knowledge, no Mg toxicity to plants exists (Marschner and Marschner, 2012). This effect might be linked to Cu toxicity as Mg is known to alleviate Cu toxic effects due to competition for adsorption site on roots (Juang et al., 2014). At higher Cu concentrations photosynthesis can be reduced by an inhibitory effect on photosystem II were both Mg and Cu play important roles (Arellano et al., 1995; Barón et al., 1995; Marschner and Marschner, 2012). Replacement of Mg by Cu in chlorophyll molecules is one mechanism of Cu toxicity (Küpper et al., 1996).

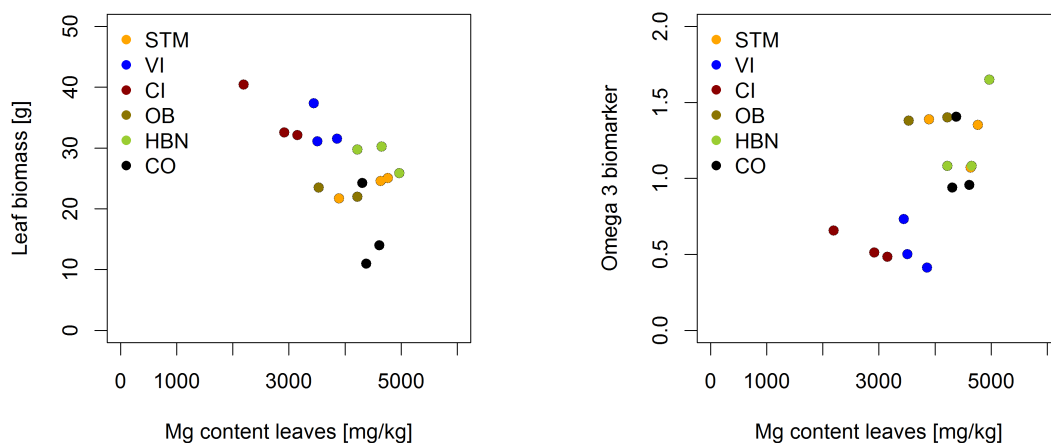


Figure 7.5 – Relation between Mg leaf contents, leaf biomass and plant Omega-3 biomarker.

A link between plant health and Mg contents in the leaf is confirmed looking at the relation between Omega-3 biomarker and Mg content in leaves. The Omega-3 biomarker appears not to respond to Cu stress even though plants on CO modality show signs of toxicity as lower biomass production. Even though studies on other plant species show that changes in total chlorophyll content can occur as part of Cu toxicity, in grapevine an increase of photosynthetic pigments and leaf fatty acids was observed after Cu exposure (Reichman, 2002; Romeu-Moreno and Mas, 1999; Rousos et al., 1989). This would explain the elevated Omega-3 values at high Cu exposure.

7.5.2 Influence of OM on Cu release

Copper content in soil solutions of the different modalities is not principally dependent on bulk soil Cu concentration, as it is common knowledge in soil sciences (e.g. Kabata-Pendias, 2004; Ruyters et al., 2013). The CO soil with 115 mg kg^{-1} Cu in the solid phase gives by far the highest Cu concentrations in solution with $2509 \mu \text{ L}^{-1}$ on average whereas VI soil with 229 mg Kg^{-1} Cu in solid phase gives only $37 \mu \text{g L}^{-1}$ in solution.

The variations in our experimentation cannot be assigned to typical variables as pH which is quite similar in all modalities except STM. Organic matter content and mobility towards the soil solution appears to play an important role, as mean relative Cu release increases dramatically with mean relative organic matter release to solution (Figure 7.6). Organic matter is released to a higher proportion in soils with the lowest organic matter contents. A higher organic matter release can either be due to higher hydrophilic organic matter content or stronger binding properties of soil minerals towards organic matter (Gallet and Keller, 1999; Ren et al., 2015; Schneider et al., 2016). Adsorption of organic matter on clay minerals would furthermore increase the stability of metal complexes on humic acids (Arias et al., 2002).

Higher binding of organic matter on clay surfaces was proposed as a mechanism making Cu less mobile in VI soils compared to CI soils in **Chapter 6**. The observation of lesser mobility of Cu in VI soils is confirmed in the experiment as Cu concentration in VI soil solutions is only half ($37 \mu \text{g L}^{-1}$) of concentration in CI soil solutions ($76 \mu \text{g L}^{-1}$).

The relationship between organic matter release and Cu release does not hold true in the non-contaminated STM soil indicating a stronger influence of solid phase speciation on Cu release to the soil solution. This is consistent with the observation that Cu in contaminated soils is mainly organic matter bound (Boudesocque et al., 2007; Strawn and Baker, 2008, 2009). Note that relative variation of Cu release is much greater than variation of organic matter release. Also the examination of mean solution values has the drawback of not taking into account variations over time.

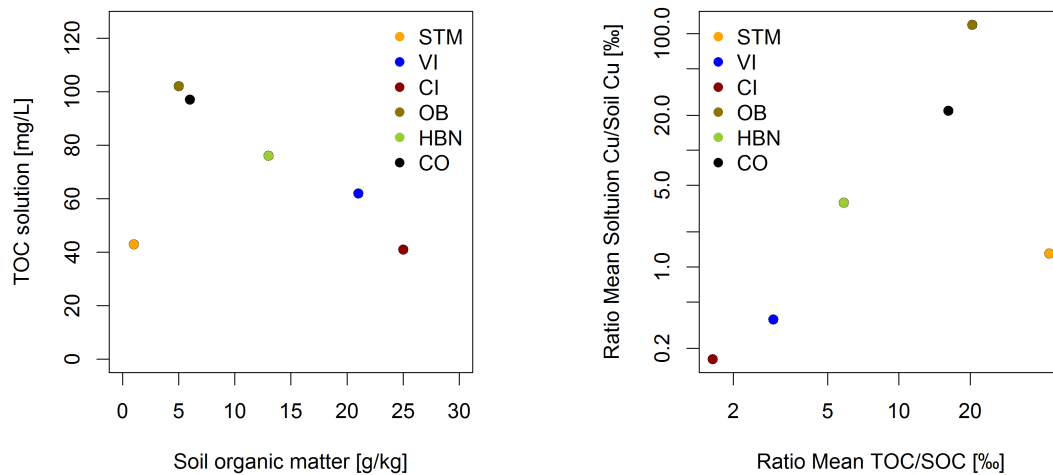


Figure 7.6 – Relationship between organic matter in solid and soil solution. Relative release of organic matter and Cu from soils to mean soil solutions.

7.5.3 Evolution of elemental contents in soil solutions over time

Elemental contents in soil solution of the different modalities evolve differently and most of the time different elements also evolve differently (Figure 7.2). Samples were always taken at field capacity. We observe elements with increasing concentration at the same time as elements with decreasing concentration so that we can exclude effects of dilution or concentration due to the sampling method. Changes in soil solution concentration appear to be due to plant action. As no global trend is obvious, evolutions of soil solution chemistry will be discussed separately for each soil modality.

STM soil. Alkalization of the rhizosphere would indicate either higher anion than cation uptake by the plant as it would be the case for N uptake in form of NO_3^- or a protection mechanism against metal stress (Bravin et al., 2009, 2012; Marschner and Marschner, 2012). Actually, in STM soil, a trend for higher pH and DOC values in later samples was observed. Moreover, in this soil modality, as metal concentrations are low, a plant nutrition on NO_3^- is suggested. Cu solution concentration declines until the 6 weeks sample staying about constant in the end of the experiment. This decline is possibly due to plant absorption as STM roots contain about 300 μg of Cu equivalent of 23 L of soil solution. In the same manner, other major plant nutrient concentrations as Mg and K decline over time, whereas Ca and P remain approximately constant, suggesting that either the solid soil phase is able to buffer plant uptake or that the plants were able to actively enhance the release of these nutrients (Hinsinger, 2001). Fe does not appear to follow the trend of Cu. Fe concentration in solution stays more or less constant except a sharp increase after ten weeks. This increase in concen-

tration falls together with relatively high organic carbon content in solution but might be as well induced by the presence of Fe colloids (Kretzschmar et al., 1994; Kretzschmar and Sticher, 1997). However the strong increase in Fe does not affect Cu indicating a different speciation of both metals in solution. In contrast As concentrations closely follow the variations of Fe concentrations. In literature iron minerals are often reported as host phases for As transport (Bowell, 1994; Nickson et al., 2000).

For **VI soil**, elemental concentrations as well as pH and organic matter contents do not show much variations. This observation is consistent with buffering of the soil solution through the high CEC values in this soil. Organic matter contents in solution show a tendency for lower values at the end of the experience also visible in Cu, indicating a role of organic matter mobility (Chopin et al., 2008; Ren et al., 2015; Strawn and Baker, 2008). However Fe concentrations peak in the ten weeks sample. Interestingly in comparison to STM, As concentrations are not affected by the Fe peak. This indicates that the peaks are not due to a common pollution but specific to their respective soil functioning. Again Cu concentrations are not affected by the iron peak suggesting a different speciation of Cu and Fe in the VI soil solutions.

In **CI solutions**, we observe an increase in pH, DOC and most metal cation concentrations. The increase in pH point towards NO₃ uptake by the plant and increasing DOC values are likely also due to plant activity like root exudates that enhance microbial activity (Hinsinger, 1998; Marschner and Marschner, 2012). The increase of Fe and Cu concentrations stands in contrast to increasing pH values as most common minerals of these metals are more soluble at low pHs and organic matter adsorption is also lower at low pH values (Kabata-Pendias, 2004). Their increase is thus likely due to an increase of DOC content. The exudation of chelating agents is a well-documented phenomenon in environments poor in Fe as calcareous soils and most of them have relatively high affinity also to other transition metals (i.e. Kraemer, 2004). The highest increases are observed for Mg concentrations which almost double in the course of the experiment. This is surprising as Mg is a major plant nutrient and we would expect the same tendency in soil solution as K and P showing decrease only slightly over time. In literature it was reported that banana plants were able to increase Mg mobility through weathering of smectites (Rufyikiri et al., 2004). As smectites are one of the main minerals in the CI soils this is a likely mechanism to explain higher Mg mobility in the end of the experiment. Also other main nutrient concentrations as K and P decrease only slightly over time. Thus in this modality the plant actively increase solubility of different nutrient elements alongside with Cu either by exudation of organic carbon or enhanced weathering of minerals.

In **OB soil**, Cu concentration decreases strongly over time. Fe, also K, Mg and Ca follow similar patterns. The decline of those element concentrations might be due to pH increase coupled with DOC decrease and plant absorption of those elements as nutrients. The strong decrease in DOC content is likely due to microbial activity. Even though P is much more concentrated in solutions from Bordeaux soils, we observe an increase in P concentration by more than a factor 2 during the experiment. As the peak in P concentration has the same shape as the As concentrations in solution but not with Fe we suggest a desorption of both elements in relation with pH increase or due to plant action (Hinsinger, 2001).

In **HBN soil**, the pattern of DOC and elemental release to the soil solution is somewhat more complex than in OB. In HBN modality DOC concentrations fluctuate over time. The DOC peak in the 10 weeks sample appears to have no influence on Cu concentrations making DOC not the only controlling factor of Cu concentrations. K and Ca concentrations follow the general curve shape of Cu but slightly increase after ten weeks when high DOC contents are measured. The ten weeks sample is also the onset of increase in P and As concentrations. Again these two elements appear to be linked in their evolution but this time no pH increase is detected. Furthermore Fe and Mg concentrations increase in the 12 and 14 weeks sample respectively suggesting a more complex plant action for nutrient release than for the other modalities.

Finally, in the **CO soil**, all element concentrations decrease over time and only P concentration reincrease after an initial decline to reproach 4 weeks values in the end of the experiment. An increase in DOC concentration after 8 weeks coincides with a slight reincrease in Mg, K and Ca values. Otherwise all elements decline from the 4 to the 6 weeks sample and then remain constant over time. This pattern likely reflects negative influence on biological activity by the high Cu and As values in the soil solution.

7.5.4 Cu speciation in soil solutions

As nitrate was detected in all our soils, solution influence of metal reduction processes can be excluded due the onset of Fe reduction needs lower Eh than values allowing NO₃ stability (Blume et al., 2016). Results from different modelling approaches give largely different results depending on the way organic matter is implemented in the model.

If the organic matter is implemented through a Nica-donnan model virtually all Cu in solution is organic matter bound (Table 7.3). This underlines the crucial role of

organic matter in metal speciation in soils state in literature (e.g. Kabala et al., 2014; Ponthieu et al., 2016; Ren et al., 2015; Schneider et al., 2016). In most soil modalities we actually observe a coevolution of DOC and Cu contents as discussed above. However this is not the case for all soil modalities. Also, in the Nica-Donnan model, Fe minerals are undersaturated in all modalities and even though Fe is abundant especially in Bordeaux soils Fe content in soil solution is not increasing in those soils over time. This suggests that the model might overestimates Fe binding properties of the actual organic matter even though highly specific siderophores can occur in soil organic matter (Kraemer, 2004). Overestimation of organic bound Cu is a known issue of default parameters of the Nica-Donnan model. For example Ren et al. (2015) state that free Cu is underestimated by up to 3.4 log units in soil solutions using standard parametrization. Also Bravin et al. (2012) obtained model results closest to measured free Cu^{2+} values with only 42 % of DOM acting as fulvic acids in bulk soils and even lower values in the rhizosphere as organic matter exuded by roots are mainly low molecular weight sugars and acids with relatively low metal complexing abilities (Degryse et al., 2008; Kim et al., 2010; Oburger et al., 2009). In other words the values modelled here can only give an indication as we do not have precise data on the nature of organic matter. The Nica-Donnan model gives a maximum estimate of organic matter reactivity and shows that it is possible that essentially all Cu in the studied soil solutions can be OM bound. This might represent an overestimation but clearly shows that under certain conditions Cu can be mobilized even at high concentrations in carbonated environments through its high affinity to organic matter. Only in modalities containing the highest amounts of Cu, free and carbonated Cu species are calculated.

In contrast, using a Gaussian DOM model, we calculate significant amounts of inorganic Cu species in the soil solutions (Table 7.3). However concentrations of free Cu make up for less than 10 % of total Cu in all modalities. Cu carbonates are the most abandoned inorganic species in all soil solutions with $\text{pH} > 7$. This is in line with oversaturation of several Cu-carbonated phases and limited Cu mobility in carbonated environments (Duplay et al., 2014; Pietrzak and McPhail, 2004; Ponizovsky et al., 2007). Nevertheless also cupric ferrite and iron minerals in general are oversaturated indicating either the presence of Fe-colloids in the soil solutions or the underestimation of metal binding capacities of organic matter.

Considering the large uncertainties associated with the thermodynamic models we cannot conclude neither on differences in speciation between soil solutions nor on actual speciation of Cu. However the two models show that at pH values > 7 the Cu speciation in solution is controlled through an interplay between organic matter and carbonate species as stated in **Chapter 6**. To get a better impression of Cu speciation, a more

precise parametrization of organic matter in soil solutions is needed (Bravin et al., 2012; Kabala et al., 2014; Ponthieu et al., 2016; Ren et al., 2015; Schneider et al., 2016).

7.5.5 Liberation of Cu to the soil solution – isotopic insights

Isotope fractionation between soil and soil solution indicates a difference in Cu mobilization between the Bordeaux soils and the others (Figure 7.7). In all soils but STM Cu release is controlled by organic matter release to the solution as discussed earlier. In Bordeaux soils isotopic ratios in soils and soil solution are the same, suggesting that no equilibrium fractionation occurs between the two phases. This likely means that speciation is the same and organic matter bound Cu is the major speciation also in the solid phase. Decline of isotopic ratios over time in HBN and CO soil solutions might reflect the uptake of heavy Cu by plant roots. However roots are expected to grow over the whole growth period whereas a decline in isotopic ratios is only detected between 4 and 8 weeks. In OB no isotope variation occurs even despite high variations in concentration.

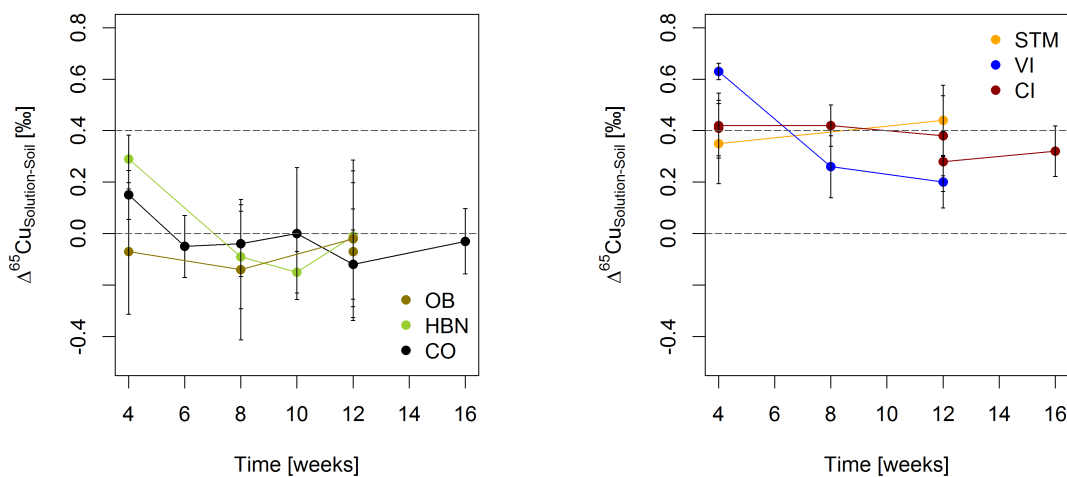


Figure 7.7 – Evolution of Cu isotopic ratios in soil solutions with respect to bulk soil isotopic ratios for the different soil modalities (STM, VI, CI, OB, HBN, CO).

For STM, VI and CI soils, soil solutions are systematically heavier than bulk soils (Figure 7.7). This is in line with values reported in literature showing that soil solutions are isotopically heavier than their respective bulk soil (Kusonwiriya Wong et al., 2017; Mathur et al., 2012). The value of $\Delta^{65}\text{Cu}_{\text{solution-soil}}$ around +0.4 ‰ is surprisingly similar to values obtained by citrate extractions in **Chapter 6**. We suppose that kinetic fractionation can be excluded as not systematic evolution of Cu concentrations is observed between the three modalities. The isotope fractionation between soil and soil solution suggest that besides organic matter influence, different pools are involved in Cu liberation. Higher Cu-isotope ratios in solutions with respect to bulk soils in-

dicating that liberation involves a process preferentially affecting heavy isotopes, as for example leaching by organic matter. The other possibility is a lighter Cu pool in the soil which is not mobilized, as for example Cu bound to carbonates, clay minerals or Cu contained in primary minerals (Babcsányi et al., 2016; Bigalke et al., 2010a; Li et al., 2015; Marechal and Sheppard, 2002). In VI modality the shift towards lighter isotope ratios over time cannot be explained with uptake of heavy Cu by the plant as all measured plant compartments show lighter isotope ratios than soil solution (Figure 7.10). Carbonate dissolution appears to be one possibility to explain these changes supported by fluctuation of pH values around 8 with a slight tendency to decrease and Ca concentration in solution slightly increasing over time (Marechal and Sheppard, 2002).

For STM as the only soil where Cu mobility is not depending on organic matter release, Cu might not be contained in organic matter in the first place. STM soil has not been treated with Cu pesticides for over ten years and relatively few Cu is present in the bulk soil even compared to geogenic background (Lado et al., 2008). This means that Cu might be contained in primary minerals that are leached by organic matter. This mechanism would be in agreement with heavier isotopic ratios in solution compared to bulk soil as well as with relatively high percentages of organic matter bound Cu in both speciation models (Bigalke et al., 2010a; Kusonwiriawong et al., 2017). In contrast to reports from literature, Fe-bearing minerals appear not to be involved in Cu mobility in STM and VI soils because Fe peak concentrations in solution does not coincide with Cu peaks (Bradl, 2004; Kretzschmar and Sticher, 1997; McCarthy and Zachara, 1989; Sayen and Guillon, 2010).

7.5.6 Mechanisms of Cu root uptake and translocation through the grapevine plants

Cu uptake. Root Cu content increases with increasing solution Cu content. For STM, VI and CI the relation between mean solution Cu and root Cu appears to be proportional ($R^2=0.88$, $p<0.001$). If solution content after 12 weeks is taken rather than mean solution content 2 out of 3 samples of the OB modality also fall close to the regression line whereas the proportionality is stays unchanged ($R^2=0.85$, $p<0.001$) for STM, VI and CI (Figure 7.8). The fact that late Cu concentration is a better fit than mean Cu concentration might suggest a dynamic adjustment of roots to the actual solution concentration. Possible mechanisms will be discussed later. In both cases (mean solution Cu and 12 weeks solution Cu) HBN and particularly CO samples lie not on the regression line. Both deviate in a way that Cu concentrations in roots are smaller than would be expected from solution content. This is also true for the third OB sample. This effect could be explained by a less bioavailable form of Cu in solution as release

mechanisms are different in Bordeaux soils from the other modalities. However the Cu release mechanisms should be the same between HBN and CO samples. Still there is as less Cu in CO roots compared to HBN roots even though Cu concentrations in solution are higher in CO samples. This contradicts the hypothesis of different bioavailabilities in solution and rather points towards a mechanism linked to Cu concentration. This mechanism might be saturation of Cu adsorption sites in roots or detoxification by the plants (Bravin et al., 2010).

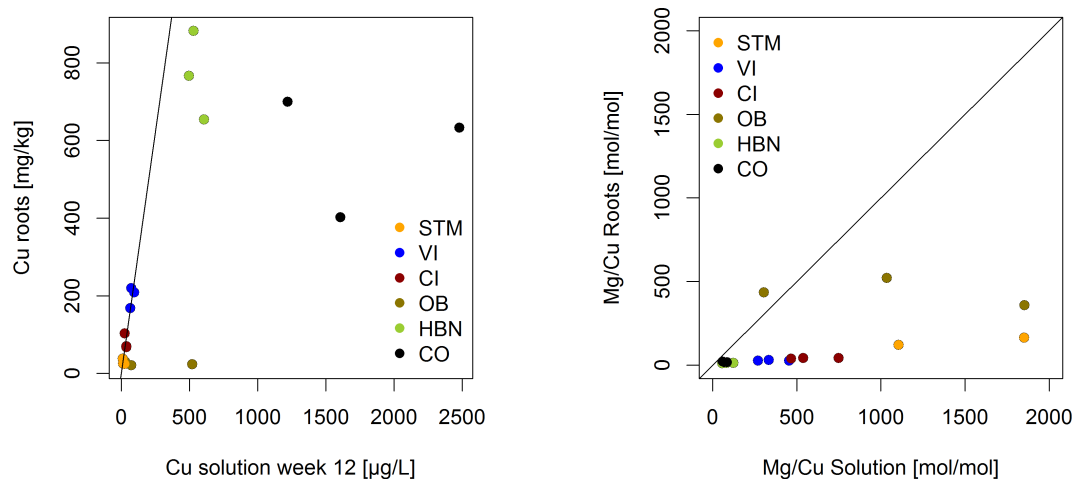


Figure 7.8 – *On the left:* Root Cu contents plotted against the Cu contents in the 12 weeks solution. The black line is a linear regression of STM, CI and VI Cu concentration. *On the right:* Mg/Cu ratios in roots plotted against Mg/Cu ratios in the 12 solutions.

Isotope ratios in roots all have similar values around 0.3 ‰, but isotope ratios in solution vary so that different fractionation patterns appear for different modalities (Figures 7.9 and 7.10). In STM, VI and CI isotope ratios in roots are lighter than in solution. In OB, HBN and CO isotope ratios are equal or heavier than solution ratios. There is a trend towards heavier isotope ratios with increasing Cu content (Figure 7.10). This would be in agreement with an increasing ratio of adsorbed-Cu/uptaken-Cu, and adsorbed Cu being heavier than Cu within plant cells and even soil solution whereas absorbed Cu is lighter than solution Cu (Bigalke et al., 2010a; Bravin et al., 2009, 2010; Jouvin et al., 2012; Ryan et al., 2013). Slightly lower isotope ratios in CO roots with the highest Cu content might indicate a change in the isotope fractionation pattern due to Cu toxicity, this falls together with lower Cu contents relative to the solution. A possible mechanism is the detoxification of Cu in Cu(I)-S groups as reduction processes favor light isotopes (Collin et al., 2014; Ehrlich et al., 2004; Jouvin et al., 2012; Ryan et al., 2013). Yet differences in isotope ratios in this modality are not significantly lower than in HBN so that this effect might be due to measurement uncertainty.

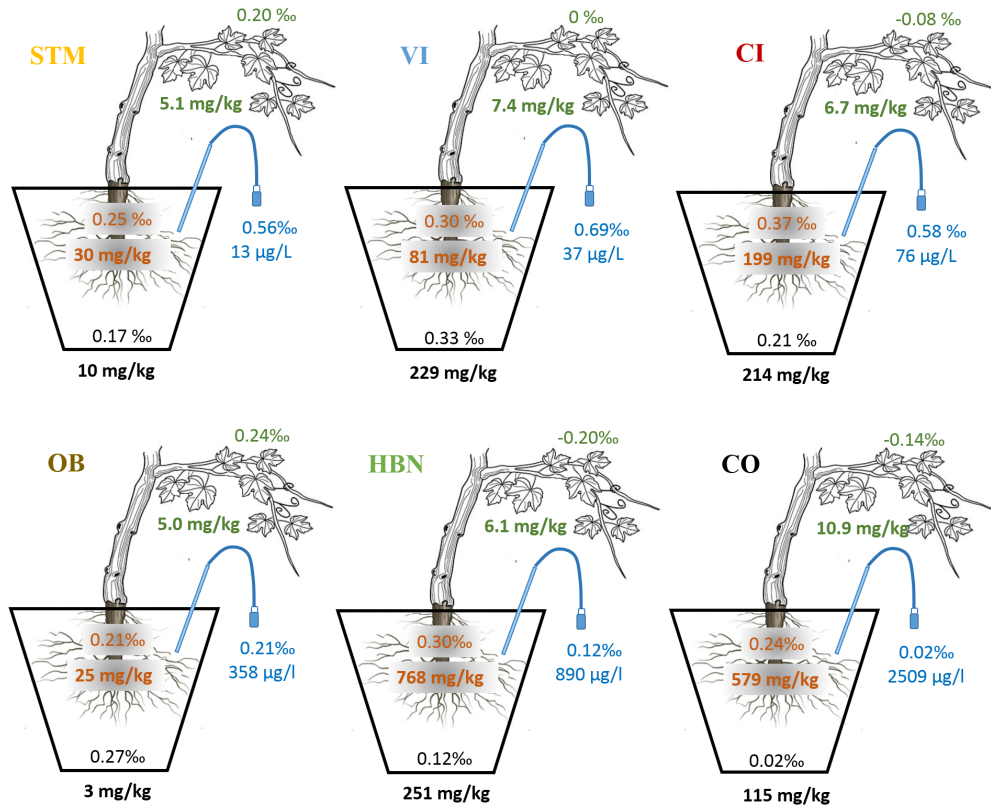


Figure 7.9 – Mean Cu contents and isotope ratios in bulk soils, soil solutions, roots and leaves of different soil modalities.

The other soil modality leaving the trend for higher isotope ratios with higher Cu concentration is OB. OB is also the modality with the highest variation in Cu concentration over time. It was discussed earlier that root concentrations rather fit low final Cu concentration in solution than high initial concentrations. The initial Cu concentration in the soil solution in OB modality was between CI and HBN modality. The isotope ratios measured in OB roots would fit this position however root concentration is lower. As root concentrations are average values of all roots there might be older roots that have seen high Cu concentration containing most Cu and dominating overall isotope ratios, whereas Cu in newer roots Cu concentration is low. One could also imagine a redistribution of Cu taken up at the beginning of the experiment redistributed across the roots (Garnett and Graham, 2005; Loneragan, 1981). Whatever the mechanism, observed Cu isotope ratios seem to be due to a dilution effect. Furthermore Mg/Cu ratios in roots appear to be relatively independent of Mg/Cu ratios in soil solution (Figure 7.8), contradicting competitive absorption of those two elements (Juang et al., 2014). Only OB samples have higher Mg/Cu ratios. If Mg was taken up in the same ratio with Cu as in other modalities but at a moment with high solution concentration of Cu, a redistribution of Cu to other cells might show this pattern.

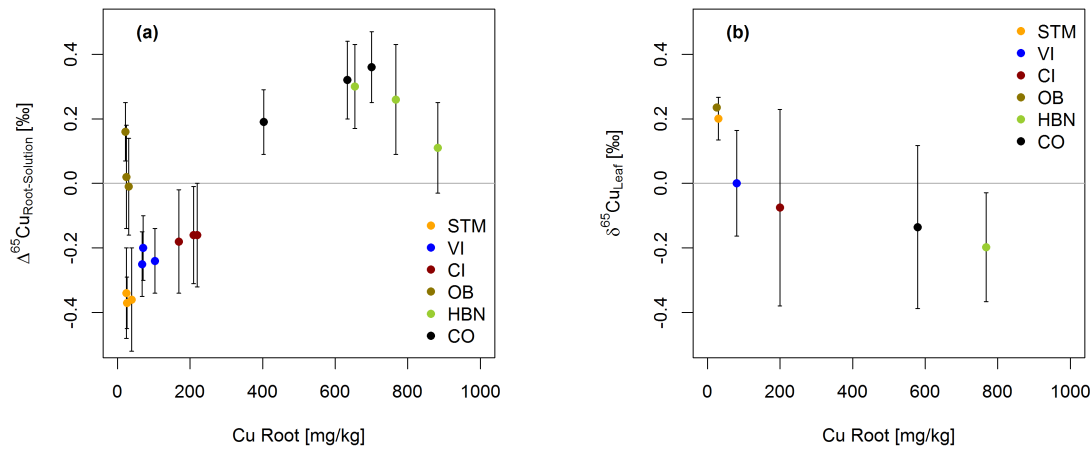


Figure 7.10 – (a) *Cu* isotope fractionation between root and soil solutions as function of root *Cu* content. (b) *Cu* isotope ratios in leaves as function of root *Cu* content.

Cu translocation. *Cu* concentrations in leaves are relatively constant, between 5.0 and 7.4 mg kg^{-1} . Only leaves grown on CO soils are more concentrated in *Cu* with 10.9 mg kg^{-1} . This is surprising as roots from CO did not have the highest *Cu* concentrations and one would expect increasing shoot concentrations with increasing *Cu* exposition (Anatole-Monnier, 2014; Bravin et al., 2010). Generally neither root nor solution *Cu* concentrations seem to influence leaf concentrations of *Cu*, probably due to homeostatic control by the plant. However *Cu*-isotope ratios in leaves are higher in plants that have low *Cu* content in their roots and lighter in leaves of modalities with high root *Cu* content (Figure 7.10). This suggests a difference in transport mechanisms depending on the *Cu* content in roots.

In STM and OB modalities with the lowest root *Cu* concentration *Cu* isotope ratio in leaves is the same as in roots. Quantitative and thus not fractionating translocation of *Cu* might explain this observation. In all other modalities *Cu* in leaves is isotopically lighter than in roots, suggesting light fractionation during translocation. In our study CO samples are the only ones which show signs of *Cu* toxicity with increased *Cu* contents in leaves and lesser leaf biomass production. Yet they show similar fractionation patterns to HBN samples that do not show sign of toxicity. *Cu* isotope fractionation thus appears to depend on *Cu* content but not on toxicity. Light to neutral isotope fractionation between roots and leaves is in agreement with data from other *Cu* isotope studies on plants (Jouvin et al., 2012; Li et al., 2016; Weinstein et al., 2011). However some studies argue that a large part of root *Cu* is adsorbed to the cell wall, thus not contributing to translocation, this pool is supposed to be isotopically heavy compared to absorbed *Cu* so that plants might even favor translocation of heavy *Cu* (Bigalke et

al., 2010a; Li et al., 2016; Ryan et al., 2013). There is no direct evidence for apoplastic Cu being isotopically heavy, this is inferred from experimental studies on complexation of Cu with organic matter (Bigalke et al., 2010a). Nevertheless the only isotope ratio measurements on roots after apoplast desorption support this theory but desorbed roots still contained high amounts of Cu (448 to 1093 mg kg⁻¹) and were grown only with metallic Cu at low pH 4.5, so that detoxification mechanisms might have played a role especially as root Cu was mainly Cu(I) bound to S ligands (Collin et al., 2014; Ryan et al., 2013).

In field grown leaves on VI and CI soils isotope ratios were much lower than in pot grown plants (Figure 7.11). This observation is in agreement with a study showing that leaves on higher positions are isotopically lighter (Weinstein et al., 2011). In that study an equation linking leaf height to isotope ratios was proposed (Weinstein et al., 2011). The equation predicts surprisingly well (-1.57 ‰) isotope ratios measured in field grown grapevines (-0.9 to -1.8 ‰). However this equation does not work in pot grown plants as a negative plant height would be necessary for positive isotope ratios in leaves.

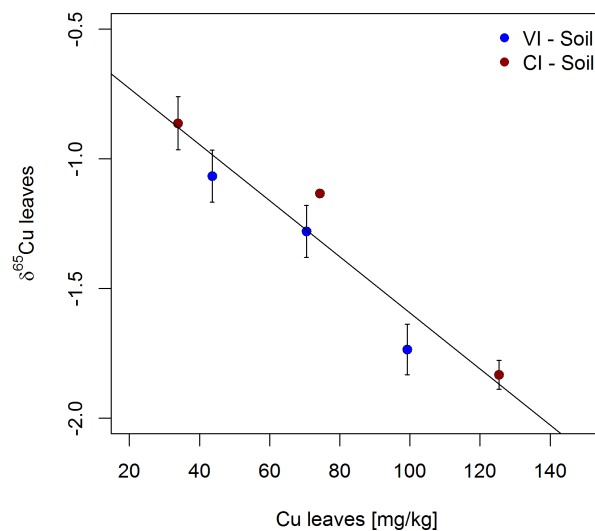


Figure 7.11 – *Cu isotope ratios in field grown leaves (from CI and VI soils) as a function of their Cu-content.*

Also in field grown leaves we observe a link between Cu concentration and isotope ratios: the higher the leaf content of Cu, the lighter the Cu (Figure 7.11). This is in good agreement with observations from the pot experiment however in the field we observe variable Cu contents in leaves. Pesticides sprayed in the vineyard have heavy Cu isotopes ratio (+0.33 ‰ in average), so that simple contamination by Cu pesticides can be excluded especially as leaves containing most Cu are isotopically light. This observation also contradicts leaf uptake of pesticides. As variations in soil isotope ratios

are small with respect to variations in leaf isotope ratios (**Chapter 6**), the observed pattern must be due to isotope fractionation within the plant. Hence an influence of Cu concentration on Cu transport mechanisms in the plant is also visible in field grown plants. Again, a possible mechanisms would be the immobilization of Cu as Cu(I)-S groups as reduction favors light isotope enrichment (Collin et al., 2014; Ehrlich et al., 2004; Jouvin et al., 2012; Mathur et al., 2005; Zhu et al., 2002).

7.6 Conclusion

In this chapter the mechanism of elemental transfer between soil, soil solution and plant were investigated in a greenhouse experiment. Grapevine plants were grown on different soils with different degrees of Cu contamination. Cu concentration in bulk soils did not correlate with Cu concentration in solution. The mobility of Cu was rather controlled by the mobility organic matter. The more organic matter was released to the soil solution relative to the bulk soil organic matter content, the more Cu was released. However, elemental concentrations in the soil solution varied over time. The variations depended on soil type and likely plant action. Fractionation of Cu isotopes between soil and soil solution indicates that in different soil types Cu speciation differs.

The Cu concentration in soil solution at the end of the experiment correlated well with Cu root content in less contaminated samples. However, in the most contaminated samples less Cu was contained in roots than would have been expected from solution contents. Leaf Cu-contents were constant in all but the soil modality with highest solution content of Cu. Cu isotope fractionation by plants appeared to depend on Cu contamination. The higher the root-Cu content the more Cu was isotopically heavy. In contrast the Cu contained in leaves was lighter when root-Cu content was high. This observation was confirmed in field measurements where leaf isotope ratios were lighter in leaves containing more Cu. However, isotope ratios in leaves of field grown plants were much lighter than leaves of pot grown plants even compared to pots with the same soil. Finally plant health indicators as biomass production and fatty acid ratios seem to depend on Mg content in leaves confirming a role of Mg in Cu toxicity. availability on uptake mechanisms.

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Chapter 8

Conclusion and perspectives

In cultivated ecosystems and in vineyards especially, a large spectrum of interactions between plants and soil geochemistry exists. This work aimed to answer several main questions regarding the influence of soil on the chemical signature of soil solution, plant organs and wine. This thesis particularly focuses on the traceability of wines due to soil properties, the existence of chemical tracer of the “terroir effect” in soils, and the element transfer in the soil – soil solution – plant continuum. A particular interest lies on the fate of Cu pesticides, still largely sprayed in viticulture, in vineyard soils. This conclusion section will develop a synthesis of the obtained results and propose perspectives for further investigations to advance understanding of the role of soil biogeochemistry in viticulture.

8.1 Main conclusions

8.1.1 The role of soil on element signature of wines

In the **fourth chapter**, elemental contents of Mg, Ca, Mn, Sr and Ba were analyzed in over 200 wines from Western Europe. This data was combined with information on the soil type (calcareous *vs.* non-calcareous soils) as well as climatic parameters (precipitations and temperature values) recorded in the respective growth periods.

Pedological and meteorological conditions cause differences in elemental profiles of wines, and Mg, Ba, Ca and Sr contents are relevant tracers of soil influence on wine chemistry.

Elemental concentrations of wines were significantly influenced by wine style (e.g. red, white, rosé and “vin gris”), most visible in the Mg/Ca ratios. The soil type also had an influence on the elemental composition of wines. Wines, made from grapes grown on

non-calcareous soils tended to have higher Mg and Ba contents than wines from grapes grown on calcareous soils. Climatic conditions also had an influence on the elemental composition of wines. Wines coming from regions with high precipitations during summer months tended to have high contents in Mn and Ba, whereas wines from warm and dry regions had high contents in Sr. These observations confirmed the assumption that variations in wine elemental profiles are caused by differences in soil chemistry and meteorological conditions (Coetzee et al., 2014; Greenough et al., 2005). However, a high percentage of the variability of Sr contents in the dataset remains unexplained. This is rather surprising as Sr is one of the most used elements in origin tracing schemes. A possible explanation is the fact that Ca/Sr ratios in carbonated rocks depend on their geological age, so that differences do not depend on soil or meteorological conditions but on the underlying geological formation.

8.1.2 Relevance of soil parameters for elemental transfer between soil and plant

The **fifth chapter** reports a study performed in field conditions. The focus was on the influence of soil type on plant properties that are relevant for winemaking. Two different soil types (calcaric cambisol and vertic cambisol) in the Soave region supposedly formed on different bedrocks were investigated. Field observations showed obvious differences in soil morphology.

Different soil morphologies will not necessarily induce differences in soil elemental signatures nor in elemental contents of plants

Nevertheless, mineralogical and chemical investigations showed evidence for complex soil forming mechanisms leading to similar chemical properties. Sr isotope ratios of leaves and to some extent Ca/Sr ratios and Mo contents allow the identification of the corresponding soil, indicating possible influences of soil type on Ca nutrition for plants. There was a general tendency for higher elemental contents in plants grown on soils more influenced by limestone. Investigation of biochemical markers such as fatty acid ratios in leaves and sugar content in grapes did not show significant differences between the two soil types. Variability between plant samples taken from one soil sampling spot or between different sampling years was much greater than differences between soil types.

8.1.3 Behavior of Cu pesticides in soils and consequences for soil – plant transfer of Cu in viticultural soils

In **Chapter 6**, calcareic and vertic cambisols from Soave appellation were examined again to analyse the behavior of Cu in vineyard soils.

Organic matter and carbonates influence Cu transport in soils, and Cu can be lost from soils even in a carbonated environment. Cu isotope signatures reflect retention/transfer processes of Cu in soils rather than Cu origin.

In contrast with former investigations (Duplay et al., 2014; Komárek et al., 2010; Pietrzak and McPhail, 2004), mass balance calculations showed that Cu is vertically mobile even in carbonated environments. Isotope ratios suggest a loss of heavy organic bound Cu from the carbonated soils. EPR analysis of bulk and decarbonated soil samples showed a contribution of carbonates to the Cu binding in calcareic soils that was absent in vertic soils. Thermodynamic modelling showed that Cu carbonates can be formed under soil conditions but can also be dissolved by organic ligands in solution even at high pH values. This suggests that Cu mobility is controlled by organic matter and raises questions on the role of amendments used in organic agriculture in metal transport. Finally Cu pesticides showed large variations of isotopic ratios covering the isotopic ratios reported in soils and making source tracing impossible.

8.1.4 Study of mechanisms of Cu transfer in the soil – soil solution – plant continuum using stable Cu isotopes

The investigation of Cu transport on a smaller scale like in the greenhouse experiment described in **Chapter 7** showed that the release of Cu from bulk soil to the soil solution was also strongly influenced by organic matter dynamics. More Cu was released to the solution if the relative release of organic matter was high, especially in contaminated soils.

Cu contamination influences plant health and nutrition. Cu content and isotopic signature in soil solution can change over time and reflect the major role of organic matter.

In soils with high Cu release to the solution, Cu toxicity appeared to be one of the factors causing lower biomass production of grapevine plants. Over time concentra-

tion of different elements in soil solution evolved differently, probably due to activity of plants. For example P concentrations in soil solution increased in some modalities despite plant uptake of P as an essential nutrient. Also metal concentrations evolved differently over time. In most but not all investigated modalities, Cu concentration in solution appears to be linked to the evolution of DOC content rather than pH.

Isotopic fractionation between bulk soils and soil solution was also variable. In soils from the Bordeaux region, the same isotope ratios were measured in solution and bulk soils, whereas in other soils, Cu isotope ratio was heavier in solutions than in bulk soils. In soils from Soave, fractionation between bulk soils and soil solutions is similar (around +0.4 ‰) to the fractionation determined by citrate extraction in **Chapter 6**. In some cases, soil solutions showed significant variations in their isotope ratios over time. In those soils, isotope ratios were lighter at the end of the experiment than in the beginning, while the inverse trend was not observed.

Cu isotope ratios in plants depend on Cu contamination level and suggest differences in uptake mechanism between modalities.

The more soluble Cu was measured in soil solutions, the more Cu was contained in plant roots. However in leaves, Cu contents were similar in 5 of the 6 soil modalities. Cu isotope fractionation between soil solutions and roots and between roots and leaves appeared to be dependent on Cu content : the higher the Cu contents in roots, the heavier the root Cu. The opposite trend was observed in leaves, with lighter isotope ratios observed in plants showing the highest Cu contents in their roots. A similar trend was observed in leaves from the Soave vineyard: Leaves with higher Cu content had lighter Cu isotope ratios. Generally leaf isotope ratios were much lower in the field than in the greenhouse experiment, confirming the observations of Weinstein et al. (2011), reporting that lighter Cu accumulates in higher leaves of the plant. Furthermore the light isotope ratios seem to contradict leaf absorption of Cu pesticides which are generally heavier and indicate that all Cu is taken up by roots. The data presented in **Chapter 7** adds to a small literature on Cu isotope fractionation in plants (Jouvin et al., 2012; Li et al., 2016; Ryan et al., 2013; Weinstein et al., 2011). Our observations suggest that uptake and translocation mechanisms of Cu in plants differ between more or less contaminated sites and that the contribution of different mechanisms can potentially be traced by Cu isotope analysis.

Finally, the underlying hypothesis of this work, that soil chemistry controls plant functioning, can only partly be confirmed. On one hand there are facts supporting this hypothesis as differences in wine composition are observed on soil types with contrasted

physico-chemical properties. Also in the greenhouse experiment, results show the influence of soil on plant functioning but in return the evolution of soil solutions chemistry appears to be controlled by plant action. On the other hand, there is also evidence for the inverse action most visible in the Soave study where soil type does not lead to large differences in elemental contents. Therefore contamination dependence of Cu isotope fractionation also indicates that plants, and especially grapevines, are able to adapt to various soil conditions, and Cu isotopes are not able to predict plant signature from soil signature.

8.2 Perspectives

8.2.1 Further investigations on the influence of soil chemistry on wine taste

Our approach does not justify differences in wine flavor (e.g. organoleptic properties) due to soil type. The high variability between vintages and even around soil sampling points observed in **Chapter 5** calls for a much larger sample size than used in this chapter. In some ways, **Chapter 4** presents such setting. Even though no thorough investigation of the respective soils are available, simple classification as calcareous or non-calcareous shows repercussion in the elemental composition data. This allows for the theoretical possibility that differences in elemental nutrition can cause either different taste properties as their contents regulate grape acidity, or catalyze the synthesis of aromatic compounds (Brunetto et al., 2015; Pohl, 2007). To identify such effects, an investigation of compounds relevant for flavor would be needed. A similar approach as in **Chapter 4** could then be used to identify the ‘taste of carbonates’ by analyzing a large panel of wines coming from known soils with known properties. High sample sizes would also allow the isolation of a soil effect from other effects such as agricultural practices, timing of harvest etc. As high throughput is needed for a such study, a promising approach would be the analysis of aroma compounds by RAMAN spectroscopy (Martin, 2015), with minimal sample preparation and measurement time in the order of minutes. These analyses could be coupled with elemental analyses to highlight which part of mineral nutrition actually causes the possible differences in aroma profiles.

8.2.2 Perspectives for further understanding of Cu mobility in soils

A reappearing factor of Cu mobility in **Chapters 6 and 7** is organic matter. A parallel investigation of organic matter and metal transport would thus be interesting. For vertical transport, the most urgent question is: *Where does the organic matter transporting Cu to depth come from – root exudates? Litter decay? Or organic amendments from viticultural practice?*

A first approach could be a study of Cu contents in soil solution over time. Therefore one could evaluate what time of a year the highest Cu concentrations are present and what treatments preceded that moment. Simultaneously organic matter parameters as the SUVA or organic acid contents could be investigated to approach the organic matter type. Also more sophisticated methods on the investigation of organic matter as EPR or nuclear magnetic resonance (NMR) could be deployed.

EPR analysis of environmental samples appears to be a promising rapid tool for speciation analysis that is less expensive than EXAFS studies. Method improvement would be necessary to use the full potential of the technique. At first, even at high pollution levels, samples should be run at low temperatures (i.e. under liquid He) to improve signal to noise ratios. This would facilitate the observation of hyperfine coupling and thus give additional information about the Cu binding environment. Secondly, a thorough investigation on interferences of Mn and Fe species commonly encountered in soils should be conducted in order to improve specificity of the signal interpretation.

8.2.3 Perspectives for the use of Cu isotope chemistry in the soil-plant continuum

Finally, we observed significant variations in Cu isotope fractionation during plant uptake, depending on Cu contamination levels. It would be interesting to see if these differences in Cu uptake mechanisms have repercussions on Fe isotope fractionation in plants, as an influence of Fe uptake mechanisms on Cu fractionation is expected (Jouvin et al., 2012; Ryan et al., 2013). Regardless, observations of isotope fractionations in plants are always mean fractionations of multiple of transport mechanisms. To move forward in the understanding of isotope fractionation by plants, it is necessary to isolate these mechanisms. A first step would be to see if root apoplast desorption actually causes lighter root isotope ratios, as suggested by Ryan et al. (2013). A next step would be the use of genetically modified plants artificially shutting down certain transport mechanisms to isolate their impact on isotope fractionation. This last point has recently been done with yeast cells and showed that, surprisingly, light isotope fractionation is caused by the import to the cell rather than the reduction step (Cadiou et al., 2017).

Chapter 9

Conclusion en français

Dans les écosystèmes cultivés et en particulier en environnement viticole, de nombreux processus d'interactions entre les sols et les plantes existent. Le travail présenté dans cette thèse tente de répondre à de nombreuses questions relevant de l'influence de la physico-chimie du sol sur la signature chimique des solutions du sol, des organes de la plantes et sur le vin. Ce travail se focalise en particulier sur la potentialité de tracer la composition chimique des vins en fonction des propriétés des sols, sur l'existence de traceurs physico-chimiques de la composante sol dans le fameux « effet terroir » et sur les transferts élémentaires dans le continuum sols – solution du sol – plante, en particulier le devenir du cuivre des pesticides cupriques largement employés en viticulture. Ce chapitre de conclusion a pour but de faire une synthèse des résultats majeurs obtenus et de proposer des pistes de réflexion pour des investigations futures dans la compréhension du rôle de la biogéochimie du sol en viticulture.

9.1 Conclusions majeures

9.1.1 Le rôle de la composante « sol » sur la signature élémentaire des vins :

Dans le **quatirème chapitre**, les teneurs élémentaires en Mg, Ca, Mn, Sr et Ba ont été analysées dans plus de 200 vins d'Europe de l'ouest. Cette base de données a été mise en relation avec le type de sol (carbonaté *vs.* non carbonaté) ainsi que les paramètres climatiques locaux (précipitations et températures) enregistrés lors des années de récolte respective pour chaque vin.

Les paramètres pédologiques et météorologiques influencent les profils élémentaires dans les vins et certains éléments tels que Mg, Ba, Ca et Sr sont les traceurs géochimiques pertinents de l'influence du type de sol sur la chimie des vins.

Les concentrations élémentaires dans les vins sont significativement influencées par le type de vin (rouge, blanc, rosé et gris), ceci est particulièrement visible au travers des rapports Mg/Ca. De la même manière, le type de sol montre une influence significative sur les compositions élémentaires dans les vins. Les vins obtenus à partir de parcelles sur sol non carbonaté ont des teneurs en Mg et Ba supérieures à celles mesurées dans les vins obtenus sur des parcelles sur sol carbonaté. Ce résultat est d'autant plus surprenant que pour la plupart des vignobles sur sol acide, les pratiques de chaulage sont courantes et ceci semble suggérer que la nutrition de la vigne provient des horizons profonds du sol qui ne sont pas affectés par les pratiques culturales. Les conditions climatiques ont aussi une influence sur les compositions élémentaires des vins. Les vins provenant de régions avec de forts taux de précipitations pendant l'été montrent des teneurs élevées en Mn et Ba alors que les vins provenant de régions au climat sec et chaud présentent des teneurs élevées en Sr. Ces observations confirment l'hypothèse d'un rôle de la chimie du sol sur le chimisme du vin (Coetzee et al., 2014; Greenough et al., 2005). Néanmoins, un pourcentage élevé de la variabilité des teneurs en Sr dans la base de données reste inexpliqué. Ceci est surprenant car le Sr est un des éléments les plus utilisés dans les méthodes de traçage d'origine. Les rapports Ca/Sr des roches carbonatées dépendent de leur âge géologique et les différences observées pourraient être davantage liées à l'âge des roches mères qu'au processus dans le sol ou au climat local.

9.1.2 Pertinence des paramètres descripteurs des sols dans l'étude des transferts élémentaires sols – plantes

Le **cinquième chapitre** de cette thèse s'appuie sur une étude réalisée à l'échelle de parcelles viticoles qui s'intéresse à l'influence du type de sol sur la vigne au travers de l'étude de paramètres importants en termes de viticulture. Deux types de sols différents (calcaric cambisol et vertic cambisol) formés aux dépens de deux formations géologiques distinctes ont été investigués dans la région de Soave en Italie.

Des propriétés des sols différentes (texturales, structurales, minéralogie, . . .) n'induisent pas forcément des différences dans les teneurs élémentaires des sols et à fortiori dans la composition chimique de la plante.

L'étude pédologique sur le terrain a confirmé l'existence de différences pédologiques entre les deux sols. En revanche, les investigations minéralogiques et géochimiques ont démontré que les mécanismes de formation et d'évolution des sols étudiés sont plus complexes qu'attendu. Il en résulte de fortes similitudes en termes de géochimie entre les deux types de sols. L'utilisation des isotopes radiogéniques du Sr a permis de relier

les signatures chimiques des plantes à celles des sols correspondants impliquant une influence possible du type de sol sur la nutrition en Ca des plantes. De façon générale il y a une tendance à plus fortes teneurs élémentaires dans les plantes poussant sur les sols plus influencés par le calcaire. Les analyses des biomarqueurs tels que les rapports des acides gras dans les feuilles ou les taux de sucre dans les baies n'ont pas réussi à démontrer l'existence de différences significatives en fonction du type de sols. La variabilité observée pour ces paramètres biochimiques entre des échantillons de plantes pris au même endroit ou entre les échantillons des différentes années est plus importante que la variabilité associée au type de sol.

9.1.3 Le devenir du Cu des pesticides dans les sols viticoles et son implication dans le transfert sol – plante du Cu

Dans le **chapitre 6**, les cambisols calcariques et vertiques de l'appellation Soave sont considérés à nouveau afin d'étudier le devenir du cuivre dans ces sols.

La matière organique et les carbonates influencent la mobilité du cuivre dans les sols qui est mobile même en environnement carbonaté. Les signatures isotopiques en cuivre reflètent les mécanismes physico-chimiques de rétentions/mobilisation du cuivre dans les sols et pas son origine.

Contrairement aux résultats des investigations menées sur cette problématique dans la littérature (Duplay et al., 2014 ; Komárek et al., 2010 ; Pietrzak and McPhail, 2004), nous démontrons à l'aide de calculs de bilans de masse à l'échelle des profils de sols que Cu est transféré verticalement même dans les sols carbonatés. Les isotopes stables du Cu suggèrent une mobilité/perte via les solutions du Cu associé à la matière organique qui appauvrirait les sols carbonatés en isotopes lourds du Cu. Les analyses EPR d'échantillons de sols, décarbonatés ou non, montrent qu'une partie du Cu est associée aux phases carbonatées dans les sols calcariques, contrairement aux sols vertiques. La modélisation thermodynamique nous indique que les carbonates de Cu peuvent se former dans les conditions du sol mais que ces formes peuvent être dissoutes par des ligands organiques en solution, même à des pH élevés. Ceci suggère que la mobilité du Cu est contrôlée par la matière organique dans les sols et ouvre la question du rôle des amendements organiques sur la mobilité des métaux. Les pesticides cupriques montrent des variations de signatures isotopiques en Cu très grandes rendant impossible l'utilisation des isotopes du Cu comme traceurs de source du Cu dans les sols.

9.1.4 Caractérisation des mécanismes de transferts du Cu dans le continuum sol – solution du sol – plante à l’aide des isotopes stables du Cu

Les investigations sur le transport du Cu à plus petite échelle dans le cadre d’une expérimentation en serre, comme décrite dans le **chapitre 7**, montrent que la perte du Cu via les solutions du sol est effectivement liée à la dynamique de la matière organique. Plus le rapport de MO mobile est élevée plus le teneur en Cu en solution est importante, en particulier dans les sols contaminés.

La présence de cuivre dans les sols viticoles influence les modalités de nutrition et la santé de la plante. La composition chimique et isotopique en Cu des solutions du sol est variable avec le temps et montre le rôle majeur de la matière organique.

Dans les sols avec des taux de relargage du Cu élevés, la toxicité du Cu apparaît comme l’un des facteurs clefs responsable de la perte de biomasse des plants de vigne. Avec le temps, les teneurs élémentaires dans la solution du sol évoluent différemment en fonction des sols et des éléments, en relation avec l’activité de la plante. Par exemple, les teneurs en P dans la solution du sol augmentent dans certaines modalités alors que le P est un nutriment qui est prélevé par la plante. Les teneurs en métaux évoluent différemment avec le temps dans les solutions du sol. Dans la majorité des modalités étudiées, les teneurs en Cu dans la solution semblent être directement liées aux teneurs en TOC plutôt qu’au pH. Les fractionnements isotopiques entre les sols et les solutions du sol sont différents selon les modalités étudiés. Dans les sols de Bordeaux, les mêmes rapports isotopiques sont mesurés dans les sols et les solutions des sols, alors que pour les autres modalités, les solutions sont plus lourdes isotopiquement. Dans les sols de la région de Soave, les fractionnements observés entre les sols et les solutions sont semblables (autour de +0.4 ‰) et semblables au fractionnement déterminé lors des extractions au citrate du **chapitre 6** sur les mêmes sols. Dans certains cas, les solutions du sol montrent des variations significatives dans les rapports isotopiques en Cu au cours du temps. Dans ces solutions, les rapports isotopiques sont plus légers à la fin des expérimentations.

Les signatures isotopiques en Cu des organes des plantes suggèrent que les plantes mettent en œuvre différents mécanismes d’incorporation du Cu en fonction du sol.

Plus les teneurs en Cu sont importantes dans la solution du sol, plus les racines des vignes contiennent du Cu. Dans les feuilles, par contre, les teneurs en Cu sont semblables pour 5 des modalités sur les 6. Le fractionnement des isotopes du Cu entre les différents compartiments de la plante dépend du niveau de contamination en Cu. Plus les teneurs en Cu sont importantes dans les racines, plus le rapport isotopique en Cu associé montre un fractionnement vers les isotopes lourds dans les racines et vers les isotopes légers dans les feuilles. Les signatures isotopiques en Cu mesurées dans des feuilles du terroir de Soave prélevées sur le terrain sont plus légères que celles des vignes poussant sur les mêmes sols de l'expérience en serre, en accord avec Weinstein et al. (2011). Ce constat contredit l'hypothèse d'une absorption foliaire du Cu car le Cu des pesticides pour ces parcelles est isotopiquement plus lourd. Ces données s'ajoutent à la littérature restreinte et controversée sur l'isotopie du Cu dans les plantes (Jouvin et al., 2012; Li et al., 2016; Ryan et al., 2013; Weinstein et al., 2011). Nos observations suggèrent que le fractionnement isotopique du Cu dans la plante dépend de la contamination en Cu dans le sol et peut ainsi être utilisé afin de tracer les mécanismes en œuvre dans la nutrition des plantes.

Finalement, l'hypothèse globale que les propriétés du sol influencent le fonctionnement de la plante ne peut être confirmée qu'en partie par ce travail. Le fait que, statistiquement sur un panel de plus de 200 vins, certaines propriétés physico-chimiques des sols tels que la présence de carbonates peuvent expliquer la composition élémentaire du vin va dans le sens de cette hypothèse. Les résultats obtenus dans le cadre de l'expérience en serre montrent que les sols influencent le fonctionnement des plantes mais qu'en retour les plantes impactent de manière non négligeable la chimie des solutions des sols. Mais cette relation entre le sol et la plante n'est plus vérifiée avec le changement d'échelle. En effet, à Soave, les différents types de sols identifiés n'amènent pas à une composition de plantes différente. Finalement les variations dans le fractionnement isotopique du Cu dans le continuum sol – solution du sol – plante pour des niveaux de contamination en Cu différents indiquent que les plantes s'adaptent fortement à leur environnement et que les isotopes du Cu ne permettent pas de prédire la signature des plantes à partir de celle du sol.

9.2 Perspectives

9.2.1 Pistes à suivre pour poursuivre les investigations sur l'influence du sol dans le goût du vin

Nos approches ne permettent pas de justifier les différences sensorielles reconnues (goût du vin) en fonction du sol. La grande variabilité interannuelle et interindividuelle nécessite un très grand nombre d'échantillons. Le dispositif du **chapitre 4** par exemple pourrait être traduit dans ce sens. L'étude des compositions élémentaires montre des différences entre sols calcaires et sols non calcaires. Cela implique une possibilité théorique d'influence du sol sur les propriétés organoleptiques du vin car des différences sont observées dans des nutriments comme le Mg. Certains nutriments comme le K et le Ca régulent l'acidité du raisin ou catalysent la synthèse de composés organiques (Brunetto et al., 2015; Pohl, 2007). Pour identifier un tel effet sur le goût du vin une étude comme présentée en **chapitre 4** devrait être couplée à des analyses de composés organiques du vin (précurseurs d'arômes,...). Comme un grand nombre d'échantillons est nécessaire une étude par spectrométrie RAMAN semble prometteuse les analyses sont rapides avec une préparation minimale de l'échantillon (Martin, 2015).

9.2.2 Pistes pour approfondir l'étude de la mobilité du Cu dans les sols

Un facteur déterminant dans la mobilité du Cu est la matière organique. Par contre il apparaît que des différences dans la mobilité du Cu existent suivant le type de matière organique. Pour comprendre le transport vertical du Cu étudié dans le **chapitre 6** la question de l'origine de la matière organique se pose. *S'agit-il d'exsudats racinaires ? de matière organique originaire de la dégradation des feuilles ou des amendements organiques de la viticulture biologique ?*

Une première approche pourrait être l'étude des teneurs en Cu dans la solution de sol en fonction du temps. Ceci permettrait d'évaluer à quel moment le Cu est le plus mobile et si cette mobilité est liée à des apports de matière organique ou à un processus saisonnier. En parallèle, une étude biogéochimique sur les types de matière organique dans la solution du sol pourrait donner des indications sur la nature de la matière organique responsable de la mobilité du Cu. Des analyses du SUVA ou des acides organiques en solution pourraient être un point de départ. De plus, des méthodes plus sophistiquées comme l'EPR ou la RMN pourraient être utilisées afin d'identifier le type de matière organique associée aux moments de plus grande mobilité du Cu.

L'analyse des échantillons environnementaux par RPE semble être un outil prometteur pour l'étude de la spéciation des métaux de transition dans différentes matrices car cette méthode est plus accessible et moins chère que l'EXAFS. Par contre, pour avoir des renseignements plus précis sur la spéciation, il faudrait améliorer le protocole présenté dans cette thèse. En premier lieu, les mesures devraient être conduites à basse température (sous He liquide) afin d'améliorer le rapport signal sur bruit. Cela faciliterait l'identification des interactions hyperfines et donnerait des informations plus précises sur les liaisons impliquées entre le Cu et les autres composants. Deuxièmement une étude sur la spéciation du Fe et du Mn dans les sols devrait être conduite afin d'identifier la contribution de ces éléments à la spéciation globale du Cu car ils sont souvent beaucoup plus concentrés dans les sols que le Cu.

Enfin, des variations dans le fractionnement isotopique du Cu dans les plantes en fonction du niveau de contamination en Cu ont été observées. Il serait intéressant d'investiguer si le niveau de contamination en Cu aurait une répercussion sur les rapports isotopiques en Fe car l'inverse a toujours été supposé dans la littérature (Jouvin et al., 2012; Ryan et al., 2013). Pour davantage développer l'outil isotopique dans le continuum sol – solution du sol – plante, il serait nécessaire de décomposer le signal moyen des mesures faites dans cette thèse mais aussi dans la littérature en général. Un premier pas serait de vérifier si la désorption du cuivre depuis l'apoplaste est responsable de rapports isotopiques plus légers dans les racines comme suggéré par Ryan et al. (2013). Une prochaine étape serait de mettre en place une expérimentation avec des plantes génétiquement modifiées en désactivant artificiellement les mécanismes de transport afin d'observer les répercussions sur les rapports isotopiques du Cu. Ce dernier point a été étudié dans les levures et a donné le résultat surprenant que ce n'est pas la réduction du Cu mais son transport vers l'intérieur de la cellule qui rend le Cu plus léger (Cadiou et al., 2017).

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Appendix

Appendix A – Wine Data

ID	Country	Appellation	Greater Wine Region	Climate	Color	Vintage	Calcareous
1	France	AOC Côtes de Provence	Provence	Mediterranean	Rosé	2015	Yes
2	France	IGP Val de Loire	Loire	Atlantic	White	2015	No
3	France	AOC Côtes du Rhône	C.d.Rhône	Mediterranean	White	2015	Yes
4	France	AOC Côtes Catalanes	Corb.- Rouss.	Mediterranean	Rosé	2015	Yes
5	Spain	DOC La Rioja	La Rioja	Mediterranean	Red	2012	Yes
6	Spain	DO Terra Alta	-	Mediterranean	Red	2015	Yes
7	France	IGP Pays Cathare	Corb.- Rouss.	Mediterranean	Red	2013	No
8	France	AOC Côtes de Bourg	Bordeaux	Atlantic	Red	2014	Yes
9	France	AOC Fronton	Fro.-Gaill.	Atlantic	Red	-	No
10	Spain	DOC La Rioja	La Rioja	Mediterranean	Red	2012	Yes
11	France	IGP Côtes de Gascogne	Gers	Atlantic	Red	2015	Yes
12	Spain	DOC La Rioja	La Rioja	Mediterranean	Red	2013	Yes
15	Germany	Franken	-	Continental	White	2014	-
18	France	IGP Côtes de Gascogne	Gers	Atlantic	Red	2015	No
19	France	IGP Pays d'Oc	Corb.- Rouss.	Mediterranean	Red	2014	Yes
22	France	AOC Bourgeuil	Loire	Atlantic	Red	2009	-
27	Spain	DO Navarra	La Rioja	Mediterranean	White	2015	Yes
28	France	AOC Pommard	Rhône.Nord	Continental	Red	1995	Yes
29	Italy	IGT Lambrusco Emilia	-	-	Red	-	No
32	France	AOC Blaye-côtes-de-Bordeaux	Bordeaux	Atlantic	Red	2000	Yes
33	France	AOC Gigondas	C.d.Rhône	Mediterranean	Red	2000	Yes
34	France	AOC Côtes du Rhone - Cairanne	C.d.Rhône	Mediterranean	Red	2011	-
35	France	AOC Maury	Corb.- Rouss.	Mediterranean	Red	2011	No
36	France	AOC Côtes du Roussillon	Corb.- Rouss.	Mediterranean	Red	2007	Yes
37	France	AOC Corbières	Corb.- Rouss.	Mediterranean	Red	2004	Yes
38	France	AOC Fronton	Fro.-Gaill.	Atlantic	Red	2000	No
39	France	AOC Côtes de Toul	-	Continental	Gris	2014	Yes
40	France	AOC Fitou	Corb.- Rouss.	Mediterranean	Red	2013	No
41	France	AOC Cotes du Roussilon Villages	Corb.- Rouss.	Mediterranean	Red	2014	No
42	France	AOC Muscat de Mireval	Languedoc	Mediterranean	White	2001	Yes
43	Germany	Württemberg	Rhine.V.	Continental	Red	2014	Yes
44	Germany	Pfalz	Rhine.V.	Continental	Red	2014	No
45	Germany	Nahe	R.Hessen	Continental	Red	2008	Yes
46	Germany	Pfalz	R.Hessen	Continental	White	2015	-
47	Germany	Baden	Rhine.V.	Continental	White	2014	-
48	Germany	Rheinhessen	R.Hessen	Continental	White	2015	-
49	Italy	DOC Nebbiolo	Ligure	Mediterranean	Red	2010	Yes
51	France	IGP Sable de Carmague	Languedoc	Mediterranean	Gris	2015	Yes
52	Spain	DOC La Rioja	La Rioja	Mediterranean	Rosé	2015	-
53	France	AOC Cote Rotie	Rhône.Nord	Continental	Red	2013	No
56	France	AOC Tavel	C.d.Rhône	Mediterranean	Rosé	2015	Yes

ID	Country	Appellation	Greater Wine Region	Climate	Color	Vintage	Calcareous
58	France	AOC Chignin	Rhône.Nord	Continental	White	2015	Yes
59	France	AOC Savoie	Rhône.Nord	Continental	Red	-	Yes
60	France	AOC Morgon	Rhône.Nord	Continental	Red	2012	No
61	France	AOC Bandol	Provence	Mediterranean	Rosé	2015	Yes
62	France	AOC Maury	Corb.- Rouss.	Mediterranean	Red	2015	-
63	France	AOC Alsace	Rhine.V.	Continental	White	2015	-
64	France	AOC Alsace	Rhine.V.	Continental	White	2014	-
65	France	AOC Picpoul de Pinet	Languedoc	Mediterranean	White	2015	-
66	France	AOC Alsace	Rhine.V.	Continental	White	2015	-
67	France	AOC Banyuls	Corb.- Rouss.	Mediterranean	Red	2008	No
68	France	AOP Gaillac	Fro.-Gaill.	Atlantic	Rosé	-	Yes
69	France	AOC Bergerac	Bordeaux	Atlantic	Rosé	2014	Yes
70	France	AOC Fitou	Corb.- Rouss.	Mediterranean	Red	-	Yes
71	France	AOC Beaujolais	Rhône.Nord	Continental	White	2015	-
72	France	IGP Gard	C.d.Rhône	Mediterranean	Rosé	-	Yes
73	France	AOC Chabils	Loire	Atlantic	White	-	Yes
74	France	AOC Sancerre	Loire	Atlantic	White	2014	Yes
75	France	IGP Ardeche	Languedoc	Mediterranean	Rosé	-	Yes
77	France	IGP Pays d'Oc	Languedoc	Mediterranean	Red	2011	No
78	France	AOC Corse - Porto Vecchio	Corse	Mediterranean	Red	-	No
80	France	AOP Gaillac	Fro.-Gaill.	Atlantic	Red	2014	-
81	France	AOC Juracon	P.Atlantiques	Mountain	White	2014	-
82	France	IGP Côtes de Gascogne	Gers	Atlantic	White	2015	Yes
83	France	AOP Gaillac	Frgaill	Atlantic	White	2011	-
84	France	AOC Pessac Léognan	Bordeaux	Atlantic	Red	2014	No
85	France	AOC Saint Chinian	Languedoc	Mediterranean	Red	2008	Yes
86	France	AOC Buzet	Gers	Atlantic	Red	2012	-
87	France	AOC Fronton	Fro.-Gaill.	Atlantic	Red	2014	No
88	France	IGP Côtes de Gascogne	Gers	Atlantic	White	2016	-
89	France	AOC Madiran	Gers	Atlantic	Red	2010	-
90	France	AOC Lussac Saint-Emilion	Bordeaux	Atlantic	Red	2012	No
91	France	AOC Cote Rotie	Rhône.Nord	Continental	Red	2002	No
92	France	AOC Côtes de Provence	Provence	Mediterranean	Rosé	2014	Yes
93	France	AOC Saint Julien	Bordeaux	Atlantic	Red	2009	-
94	France	AOP Gaillac	Fro.-Gaill.	Atlantic	Red	2015	-
95	France	AOC Limoux	Corb.- Rouss.	Mediterranean	Red	2012	Yes
96	France	AOC Beaujolais	Rhône.Nord	Continental	Red	2010	Yes
97	France	AOC Bourgogne	Rhône.Nord	Continental	White	2001	-
98	France	AOC Patrimonio	Corse	Mediterranean	White	2014	Yes
99	Germany	Rheinhessen	R.Hessen	Continental	White	2015	Yes
100	Germany	Rheinhessen	R.Hessen	Continental	White	2014	-

ID	Country	Appellation	Greater Wine Region	Climate	Color	Vintage	Calcareous
101	Germany	Rheinhessen	R.Hessen	Continental	White	2015	Yes
102	France	AOC Côtes du Roussillon	Corb.- Rouss.	Mediterranean	Red	2011	Yes
103	France	AOC Brulhois	Gers	Atlantic	White	2013	No
104	France	AOC Alsace	Rhine.V.	Continental	White	2012	-
105	France	IGP Côtes de Gascogne	Gers	Atlantic	White	2014	-
106	France	Roussillon	Corb.- Rouss.	Mediterranean	White	2010	Yes
107	France	AOC Corbières	Corb.- Rouss.	Mediterranean	Red	2010	-
108	France	AOC Coteaux du Languedoc	Languedoc	Mediterranean	Red	2010	Yes
109	France	IGP Pays d'Oc	Languedoc	Mediterranean	Red	2013	-
110	France	AOC Brulhois	Gers	Atlantic	Red	2012	No
111	France	AOC Coteaux du Languedoc	Corb.- Rouss.	Mediterranean	Red	2009	Yes
112	France	AOC Corbières	Corb.- Rouss.	Mediterranean	Red	2011	-
113	France	AOC Coteaux du Languedoc	Languedoc	Mediterranean	Red	2013	No
114	France	AOC Duche d'Uzes	Languedoc	Mediterranean	Red	2014	-
115	France	AOC Côtes du Forez	Rhône.Nord	Continental	Red	2015	No
116	France	IGP Val de Loire	Loire	Atlantic	Red	-	-
120	Italy	DOP Chianti - Colli Sensi	Toscane	Mediterranean	Red	2014	Yes
121	Italy	IGT Toscana Centrale	Toscane	Mediterranean	Red	-	-
122	Italy	DOC Chianti	-	Mediterranean	Red	2012	-
123	France	AOC Saumu et Cabernet-de-Saumur	Loire	Atlantic	White	-	Yes
124	France	AOC Chinon	Loire	Atlantic	Red	2010	Yes
125	Germany	Nahe	R.Hessen	Continental	White	2013	No
126	Germany	Nahe	R.Hessen	Continental	White	2014	No
127	Germany	Nahe	R.Hessen	Continental	White	2015	No
128	Germany	Nahe	R.Hessen	Continental	Red	2012	No
129	Germany	Württemberg	Rhine.V.	Continental	Red	2014	-
130	Spain	DOP Pago del Otazu	La Rioja	Mediterranean	White	2015	Yes
131	France	AOC Alsace	Rhine.V.	Continental	White	2015	-
132	France	AOC Saint Mont	Gers	Atlantic	Rosé	2015	-
133	France	AOC Mâcon Lugny	Rhône.Nord	Continental	White	2013	Yes
134	France	AOC Madiran	Gers	Atlantic	White	2013	No
135	France	IGP Pays d'Oc	Languedoc	Mediterranean	Red	2012	Yes
136	France	AOC Chinon	Loire	Atlantic	Red	2010	-
138	France	AOC Coteaux de Giennois	Loire	Atlantic	Red	2010	Yes
141	France	AOC Saint Emilion - Grand Cru	Bordeaux	Atlantic	Red	2005	Yes
142	France	AOC Iroulégu	P.Atlantiques	Mountain	Red	2014	No
143	France	AOP Gaillac	Fro.-Gaill.	Atlantic	Red	2011	-
144	France	AOC Beaujolais-Villages	Rhône.Nord	Continental	Red	2014	No
145	Spain	DOC La Rioja	La Rioja	Mediterranean	Red	2012	-
146	Italy	DOC Vallée d'Aoste	Aoste V.	Mountain	White	2014	Yes
147	France	AOC Corbières	Corb.- Rouss.	Mediterranean	White	2015	Yes
148	France	AOC Juracon	P.Atlantiques	Mountain	White	2015	-
149	France	AOC Alsace	Rhine.V.	Continental	White	2014	-
154	Italy	DOCG Moscato d'asti	Ligure	Mediterranean	White	2015	Yes

ID	Country	Appellation	Greater Wine Region	Climate	Color	Vintage	Calcareous
155	France	AOC Faugères	Languedoc	Mediterranean	Red	2003	No
159	France	AOC Beaune	Rhône.Nord	Continental	Red	2014	Yes
160	France	AOC Margaux	Bordeaux	Atlantic	Red	2014	Yes
161	France	AOC Pic St. Loup	Languedoc	Mediterranean	Red	2004	Yes
162	France	AOC Puisseguin Saint Emilion	Bordeaux	Atlantic	Red	2015	-
163	France	Montfort	-	-	Red	-	-
166	Spain	DO Somontano	-	Mediterranean	Red	2009	No
167	Spain	DOC La Mancha	-	Mediterranean	Red	2011	Yes
168	Italy	IGT Toscana Centrale	Toscane	Mediterranean	Red	2011	Yes
169	Italy	DOC Vallée d'Aoste	Aoste V.	Mountain	Red	2013	No
170	Italy	IGT Colli della Toscana Centrale	Toscane	Mediterranean	Red	2013	-
171	France	AOC Saint Chinian	Languedoc	Mediterranean	Red	2015	No
172	France	AOC Madiran	Gers	Atlantic	Red	2009	-
173	Italy	Soave	Soave	Atlantic	White	2016	Yes
174	Italy	Soave	Soave	Atlantic	White	2016	Yes
175	Italy	Soave	Soave	Atlantic	White	2016	Yes
176	Italy	DOC Soave	Soave	Atlantic	White	2015	Yes
177	France	AOP Gaillac	Fro.-Gaill.	Atlantic	White	2013	Yes
178	France	AOC Saint Joseph	Rhône.Nord	Continental	White	2015	-
179	France	AOC Sancerre	Loire	Atlantic	White	2015	Yes
180	France	AOC Saint Mont	Gers	Atlantic	Red	2013	-
182	Germany	Rheinhessen	R.Hessen	Continental	White	2015	Yes
184	France	AOC Pouilly-Fumé	Loire	Atlantic	White	2014	-
185	France	AOC Iroulégu	P.Atlantiques	Mountain	White	2013	No
187	France	AOC Côtes du Rhone - Cairanne	C.d.Rhône	Mediterranean	Red	2015	-
188	France	AOC Languedoc - Terrases du Larzac	Languedoc	Mediterranean	Red	2013	-
189	France	AOC Coteaux du Languedoc-Pezenas	Languedoc	Mediterranean	Red	2010	-
190	France	AOC Coteaux du Languedoc	Languedoc	Mediterranean	White	2011	-
191	France	La Clape	Corb.-Rouss.	Mediterranean	Red	2013	Yes
192	France	La Clape	Corb.-Rouss.	Mediterranean	Red	2013	Yes
193	France	La Clape	Corb.-Rouss.	Mediterranean	Red	2012	Yes
194	France	La Clape	Corb.-Rouss.	Mediterranean	Red	2013	Yes
195	France	La Clape	Corb.-Rouss.	Mediterranean	Red	2014	Yes
196	France	La Clape	Corb.-Rouss.	Mediterranean	Red	2012	Yes
197	France	La Clape	Corb.-Rouss.	Mediterranean	Red	2013	Yes
198	France	La Clape	Corb.-Rouss.	Mediterranean	Red	2014	Yes
199	Italy	Soave	Soave	Atlantic	White	2015	Yes
200	Italy	Soave	Soave	Atlantic	White	2015	Yes
201	Italy	Soave	Soave	Atlantic	White	2015	Yes
202	Italy	DOC Soave	Soave	Atlantic	White	2014	Yes
203	Italy	DOC Soave	Soave	Atlantic	White	2013	Yes
204	Italy	DOC Soave	Soave	Atlantic	White	2012	Yes
205	Italy	IGT Verona Garganega	Soave	Atlantic	White	2013	Yes
206	Italy	DOC Soave	Soave	Atlantic	White	2008	Yes

ID	Country	Appellation	Greater Wine Region	Climate	Color	Vintage	Calcareous
207	France	Pessac Léognan	Bordeaux	Atlantic	Red	2015	No
208	France	Pessac Léognan	Bordeaux	Atlantic	Red	2015	No
209	Germany	Rheinhessen	R.Hessen	Continental	White	2015	No
210	France	AOC Alsace	Rhine.V.	Continental	White	2015	No
211	France	AOC Alsace	Rhine.V.	Continental	White	2015	No
212	France	AOC Alsace	Rhine.V.	Continental	White	2015	Yes
213	France	AOC Alsace	Rhine.V.	Continental	White	2015	Yes
214	France	AOC Alsace	Rhine.V.	Continental	White	2011	-
215	France	AOC Alsace	Rhine.V.	Continental	White	2015	-
216	France	AOC Bergerac	Bordeaux	Atlantic	White	2015	Yes
217	France	IGP Val de Montferrand	Languedoc	Mediterranean	White	2014	Yes
218	Italy	DOCG Barolo	Ligure	Mediterranean	Red	2012	Yes
219	Italy	DOC Colli di Luni	Ligure	Mediterranean	Red	2012	-
220	France	AOC Coteaux du Languedoc	Corb.- Rouss.	Mediterranean	Red	2013	No
221	France	AOC Muscadet-sevre- et-maine	Loire	Atlantic	White	2014	No
222	France	AOC Bordeaux	Bordeaux	Atlantic	Red	2015	Yes
223	France	AOC Fitou	Corb.- Rouss.	Mediterranean	Red	2014	No
224	Germany	Baden	Rhine.V.	Continental	Red	2014	No
225	France	AOC Buzet	Gers	Atlantic	Red	2013	-
226	France	AOC Cahors	Fro.-Gaill.	Atlantic	Red	-	No
227	France	AOC Côte du Rhone Villages	C.d.Rhône	Mediterranean	Red	2015	Yes
28	France	AOC Saumur-Champigny	Loire	Atlantic	Red	2015	-
229	France	IGP Côtes Catalanes	Corb.- Rouss.	Mediterranean	Red	2011	No
230	France	AOC Saint Estèphe	Bordeaux	Atlantic	Red	2000	No
231	France	AOC Pauillac	Bordeaux	Atlantic	Red	2005	Yes
232	France	AOC Beaumes de Venise	C.d.Rhône	Mediterranean	Red	2015	Yes
234	France	AOC Languedoc - Terrasses du Larzac	Languedoc	Mediterranean	Red	2014	Yes
235	France	IGP Côtes de Gascogne	Gers	Atlantic	Red	2013	Yes
236	France	AOP Gaillac	Fro-Gaill.	Atlantic	Red	2014	Yes
237	Italy	DOC Firriato	Sicilie	Mediterranean	White	2013	No
238	France	AOC Sartène	Corse	Mediterranean	White	2014	No
239	France	AOC Anjou	Loire	Atlantic	Red	2015	-
240	France	AOC Saint Chinian	Languedoc	Mediterranean	Red	2013	No
242	Germany	Rheinhessen	R.Hessen	-	White	-	-
243	France	AOC Montbazillac	Bordeaux	Atlantic	White	1947	Yes
244	France	AOC Côtes du Rhone	C.d.Rhône	Mediterranean	Red	2014	-
245	France	AOC Côtes du Rhone	C.d.Rhône	Mediterranean	Red	2014	Yes
246	France	AOC Côtes du Rhone - Cairanne	C.d.Rhône	Mediterranean	Red	2013	-
247	France	AOC Côtes du Rhone	C.d.Rhône	Mediterranean	White	2014	-

ID	Country	Appellation	Greater Wine Region	Climate	Color	Vintage	Calcareous
247	France	AOC Côtes du Rhone	C.d.Rhône	Mediterranean	White	2014	-
248	France	AOC Saint Joseph	C.d.Rhône	Mediterranean	Red	2015	No
249	France	AOC Gigondas	C.d.Rhône	Mediterranean	Red	2013	Yes
250	France	AOC Ventoux	C.d.Rhône	Mediterranean	Red	2011	Yes
251	France	AOC Côtes du Rhone - Cairanne	C.d.Rhône	Mediterranean	Red	2015	-
252	France	AOC Ventoux	C.d.Rhône	Mediterranean	Red	2015	-
253	France	AOC Ventoux	C.d.Rhône	Mediterranean	Red	2013	Yes

ID	Ca	Mg	Mn	Sr	Ba
	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹
1	59.423	74.014	0.803	0.386	0.102
2	68.211	56.059	1.372	0.155	0.093
3	81.677	76.202	0.394	0.368	0.036
4	82.772	68.71	0.710	0.271	0.079
5	52.150	98.038	0.725	1.123	0.049
6	77.974	107.557	0.826	1.9776	0.0658
7	53.492	93.491	0.814	0.462	0.103
8	59.758	92.528	0.938	0.309	0.156
9	82.890	97.239	2.358	0.586	0.231
10	61.018	102.431	0.967	1.548	0.056
11	73.551	87.354	0.701	0.285	0.056
12	51.761	102.730	0.512	1.876	0.047
15	104.939	80.846	0.820	0.361	0.116
18	69.416	91.544	1.075	0.698	0.090
19	62.224	86.404	0.549	0.327	0.066
22	56.465	74.665	1.180	0.262	0.115
27	90.677	94.972	0.915	1.704	0.032
28	56.721	82.132	1.186	0.106	0.042
29	141.689	98.750	1.362	0.965	0.094
32	59.241	70.764	0.470	0.209	0.039
33	44.313	98.572	1.032	0.346	0.046
34	45.761	102.262	0.837	0.468	0.077
35	48.696	104.079	1.59	1.296	0.197
36	45.742	91.369	0.790	0.446	0.048
37	34.586	78.946	0.504	0.178	0.102
38	36.937	87.923	2.571	0.304	0.187
39	91.638	71.338	0.504	0.219	0.024
40	64.774	91.371	0.622	0.251	0.054
41	50.02	105.555	2.02	0.451	0.161
42	37.737	80.489	0.468	0.098	0.023
43	61.348	75.843	0.610	0.229	0.102
44	44.912	98.052	1.011	0.242	0.091
45	68.260	71.905	0.895	0.310	0.085
46	103.731	83.235	0.992	0.343	0.054
47	83.884	65.005	0.725	0.257	0.094
48	85.578	86.621	0.964	0.463	0.187
49	59.973	92.702	0.579	0.52	0.049
51	89.326	60.009	0.402	0.396	0.036
52	79.303	78.123	0.730	0.703	0.011
53	51.309	92.638	1.653	0.262	0.091
56	53.083	63.645	0.505	0.288	0.032
58	82.566	63.893	0.684	0.470	0.084
59	70.954	74.416	1.021	0.486	0.087
60	57.269	102.805	3.894	0.377	0.180

ID	Ca	Mg	Mn	Sr	Ba
	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹
61	58.346	66.911	0.233	0.558	0.024
62	62.426	99.927	1.802	0.540	0.078
63	92.171	80.222	1.087	0.228	0.169
64	119.405	92.692	1.507	0.248	0.148
65	90.102	70.746	0.848	0.146	0.043
66	55.509	70.185	0.672	0.167	0.153
67	63.246	130.835	3.552	0.795	0.109
68	73.782	70.890	0.436	0.179	0.053
69	90.668	69.020	0.680	0.139	0.095
70	71.555	114.877	0.969	0.288	0.065
71	38.800	93.967	4.971	0.238	0.160
72	59.409	69.337	0.492	0.289	0.049
73	69.061	68.609	0.680	0.113	0.017
74	80.266	65.143	0.824	0.154	0.048
75	69.665	67.315	0.928	0.298	0.103
77	66.021	100.826	0.682	1.194	0.215
78	59.029	134.45	2.339	0.861	0.228
80	78.765	90.570	0.845	0.265	0.134
81	83.849	76.864	0.985	0.173	0.071
82	83.621	63.727	0.854	0.197	0.063
83	47.648	56.231	0.681	0.192	0.074
84	74.035	104.106	1.075	0.359	0.200
85	43.343	103.148	0.766	0.149	0.049
86	54.047	86.390	0.612	0.236	0.081
87	73.479	88.073	2.245	0.497	0.178
88	105.562	80.851	1.32	0.47	0.123
89	69.720	88.991	2.677	0.198	0.110
90	60.276	86.455	1.404	0.292	0.152
91	64.868	94.453	2.766	0.354	0.126
92	54.813	66.852	0.824	0.202	0.086
93	56.146	94.432	0.919	0.167	0.101
94	60.128	89.384	1.121	0.236	0.133
95	58.829	99.160	1.094	0.562	0.118
96	58.157	95.826	5.758	0.414	0.342
97	69.081	68.493	1.036	0.116	0.069
98	60.468	55.927	0.218	0.112	0.029
99	84.362	81.783	0.948	0.448	0.066
100	113.278	78.055	1.055	0.352	0.084
101	61.119	71.295	0.77	0.218	0.077
102	59.344	117.059	0.962	0.469	0.042
103	133.731	102.227	2.863	0.464	0.207
104	81.199	68.133	1.041	0.271	0.168
105	101.078	77.358	1.848	0.333	0.124
106	51.014	67.316	0.627	0.124	0.023
107	53.382	120.559	0.965	1.113	0.108
108	49.133	115.873	0.471	0.177	0.042
109	64.183	98.495	1.471	0.405	0.160
110	51.073	80.552	1.456	0.465	0.285
111	51.717	93.920	0.674	0.345	0.069

ID	Ca	Mg	Mn	Sr	Ba
	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹
112	85.217	102.55	0.878	0.345	0.061
113	56.367	104.190	0.813	0.302	0.108
114	57.179	91.458	0.818	0.406	0.082
115	55.005	101.706	1.259	0.515	0.138
116	60.517	129.870	2.929	0.437	0.145
120	75.942	95.269	0.64	0.715	0.053
121	68.439	56.193	0.865	0.36	0.070
122	65.533	120.775	1.058	0.699	0.329
123	85.230	62.670	0.585	0.122	0.039
124	45.362	75.391	0.416	0.119	0.042
125	111.720	86.126	0.818	0.515	0.187
126	131.653	83.921	0.870	0.543	0.188
127	64.020	70.475	0.671	0.295	0.138
28	31.132	87.021	0.711	0.207	0.187
129	79.669	81.732	0.592	0.190	0.104
130	36.469	57.899	0.928	0.531	0.053
131	53.590	65.370	0.588	0.152	0.117
132	80.843	69.278	1.401	0.164	0.093
133	87.005	72.783	0.988	0.137	0.088
134	93.805	94.895	5.041	0.253	0.095
135	56.378	81.140	0.522	0.871	0.065
136	45.113	75.519	0.424	0.107	0.041
138	52.740	95.099	0.623	0.187	0.037
141	57.820	81.687	0.726	0.291	0.119
142	64.533	93.930	1.451	0.218	0.273
143	59.733	126.171	0.658	0.266	0.072
144	66.151	93.449	1.988	0.376	0.199
145	60.539	104.65	0.882	1.636	0.077
146	67.041	85.061	0.743	0.701	0.026
147	71.642	54.951	0.405	0.071	0.033
148	67.741	60.330	1.265	0.160	0.057
149	64.743	63.825	0.755	0.166	0.059
154	108.478	75.459	0.779	0.454	0.087
155	43.937	110.711	1.355	0.326	0.072
159	74.987	84.137	1.102	0.164	0.084
160	86.644	90.828	1.086	0.325	0.105
161	65.649	101.963	0.878	0.522	0.029
162	40.117	141.867	0.858	0.705	0.217
163	6.233	56.673	0.199	0.162	0.260
166	67.710	109.053	0.826	0.945	0.139
167	59.393	94.705	0.672	0.281	0.094
168	51.302	140.837	0.805	0.772	0.031
169	46.212	109.745	0.827	0.657	0.054
170	86.643	100.420	1.144	0.499	0.144
171	52.284	113.02	2.905	0.395	0.174
172	79.077	98.747	1.901	0.217	0.088
173	45.477	46.106	0.136	0.059	0.037
174	64.078	44.257	0.131	0.075	0.047
175	56.532	59.752	0.135	0.075	0.032
176	58.701	77.310	0.230	0.146	0.027
177	55.827	80.762	0.844	0.161	0.088
178	55.657	73.847	0.963	0.326	0.123
179	50.913	64.820	0.797	0.125	0.079
180	71.985	88.07	1.831	0.237	0.106
182	64.094	69.048	0.837	0.215	0.075
184	79.418	61.654	0.713	0.167	0.071
185	69.453	109.324	1.985	0.258	0.2
187	42.243	89.214	0.510	0.421	0.073

ID	Ca	Mg	Mn	Sr	Ba
	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹
188	42.263	94.223	0.542	0.102	0.027
189	58.747	126.924	1.838	0.498	0.277
190	41.046	86.133	2.346	0.24	0.132
199	98.717	83.645	0.213	0.108	0.027
200	92.520	92.983	0.204	0.090	0.016
201	97.872	79.951	0.211	0.081	0.030
202	61.366	77.688	0.262	0.156	0.044
203	60.369	67.371	0.211	0.131	0.024
204	62.876	94.582	0.275	0.184	0.064
205	51.286	69.789	0.197	0.110	0.033
206	62.350	88.212	0.287	0.133	0.039
207	35.626	85.326	0.596	0.219	0.184
208	35.562	90.306	0.444	0.296	0.224
209	76.728	86.144	1.218	0.427	0.098
210	114.687	97.677	1.808	0.235	0.111
211	65.697	91.696	1.492	0.336	0.131
212	63.440	76.981	0.643	0.226	0.121
213	102.842	91.424	0.999	0.191	0.116
214	65.598	60.807	0.557	0.134	0.044
215	50.742	80.339	0.787	0.180	0.114
216	61.979	77.203	0.605	0.175	0.078
217	65.888	77.884	0.604	0.300	0.114
218	54.510	94.551	0.878	0.907	0.079
219	73.291	102.233	1.360	0.889	0.173
220	56.627	99.385	1.29	0.350	0.220
221	85.118	67.929	1.621	0.317	0.113
222	56.768	109.05	1.192	1.016	0.081
223	60.835	83.302	0.739	0.27	0.099
224	80.001	95.632	1.619	0.281	0.132
225	36.198	69.855	1.109	0.32	0.141
226	59.926	85.221	1.388	0.304	0.237
227	65.241	91.704	0.700	0.540	0.056
2228	61.054	85.869	0.845	0.307	0.125
229	54.564	111.333	1.077	0.651	0.111
230	47.808	89.615	0.694	0.224	0.085
231	43.680	79.200	0.610	0.164	0.067
232	49.94	98.864	0.481	0.788	0.059
234	46.248	101.997	0.864	0.144	0.051
235	73.238	87.520	0.779	0.608	0.144
236	49.594	71.14	0.365	0.212	0.111
237	64.292	92.474	0.686	0.905	0.057
238	71.46	81.898	1.051	0.268	0.083
239	47.936	90.867	0.926	0.188	0.086
240	40.076	122.535	3.799	0.29	0.17
242	97.026	83.717	1.117	0.487	0.114
243	126.876	75.080	0.951	0.197	0.017
244	72.949	109.284	0.93	0.823	0.083
245	68.404	129.027	0.764	0.749	0.070
246	52.718	103.791	0.868	0.58	0.083
247	108.168	74.271	0.681	0.408	0.069
248	51.042	114.821	1.186	0.355	0.155
249	56.081	100.853	0.777	0.377	0.091
250	47.095	103.71	0.715	0.711	0.048
251	68.866	98.711	0.694	0.584	0.103
252	67.245	100.306	0.700	0.577	0.103
253	67.267	105.408	0.654	0.94	0.067

ID	Weather Station	Data Source	Rainfall March	Rainfall April	Rainfall May	Rainfall June	Rainfall July	Rainfall August	Rainfall September	Temperature March	Temperature April	Temperature May	Temperature June	Temperature July	Temperature August	Temperature September
			mm	mm	mm	mm	mm	mm	mm	°C	°C	°C	°C	°C	°C	°C
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Avignon	INRA	61	82.5	2.5	82	8.5	68.5	37.5	11.6	14.4	19	23.5	27.2	24.5	19.5
4	Perpignan	NOAA	148.8	32.7	13	35.6	22	13.7	24	12.6	14.9	18.7	23.8	26.3	24	20.5
5	Logrono	Amet	13.4	55.8	43.6	19.3	14.7	11.8	30.1	11.2	11.5	17.9	22.3	22.7	24.7	19.9
6	Reus	Amet	37.6	14.4	1.5	27.2	18.1	27.8	130.8	13.2	14.6	19.5	23.1	27.1	26.1	21.5
7	Gruissan	INRA	133.5	93.5	46.5	4.5	18	23	59	9.7	11.6	15	19	23.7	23.8	20.4
8	Merignac	NOAA	88	82.7	71.2	67.1	50.8	79.5	21.9	10.9	14.3	15	20.9	21.3	19.8	20.7
9	Albi - Le Séquestre	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Logrono	Amet	13.4	55.8	43.6	19.3	14.7	11.8	30.1	11.2	11.5	17.9	22.3	22.7	24.7	19.9
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Logrono	Amet	102	48.6	50.9	63.9	23.1	7.2	41	9.7	11.7	12.5	17.9	24.3	22.6	19.5
15	Nuremberg	dwd	5.1	22.8	89.9	15.9	103.6	104	108.5	7.5	11.7	13.1	17.5	19.9	16.7	15.2
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	Montreuil-Bellay	INRA	19.5	55	39.7	73.5	34.5	9.5	61.5	8.7	11.9	15.9	18.6	19.8	21	17.4
27	Logrono	Amet	76.8	13.1	1.1	79.7	60.1	35.9	18.2	10.9	13.6	17.4	21.6	25	22.8	18
28	Bretenieres	INRA	55	81.5	132.5	28	47	39.5	91.5	5.8	10	13.7	16.1	21.9	20.2	13.6
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	Merignac	NOAA	52.4	153.6	99	55.2	59	26	43	10	12.6	17.6	20	20.2	22.4	19.4
33	Avignon	INRA	25.5	92	51	50.5	59	13.5	70	11.8	13.8	19	22.4	22.6	24.7	20.7
34	Avignon	INRA	75	35.5	4	70	52	62	47	10.7	16.4	19.6	21.3	22.5	23.9	21.8
35	Perpignan	NOAA	149.9	68.1	9.6	46.4	19.8	14.4	4	11.7	16.3	19.3	21	23	24.2	22.7
36	Perpignan	NOAA	43.6	80.2	47.8	6.8	4.6	17	22.6	11.8	15.8	18.5	22	23.9	22.9	20.4
37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	Blagnac	NOAA	48	85	64.4	116.8	58.6	53.4	31	9.6	12.2	17.5	20.9	21	22.9	20.1
39	Chapenoux	INRA	16.5	10	80	31	84	82.5	43.5	8.1	11.7	13.2	17.8	19.6	16.8	16.4
40	Gruissan	INRA	133.5	93.5	46.5	4.5	18	23	59	9.7	11.6	15	19	23.7	23.8	20.4
41	Perpignan	NOAA	13.4	63.3	9.2	5.4	61.1	47	109.9	12.5	16.2	17.5	23.1	23.4	23.3	22.4
42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	Rheinstetten	dwd	13.4	29.6	61.1	20.3	184.9	101.8	51.1	8.7	12.7	14.3	19.3	20.4	17.8	16.6
44	Rheinstetten	dwd	13.4	29.6	61.1	20.3	184.9	101.8	51.1	8.7	12.7	14.3	19.3	20.4	17.8	16.6
45	Geisenheim	Uni Geisenheim	65.8	34.2	36.5	78.2	63.3	55.9	28	6.4	9.4	17.7	19.2	19.8	19	13.7
46	Geisenheim	Uni Geisenheim	23.5	23	7.1	83.6	13.3	27.4	58.6	7	10.9	14.8	18.1	22	21.5	14.6
47	Strasbourg	NOAA	4.4	22.3	65.6	17.4	202.2	103.9	23.1	9.2	13	15.3	20	20.6	18.4	17.2
48	Geisenheim	Uni Geisenheim	23.5	23	7.1	83.6	13.3	27.4	58.6	7	10.9	14.8	18.1	22	21.5	14.6
49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	Montpellier	INRA	67.5	92	1.5	62.5	6	242	23.5	11.6	13.9	18.3	22.7	25.6	22.9	18.6
52	Logrono	Amet	76.8	13.1	1.1	79.7	60.1	35.9	18.2	10.9	13.6	17.4	21.6	25	22.8	18
53	Lyon-Bron	NOAA	86.1	94.9	202.2	53.1	52.7	60.1	91.5	7.2	11.6	12.7	18.6	23.4	20.8	17.6
56	Avignon	INRA	61	82.5	2.5	82	8.5	68.5	37.5	11.6	14.4	19	23.5	27.2	24.5	19.5
58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ID	Weather Station	Data Source	Rainfall March	Rainfall April	Rainfall May	Rainfall June	Rainfall July	Rainfall August	Rainfall September	Temperature March	Temperature April	Temperature May	Temperature June	Temperature July	Temperature August	Temperature September
			mm	mm	mm	mm	mm	mm	mm	mm	°C	°C	°C	°C	°C	°C
61	Cap Cepet	NOAA	34.2	36.5	1.7	48.8	0.9	8.4	12.9	12.2	14.4	18.5	22.7	26.2	24.2	20.9
62	Perpignan	NOAA	148.8	32.7	13	35.6	22	13.7	24	12.6	14.9	18.7	23.8	26.3	24	20.5
	Strasbourg	NOAA	20	51.8	64.5	31	19.4	60.4	50.5	7.9	11.5	15.5	19	23.4	22.1	15.4
64	Strasbourg	NOAA	4.4	22.3	65.6	17.4	202.2	103.9	23.1	9.2	13	15.3	20	20.6	18.4	17.2
65	Roujan	INRA	39	36.5	8.5	37	6	91.5	27.5	10.9	13.2	17.7	21.8	24.7	21.8	17.8
66	Strasbourg	NOAA	20	51.8	64.5	31	19.4	60.4	50.5	7.9	11.5	15.5	19	23.4	22.1	15.4
67	Perpignan	NOAA	19.4	17.8	70.6	8	14.2	6.6	15.6	12	14.3	17.9	22.2	23.9	24.1	19.9
68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
77	Roujan	INRA	217	25.5	6.5	45	43	11.5	4.5	10.4	15.5	17.9	19.7	21.2	22.6	20.9
78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
84	Villenave	INRA	70.5	64.5	55.5	71.5	61.5	69	26.5	11	14.5	15.3	21.1	21.5	20	20.8
85	Roujan	INRA	12.5	38.5	85.5	87.5	34	25.5	50	10.4	13	16.8	20.9	22.5	22.3	18.2
86	Bourran	INRA	20.5	120.5	84.5	62	22.5	11.5	23	10.3	10.4	16.4	19.6	19.2	22.3	18.2
87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	Merignac	NOAA	31.3	178.8	28.3	64.8	46.8	18.7	59.4	11.5	11	17.3	19.8	20	22.8	19.3
91	Lyon-Bron	NOAA	26.4	16.4	141.8	61.8	125.4	88	86.8	9.8	12	14.3	21.1	20.2	19.8	16.2
92	Cap Cepet	NOAA	45.2	5.3	12.6	46.2	1.4	14.9	12.1	12.3	15.5	17.3	22	23.1	23.4	22
93	Merignac	NOAA	31	115.8	78.4	75	46.6	23.6	48.6	10.1	12.4	17.3	20.3	21.5	22.3	19.1
94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99	Geisenheim	Uni Geisenheim	23.5	23	7.1	83.6	13.3	27.4	58.6	7	10.9	14.8	18.1	22	21.5	14.6
100	Geisenheim	Uni Geisenheim	5	38.7	43	23.9	133.1	114.7	38.5	8.6	13.4	14.5	18.4	20.9	17.3	16.5
1011	Geisenheim	Uni Geisenheim	23.5	23	7.1	83.6	13.3	27.4	58.6	7	10.9	14.8	18.1	22	21.5	14.6
102	Perpignan	NOAA	149.9	68.1	9.6	46.4	19.8	14.4	4	11.7	16.3	19.3	21	23	24.2	22.7
103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	Strasbourg	NOAA	16.7	39.4	62.6	74.1	73.8	40.2	55.4	9.5	10.7	16.8	18.7	19.6	21.1	16.1
105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
106	Perpignan	NOAA	110.6	21.4	95.1	16.6	15.3	4.4	19.3	9.9	14.7	16.9	21.5	25.9	24.5	20.4

ID	Weather Station	Data Source	Rainfall March	Rainfall April	Rainfall May	Rainfall June	Rainfall July	Rainfall August	Rainfall September	Temperature March	Temperature April	Temperature May	Temperature June	Temperature July	Temperature August	Temperature September
			mm	mm	mm	mm	mm	mm	mm	mm	°C	°C	°C	°C	°C	°C
107	Gruissan	INRA	56	8	97.5	14	7.5	24.5	51	8.8	13.7	15.6	20.3	24.3	22.7	18.9
108	Roujan	INRA	57	2.5	74.5	34	4	6.5	42.5	9.1	14.2	16.2	20.5	24.8	23.4	18.6
109	Montpellier	INRA	271.5	70	21	58.5	14	18	12.5	9.8	12.2	15	19.9	24.5	23.1	19.8
110	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
111	Gruissan	INRA	35	95.5	14.5	14	6	8.5	27.5	10.7	12.6	17.6	21.5	23.3	24.1	19.9
112	Gruissan	INRA	67	43	15.5	29	24.5	30.5	7	10.8	15.2	17.8	19.5	21.6	22.2	21.1
113	Montpellier	INRA	271.5	70	21	58.5	14	18	12.5	9.8	12.2	15	19.9	24.5	23.1	19.8
114	Avignon	INRA	34	20.5	14.5	62	93.5	42	86	11.4	15.5	17.6	23.2	22.8	23	21.3
115	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
116	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
121	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
122	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
124	Montreuil-Bellay	INRA	52	10	23.5	29.5	27.5	24	22	8	12.3	14.1	18.9	21.5	19.8	16.7
125	Geisenheim	Uni Geisenheim	36.5	49	89	54.4	22.5	39.7	45.5	3	10.2	13.1	17.6	22.1	19.6	15.3
126	Geisenheim	Uni Geisenheim	5	38.7	43	23.9	133.1	114.7	38.5	8.6	13.4	14.5	18.4	20.9	17.3	16.5
127	Geisenheim	Uni Geisenheim	23.5	23	7.1	83.6	13.3	27.4	58.6	7	10.9	14.8	18.1	22	21.5	14.6
128	Geisenheim	Uni Geisenheim	9.4	17.9	49.9	74.4	62.8	29.5	31.5	9.1	9.9	16.7	17.2	19.2	20.8	15
129	Oehringen	dwd	11.5	46.7	52.7	26.1	107.7	97.1	69.5	8.6	12.4	13.7	18.5	20.1	17.3	15.7
130	Pamplona	Amet	89	39.3	7.3	111.3	42.4	76.4	33.5	9.7	13.5	16.1	20.6	23.2	21.9	17
131	Strasbourg	NOAA	20	51.8	64.5	31	19.4	60.4	50.5	7.9	11.5	15.5	19	23.4	22.1	15.4
132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
133	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
134	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
135	Montpellier	INRA	4.5	52.5	64.5	15	38.5	88.5	68.5	12	13.4	17.4	21.5	23	24.5	19.7
136	Montreuil-Bellay	INRA	52	10	23.5	29.5	27.5	24	22	8	12.3	14.1	18.9	21.5	19.8	16.7
138	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
141	Merignac	NOAA	38.2	90.4	16.2	32.2	20	14.4	56.2	9.8	13.1	17.1	22	22.3	21.2	18.6
142	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
143	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
144	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
145	Logrono	Amet	13.4	55.8	43.6	19.3	14.7	11.8	30.1	11.2	11.5	17.9	22.3	22.7	24.7	19.9
146	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
148	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
149	NOAA	4.4	22.3	65.6	17.4	202.2	103.9	23.1	9.2	13	15.3	20	20.6	18.4	17.2	
154	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
159	Bretenieres	INRA	15	19.5	27	41	105	141	25	8.9	12	14.1	19.7	19.8	17.8	17.4
160	Merignac	NOAA	88	82.7	71.2	67.1	50.8	79.5	21.9	10.9	14.3	15	20.9	21.3	19.8	20.7
161	INRA	71.8	101.8	13.6	26.8	1.8	55.2	84.4	9.5	12.6	16	21.4	23.1	22.9	19.7	
162	Villenave	INRA	33.5	31.5	26	40.1	26	109.5	24.5	10.5	14.6	17	21.5	23.6	22.3	17.9

ID	Weather Station	Data Source	Rainfall March	Rainfall April	Rainfall May	Rainfall June	Rainfall July	Rainfall August	Rainfall September	Temp. March	Temp. April	Temp. May	Temp. June	Temp. July	Temp. August	Temp. September
			mm	mm	mm	mm	mm	mm	mm	mm	°C	°C	°C	°C	°C	°C
163	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
166	Huesca	Amet	36.3	93.1	27.7	6.8	11.4	51.5	45.4	10.7	11.8	18.6	23	24.8	25	20
167	Ciudad Real	Amet	29.7	49.7	77.1	4.6	0.1	2.1	2	10.6	16.7	19.7	24.3	26.4	27.1	23.2
168	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
170	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
171	Roujan	INRA	39	36.5	8.5	37	6	91.5	27.5	10.9	13.2	17.7	21.8	24.7	21.8	17.8
172	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
173	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
174	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
175	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
178	Saint Marcel	INRA	40	56.5	28.5	106.5	16	45.5	172	9.5	13.4	17.1	21.6	25	22.9	17.1
179	Bourges Aerodrome	NOAA	41	57.3	67.7	78.9	12.8	84.8	44.3	8.4	12.5	14.9	19.5	22	21	14.9
180	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
182	Geisenheim	Uni Geisenheim	23.5	23	7.1	83.6	13.3	27.4	58.6	7	10.9	14.8	18.1	22	21.5	14.6
184	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
185	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
187	Avignon	INRA	61	82.5	2.5	82	8.5	68.5	37.5	11.6	14.4	19	23.5	27.2	24.5	19.5
188	Montpellier	INRA	271.5	70	21	58.5	14	18	12.5	9.8	12.2	15	19.9	24.5	23.1	19.8
189	Roujan	INRA	57	2.5	74.5	34	4	6.5	42.5	9.1	14.2	16.2	20.5	24.8	23.4	18.6
190	Roujan	INRA	217	25.5	6.5	45	43	11.5	4.5	10.4	15.5	17.9	19.7	21.2	22.6	20.9
191	Gruissan	INRA	23.2	69.1	32.5	2.2	2.6	22.4	37.3	10.8	12.8	15.7	19.7	24.8	24.2	20.1
192	Gruissan	INRA	23.2	93.5	46.5	4.5	18	23	59	10.8	11.6	14.6	18.9	23.8	23.8	20.1
193	Gruissan	INRA	21	29.5	27.5	28	11.5	51.5	72.5	12	12.7	16.8	21	22.3	23.2	18.9
194	Gruissan	INRA	133.5	93.5	46.5	4.5	18	23	59	9.7	11.6	15	19	23.7	23.8	20.4
195	Gruissan	INRA	14	69	13.5	43	30.5	34.5	111	12.1	15.6	17.2	22.2	22.9	22.7	21.3
196	Gruissan	INRA	21	29.5	27.5	28	11.5	51.5	72.5	12	12.7	16.8	21	22.3	23.2	18.9
197	Gruissan	INRA	133.5	93.5	46.5	4.5	18	23	59	9.7	11.6	15	19	23.7	23.8	20.4
198	Gruissan	INRA	14	69	13.5	43	30.5	34.5	111	12.1	15.6	17.2	22.2	22.9	22.7	21.3
199	Filippo	GET	72.1	40.1	67.8	7.5	2.6	4.4	103.1	9.6	13.5	17.9	22.2	26.8	24.9	19.6
200	Filippo	GET	72.1	40.1	67.8	7.5	2.6	4.4	103.1	9.6	13.5	17.9	22.2	26.8	24.9	19.6
201	Filippo	GET	72.1	40.1	67.8	7.5	2.6	4.4	103.1	9.6	13.5	17.9	22.2	26.8	24.9	19.6
202	Filippo	GET	50.8	61.6	72.4	110.6	170.8	105	107	11.6	14.2	16.8	21.6	21.5	21.1	18.5
203	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
204	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
205	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
206	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
207	Merignac	INRA	40.2	26.9	33.5	43.8	35.3	89.7	35.4	10.4	14.5	16.5	21	23.1	22.1	17.5
208	Merignac	NOAA	40.2	26.9	33.5	43.8	35.3	89.7	35.4	10.4	14.5	16.5	21	23.1	22.1	17.5
209	Geisenheim	Uni Geisenheim	23.5	23	7.1	83.6	13.3	27.4	58.6	7	10.9	14.8	18.1	22	21.5	14.6
210	Strasbourg	NOAA	20	51.8	64.5	31	19.4	60.4	50.5	7.9	11.5	15.5	19	23.4	22.1	15.4
211	Strasbourg	NOAA	20	51.8	64.5	31	19.4	60.4	50.5	7.9	11.5	15.5	19	23.4	22.1	15.4
212	Colmar	INRA	19	40	61.5	32	18	69.5	66.5	8	11.6	15.7	19	22.4	21.3	15.2
213	Colmar	INRA	19	40	61.5	32	18	69.5	66.5	8	11.6	15.7	19	22.4	21.3	15.2
214	Strasbourg	NOAA	18.3	13.9	26.5	74.8	100.9	93	33.1	8.3	14.3	16.7	18.8	17.8	20.1	18
215	Colmar	INRA	19	40	61.5	32	18	69.5	66.5	8	11.6	15.7	19	22.4	21.3	15.2
216	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
217	Montpellier	INRA	16	26	27	33.5	34.5	37	245	11.1	15	16.7	22	23.3	22.3	20.4
218	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ID	Weather Station	Data Source	Rainfall March	Rainfall April	Rainfall May	Rainfall June	Rainfall July	Rainfall August	Rainfall September	Temperature March	Temperature April	Temperature May	Temperature June	Temperature July	Temperature August	Temperature September
			mm	mm	mm	mm	mm	mm	mm	mm	°C	°C	°C	°C	°C	°C
219	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
221	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
222	Villeneuve	INRA	33.5	31.5	26	40.1	26	109.5	24.5	10.5	14.6	17	21.5	23.6	22.3	17.9
223	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
224	Lahr	dwd	18.4	26.7	91.3	24.5	305.3	136.6	21.8	8.4	12.3	14.5	19.3	19.8	17.8	16.1
225	Bourran	INRA	93	39	126.5	75.5	17.5	36	66	9.9	12.3	13.2	17.6	24.1	21.3	18.8
226	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
227	Avignon	INRA	61	82.5	2.5	82	8.5	68.5	37.5	11.6	14.4	19	23.5	27.2	24.5	19.5
228	Montreuil-Bellay	INRA	37	48.5	47	36.5	15	107	77	8.9	13.3	15	19.1	21.2	20.6	15.3
229	Perpignan	NOAA	149.9	68.1	9.6	46.4	19.8	14.4	4	11.7	16.3	19.3	21	23	24.2	22.7
230	Merignac	NOAA	52.4	153.6	99	55.2	59	26	43	10	12.6	17.6	20	20.2	22.4	19.4
231	Merignac	NOAA	38.2	90.4	16.2	32.2	20	14.4	56.2	9.8	13.1	17.1	22	22.3	21.2	18.6
232	Avignon	INRA	61	82.5	2.5	82	8.5	68.5	37.5	11.6	14.4	19	23.5	27.2	24.5	19.5
234	Montpellier	INRA	16	26	27	33.5	34.5	37	245	11.1	15	16.7	22	23.3	22.3	20.4
135	Bourran	INRA	93	39	126.5	75.5	17.5	36	66	9.9	12.3	13.2	17.6	24.1	21.3	18.8
236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
237	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
238	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
239	Montreuil-Bellay	INRA	37	48.5	47	36.5	15	107	77	8.9	13.3	15	19.1	21.2	20.6	15.3
240	Roujan	INRA	187	71	42	57.5	15.5	22.5	40.5	9.2	11.5	14	18.8	23.5	22.3	18.8
242	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
243	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
244	INRA	Avignon	34	20.5	14.5	62	93.5	42	86	11.4	15.5	17.6	23.2	22.8	23	21.3
245	Avignon	INRA	34	20.5	14.5	62	93.5	42	86	11.4	15.5	17.6	23.2	22.8	23	21.3
246	Avignon	INRA	95.5	80	96	4	80	15	100	9.8	12.8	15.1	20.7	25.7	23.9	20.6
247	Avignon	INRA	34	20.5	14.5	62	93.5	42	86	11.4	15.5	17.6	23.2	22.8	23	21.3
248	Saint Marcel	INRA	40	56.5	28.5	106.5	16	45.5	172	9.5	13.4	17.1	21.6	25	22.9	17.1
249	Avignon	INRA	95.5	80	96	4	80	15	100	9.8	12.8	15.1	20.7	25.7	23.9	20.6
250	Avignon	INRA	75	35.5	4	70	52	62	47	10.7	16.4	19.6	21.3	22.5	23.9	21.8
251	Avignon	INRA	61	82.5	2.5	82	8.5	68.5	37.5	11.6	14.4	19	23.5	27.2	24.5	19.5
251	Aivgnon	INRA	61	82.5	2.5	82	8.5	68.5	37.5	11.6	14.4	19	23.5	27.2	24.5	19.5
253	Avignon	INRA	95.5	80	96	4	80	15	100	9.8	12.8	15.1	20.7	25.7	23.9	20.6

Appendix B – Statistical Code Chapter 4

```
library(MASS) # lda
library(FactoMineR) # PCA
library(ade4) # s.class
library(vegan) # rda

#####
# DATA
#####

# xls file was exported using libreoffice (encoding=UTF8; separator=';'; with
# quotes)

WINES=read.table("Supplementary_Data_1_wines.txt", sep=';', dec='.',encoding
='UTF-8',header=TRUE,skip=1, quote= "")

# concentration data
# now in a log10 scale
CONC=log10(WINES[c("Ca","Mg","Mn","Sr","Ba")])

# meteorological data
METEO=WINES[c(grep('Rainfall',names(WINES)), grep('Temperature',names(WINES))
)]

# factors
FACTORS=WINES[c("Country","Appellation","Greater.Wine.Region","Climate","
Color","Vintage","Calcareous")]

# grouping 'White/Rose/Gris'
FACTORS$Color=factor(FACTORS$Color,levels=c('Red','White','Ros','Gris'))
COL_color=c('red','green','magenta','grey')
FACTORS$Color2=as.character(FACTORS$Color)
FACTORS$Color2[FACTORS$Color2!='Red']='White/Ros/Gris'
FACTORS$Color2=factor(FACTORS$Color2)

# sample size
table(FACTORS$Country)

# France Germany Italy Spain
```

```

#      161      19      25      10
nrow(CONC)
#215

# splitting the dataset for cross-validation
# selection 1 :
data with both meteorological and soil data (91 samples)
# selection 2 :
data with meteorological and without soil data (41 samples)
# selection 3 :
data without meteorological data and with soil data (61 samples)

table(c('meteo','NA')[1+is.na(METEO$Temperature_August)],
c('soil','NA')[1+is.na(FACTORS$Calc)])

#      NA soil
#meteo 41  91
#NA     22  61
SELECT1=!is.na(WINES$Temperature_August) & !is.na(WINES$Calc)

CONC1=CONC[SELECT1,] METEO1=METEO[SELECT1,] FACTORS1=FACTORS[SELECT1,] nrow(
  CONC1)

#91
SELECT2=!SELECT1 CONC2=CONC[SELECT2,] FACTORS2=FACTORS[SELECT2,] nrow(CONC2)

#124
SELECT3=!SELECT1 & !is.na(FACTORS$Calc) CONC3=CONC[SELECT3,] FACTORS3=FACTORS
  [SELECT3,] nrow(CONC3)
#61

#####
# Concentration ~ Color (LDA)
#####

# LDA (with 4 color classes)
ALD=lda(CONC1,FACTORS1$Color)
ALD$scaling
#      LD1      LD2      LD3
#Ca -6.6819890 6.2291497 3.401308

```

```

#Mg 13.4799260 9.1570915 3.690893
#Mn 0.3912696 -0.3225633 1.733381
#Sr 0.2098493 -2.3947138 1.147356
#Ba 0.5398619 1.4738982 -3.142334

PROJ=as.matrix(CONC1) %*%
as.matrix(ALD$scaling)

# plots
plot(PROJ,col=COL_color[unclass(FACTORS1$Color)],pch=19)

text(PROJ[,1],PROJ[,2]-0.1,WINES$ID[SELECT1])
pred = predict(ALD,CONC1)
table(FACTORS1$Color,pred$class)
#      Red White Ros Gris
#Red   58    5   0   0
#White  2   19   1   0
# Ros   0    2   2   0
#Gris   0    1   0   1

# LDA (with 2 color classes)
ALD=lda(CONC1,FACTORS1$Color2)
ALD$scaling
#      LD1
#Ca  6.8748035
#Mg -12.5121089
#Mn -0.4998234
#Sr -0.4745924
#Ba -0.2319520

PROJ=as.matrix(CONC1) %*% as.matrix(ALD$scaling)

# plots
plot(PROJ,col=COL_color[unclass(FACTORS1$Color)],pch=19,xlab='Index',ylab='
LDA1')
text(1:length(PROJ),PROJ-0.1,WINES$ID[SELECT1])
DISCRIM=-PROJ
BREAKS=seq(5,12,0.5)
DISCRIM_class=cut(DISCRIM,BREAKS)
barplot(t(table(DISCRIM_class,FACTORS1$Color)),col=COL_color,xlab='LDA1',ylab
='Frequency')

```



```

legend('topright',col=COL_color,legend=levels(FACTORS$Color),pch=15,cex=1.3)

# efficiency
pred = predict(ALD,CONC1)
CLASSIF=table(FACTORS1$Color2,pred$class)
CLASSIF

#
      Red White/Ros/Gris
# Red      58          5
# White/Ros/Gris  2          26
sum(diag(CLASSIF))/sum(CLASSIF)*100
# 92.30769
# cross-validation
pred = predict(ALD,CONC2)
CLASSIF=table(FACTORS2$Color2,pred$class)
CLASSIF
#
      Red White/Ros/Gris
# Red      55          8
# White/Ros/Gris 11          50
sum(diag(CLASSIF))/sum(CLASSIF)*100
# 84.67742

# LDA1= $\sim 2 \cdot \log_{10}(\text{Mg}) - \log_{10}(\text{Ca}) = \log_{10}(\text{Mg}/\text{Ca})$ 
plot(1:length(PROJ),2*CONC1$Mg-CONC1$Ca,col=COL_color[unclass(FACTORS1$Color)
],pch=19,xlab='Index',ylab='LDA1')
text(1:length(PROJ),2*CONC1$Mg-CONC1$Ca-0.05,WINES$ID)

# efficiency (using all available samples)
CONC12=rbind(CONC1,CONC2)
FACTORS12=rbind(FACTORS1,FACTORS2)
combi=2*CONC12$Mg-CONC12$Ca
BREAKS=seq(1.4,2.8,0.05)
DISCRIM_class=cut(combi,BREAKS)
barplot(t(table(DISCRIM_class,FACTORS12$Color2)),col=c('red','green'),xlab='
LDA1',ylab='Frequency')

for(x in BREAKS){
  pred=rep('Red',nrow(FACTORS12))
  pred[combi<x]='White/Ros/Gris'
  pred=factor(pred,levels=levels(FACTORS12$Color2))
  CLASSIF=table(FACTORS12$Color2,pred)
}

```

```

cat(x,':',sum(diag(CLASSIF))/sum(CLASSIF)*100,'%\n')
}

# classification rule is
# if Mg /Ca<100 => White/Ros/Gris
# if Mg /Ca>=100 => Red
# efficiency is 89.8 % using the 215 wine samples

# plot presented in the paper
pdf('LDA_color.pdf',width=10,height=6)
par(mar=c(6,6,1,1))
barplot(t(table(DISCrim_class,FACTORS12$Color)),col=COL_color,axes=FALSE,ylim
=c(0,20),names.arg='')

#lab_BREAKS=BREAKS
#lab_BREAKS[abs(BREAKS-round(BREAKS,1))>0.01]=''
axis(1,at=0.1+seq(0,(length(BREAKS)-1)*1.2, length=length(BREAKS)),labels=
BREAKS,cex.axis=1.3)

# default value for space between bars is 0.2

mtext(expression(log[10](Mg^2/Ca)),side=1,line=4,cex=1.3)
axis(2,at=seq(0,30,5),cex.axis=1.3)
mtext('Frequency',side=2,line=4,cex=1.3)
legend('topright',col=COL_color,
legend=levels(FACTORS$Color),pch=15,cex=1.3)
dev.off()

#####
# Concentration Calcareous (LDA)
#####

# LDA

ALD=lda(CONC1,FACTORS1$Calc)
ALD$scaling
#      LD1
#Ca -0.1754243
#Mg -3.8052911
#Mn -1.5488679
#Sr 0.7090400

```

```

#Ba -3.4525619

PROJ=as.matrix(CONC1) %*% as.matrix(ALD$scaling)
DISCRIM=-PROJ
BREAKS=seq(0,8,0.25)
DISCRIM_class_sol=cut(DISCRIM,BREAKS)

barplot(t(table(DISCRIM_class_sol,FACTORS1$Calc)), col=c('darkgrey','white'),
        ,xlab='LDA1',ylab='Frequency')

# efficiency

pred = predict(ALD,CONC1)
CLASSIF=table(FACTORS1$Calc,pred$class)

# calcaire mesur V. calcaire prdit de LDA1

CLASSIF
#      No Yes
# No  24  5
# Yes  5  57

sum(diag(CLASSIF))/sum(CLASSIF)*100
# 89.01099
# cross-validation
pred = predict(ALD,CONC3)
CLASSIF=table(FACTORS3$Calc,pred$class)
CLASSIF
sum(diag(CLASSIF))/sum(CLASSIF)*100

# 85.2459
# LDA1=~log10(Mg)+log10(Ba)=log10(Mg*Ba)
plot(1:length(PROJ),CONC1$Mg+CONC1$Ba,
     pch=c(19,1)[unclass(FACTORS1$Calc)])
text(1:length(PROJ),CONC1$Mg+CONC1$Ba-0.05, WINES$ID[SELECT1])

# efficiency (using all available samples)
CONC13=rbind(CONC1,CONC3)

```

```

FACTORS13=rbind(FACTORS1,FACTORS3)
combi=CONC13$Mg+CONC13$Ba
BREAKS=seq(0,1.6,0.05)
DISCRIM_class=cut(combi,BREAKS)

barplot(t(table(DISCRIM_class,FACTORS13$Calc)), col=c('darkgrey','white'),
        xlab=expression(log[10](Mg~Ba)),ylab='Frequency')

for(x in BREAKS){
  pred=rep('No',nrow(FACTORS13))
  pred[combi<x]='Yes'
  pred=factor(pred,levels=levels(FACTORS13$Calc))
  CLASSIF=table(FACTORS13$Calc,pred)
  cat(x,':',sum(diag(CLASSIF))/sum(CLASSIF)*100,'%\n')
}

# classification rule is
# si Mg*Ba<10 => Calcareous
# si Mg*Ba>=10 => Non-calcareous
# efficiency is 84.2 % using the 152 wine samples

# plot presented in the paper

pdf('LDA_calc.pdf',width=10,height=6)
par(mar=c(6,6,1,1))
barplot(t(table(DISCRIM_class,FACTORS13$Calc)), col=c('darkgrey','white'),
        axes=FALSE,ylim=c(0,15),names.arg='')

#lab_BREAKS=BREAKS
#lab_BREAKS[abs(BREAKS-round(BREAKS,1))>0.01]=''
axis(1,at=0.1+seq(0,(length(BREAKS)-1)*1.2, length=length(BREAKS)), labels=
     BREAKS,cex.axis=1.3)

# default value for space between bars is 0.2

mtext(expression(log[10](Mg~Ba)),side=1,line=4,cex=1.3)
axis(2,at=seq(0,15,5),cex.axis=1.3)
mtext('Frequency',side=2,line=4,cex=1.3)

legend('topright',col=c('darkgrey','black'), legend=c('Non-calcareous','
     Calcareous'), pch=c(15,0),cex=1.3)

```

```

dev.off()

#####
# Concentration ~ Meteo (RDA)
#####

# RDA

RDA=rda(CONC1~.,data=METE01)
summary(RDA)$constr.chi/summary(RDA)$tot.chi

# 0.2412444

#####
# Concentration ~ Color + Calcareous (RDA)
#####

# RDA
RDA1=rda(CONC1~.,data=data.frame(Color=unclass
(FACTORS1$Color2)-1, Calcareous=unclass(FACTORS1$Calc)-1))

# percentage of explained variance
summary(RDA1)$constr.chi/summary(RDA1)$tot.chi

# 0.2849746
# plot presented in the paper
pdf('RDA_calc_color.pdf',width=10,height=10)
plot(summary(RDA1)$sites[,1:2],bg=c('khaki','pink','lightgreen','aliceblue')
[unclass(FACTORS1$Color2:FACTORS1$Calc)],pch=21,cex=1.5,xlim=c(-1.2,1),ylim=c
(-1.2,1))
s.class(summary(RDA1)$sites[,1:2],FACTORS1
$Color2:FACTORS1$Calc,

label=c('Red/Non-calcareous','Red/Calcareous','Non-Red/Non-calcareous','Non-
Red/Calcareous'),add.plot=TRUE,cellipse=0,cpoint=0,clabel=1)

arrows(c(0,0),c(0,0),summary(RDA1)$biplot[,1],summary(RDA1)$biplot[,2],lwd=2,
col='darkblue')

text(1.1*summary(RDA1)$biplot[,1],1.1*summary(RDA1)$biplot[,2],rownames(
summary(RDA1)$biplot),cex=2,col='darkblue')

```

```

arrows(rep(0,5),rep(0,5),summary(RDA1)$species[,1],summary(RDA1)$species[,2],
       lwd=2,col='darkred')

text(1.1*summary(RDA1)$species[,1],1.1*summary(RDA1)$species[,2],rownames(
      summary(RDA1)$species),cex=2,col='darkred')
dev.off()

# residuals
RESID1=residuals(RDA1)

#####
# CONC ~ METEO (partial RDA)
#####

# RDA
RDA2=rda(RESID1~.,data=METE01)

# percentage of explained variance
summary(RDA2)$constr.chi/summary(RDA2)$tot.chi

# 0.2339948
# plot presented in the paper

pdf('RDAPartial_meteo.pdf',
    width=12,height=12)
plot(summary(RDA2)$sites[,1:2],pch=21, cex=1.5,xlim=c(-1,1),ylim=c(-1,1))
arrows(rep(0,14),rep(0,14), summary(RDA2)$biplot[,1],summary(RDA2)
       $biplot[,2],lwd=2,col='darkblue')

text(1.1*summary(RDA2)$biplot[,1], 1.1*summary(RDA2)$biplot[,2], rownames(
      summary(RDA2)$biplot), cex=1,col='darkblue')

arrows(rep(0,5),rep(0,5),
       summary(RDA2)$species[,1],summary(RDA2)
       $species[,2],lwd=2,col='darkred')
text(1.1*summary(RDA2)$species[,1], 1.1*summary(RDA2)
       $species[,2],rownames(summary(RDA2)$species),cex=2,col='darkred')

dev.off()

```

```

# residuals
RESID2=residuals(RDA2)

#####
# CONC (PCA)
#####

# unscaled PCA
PCA1=PCA(RESID2,scale=FALSE,graph=FALSE)

# percentage of explained variance
PCA1$eig$perc

# plot presented in the paper
pdf('PCA_residuals.pdf',width=10,height=10)
plot(NA,NA,xlim=c(-0.1,0.3),ylim=c(-0.2,0.2),
xlab='PCA1',ylab='PCA2')
arrows(rep(0,5),rep(0,5),PCA1$var$coord[,1], PCA1$var$coord[,2],lwd=2,col='
  darkred')

text(1.1*PCA1$var$coord[,1],1.1*PCA1$var$coord[,2], rownames(PCA1$var$coord),
  cex=2,col='darkred')
dev.off()

#####
# percentage of explained variance
#####

# first RDA (~Color + Calcareous)

prop1=summary(RDA1)$constr.chi/summary(RDA1)$tot.chi
prop1*100

# 28.49746
# second RDA (~Meteo)

prop2=(1-prop1)*summary(RDA2)$constr.chi/summary(RDA2)$tot.chi
prop2*100

# 16.73122
# first 2 axes of the PCA
prop3=(1-prop1-prop2)*PCA1$eig[2,3]/100

```

```

prop3*100
# 41.3226

# unexplained
(1-prop1-prop2-prop3)*100

# 13.44872

#####
# Concentration ~ Color + Calcareous (LDA)
#####

library(klaR) # drawparti

# LDA
ALD=lda(CONC1,FACTORS1$Color2:FACTORS1$Calc)
ALD$scaling

#          LD1          LD2          LD3
#Ca -5.282383941  5.3483010  6.39540355
#Mg 12.029350485 -4.3079657  6.44688734
#Mn  1.237314728  0.9614026 -0.85150515
#Sr -0.003059864 -0.8214741  1.27564193
#Ba  2.077279644  2.9173748  0.03246679

PROJ=as.matrix(CONC1) %*% as.matrix(ALD$scaling)

# efficiency

pred = predict(ALD,CONC1)
CLASSIF=table(FACTORS1$Color2:FACTORS1$Calc,pred$class)
CLASSIF
sum(diag(CLASSIF))/sum(CLASSIF)*100

# 82.41758
# cross-validation

```



```

pred = predict(ALD,CONC3)
CLASSIF=table(FACTORS3$Color2:FACTORS3$Calc,pred$class)
CLASSIF
sum(diag(CLASSIF))/sum(CLASSIF)*100
# 70.4918

# plot presented in the paper

pdf('LDA_calc_color.pdf',width=8,height=8)
par(mar=c(5,5,1,1))

klaR::drawparti(FACTORS1$Color2:
FACTORS1$Calc,PROJ[,1],PROJ[,2], gs=rep(19,4), image.colors=c('khaki','pink
','lightgreen','aliceblue'),
col.wrong='black',xlab='LDA1',ylab='LDA2', print.err=0,prec=500)

points(PROJ,lwd=1,bg=c('khaki','pink','lightgreen','aliceblue')
[unclass(FACTORS1$Color2:FACTORS1$Calc)], pch=21,cex=1.5)

s.class(PROJ[,1:2],FACTORS1$Color2:FACTORS1$Calc, label=c('Red/Non-calcareous
','Red/Calcareous', 'Non-Red/Non-calcareous','Non-Red/Calcareous'),
add.plot=TRUE,cellipse=0,cpoint=0,clabel=1)

dev.off()

```

Appendix C – Adaptation of Cu isotope separation method for environmental samples

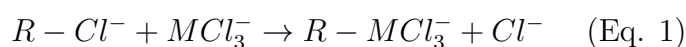
Introduction

Importance of sample purification step in Cu separation

Cu-isotope ratios at natural abundances are typically measured on a Multiple Collector – Inductively Coupled Plasma - Mass Spectrometer (MC-ICP-MS). The measurement methods are relatively straight forward and most working groups rely on the method published by Maréchal et al. (1999). However as relatively small differences are measured, in the order of the tenth per mil variance in ^{63}Cu to ^{65}Cu ratio, measurement requires high purity of the sample and correction of the internal machine fractionation (Maréchal et al., 1999). While mass bias correction by either Ni or Zn spikes is routinely performed, the sample purification, especially for environmental samples, for Cu isotope ratio measurements remains critical (Babcsanyi, 2015; Chapman et al., 2006; Ehrlich et al., 2004; Maréchal et al., 1999; Zhu et al., 2015). Sample purification is important to keep ionization and transport effects in the machine comparable to the standards. Furthermore sample purification assures the absence of isobaric interferences on Cu and Zn isotopes. Isobaric interferences on the different Cu and Zn isotopes are listed in Table 9.1. During the measurement, Ar is present from plasma, N from nitric acid and H and O from water and air. These elements only cause problems during the measurement when forming polyatomic species in the spectrometer, for example with P, Ti, Na or Mg. The elements S, Ni and Cl can also cause single or polyatomic interferences even without being combined to other atoms This means special care needs to be taken to eliminate the former mentioned elements from the samples. However other interferences are also imaginable, for example due to rest of organic matter.

The traditional method of Cu separation

Typically Cu separation from a digested sample is achieved using anion exchange resin columns. The resin used in this study is the AG-MP 1 resin in 100-200 μm mesh size by biorad. It consists of positively charged, quaternary ammonium groups chemically bound to styrene-divinyl-benzene copolymers (Marechal and Albarede 2002). The ammonium groups readily bind counter ions such as chloride. In presence of metal-chloroanions, the chloride ions attached to the resin can be exchanged so that metal-chloroanions are more or less strongly retained (Marechal and Albarede 2002) as expressed in Equation 1 :



The reaction constant K_a of the retention reaction Eq. 1 depends on the nature of the complexed ion and thus separation of the different elements is possible. Empirical constants are displayed in Figure 9.1.

Table 9.1 – Possible isobaric interferences on relevant isotopes during Cu isotope measurement (modified from May and Wiedmeyer, 1998).

Isotope	Isobaric interference
^{63}Cu	$^{31}\text{P}^{16}\text{O}_2^+$, $^{40}\text{Ar}^{23}\text{Na}^+$, $^{47}\text{Ti}^{16}\text{O}^+$, $^{23}\text{Na}^{40}\text{Ca}^+$, $^{36}\text{Ar}^{12}\text{C}^{14}\text{N}^1\text{H}^+$, $^{14}\text{N}^{12}\text{C}^{37}\text{Cl}^+$, $^{12}\text{C}^{16}\text{O}^{35}\text{Cl}^+$
^{65}Cu	$^{49}\text{Ti}^{16}\text{O}^+$, $^{32}\text{S}^{16}\text{O}^{21}\text{H}^+$, $^{40}\text{Ar}^{25}\text{Mg}^+$, $^{40}\text{Ca}^{16}\text{O}^1\text{H}^+$, $^{36}\text{Ar}^{14}\text{N}^{21}\text{H}^+$, $^{32}\text{S}^{33}\text{S}^+$, $^{32}\text{S}^{16}\text{O}^{17}\text{O}$, $^{32}\text{S}^{16}\text{O}_2$, $^{12}\text{C}^{16}\text{O}^{37}\text{Cl}^+$, $^{12}\text{C}^{18}\text{O}^{35}\text{Cl}^+$, $^{31}\text{P}^{16}\text{O}^{18}\text{O}^+$
^{64}Zn	^{64}Ni , $^{32}\text{S}^{16}\text{O}_2^+$, $^{48}\text{Ti}^{16}\text{O}^+$, $^{31}\text{P}^{16}\text{O}^{21}\text{H}^+$, $^{48}\text{Ca}^{16}\text{O}^+$, $^{32}\text{S}_2^+$, $^{31}\text{P}^{16}\text{O}^{17}\text{O}^+$, $^{34}\text{S}^{16}\text{O}_2^+$, $^{36}\text{Ar}^{14}\text{N}_2^+$
^{66}Zn	$^{50}\text{Ti}^{16}\text{O}^+$, $^{34}\text{S}^{16}\text{O}_2^+$, $^{33}\text{S}^{16}\text{O}^{21}\text{H}^+$, $^{32}\text{S}^{16}\text{O}^{18}\text{O}^+$, $^{32}\text{S}^{17}\text{O}_2^+$, $^{33}\text{S}^{16}\text{O}^{17}\text{O}^+$, $^{32}\text{S}^{34}\text{S}^+$, $^{33}\text{S}_2^+$
^{67}Zn	$^{35}\text{Cl}^{16}\text{O}_2^+$, $^{33}\text{S}^{34}\text{S}^+$, $^{34}\text{S}^{16}\text{O}^{21}\text{H}^+$, $^{32}\text{S}^{16}\text{O}^{18}\text{O}^1\text{H}^+$, $^{33}\text{S}^{34}\text{S}^+$, $^{34}\text{S}^{16}\text{O}^{17}\text{O}^+$, $^{33}\text{S}^{16}\text{O}^{18}\text{O}^+$, $^{32}\text{S}^{17}\text{O}_2^+$, $^{35}\text{Cl}^{16}\text{O}^+$
^{68}Zn	$^{35}\text{Cl}_2^+$, $^{40}\text{Ar}^{14}\text{N}^{16}\text{O}^+$, $^{35}\text{Cl}^{17}\text{O}^{18}\text{O}^+$, $^{37}\text{Cl}^{16}\text{O}^{17}\text{O}^+$, $^{34}\text{S}^{18}\text{O}_2^+$, $^{36}\text{S}^{16}\text{O}^{18}\text{O}^+$, $^{36}\text{S}^{17}\text{O}_2^+$, $^{34}\text{S}^{36}\text{S}^+$, $^{36}\text{Ar}^{34}\text{S}$, $^{38}\text{Ar}^{32}\text{S}^+$

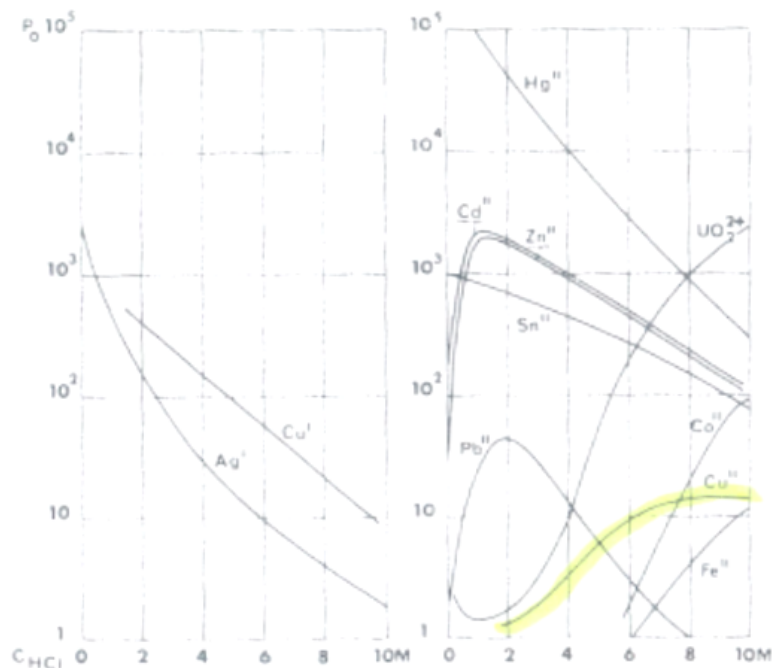


Figure 9.1 – Distribution coefficients of AG-MP 1 resin and different metal species depending on HCl concentration (from Tremillon, 1965)

Distribution coefficients of Cu (I) and Cu (II) evolve inversely with HCl concentration. For Cu (I) the distribution coefficient approaches 10^3 at HCl concentration below 2 M and falls to 10 at about 10 M HCl. Dissolved and resin bound Cu(II) is almost equally distributed at in an 2 M HCl environment whereas the distribution coefficient rises to above 10 at about 8 M. This is delicate as redox reactions are some of the most isotope fractionating processes for Cu isotopes but can also be exploited for separation purposes (Larner et al., 2011). Note as well that affinity for resin binding never rises as high for Cu as for example for Zn (log – scale) explaining a somewhat fragile separation procedure. Also it is relatively complicated to separate Cu from elements as Co because retention maxima have a similar evolution (Tremillon, 1965).

Table 9.2 – *Cu separation protocol from Marechal et al. (1999)*

Purpose	Volume	Eluant
Washing	10 mL	H ₂ O
Washing	7 mL	0.5 M HNO ₃
Washing	2 mL	H ₂ O
Washing	7 mL	0.5 M HNO ₃
Washing	2 mL	H ₂ O
Washing	7 mL	0.5 M HNO ₃
Washing	2 mL	H ₂ O
Sample loading	1 mL	7 M HCl + 0.001 % H ₂ O ₂
Matrix elution	10 mL	7 M HCl + 0.001 % H ₂ O ₂
Cu elution	20 mL	7 M HCl + 0.001 % H ₂ O ₂

The Cu retention thus depends on the formation of chloro-anions of a given element at the physico-chemical environment of the injected solution (i.e. pH, competing ions, ionic strength, etc.). This implies that the column process can be disturbed by (a) the exchange of the counter ion due to presences undesired anions, or (b) by the presence of high quantities of other metal-chloro-complexes competing for resin adsorption sites with the specie desired to be retained or (c) competition for the formation of chloro-ions. As column separation typically is performed at high HCl concentrations ($> 7 \text{ mol L}^{-1}$), the last point (c) does not seem to be of major concern.

Elution steps of the traditional protocol are denoted in Table 9.2. The same acid is used for matrix and Cu elution, implying that Cu elution is a matter of timing. For natural samples with low Cu abundance relative to matrix it has been shown that early elution can occur (Chapman et al., 2006). Elution was found to be as early as after 2 mL of matrix elution, thus not achieving sufficient matrix separation (Chapman et al., 2006).

The earliest elution was observed for reference materials containing high amounts of Fe and P elution probably due to competition between Fe and Cu – chlorocomplexes for resin sites and occupation of resin sites by negatively charged PO₄ species (Chapman et al., 2006).

Early elution is not only a problem because of a lack of matrix separation but also for possible incomplete recovery of Cu. The resin passage is known to fractionate Cu isotopes much more (≈ 19 ‰) between first and last mL of Cu elution than typical fractionation observed in environmental samples (≈ 2 ‰) (Dong et al., 2013; Fekiacova et al., 2015; Maréchal and Albarède, 2002; Maréchal et al., 1999; Vance et al., 2008). Empirical evidence shows that, in the first milliliters of Cu elution, a heavy Cu fraction is collected followed by a light fraction (Maréchal and Albarède, 2002). Early elution thus results in recovery of an isotopically lighter sample. On the basis of data from Maréchal and Albarède 2002, one can calculate that in the chalcopyrite sample used, missing the first 5 % (m/m) of copper elution would result in a $\Delta^{65}\text{Cu}$ of -0.3 ‰ with respect to the complete sample. Missing the last 5 % would result in $\Delta^{65}\text{Cu} = +0.2$ ‰. This illustrates that typical limits of acceptance of 100 ± 5 % for Cu recovery in the resin protocol are not sufficient to assure result quality as fractionation by loss of 5 % Cu is much higher than typically reported external precision of about 0.1 ‰. The recovery target from resin procedure thus needs to be 100 % even though typical precision of concentration analysis do not allow verification. At best this is verified by calibrating elution curves on each sample type.

Modifications of the Cu separation method

All the previous considerations led to the situation that almost every working group measuring Cu isotope ratios has developed their own slightly modified column procedure. For soil investigations, typical modifications are increased resin volume to 2 mL (Bigalke et al., 2010) or even 3 mL (Babcsanyi, 2015) to increase the number of exchange sites available on the resin thus avoiding competition of Cu with other ions. One backdrop of this modification is prolonged elution of Cu leading to higher acid consumption and long Cu elution tails (Babcsanyi, 2015; Bigalke et al., 2010). Other studies have increased HCl molarity to increase Cu retention with respect to most other matrix elements (Borrok et al., 2007; Liu et al., 2014). In loading and matrix elution protocol proposed by Borrok et al. (2007), molarity is increased to 10 M HCl and Cu is eluted with 5 M HCl as Cu retention is significantly lower at lower molarities. For plant studies, a fairly traditional protocol is often used lowering matrix elution volume and increasing number of column passages (Jouvin et al., 2012; Ryan et al., 2013). This was also proposed by Chapman et al (2006) for geological matrices.

A few radically different approaches exist. Vance and Archer combine the classical AG-MP 1 procedure on longer and thinner columns with a second passage on a TruSpec column effectively eliminating Fe and Ti. This method has been calibrated for silicate rocks and it is unclear if it is suitable for biological samples containing large amounts of P and samples containing high amounts of alkaline earth elements. Larner et al. (2011) tried to exploit retention differences between Cu(I) and Cu(II) for matrix separation. An external precision of 0.15 ‰ (2 SD) on foetal bovine serum was reached in their study. Babcsanyi (2015) tested this method on problematic environmental samples ($\text{Ca} \gg \text{Cu}$) and found 9 % of Cu eluted during matrix elution step, thus discarding this method. This group also investigated the possibility of Cu concentration through co-precipitation through $\text{Mg}(\text{OH})_2$ and KMnO_4 in both cases recovery was judged insufficient. Leaving Cu isotope ratios in samples with Ca/Cu ratios $\lesssim 10000$ not measurable, however this might be due to the high vulnerability of the Ni spike to isobaric Ca-O(H) interferences.

In this manuscript, Cu isotope ratios are measured in various matrices. We thus aim to develop a robust first separation step that efficiently reduces most matrix elements but robustly recovers all the Cu. In this way, a recalibration of the separation protocol is not necessary for every matrix and concentration levels, and different matrices could be treated at the same time.

Materials and Methods

Experiment 1: Matrix separation

In a first step, matrix separation was evaluated for four different matrix types. A soil sample from a calcareic cambisol a bulk soil sample from a vertic cambisols, a citrate soil extract and a leaf sample. The calcareic cambisols sample came from the lowest horizon (50-60 cm) of the C220 sample spot of the Soave vineyard described in **Chapter 5**. The vertic cambisols sample was B20 110-120 cm. Deepest horizons were chosen as they contain lowest Cu concentrations and are most contrasted in their mineralogy. 100 mg of ground soil samples and 200 mg of ground leaf sample were digested in a CEM MARS 5 microwave oven using ultrapure acids (9 mL HNO₃ : 2 mL HCl : 3mL HF). Na-citrate extractions (Labanowski et al., 2008) were performed on a vertic cambisols sample using 0.1 molar tri-sodiumcitrate solution (citric acid trisodium salt dehydrate, ACS reagent, Acros Organics). 3 g of ground soil sample was shaken with 30 mL of solution for 6 h and then centrifuged. The supernatant filtered to 0.22 µm. Samples were then transferred to teflon vessels, evaporated to dryness and digested twice on a hotplate using HNO₃ and aqua regia (both 120°C for 12h). Digests were redissolved in 0.2 % (v/v) nitric acid for and spiked with In/Re solution and measured on an Agilent 7500ce Q-ICP-MS.

Aliquots containing 500 ng of Cu were taken from digests, evaporated on a hotplate at 90 °C and redissolved in 1 mL 7 M HCl containing 0.01 % H₂O₂. The soil and extract samples were loaded on 2.5 mL of AG-MP-1 resin on in a Biorad column. The leaf sample was loaded on 2 mL of resin. These higher resin volumes were chosen as preliminary tests showed that Cu recovery was not quantitative. Separation was conducted 7 M HCl containing 0.01 % H₂O₂ following to Maréchal et al. (1999). The eluted solution was collected in different Savillex vessels that were changed after 2, 4, 6, 8, 10, 15, 20, 23, 26, 29, 32, 35 mL. Recovered solution was evaporated and redissolved and elemental contents were measured on an Agilent 7500ce Q-ICP-MS, as described above.

Experiment 2: Cu recovery

In the following experiment, two vertic soil samples (3 and 5 M elution) and one sample of a citrate extraction (4 M elution) were loaded on a separation column as described above. The elution protocol was interrupted after the matrix elution, at 10 mL percolated 7 M HCL + 0.01 % H₂O₂. Subsequently Cu was eluted using 3, 4 and 5 M HCl with 0.01 % H₂O₂. Again eluted solutions were sampled at 3, 6, 9, 12, 15, 18 mL of the respective acid treatment. Recovered solutions were evaporated to dryness, redissolved and Cu contents measured on a AAnalyst600 (Perkin Elmer).

Results and Discussion

Results of the matrix separation experiment are reported in Figure 9.2. Concentrations are reported for the last mL of sampled solution. For example the measured value for the sample step 0-2 mL is reported at 2 mL. Elution curves are shown for elements expected to cause major interferences during Cu isotope ratio measurement. For the B20 110-120 sample a good separation of Na, Mg, Ca and Ti is achieved and more than 99 % of the initial mass of those elements are eluted before 10 mL. Cu elution begins slowly in the 10-15 mL sample and did not drop to 0 after after 35 mL of percolated acid, this is also true for the C220 50-60 and citrate extract samples. In the C220 50-60 more than 99 % of Na, Mg and Ca were eluted before 10 mL. For P and Ti, respectively 84 and 93 % were eluted before 10 mL of acid percolation. Cu breakthrough begins in the 10-15 mL sample containing about 1.5 % of total Cu. In the citrate extract more than 99 % of Na, Mg, Ca and Ti was eluted before 10 mL. Also 96 % of P was eluted before 10 mL of acid percolation. For the leaf sample more than 99 % of initial mass was eluted before 10 mL of percolated acid for Mg, Ca and P. About 90 % of Ti and 75 % of Na were eluted before 10 mL. Cu elution started in the 10-12 mL sample elution 13 % of Cu in this sample.

Cu elution from 2.5 resin was latest when 5 M HCl was used. Only 1 % of Cu was eluted with the first 3 mL of percolated acid and only 90 % of Cu were recovered after 28 mL of percolated acid. When 4 M HCl was used 13 % of Cu were eluted in the first 3 mL of percolated acid. Between 22-25 mL of acid only 0.6 % of Cu were eluted and in the 28 mL sample no more Cu was detected. Elution using 3 M HCl was even earlier with 22 % in the first 3 mL. However, there were still 1 % of Cu detected in the 22-25 mL sample. No Cu was detected in the 28 mL sample.

For the leaf sample, a good separation of Cu from potentially interfering matrix elements was achieved. Even though some Ti was detected after 10 mL of percolated acid this did not represent a significant quantity with respect to Cu and might be due to inaccuracy in the measurement due to concentration close to detection limit. As Cu elution is relatively high just after 10 mL we started to collect Cu after 9 mL for sample measurements, to be sure that all Cu was recovered even if the elution curve slightly varied. Tests on field grown leaf samples that were run once and twice by the column procedure showed no significant fractionation so that one column passage was judged sufficient for field grown leaf samples (Data not show).

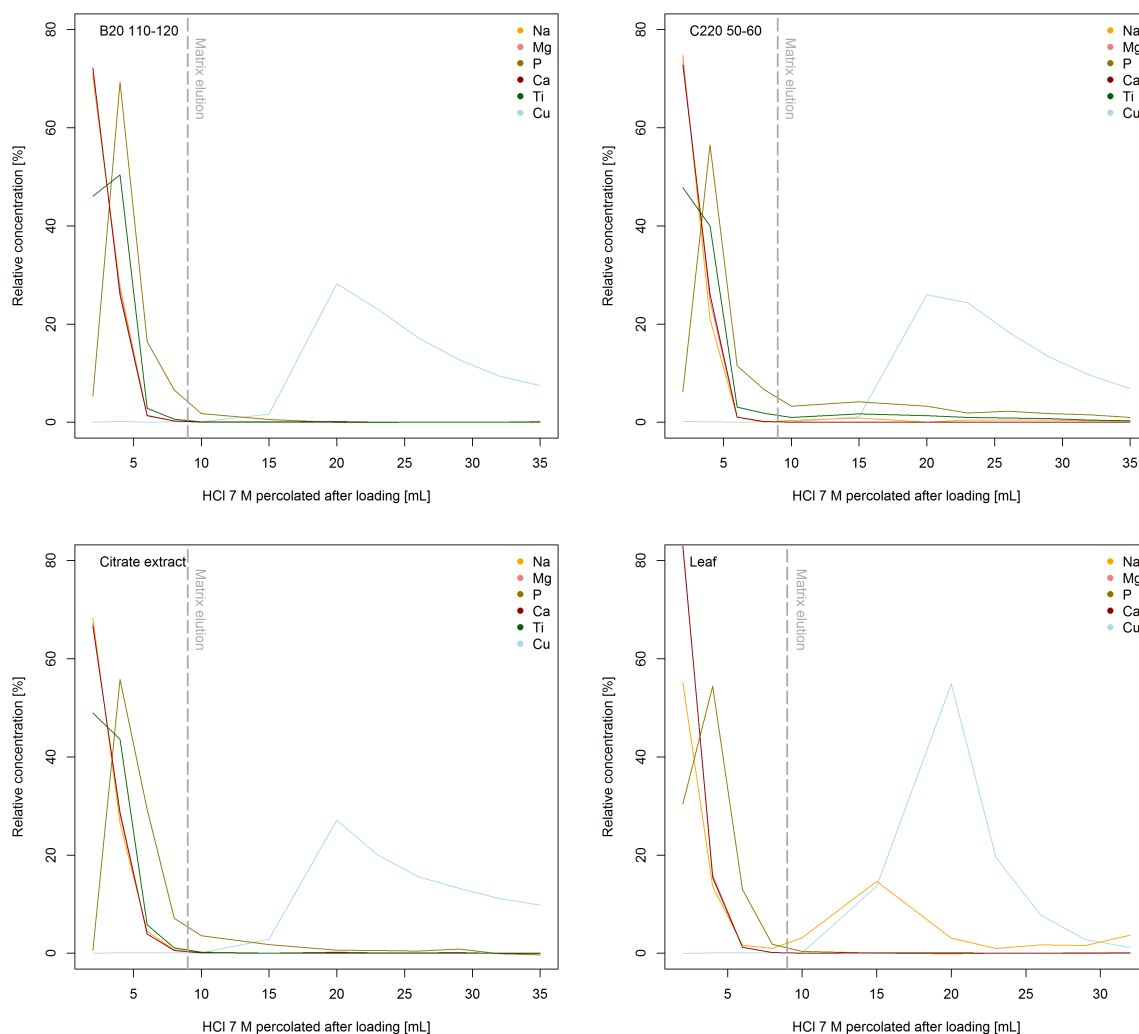


Figure 9.2 – Elution curves of Cu and main interfering elements (Na, Mg, P, Ca and Ti). Elution for the two soil samples and the citrate extract were done using 2.5 mL of AG MP-1 resin. For the leaf sample 2 mL of resin

The sample content of alkaline earth elements and Na were reduced by more than a factor 100 in the all three matrices using 2.5 mL of resin after 10 mL of acid percolation. Very early elution of these elements suggest that they are virtually not retained by the column. However, as the content of these elements can be high with respect Cu the remaining mass after one column passage can still be a multiple of Cu content so that a second column passage is still necessary. For example the ratios between Ca/Cu in soil samples treated in this manuscript lie between 75 and 1000 (m/m). These values illustrate that one column passage will not be enough to bring down Ca concentration to below Cu concentration. However after two column passages it should be around 0.1 and thus acceptable.

In the Geosciences Environment Toulouse (GET) laboratory, the Q-ICP-MS is used for measurements on variable matrices, making precise determination of trace amounts

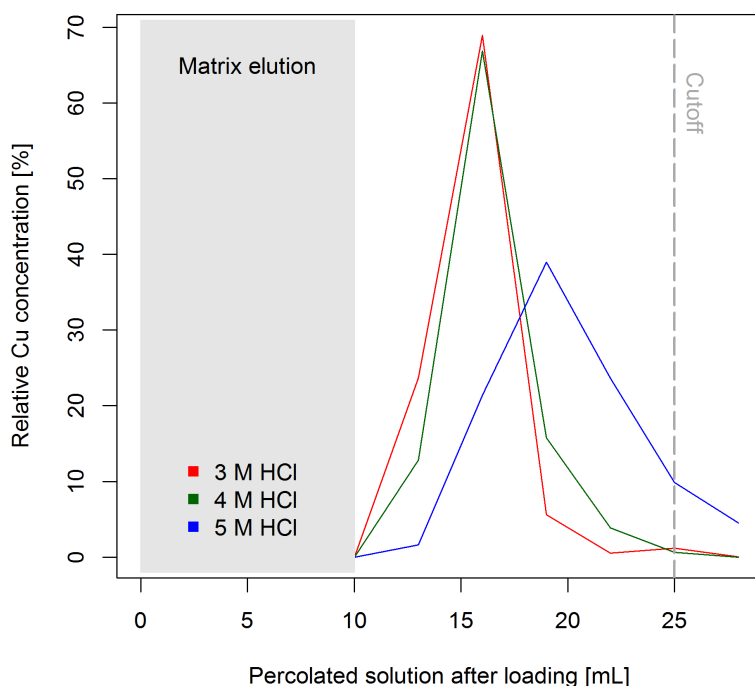


Figure 9.3 – Cu elution from 2.5 mL of AG MP-1 resin using HCl + 0.01 % H₂O₂ solutions at different molarities.

of major elements challenging. So that tests were carried out directly during isotope measurements, spiking standard Cu/Zn standard solutions with Ca. At a Ca/Cu ratio of 20 only a $\Delta^{65}\text{Cu}$ of 0.03 ‰ was observed (oral communication with Remy Freydier, from HydroSciences Montpellier (HSM) laboratory). For Ti, the Ti/Cu ratios can reach up to 440 (m/m) implying that a reduction by 93 % still leaves more Ti than Cu after one separation passage. However, after a second column passage, Ti was systematically below detection limit in Q-ICP-MS measurements. Only leftovers of P were frequently detected in samples after a second column passage. Test with spiked standard solutions indicated that up to P/Cu = 2, no significant fractionation occurred (data not shown).

Cu elution from columns packed with 2.5 mL of resin was not finished after 35 mL of 7 M HCl. This corresponds to a later elution than measured by Babcsanyi (2015). To shorten Cu elution a second experiment was performed using lower acid molarities. Cu elution was not quantitative after 15 mL of 5 M HCl added after matrix elution. For 3 and 4 M HCl total elution of Cu was achieved. In the recovered Cu solution, the yellow color indicated the recovery of Fe especially in the 3 M HCl elution so that 4 M elution was retained.

In the final protocol matrix was eluted until 9 mL of percolated 7 M HCl to leave space for possible early elution due matrix effects. The final protocol presented for leaf

separation was then used as a second elution step. A second separation step using high HCl molarity was necessary to eliminate elements that are more strongly retained in the resin such as Fe and Zn. The final protocol is shown in Table 9.3.

Table 9.3 – *Modifications of the Cu separation procedure*

	Plant samples	Soil samples	
		First step	Second step
Resin Volume	2 mL	2.5 mL	2 mL
Conditioning	6 mL 7 M HCl, 0.01% H ₂ O ₂	6 mL 7 M HCl, 0.01% H ₂ O ₂	6 mL 7 M HCl, 0.01% H ₂ O ₂
Sample Loading	1 mL 7 M HCl, 0.01% H ₂ O ₂	1 mL 7 M HCl, 0.01% H ₂ O ₂	1 mL 7 M HCl, 0.01% H ₂ O ₂
Matrix elution	9 mL 7 M HCl, 0.01% H ₂ O ₂	9 mL 7 M HCl, 0.01% H ₂ O ₂	9 mL 7 M HCl, 0.01% H ₂ O ₂
Cu elution	24 mL 7 M HCl, 0.01% H ₂ O ₂	15 mL 4 M HCl, 0.01% H ₂ O ₂	23 mL 7 M HCl, 0.01% H ₂ O ₂

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