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Resumo

Neste trabalho, que foi pioneiro em Portugal no uso da técnica analítica de espectroscopia de massa com plasma acoplado por indução (ICP-MS), as duas potencialidades mais relevantes desta técnica, determinação de razões isotópicas e análise multi-elementar, foram exploradas no sentido de alcançar os seguintes objectivos finais: (1) identificação das fontes mais importantes de contaminação por chumbo em vinhos Portugueses; e (2) estudo da utilidade da razão $^{87}Sr/^{86}Sr$ e/ou da composição multi-elementar para a determinação da proveniência de vinhos com vista à detecção ou prevenção de fraudes. A implementação/optimização de metodologias analíticas adequadas quer para o pré-tratamento quer para a análise das amostras nos estudos propostos constituíram objectivos intermédios, embora relevantes.

A maior parte dos estudos incidiram sobre dois vinhos da região do Douro, Portugal, um vinho de mesa tinto e um vinho tinto licoroso (idêntico ao designado vinho do Porto). O vinho de mesa foi produzido segundo um moderno processo de vinificação com uvas de uma vinha nova, plantada há dez anos numa área até aí de floresta. Em contraste, o vinho licoroso foi produzido com uvas de uma vinha muito mais velha, com sessenta a setenta anos, usando um processo de vinificação tradicional. Durante um ciclo anual de produção vinícola (ano de 2000) foram mensalmente colhidas e analisadas amostras de aerossóis atmosféricos da área das vinhas, solo das mesmas, folhas das videiras e uvas, bem como, amostras dos passos intermédios dos processos de vinificação e dos vinhos produzidos.

A razão $^{87}Sr/^{86}Sr$ foi também determinada em oito vinhos provenientes de cinco regiões Portuguesas e em dois vinhos da região de Bordéus, França.

Em relação às metodologias usadas, foram implementados/optimizados procedimentos de pré-tratamento de irradiação por UV e de digestão assistida por microondas a alta pressão (apenas para comparação) para vinhos e mostos. Digestões a alta pressão foram também optimizadas para as amostras sólidas. Para a determinação da razão $^{87}Sr/^{86}Sr$ quer em vinhos quer em solos, foi optimizado um procedimento de cromatografia por permuta iónica para a eliminação prévia do interferente rubídio. Este procedimento foi efectuado após o pré-tratamento geral atrás mencionado. Para as determinações por ICP-MS foi ainda necessário optimizar procedimentos analíticos adequados tanto para as razões isotópicas de chumbo e de estrôncio como para a composição multi-elementar.

Relativamente às medições da razão $^{87}Sr/^{86}Sr$, um outro método analítico foi implementado/optimizado usando um ICP-MS com tecnologia de reacção dinâmica (DRC-ICP-MS) existente no Laboratório de Química Analítica da Universidade de Ghent, Bélgica. Os resultados obtidos

foram concordantes com os obtidos com o ICP-MS normalmente usado neste trabalho, o qual possuí um quadrupolo (Q) como espectrómetro de massa. O equipamento DRC-ICP-MS mostrou ser mais expedito na determinação da razão $^{87}Sr/^{86}Sr$, por não exigir o longo e aborrecido processo de remoção do rubídio. O conjunto de resultados obtidos com os dois referidos equipamentos foram ainda comparados com os obtidos, após separação do rubídio, no "Institute for Reference Materials and Mesurements", Geel, Bélgica, usando um ICP-MS de alta resolução. Obteve-se uma boa concordância com os resultados obtidos com o instrumento Q-ICP-MS.

O equipamento Q-ICP-MS usado neste trabalho pode operar em duas formas distintas, conhecidas por modo "quantitativo" e modo "semi-quantitativo". Para controlo da qualidade analítica, os resultados obtidos, para alguns vinhos seleccionados, usando o modo "quantitativo" foram comparados com os obtidos, para os mesmos vinhos, no Laboratório da "Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes", França, usando uma metodologia diferente e um equipamento Q-ICP-MS de outra marca. Foram obtidos resultados estatisticamente iguais para um conjunto de dez elementos. Por outro lado, a eficiência do modo "semi-quantitativo" foi testada por comparação com os resultados obtidos para os mesmos vinhos usando o modo "quantitativo" de análise. Resultados estatisticamente idênticos foram obtidos para a maioria dos elementos medidos. Como o modo "semi-quantitativo" apresenta algumas vantagens (é mais rápido e consume menos reagentes), em quase todos os estudos multi-elementares posteriores usou-se este modo de análise.

Quanto à contaminação por chumbo em vinhos, observou-se que as fontes mais relevantes estavam no sistema de vinificação, e que o sistema mais tradicional introduzia mais chumbo no vinho do que o sistema moderno. Apenas 1/4 (vinho licoroso) e 1/3 (vinho de mesa) do total de chumbo presente no vinho provieram do solo e de deposição atmosférica. Portanto, serão de esperar reduções acentuadas nos níveis de chumbo em vinhos se as fontes deste metal forem removidas dos tubos e recipientes usados no sistema de vinificação, em particular passando a utilizar apenas ligas metálicas praticamente isentas de chumbo. Apesar da contaminação, a concentração deste metal foi de apenas 17.2 $\mu g\ l^{-1}$ e 13.1 $\mu g\ l^{-1}$ nos vinhos licoroso e de mesa, respectivamente, portanto, muito abaixo do limite máximo estabelecido pela Organização Internacional do Vinho e da Vinha (200 $\mu g\ l^{-1}$).

As potencialidades da razão isotópica de estrôncio $^{87}Sr/^{86}Sr$ para a determinação da proveniência de um vinho foram avaliadas de dois modos distintos e complementares. O primeiro envolveu vinhos de cinco regiões Portuguesas e de uma região Francesa, e permitiu detectar diferenças significativas neste parâmetro em vinhos de diferentes origens. O segundo incidiu nos dois vinhos da região do Douro cujos processos de vinificação foram monitorizados. A razão $^{87}Sr/^{86}Sr$ foi estatisticamente idêntica nos vinhos,

nos respectivos sumos de uva (preparado no laboratório e, portanto, sem qualquer contacto com o sistema de vinificação) e no solo de proveniência. Estes resultados indicam que a razão $^{87}Sr/^{86}Sr$ é uma ferramenta promissora para a determinação da proveniência de um vinho.

No que se refere à composição multi-elementar dos vinhos e dos seus precursores incluindo o solo de proveniência, observou-se que ambos os processos de vinificação estudados influenciaram a composição multi-elementar dos vinhos produzidos. Evidência efectiva de contaminação foi observada para Cd, Cr, Cu, Fe, Ni, Pb, V e Zn no vinho licoroso e para Al, Cr, Fe, Ni, Pb and V no vinho de mesa durante o esmagamento das uvas, fermentação e/ou envelhecimento dos vinhos (dependendo dos elementos). Apesar disso, foram obtidas correlações significativas ($P < 0.01$) entre a composição multi-elementar dos vinhos produzidos e a dos respectivos sumo de uva, bem como, entre a dos vinhos e a do solo de proveniência. Estes resultados são também promissores quanto à utilização da composição multi-elementar como impressão digital da origem dos vinhos.

Porém, muitos mais vinhos e respectivos solos de proveniência têm de ser estudados no sentido de validar a utilidade dos parâmetros testados como ferramentas para a identificação da região de origem dos vinhos.

Summary

In this work, which was pioneer in using in Portugal inductively coupled plasma mass spectrometry (ICP-MS), the two relevant potentialities of this technique, determination of elemental isotopic ratios and multi-element analysis, were explored in order to attain the two final goals as follows: (1) identification of major sources of lead contamination in Portuguese wines; and (2) study of the suitability of the $^{87}Sr/^{86}Sr$ and/or of the multi-element composition as tools for the determination of the provenance of wines and for detecting/preventing wine fraud. The implementation/optimisation of methodologies suitable to carry out both samples pre-treatments and analysis in the purposed studies constituted intermediary, but relevant, aims of this work.

In the main studies, two wines from the Douro Portuguese region, one red table wine and one red fortified wine (similar to Port), were used. The table wine was produced in a very modern winery with grapes from a new vineyard, which was raised ten years ago in a forest area. In contrast, the fortified wine was produced with grapes from a sixty to seventy years old vineyard and was made by a traditional vinification process. An annual cycle of wine production (year of 2000) was followed: aerosols from the vineyards atmosphere, vineyards soil, vine leaves and grapes were collected in different months and analysed, as well as samples from the intermediary steps of the winemaking processes and produced wines.

The $^{87}Sr/^{86}Sr$ was also determined in eight wines from five Portuguese regions (Douro, Dão, Bairrada, Borba and Madeira) and in two wines from the French region of Bordeaux.

As concerns methodologies, for wine and musts both an UV-irradiation and a high-pressure microwave digestion (only for comparison purposes) pre-treatments were implemented/optimised. Suitable high-pressure microwave digestions were also optimised for solid samples. A cation-exchange chromatographic procedure, for the elimination of rubidium, was optimised to permit the determination of $^{87}Sr/^{86}Sr$ in both wines and soils. This procedure was carried out after the more general mentioned pre-treatments. For the ICP-MS measurements, analytical procedures had to be optimised, namely, for determination of isotope ratios (both of lead and strontium) and for multi-element determinations.

Regarding $^{87}Sr/^{86}Sr$ measurements, another analytical method was implemented/optimised for an ICP-MS equipment with dynamic reaction technology (DRC-ICP-MS), that it is at the Laboratory of Analytical Chemistry of Ghent University, Belgium. The obtained results were comparable with those obtained with the quadrupole-based (Q) ICP-MS instrument usually use in the present work. The major advantage was the possibility of measuring $^{87}Sr/^{86}Sr$ without having to carry out the long and tedious

separation procedures for removing rubidium. Both sets of results were also compared with those obtained for the same wines (after rubidium separation) at the Institute for Reference Materials and Measurements, Geel, Belgium, using multi-collector double-focusing sector field ICP-MS. Some differences were observed between this latter set of results with that obtained by DRC-ICP-MS but not with the set of results obtained by Q-ICP-MS.

The Q-ICP-MS instrument used can operate in two different modes for multi-element analysis, namely, "quantitative" and "semi-quantitative". For analytical quality control, results obtained for some wines by using the "quantitative" mode were compared with those obtained in Laboratory of the "Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes", France, by using a different methodology and a Q-ICP-MS apparatus of a different brand. Comparable results were obtained for a set of ten elements. In addition, results obtained for some wines with the Q-ICP-MS "quantitative" mode of analysis were compared with those obtained in parallel by using the "semi-quantitative" one. Comparable results were obtained for a set of thirteen elements. As the "semi-quantitative" mode showed to have some advantages (it is much faster and requires fewer reagents), most of the further multi-element studies were carried out by using this mode of analysis.

Regarding the study of lead contamination in wines, it was observed that the major sources of lead were in the vinification system, the more traditional one introducing more lead than the modern one. Only about 1/4 (fortified wine) and 1/3 (table wine) of the lead total content of the final products came from soil and atmospheric deposition. Therefore, it is expected that marked reductions of the lead content in the wines would occur if the sources of lead were removed of the tubes and containers used in the vinification system, particularly by using welding alloys and small fittings free of lead. Despite the releasing of lead from the vinification system, the lead concentration was only 17.2 $\mu g\ l^{-1}$ and 13.1 $\mu g\ l^{-1}$ in the fortified and table wines, respectively, well below the respective threshold limit value established by the International Office of Vine and Wine (200 $\mu g\ l^{-1}$).

The potential of the strontium isotope ratio $^{87}Sr/^{86}Sr$ for wine provenance determination was evaluated by two complementary approaches. The first one involved wines of five Portuguese and one French regions, being detected significant differences in the $^{87}Sr/^{86}Sr$ among wines of different origins. The second approach involved the two selected winemaking processes of Douro region. The $^{87}Sr/^{86}Sr$ was statistically identical in the wine, in the provenance grape juice (which was prepared in the laboratory and therefore, did not have any contact with the vinification system) and in the provenance soil. These results indicate that $^{87}Sr/^{86}Sr$ ratio could be a promising fingerprint of the wine provenance.

As concerns the studies of multi-composition of wines and their precursors including provenance soil, it was observed that both studied vinification processes influenced the multi-element composition of

the produced wines. Evidence of effective contamination during grape pressing, fermentation and/or fining of wines (depending on the element) was observed for Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in the fortified wine and Al, Cr, Fe, Ni, Pb and V in the table wine. Notwithstanding, significant correlations ($P < 0.01$) were obtained between the multi-element composition of the produced wine and the respective grape juice, as well as between those in wines and the provenance soil. These results are also promissory as concerns the usefulness of the multi-element composition as fingerprint of the wines origin.

However, much more wines/provenance soils must be studied in order to substantiate the suitability of the tested parameters as tools for the identification of wine origin region.

Résumé

Au cours de ce travail, pionnier au Portugal dans l'usage de la technique de ICP-MS, on a exploré les deux principales potentialités de cette technique, détermination des rapports isotopiques et analyse multi-élémentaire, pour atteindre les objectifs suivants: (1) identification des sources de contamination plus importantes par le plomb dans les vins Portugaises; et (2) étude de l'utilité du rapport $^{87}Sr/^{86}Sr$ et/ou de la composition multi-élémentaire pour la détermination de la provenance des vins en vue de la détection ou prévention de fraudes. On a considéré comme objectifs intermédiaires, bien qu'importants, l'implémentation / optimisation de méthodologies appropriées pour le pré-traitement et analyses des échantillons dont on s'est servi au cours de ce travail.

Dans la plupart des études, on s'est servi de deux vins de la région du Douro, Portugal, un vin de table rouge et un vin liquoreux rouge (identique au vin dit de Porto). Le vin de table a été produit suivant un procédé de vinification moderne avec des raisins d'une vigne jeune, plantée il y a dix ans dans une région jusqu'à lors arborisée. Par contre, le vin liquoreux a été produit avec des raisins d'une vigne beaucoup plus âgée, de soixante à soixante-dix ans, et a été élaboré suivant un procédé de vinification traditionnel. Pendant un cycle de production vinicole annuel (année de 2000) on a recueilli et analysé chaque mois des échantillons d'aérosol atmosphérique dans l'aire des vignes, sols de ces vignes, feuilles et raisins, tout comme des échantillons des étapes intermédiaires du procédé de vinification et des vins produits.

Le rapport $^{87}Sr/^{86}Sr$ a également été déterminé dans huit vins provenant de cinq régions Portugaises et deux vins de la région Française de Bordeaux.

En ce qui concerne les méthodologies employées, on a implémenté/optimisé les procédés de pré-traitement par irradiation de UV et par digestion par microondes à haute pression (pour comparaison) pour les vins et les moûts. Les digestions par microondes à haute pression ont aussi été optimisées pour les échantillons solides. Pour la détermination du rapport $^{87}Sr/^{86}Sr$, soit dans les vins, soit dans les sols, on a optimisé une méthode chromatographique par échange ionique visant l'élimination préalable du rubidium. L'usage de cette méthode a été précédé du pré-traitement général mentionné au dessus. Pour les analyses par ICP-MS, il a fallu optimiser des procédés analytiques appropriés autant pour la détermination des rapports isotopiques pour le plomb et le strontium que pour les déterminations multi-élémentaires.

Pour les déterminations du rapport $^{87}Sr/^{86}Sr$, une autre méthode analytique a été implémentée/optimisée, employant un ICP-MS avec technologie de réaction dynamique (DRC-ICP-MS)

que existe dans le Laboratoire de Chimie Analytique de l'Université de Gand, Belgique. Les résultats obtenus sont en accord avec les résultats obtenus avec l' ICP-MS utilisé pour ce travail, lequel possède un quadrupole (Q) avec un spectromètre de masse. L'équipement DRC-ICP-MS s'est avéré plus expéditif pour la détermination du rapport $^{87}Sr/^{86}Sr$, puisqu'il évite le processus long et ennuyeux d'élimination du rubidium. L'ensemble des résultats obtenus avec les équipements mentionnés a également été comparé avec les résultats obtenus à "l'Institut des Matériaux et Mesures" à Geel, Belgique, par un ICP-MS de haute résolution, après séparation du rubidium. On a obtenu une bonne concordance avec les résultats obtenus avec l'instrument Q-ICP-MS.

L'équipement Q-ICP-MS utilisé dans ce travail peut opérer de deux façons distinctes connues par modes "quantitatif" et "semi-quantitatif". Pour contrôle de qualité analytique, les résultats obtenus, pour quelques vins sélectionnés, utilisant le mode "quantitatif" ont été comparés avec les résultats obtenus, pour ces vins, dans le "Laboratoire de Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes" en France, employant une méthodologie différente et un équipement Q-ICP-MS d'une autre marque. On a obtenu des résultats statistiquement identiques pour un ensemble de dix éléments. D' autre part, l'efficacité du mode "semi-quantitatif" a été testée par comparaison des résultats obtenus pour les mêmes vins utilisant le mode "quantitatif". Des résultats statistiquement égaux ont été obtenus pour la majorité des éléments mesurés. Comme le mode "semi-quantitatif" présente certains avantages (est plus rapide et consomme moins de réactants), ont a utilisé ce mode d'analyse dans la plus part des études multi-élémentaires postérieures.

Pour ce qui est de la contamination des vins par le plomb, on a observé que les sources plus importantes se trouvent dans le système de vinification, et que le système plus traditionnel introduit plus de plomb dans le vin que le système moderne. À peine 1/4 (vin liquoreux) et 1/3 (vin de table) du total de plomb présent dans le vin provient du sol et de la déposition atmosphérique. Il serait donc d'espérer des réductions accentuées des niveaux de plomb dans les vins si les sources de ce métal sont éliminées des tuyaux et récipients utilisés dans le système de vinification, en particulier à travers l'usage d'alliages métalliques pratiquement exempts de plomb. Malgré la contamination, la concentration de ce métal est d'à peine 17.2 $\mu g\ l^{-1}$ et 13.1 $\mu g\ l^{-1}$ dans les vins liquoreux et de table, respectivement, soit bien au dessous du limite établit par "l'Organisation Internationale du Vin et de la Vigne" (200 $\mu g\ l^{-1}$).

Les potentialités du rapport isotopique du strontium $^{87}Sr/^{86}Sr$ pour la détermination de la provenance d'un vin on été évaluées de deux façons différentes et complémentaires. La première a engagé des vins de cinq régions Portugaises et d'une région Française, et a permit de détecter des différences appréciables de ce paramètre dans des vins de différentes origines. La seconde a engager les deux vins de la région du Douro dont les procédés de vinification on été monitorisés. Le rapport $^{87}Sr/^{86}Sr$

a été statistiquement identique dans les vins, les jus de raisin correspondants (préparés dans le laboratoire et donc sans aucun contact avec le système de vinification) et dans le sol de provenance. Ces résultats indiquent que le rapport $^{87}Sr/^{86}Sr$ est un outil prometteur pour la détermination de la provenance d'un vin.

En ce qui concerne la composition multi-élémentaire des vins et de ses précurseurs, sol de provenance inclut, on a observé que les deux procédés de vinification étudiés on influencé la composition multi-élémentaire des vins produits. On a observé une évidence effective de contamination par le Cd, Cr, Cu, Fe, Ni, Pb, V et Zn dans le vin liquoreux et par l' Al, Cr, Fe, Ni, Pb et V dans le vin de table au cours du broyage des raisins, fermentation et/ou vieillissement des vins (suivant les éléments). Malgré tout, on a obtenus des corrélations significatives ($P < 0.01$) entre la composition multi-élémentaire des vins produits et celle des jus de raisin correspondants, bien comme entre les vins et leur sol de provenance. Ces résultats sont aussi prometteurs quant à l'utilisation de la composition multi-élémentaire comme impression digitale de l'origine des vins.

Cependant, un plus grand nombre de vins et de sols correspondants devront être étudiés pour pouvoir valider l'utilité des paramètres testés comme outils pour l'identification de la région d'origine des vins.

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APPENDIX

List of Abbreviations and Symbols

AAS – Atomic Absorption Spectrophotometry

BCR – Bureau Communautaire de Référence

DRC-ICP-MS – Dynamic Reaction Cell Inductively Coupled Plasma Mass Spectrometry

EDTA – Ethylenodiaminotetracetic Acid

ETAAS - Atomic Absorption Spectrophotometry with Electrothermal Atomisation

FAAS - Atomic Absorption Spectrophotometry with Flame

GJ – Grape Juice

HPMW – High-Pressure Microwave

ICP-MS - Inductively Coupled Plasma Mass Spectrometry

ICP-OES - Inductively Coupled Plasma Optical Emission Spectrometry

IR – Isotope Ratio

LOD – Limit of Detection

LOQ – Limit of Quantification

LSD - Least Significance Difference

MC-ICP-MS – Multi-Collector Double-Focusing Sector Field Inductively Coupled Plasma Mass Spectrometry

MW - Microwave

m/z – mass to charge ratio

NIST - National Institute of Standards and Technology

OIV – L'Organisation Internationale de la Vigne et du Vin

PTFE - Polytetrafluoroethylene

Q-ICP-MS – Quadrupole Inductively Coupled Plasma Mass Spectrometry

REE – Rare Earth Element

RF – Radio-Frequency

RSD – Relative standard deviation

SRM – Standard Reference Material

TIMS - Thermal Ionization Mass Spectrometry

UV – Ultraviolet

WF – Wine Final Product

W_F – Related to Fortified Wine

W_T – Related to Table Wine

Part I

General Introduction

Chapter 1

General Introduction

1.1. WINE

Wine is a drink that it is obtained by a controlled alcoholic fermentation of smashed grapes. It is a hydro-alcoholic solution (pH between 3 and 4), very rich in alcohol, with particles in suspension and a huge variety of polymeric organic compounds (whose proportions determine the characteristics of the wine). In highest percentage is the water, followed by the alcohol, but organic acids, sulphur compounds (sulphur oxide, thiols, etc.) and nitrogen compounds are also present. It also contains polyphenolic compounds, which are major components of grapes (whose influence in wine quality is of extreme importance, namely in the colour and organoleptic properties), and various inorganic compounds, like, acids, anionic minerals, cationic metals and other compounds in trace amounts.

Two major groups of wines can be found: table wines and fortified wines. The first ones have alcohol contents normally between 10 and 12 %. Fortified wines, in which Port wine is included, have a much more complex matrix and its composition differs considerably from traditional table wines, especially regarding the alcohol (normally around 20 %) and sugar contents.

The composition of wines in terms of trace elements is influenced by the type of soil, wine processing equipment, vinification methods and fungicides, insecticides, fertilizers, etc., used in wine industry [1].

Several of the elements present in the wines, like sodium, potassium, magnesium, calcium, iron, copper, etc. are important nutrients to the human organisms although in low amounts. Nevertheless, other elements, also found in wines, as lead, cadmium and arsenic are know to be potentially toxic even in very small amounts [1]. The trace elements should be present at levels below the maxima permissible and efforts should be done in order to control the levels of these elements in wines. Therefore, methodologies should be developed in order to determine and control the source of undesirable elements, like, for instance, lead, which is of special interest due to its toxicity in case of excessive intake.

On the other hand, the content of some metals present in the wine can be used for the identification of the area where the wine comes from [2]. Labelling the origin of wine may not only help protect wineries from counterfeit wines, but also protect the consumer from buying falsified wine, and permit source confirmation for government certification. Since prestige wines are among the most imitated and falsified products [3], it deserves to be developed analytical methods for a suitable characterisation of the wines, identification of adulterations and establishment of wine origin.

1.1.1. Usefulness of isotope ratios

Normally, isotopic abundances of the elements are constant in the nature. However, there is a reduced number of elements with variable isotopic composition in which lead and strontium are included. The proportions of the isotopes of these elements vary with geological ages and consequently with the geographical locations of the mineral [4]. Therefore, measurements of isotope ratios of elements with variable isotopic abundances may be useful in the protection of prestige wines.

Such variations may be explored, for instance, to identify the source of an element in a given sample, like lead in the case of lead contamination.

Another possibility is the use of the isotope ratios of theses elements, like, for instance, the strontium isotope ratio $^{87}Sr/^{86}Sr$, as provenance indicators or tracers in the biosphere [5], since elements are taken up by plant roots with the same isotopic proportions in which they occur in the provenance soils. Several articles can be already found in the literature that report the use of stable isotope ratios for determination of wine origin, mostly with isotopes of carbon, oxygen and hydrogen [6–8] but also with those of lead [9–11] and strontium [5].

1.1.1.1. Lead

The determination of lead in wine is important because of its well-known toxicity. Most of the lead (a toxic heavy metal) intake by man comes from food and beverages, while respiration through lungs and skin contributes only to a small extent. A small part of the lead ingested can surpass the intestinal barrier, being transferred from the blood to the tissues where it will accumulate. The regular absorption of small amounts of lead may results in serious effects on human health, particularly in individuals of risk (people more susceptible to get sick by reason of lead absorption). Therefore, efforts should be made in order to be able to control the levels of the metal intake and, therefore, control the levels of the metal in the wine. The International Office of Vine and Wine (OIV) has been reducing progressively the threshold limit value of lead in wines, which is actually 200 $\mu g\ l^{-1}$.

The presence of lead in wine is due to two types of sources of contamination: one natural, soil related, and another one resulting from human activity. The latter is related with atmospheric precipitation, pesticides used in the vine, materials used to produce, transport and store the wine, etc. [12]. The role of the different lead sources on the levels of the metal in the final product is unknown but

a clarification of this issue deserves research in order to allow an efficient reduction of the lead levels in wine.

Lead is composed of four stable isotopes, three of which are of radiogenic origin: the radioactive decay of ^{238}U, ^{235}U and ^{232}Th generates, respectively, ^{206}Pb, ^{207}Pb and ^{208}Pb. The most stable isotope, ^{204}Pb, is non-radiogenic. The respective proportions of these lead isotopes, originated from the genesis of the rocks and ore deposits, vary with geological ages and consequently with geographical locations [4]. Stable isotope ratio analysis can yield information about the origin of lead in a given sample.

1.1.1.2. Strontium

The alkali-earth metal strontium has four stable, naturally occurring, isotopes: ^{84}Sr, ^{86}Sr, ^{87}Sr and ^{88}Sr. Only ^{87}Sr is radiogenic, and gradually increases in minerals due to the radioactive decay of ^{87}Rb isotope. Differences in the absolute proportion of ^{87}Sr vary with geological ages and consequently with geographical locations [4]. The stable isotope pair $^{88}Sr/^{86}Sr$ can be used as an internal monitor of both natural and analytical fractionation.

The use of the isotope ratio of strontium $^{87}Sr/^{86}Sr$ is a well established tool in geo- and cosmo-chemistry for dating and tracing the origin of rocks and minerals [4].

The use of the isotope ratio as tracers for wine origin is based on the supposition that a correlation exists between the $^{87}Sr/^{86}Sr$ ratio in the soil and in the wine.

1.1.1.3. Measurement of isotope ratios

Until recently, Thermal Ionization Mass Spectrometry (TIMS) has been considered the more suitable technique for determination of isotope ratios with precision enough to distinguish elements of different origins in a sample. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is now also a suitable and convenient technique for that purpose [13] being less time-consuming than TIMS [14].

ICP-MS has been used for determination of lead isotope ratios in different environmental [*e.g.* 15-27] and biological [*e.g.* 19-21,26] samples, in studies were these ratios were used as tracers of lead sources. It has proved to be a suitable and convenient technique for determination of these isotope ratios with precision enough to distinguish lead of different origins [28].

However, the application of ICP-MS to the determination of lead isotope ratios in wines is scarce. When the work presented in Chapter 4 of this Dissertation was carried out there were only a few results published and all on table wines [9,10]. At present, a few more works can be found on table wines [11,29,30], besides those resulted of the present work (Chapter 4) on fortified wines [31,32].

The application of ICP-MS to strontium isotope ratios measurements is still an unexplored subject, with only a few studies published about optimisation of operational conditions for measurements on standard solutions [33,34] and about determinations in archaeological [35,36] and geological samples [37–39].

At the time the work presented in Chapter 6 was developed, to our knowledge, ICP-MS had never been applied to the determination of strontium isotope ratios in wines (table or fortified). A previous work on strontium isotopes in wine had been carried out by TIMS [5]. By now, the application of ICP–MS is still scarce with only one article [40] published on the subject besides the work described in Chapter 6 [41].

1.1.2. Usefulness of wine multi-element composition

Trace elements concentrations in wines depend, among others factors, on geographical origin. This is based on the assumption that the element composition of the provenance soil is represented in the wine. For instance, Greenough *et al* [2] has shown that wine multi-elemental composition is strongly influenced by solubility of inorganic compounds of the soil.

Multi-element analysis in wines has been used to obtained information about the origin and the authenticity of a wine. Some recent studies can be found in the literature reporting results of these types of analysis in wines [3,42-46], which have been considered promising for the establishment of the wine origin. Although some authors used analysis of wines' multi-element content combined with others, like, for instance, analysis of organic compounds, as phenols [42-44] or anthocyanins [42], others used only multi-element composition for the mentioned purpose. For instance, Augagneur *et al* [3] determined rare earth elements in wines samples of France, California and Australia and concluded that their distribution pattern varied with the region of origin of the wine.; Baxter *et al* [45] have established the elemental composition of several wines by analysing major, minor and trace elements in them and observed significant differences among wines from two different countries, England and Spain; and Taylor *et al* [46] used wine multi-elemental composition for the characterisation and fingerprinting of Canadian wines.

Based on the reported results, multi-element analyses by itself can be considered promising for the establishment of the wine origin. Nevertheless, these studies are still scarce and much more wines need to be analysed, including wines from other countries, in order to substantiate the reliability of this

parameter. Besides, the use of wine multi-element composition as a fingerprint of its origin region is based on the supposition that only the movement of elements from rock to soil and from soil to grape would influence the elements concentrations in it. Therefore, it is also important to validate this assumption by analysing not only multi-element composition of wines but also that of its precursors as well as of the respective provenance soils.

1.1.2.1. Multi-element analysis

For the determination of the multi-element content of a sample several spectroscopic techniques are available. However, ICP-MS, which is a multi-element technique with vocation for analyses of liquid samples, provides high selectivity, sensitivity and lower detection limits than other multi-element ones, like inductively coupled plasma optical emission spectrometry (ICP-OES), or, in some cases, single-element techniques, like electrothermal atomisation-atomic absorption spectroscopy (ETAAS) [47]. These characteristics make of ICP-MS an excellent tool for a detailed characterisation of the elemental composition of numerous samples, including environmental, biological and food and drink samples.

Several studies can be found in the literature reporting the use of ICP-MS for multi-elemental determination in wines [2,3,45,46,48], besides those that resulted of the present work (Chapters 9 and 10) [49,50]. A few of these studies were about the origin and the authenticity of wines as mentioned before. Therefore, ICP-MS multi-elemental analysis of wines seemed suitable to be used as a main tool for detecting/preventing wine fraud, being this technique chosen to carry out most of the work described in this Dissertation.

1.2. AIMS OF THE WORK

This work had several complementary aims.

The first goal was the investigation of lead contamination sources in two Portuguese red wines, which were one table and one fortified wine, like Port.

The second goal consisted of exploring the suitability of the strontium isotope ratio $^{87}Sr/^{86}Sr$ to be used as wine's origin fingerprint.

A third goal was to study multi-element composition of two different Portuguese wines and their precursors including the respective provenance soil in order to evaluate the usefulness of that data as a tool for establishment of wine origin and for detecting/preventing wine fraud.

The optimisation of suitable methodologies for the different purposed goals constituted intermediate aims of this work. The results they provided were essential for obtaining reliable information on the main final goals.

1.3. ORGANISATION OF THE DISSERTATION

This dissertation is structured in four parts.

The first part – General Introduction - includes two chapters. The current Chapter 1 is composed of the framing of the work in which some general topic related with the developed work are covered. The aims of the work are also presented, as well as this present description of the organisation of the Dissertation. Chapter 2 gives a description of the history, principles of operation, advantages and limitations of ICP-MS (the technique that was mainly used in the work).

The second part – Experimental Section – includes a single chapter, Chapter 3, which contains aspects regarding the experimental execution, namely the materials, reagents and solutions used, samples selected, the assemblies sets, the instrumental conditions, the experimental procedures and the methods of data handling used.

In the third part – Results and Discussion – the results of the studies carried out are reported and interpreted. Each Chapter starts with an introductory section, in which specific aspects concerning the described work are focused and the specific aims are pointed out. There is also an experimental section devoted only to particular experimental details.

Results and Discussion section includes nine Chapters and is divide in two parts. III.A is devoted to studies involving isotope ratios and III.B is dedicated to multi-element composition studies. The part III.A is still divided in two sub-parts. The first one (III.A.1) pursues lead and the second one (III.A.2) devotes to strontium.

Part III.A.1 contains the studies on lead contamination in wines, being structured in two Chapters. Chapter 4 is dedicated to the implementation of methodology for ICP-MS determination of lead isotope ratios in wine. Chapter 5 is devoted to the identification of lead contamination sources in two different vinification processes (from the vineyard to the final wine product).

In III.A.2 the suitability of the strontium isotope ratio $^{87}Sr/^{86}Sr$ for wine's origin fingerprinting is explored, being organised in three Chapters, 6, 7 and 8. Chapters 6 and 7 covers methodologies optimisation and methodologies comparisons and Chapter 8 reports a case study where the possible influence of the winemaking processes in the $^{87}Sr/^{86}Sr$ of wines was evaluated.

Part III-B includes four Chapters. Chapters 9 and 10 are dedicated to methodologies optimisation and methodologies comparisons for ICP-MS multi-element analysis of wines, while Chapter 11 is devoted to the optimisation of methodology for ICP-MS multi-element analysis in soils. Finally, in Chapter 12 the relationships between the multi-element composition of wine, soil and samples of different stages of two different winemaking processes are presented and the usefulness of multi-element composition, as a tool for the establishment of a wine origin, is discussed.

The fourth part – Overall Conclusions and Final Remarks – coincides with Chapter 13 and is dedicated to the final conclusions and some considerations on future research needs.

A list of references is included at the end of each Chapter.

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Chapter 2

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

2.1. THE HISTORY OF ICP-MS

ICP-MS is a relatively new technique. Even though it can broadly determine the same suite of elements as other atomic spectroscopic techniques such as flame (F) AAS, ETAAS or ICP-OES, ICP-MS has advantages in its multi-element characteristics (it allows the "simultaneous" determination of more than seventy-five elements), speed of analysis, low detection limits (≤ 1 $\mu g\ l^{-1}$ for most elements) and isotopic capabilities [1].

Although a lot of people and organisations have contributed to the development of ICP-MS the names of A. Gray and R. S. Houk (under V. A. Fassels supervision) come up as pioneers in the development of this technique. The experimental development of ICP-MS from its original idea (that came up in 1970) is described in a publication by A. Gray [2].

The first commercial device appeared in 1983, and it included a low-resolution quadrupole as a mass spectrometer. Since then, several other ICP-MS devices with different characteristics have been developed, like the high-resolution double-focusing sector field and the time-of-flight ICP-mass spectrometers, and instruments with a collision/reaction cell, or more recently, with dynamic reaction cell technology. From all of these, instruments equipped with a quadrupole mass spectrometer are still the most commonly found [3].

Despite the multiple advantages it offers, ICP-MS is not free from problems like, for instance, low tolerance for dissolved solids and mass discrimination phenomena [3]. However, ICP-MS' most important problem has always been spectroscopic and non-spectroscopic interferences. From what has been published in the literature, it can be concluded that the history of ICP-MS is also, to a major extent, the history of a permanent fight against interferences as an aggravating, but inevitable, phenomenon [1]. As a result, there has been a permanent process of technical innovation in this technique.

ICP-MS can be applied to very diverse sample types (environmental, geochemical, biological, food, industrial, semiconductors, etc), allowing the identification and quantification of almost all elements in a sample and the distinguishing/quantification of different isotopes of the same element. In our days, ICP-MS is considered a versatile tool for the elemental characterisation of diverse samples and for obtaining information about the origin of an element, as long as it is characterised by the existence of several isotopes and a variable isotopic composition in the nature.

2.2. PRINCIPLES OF OPERATION

A number of ICP-MS instrument types are commercially available today, each with their own strengths and limitations. They all share similar components, such as the nebulizer, plasma torch, interface and detector, but can differ significantly in the design of the mass spectrometer.

The ICP-MS instrument mainly used in the present work was equipped with a quadrupole filter as a mass spectrometer. However, in the study reported in Chapter 7 different systems, namely an ICP-MS device with dynamic reaction cell technology and a multi-collector double-focusing sector field ICP-MS instrument were used.

In this section, an overview of the principles of operation of ICP-MS is given. Although the description is mainly of a quadrupole-ICP-MS, a summarised description of the two other ICP-MS used is also included.

In Fig. 2.1, a schematic diagram of a typical ICP-MS system is presented.

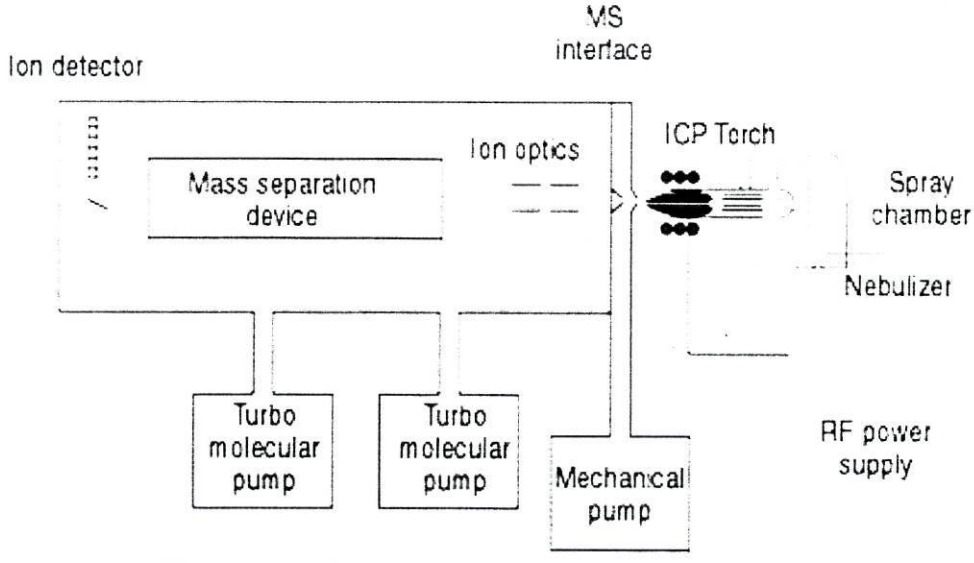

Fig. 2.1. Basic instrumental components of ICP–MS [+].

2.2.1. Sample introduction system

The sample, typically in liquid form, is pumped into the sample introduction system, which is made up of a nebulizer and a spray chamber, where it is converted in an aerosol. Then it is conducted through an injector tube into the plasma region.

The main function of the sample introduction system is to generate a fine aerosol of the sample. The sample is pumped (normally at ~1 ml min^{-1}) via a peristaltic pump into the nebulizer. There the liquid is broken up into a fine aerosol by the pneumatic action of an argon gas flow (~1 l min^{-1}). After that, it enters the spray chamber, the main function of which is to reject the larger droplets and allow only the smallest ones into the plasma.

There are several types of pneumatic nebulizers commonly used in commercial ICP-MS: cross-flow, concentric, micro-concentric and micro-flow, the first two being the most popular ones. In the cross-flow nebulizer, the argon gas is directed at a right angle with respect to the tip of a capillary tube where the sample is coming off. This is in contrast with the concentric nebulizer where the gas flow is parallel to the sample capillary. Cross-flow nebulizers are not as efficient or stable as concentric nebulizers but are more rugged for routine use dealing better with samples that contain a heavier matrix or small amounts of undissolved matter.

Regarding the spray chambers, two designs are mostly used in commercial ICP-MS instruments: double-pass and cyclonic, the first one being by far the most common. In addition, some ICP-MS spray chambers are externally cooled, as is normally used for organic solvents. The most common design for a double-pass spray chamber is the Scott design, which consists mainly of two concentric tubes. This design selects the small droplets by directing the aerosol into the central tube while the large droplets emerge from the external tube and, by gravity, exit the spray chamber via a drain tube. In the cyclonic spray chamber, droplets are discriminated according to their size by means of a vortex produced by a tangential flow of the sample aerosol and the argon gas inside the chamber. Smaller droplets are carried with the gas stream into the plasma, while the large droplets collide with the walls and fall out through the drain tube. Cyclonic spray chambers can provide, in general, a higher analyte introduction efficiency and hence, lower detection limits, while the Scott double-pass spray chambers are generally considered as the most rugged design for routine work.

Other non-standard sample introduction systems are available for commercial ICP-MS instruments, such as ultrasonic nebulization, possibly combined with a membrane desolvation unit, flow injection, electrothermal vaporisation and laser ablation (for solid samples).

In the present work, the quadrupole ICP-MS instrument used was equipped with a cross-flow nebulizer and a double-pass spray chamber, while the two other ICP-MS instruments used in the studies reported in Chapter 7 were equipped with a concentric nebulizer and a cyclonic spray chamber.

2.2.2. Plasma region

The sample emerges from the introduction system as an aerosol and is conducted through an injector tube into the plasma region, by a gas flow, the nebulizer flow (normally at ~1 l min^{-1}). That injector tube, made of alumina in the set-up used, is located in the middle of the plasma torch. The plasma torch consists of two concentric tubes, which are usually made from quartz. The gas (normally argon) used to form the plasma (plasma gas) passes between these two tubes (normally at ~15 l min^{-1}), while a second gas flow, the auxiliary gas, passes between the torch's inner tube and the injector (normally at ~1 l min^{-1}).

Surrounding the top end of the torch there is a load coil, a filament normally made out of copper, which is connected to a radio frequency (RF) generator. The plasma is the result of the transfer of RF energy to the continuous plasma gas flow and is mainly composed of argon atoms, ions and electrons. A high-voltage spark starts the ionisation of the argon flow. The resulting ions and their electrons interact with the magnetic field created by the load coil. This interaction makes ions to circulate in a circular trajectory within the filament. The resistance to this movement causes an ohmnic warming which is responsible for the high plasma temperatures (between 6000 and 8000 K). These high temperatures give origin to the desolvation, vaporisation, atomisation and ionisation of the sample during their existence/presence in the plasma region. The ions then emerge from the plasma, being directed into the interface zone.

2.2.3. Interface zone

The role of the interface is to transport the ions efficiently from the plasma, which is at atmospheric pressure (around 760 torr), to the mass spectrometer region, which requires high vacuum (pressure around 1 x 10^{-6} torr). It is, therefore, a zone already with a relatively low pressure, around 4 torr. The interface zone consists of two metallic cones, each with a central opening of about 1 mm of diameter, whose functions are to extract the sample ions. After the ions are generated in the plasma, they pass through the first cone, known as the sampler cone. From there, they travel a short distance to the second cone, the skimmer cone, which is generally sharper than the first one. Both cones are usually made of nickel, but they can be made of materials such as platinum that are more tolerant to corrosive

liquids. After passing the cones through the gate valve, the ion beam enters the mass spectrometer region.

2.2.4. Ion optics

Once the ions have been extracted from the interface zone, they are directed into the main vacuum chamber – the mass spectrometer region - by a series of electrostatic lenses called ion optics. The ion optics are positioned between the skimmer cone and the mass separation device, and their function is to take ions from the hostile environment of the plasma at atmospheric pressure via the interface cones and steer them into the mass analyser, which is under high vacuum. A secondary but very important role of the ion optics system is to stop particulates, neutral species, negatively charged species (electrons and to a less extent negative ions) and photons from reaching the mass analyser and the detector. Over the years, there have been many different optic designs. The most common one used today consists of several lens components all with a specific role, such as the one found in the quadrupole ICP-MS device used in this work. Another, more novel approach is to use just one cylindrical ion lens, as is, *e.g.*, the case in the ICP-MS system with dynamic reaction cell technology.

2.2.5. Mass separation devices

After passing the ion optics system, the ions are conducted to the mass separation device in order to be separated accordingly to their mass/charge ratio (m/z). The mass separation device is positioned between the ion optics and the detector in the mass spectrometer region.

There are, basically, three kinds of commercially available mass analysers: quadrupole, high-resolution double-focusing sector field and time-of-flight analysers, the most common being the quadrupole filter. These devices all work differently, but all serve the same basic purpose: to allow analyte ions of a particular m/z to reach the detector and to filter out all the non-analyte, interfering and matrix ions. There is also another type of ICP-MS that although with a quadrupole mass analyser it considerer as a different equipment because they have either collision/reaction or dynamic reaction cell technology. As indicated previously, in the present work the ICP-MS type mainly used was a quadrupole ICP-MS device, but both an ICP-MS with dynamic reaction cell technology and a multi-collector

double-focusing sector field ICP-MS instrument were used for the study presented in Chapter 7. A brief description of these three types of instrumentation is given below.

Quadrupole-based systems represent approximately 90 % of all ICP-MS instruments used today. The quadrupole is not more than a mass filter, consisting of four rods, of the same length and diameter, controlled by electric potentials, which forms an electric field. When a particular voltage is applied to the quadrupole rods, the analyte ions of interest are steered down the middle of the four rods to the end, where they will emerge and are subsequently converted into electric pulses by the detector. Ions of different m/z will collide with the rods, being neutralised and hence, removed from the ion beam. In Fig. 2.2 a schematic diagram of this type of spectrometer is presented.

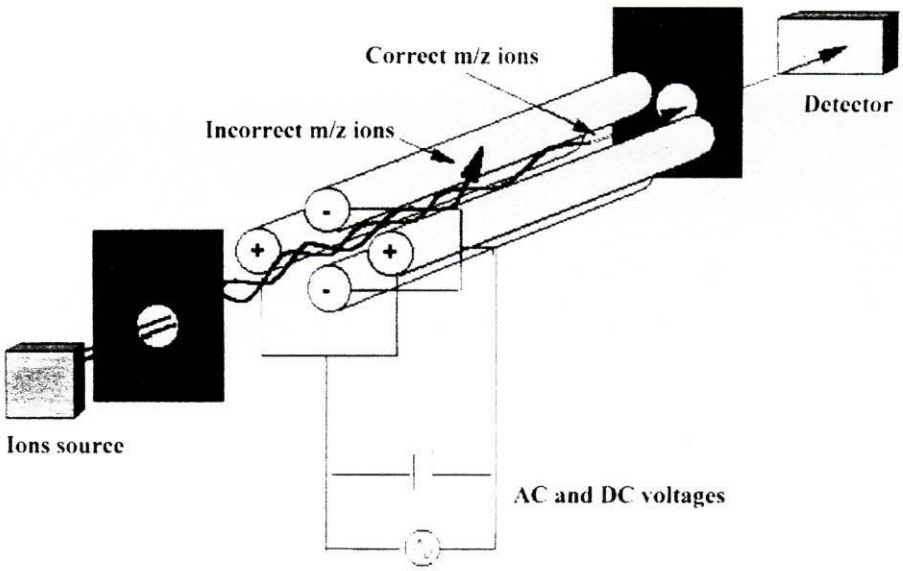

Fig. 2.2. Schematic of a quadrupole mass spectrometer [1].

Two very important performance specifications of a mass analyser govern its ability to separate an analyte peak from a spectral interference. The first is resolution – the ability to separate ions of different m/z – and the second is abundance sensitivity – the signal contribution of the tail of an adjacent peak at one mass lower and one mass higher than the analyte peak to the latter peak. The resolution is normally defined as the width of a peak at 5 % of its height, and, in practice, the quadrupole is normally operated at a resolution of 0.7-1.0 for most applications. The abundance sensitivity is affected by a combination of factors including design of the rods, frequency of the power supply and operating vacuum. Many

different designs of quadrupoles are used in ICP-MS instruments but in practice, the performance is similar for most of them.

Quadrupole instrumentation's limited resolving power led to the development of high-resolution systems based on **double-focusing sector field** mass spectrometer technology. These ICP-MS instruments offered a resolving power as high as 10 000, which is high enough to resolve the majority of spectral interferences. Nevertheless, double-focusing sector field ICP-MS equipments are not as common as the quadrupole ones, especially due to their high cost. Today, sector field ICP-MS instrumentation is based on two different mass spectrometer designs commonly refereed to as Nier-Johnson or reverse Nier-Johnson geometry. Both designs, which use the same basic principles, consist of two analysers: a traditional magnet and an electrostatic analyser (ESA). In the Nier-Johnson configuration, the ESA is positioned before the magnet, and in the reverse design (the most common) it is positioned after the magnet. The ions are sampled from the plasma in a conventional way and then accelerated into the ion optics system before they enter the magnet. The magnetic field, which is dispersive with respect to ion energy and mass, then focuses all the ions with diverging angles of motion from the entrance slit into the intermediate slit. The ESA, which is only dispersive with respect to ion energy, then focuses all the ions from the intermediate slit onto the exit slit where the detector is positioned. The combined system focuses both in angle and energy, and this is the reason why this arrangement is often called double focusing.

These systems are slightly slower than quadrupole-based instruments, but they offer the advantages of high mass resolution and a very high sensitivity combined with extremely low background levels. Besides that, another of the recognised benefits of the sector field approach is the excellent isotope ratio precision. Nevertheless, high mass resolution is not a panacea to all types of spectroscopic interferences, since some of the interferences require an even higher resolution, which can not be obtained with commercial instruments.

The detection capability of traditional quadrupole mass analysers for some critical elements is severely compromised by the formation of polyatomic spectral interferences generated by either argon, solvent or sample-based ionic species. Although there are ways to minimise these interferences – including correction equations, cool plasma conditions and matrix separation – they cannot be eliminated. Therefore, a new technology was recently developed– the **dynamic reaction cell**. With this technology, ions enter the interface in the normal manner, where they are extracted under vacuum into a dynamic reaction cell that is positioned before the quadrupole mass analyser. The dynamic reaction

cell is a quadrupole that acts like a selective filter: the ions are separated according to their masses like in a traditional quadrupole and at the same time collide and react with molecules of the reaction gas. By a number of different ion-molecule reaction mechanisms, polyatomic interfering species will be converted to harmless non-interfering species. The analyte ions, free from interferences, emerge from the cell, being directed to the quadrupole analyser for normal mass separation. The benefit of the dynamic reaction cell is that by careful selection of the reaction gas, the user takes advantages of the different rates of reaction of the analyte and the interfering species.

2.2.6. Detectors devices

After going through the mass separation device, the selected ions are detected by a specific detector. In our days, there are two types of detectors used in ICP-MS equipments: the Faraday cup and the electron multiplier (the most common).

The electron multiplier detector has the shape of a curved cone and it is coated with a material that releases electrons when the ions collide with it. These electrons collide again with the detector releasing more electrons. The process goes on until the current generated by this electron cloud is measured at the detector exit.

The Faraday cup detector is a metallic cup connected to a measuring device that measures the ions beam current, which is collected in the cup. It is less sensitive than the multi-electron detector, and sometimes is used as an alternative for the latter for zones of the elemental spectra where the elements concentrations are high.

Most commercial ICP-MS instruments, particularly the quadrupole models, use just one detector. However, specialised sector field ICP-MS instrumentation with a multiple detector configuration is available, see, *e.g.*, the one used in the study described in Chapter 7. Often referred to as multi-collector systems, these instruments offer the capability of detecting and measuring multiple ion signals at the same time. As a result they are recognised as the ultimate tools for isotope ratio measurements as they offer a markedly improved precision.

A computer, provided with appropriate software, which is associated to the ICP-MS, processes the data collected by the detector.

References. The text above was based on [1-16].

2.3. ICP-MS INTERFERENCES

As mentioned before, ICP-MS is not free from problems and one of those is the occurrence of interferences, both spectroscopic, caused by the limited resolution of the mass spectrometer used (normally a quadrupole filter), and non-spectroscopic ones, associated with sample matrix effects and therefore variable from case to case. Many different techniques, mainly hyphenated ones, have been used for the elimination or reduction of interferences. Other techniques that are provided as interface techniques, like the dynamic reaction cells, as well as new ICP-MS instruments with different designs were also developed aiming at the elimination or reduction of specific interferences.

The actual knowledge over ICP-MS interferences, as well as the methods that are being used in their elimination, is described in good review articles (*e.g.*, [17,18]). Next, some of the most relevant aspects are resumed.

2.3.1. Spectroscopic interferences

Spectroscopic interferences are caused by atomic or molecular ions with the same nominal m/z as the analyte of interest, interfering, therefore, with the analysis because they may contribute to the true analytical signal or even obscure it.

These interferences can be divided in two categories.

The first one includes the so called isobaric interferences, which are caused by the overlap, in the spectra, of ions of isobaric nuclides of different elements, *e.g.*, the overlap of $^{114}Sn^+$ in $^{114}Cd^+$ and of $^{40}Ar^+$ in $^{40}Ca^+$. These interferences are easy to predict and can be overcome either by choosing another isotope of the element in question or by mathematical correction equations. For instance, since the higher abundant calcium isotope, ^{40}Ca (96.97%), suffers interference from $^{40}Ar^+$ the isotope ^{43}Ca, which has no interference problems, can be chosen. Although ^{43}Ca has a low isotopic abundance (0.145 %), it is perfectly measurable owing to the high calcium concentration present in most of the samples. An example of a mathematical equation for the correction of the isobaric interference of $^{114}Sn^+$ in $^{114}Cd^+$ is:

$$I(^{114}Cd) = I(^{114}Cd + {}^{114}Sn) - [Ab(^{114}Sn) / Ab(^{118}Sn) * I(^{118}Sn)]$$

where I stands for the signal intensity at the isotopic mass indicated and Ab is the isotopic abundance of the nuclide in question.

The second category includes all the interferences caused by polyatomic or molecular ions overlapping elements with the same nominal m/z. These ions are formed from elements in the plasma gas (argon), in the atmospheric gases which are introduced into the plasma region (carbon, oxygen and nitrogen), in the water and acids used in the dissolution and dilution of the samples (for example, chlorine, sulphur, nitrogen, besides hydrogen and oxygen) and in the matrix of the samples. Examples of these interferences are $^{40}Ar^{40}Ar^{+}$ on $^{80}Se^{+}$, $^{12}C^{16}O^{+}$ on $^{28}Si^{+}$, $^{14}N^{16}O^{1}H^{+}$ on $^{31}P^{+}$, $^{40}Ar^{35}Cl^{+}$ on $^{75}As^{+}$ and $^{32}S^{16}O^{+}$ on $^{48}Ti^{+}$. Additionally, the analyte itself or the analytes of interest may form double charged ions (M^{2+}) (in the case of elements who have a relatively low second ionisation energy), oxides (MO^{+}) and hydroxides (MOH^{+}) (elements with a very strong MO bond), which will decrease its signal intensity and/or cause interferences for other elements with the same nominal m/z as those species. Examples of these interferences are $^{40}Ca^{16}O^{+}$ on $^{56}Fe^{+}$ and $^{138}Ba^{2+}$on $^{69}Ga^{+}$. Nevertheless, the percentage of formation of the species M^{2+}, MO^{+} and MOH^{+} can be reduced to 1 – 3 % through the optimisation of the ICP-MS instrumental conditions, for instance, the nebulizer, plasma or auxiliary gas flows and of the plasma power [18-22].

Several articles can be found in the literature where many of the species that can be formed and that cause interferences are enumerated as well the elements that are affected by those interferences. For instance, Vaughan and Horlick [19] gave a general review of the spectroscopic interferences due to the formation of M^{2+}, MO^{+} and MOH^{+} species. Tan and Horlick [23] described the background spectra for distilled water and for 5 % solution of nitric, hydrochloric and sulphuric acids. And Vanhoe *et al.* [24] described and estimated the contribution of the interfering species found in analysis of biological materials on the signals of the analytes of interest.

There are several theories in the literature that try to explain the origin of the polyatomic ions, *e.g.*, [17,21,25,26], but there is not yet a complete agreement on this issue.

Besides the methods previously mentioned for the elimination or correction of the isobaric interferences, there are many others [3,5,17,18,24], like, for instance, alternative sample preparation methods, alternative methods for introducing the sample into the plasma region and alternative instrumental approaches.

The alternative sample preparation methods include different sample dissolution procedures, for instance, dissolution with nitric acid instead of hydrochloric or perchloric acids, which will eliminate chlorine interferences [27]. They also include solvent extraction, either of the analyte of interest or the interfering elements (*e.g.*, [27-29]) as well as methods for on-line separation either using chelating resins [30,31] or other chromatographic techniques (like ion exchange size exclusion chromatography)

[32,33]. Besides the elimination of the matrix interfering elements, these approaches allow also the pre-concentration of the element of interest in the sample.

As alternative methods for the introduction of the sample into the plasma region, for instance, flow injection [34], electrothermal vaporisation [35], laser ablation (for solid samples) or sample desolvation by using various types of nebulizers [36] can be mentioned. These approaches allow the elimination or reduction of the amount of solvent and, therefore, the reduction of oxides and hydroxides formation.

The alternative instrumental approaches include the use of mixed gas plasmas, for instance argon and nitrogen, which will reduce the interferences caused by argon [37,38] and the use of cool plasma conditions that also reduces the interferences caused by molecular species with argon. The use of a collision/reaction cell or dynamic reaction cell and, of course, the use of high resolution ICP-MS equipment, as discussed in the previous section, will also reduce or eliminate most of the isobaric interferences.

2.3.2. Non-spectroscopic interferences

The non spectroscopic interferences are the second major group of ICP-MS interferences. Contrary to what happens with the spectroscopic interferences in which the signal of the analyte of interest is increased as a result of the presence of another nuclide or polyatomic specie with the same nominal m/z, a non-spectroscopic interference is characterised by a reduction or increase in the signal of the analyte of interest. This is due to factors that influence the sample transport, plasma ionisation and the ion transfer efficiencies. The nature of the sample and of the elements that are part of it, as well as the respective concentration, influence the intensity of the interfering effects [39,40]. Therefore, since these are interferences associated with matrix effects (depending of the matrix of the sample to be analysed), they are different from case to case.

The matrix effects can be classified into reversible and not reversible. The latter ones are more problematic because they can occur not only during the analysis, like the reversible ones, but also after the analysis, normally resulting from high salt concentrations in the sample, which will give origin to salts or oxides depositions either in the sample transport system or on the interface cones. Such deposits will reduce the amount of ions that enters into the mass spectrometer or even completely prevent ion transmission. One way of eliminating or reducing these deposit formations is sample dilution.

There are several methods described in the literature for the elimination or correction of non-spectroscopic interferences [17,18]. Among those, we can point out the use of internal standards [41-

43], standard addition [44,45], isotopic dilution [46-51], methods for matrix separation (already mentioned for spectroscopic interferences) and flow injection [52-55].

The use of an internal standard has proven to be an efficient way to compensate for matrix-induced suppression, as long as that standard has an isotopic mass close to that of the analyte of interest [56,57]. This method is also recommended, even for simple matrix, in order to compensate for instrumental bias.

As mentioned above, there are numerous techniques and procedures for the evaluation and elimination or correction of interferences (spectroscopic and non-spectroscopic) in ICP-MS. Nevertheless, those interferences depend significantly on the sample to be analysed, the elements to be determined, the solvents and on the measuring equipment used as well as on the quality of the argon. Each case has its own characteristics and, therefore, should be individually studied considering the objectives aimed at: possible interferences should be identified and the most adequate procedure for their elimination or correction selected. These types of problems have to be solved in a preliminary phase of any study when using ICP-MS, and thereat optimisation of different procedures were performed in the present work, with different specific purposes, in order to permit reliable results to be obtained.

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Part II

Experimental Section

Chapter 3

Experimental Procedures

3.1. MATERIAL AND REAGENTS

Suprapure concentrated HNO_3 (65 % m/m, d = 1.40 g ml^{-1}) and solution of 30 % H_2O_2, *pro analysis*, from Merck, were used without further purification.

Rh and Pb stock standard solutions from Merck and Ce, Ba and Mg stock standard solutions from BDH (Spectrosol) were used for ICP-MS sensibility assessment.

For *Pb isotope ratios (IRs) determinations*, a stock Common Pb Isotopic Standard solution (1000 mg l^{-1} of Pb) from the National Institute of Standards and Technology (NIST), USA, standard reference material, SRM-981, was prepared by dissolving a portion of the metal in 1 % v/v HNO_3 solution. A Tl standard stock solution (1000 mg l^{-1}), for ICP-MS, was obtained from Alfa.

For *Sr IRs studies*, a stock NIST SRM-987 Strontium Carbonate (Isotopic) Standard solution (1000 mg l^{-1} of Sr), was prepared by dissolving 1 g of powder in 1 % (v/v) HNO_3. A Rb stock standard solution was prepared by dissolving 0.1 g of powder $RbNO_3$ (*pro analysis*, from Aldrich) in 3 % (v/v) HNO_3.

For *multi-element determinations*, using the ICP-MS quantitative mode of analysis, a rare earth elements (REEs) (100 µg l^{-1} from La to Lu) stock standard solution from Alfa, a multi-element stock standard solution with 13 elements (100 mg l^{-1} of Al, Cd, Co, Cr, Cu, Mn, Pb, V and Zn, and 50 mg l^{-1} of As, Fe, Ni and Se), from Teknokroma, and/or a multi-element stock standard solution with 30 elements (1000 mg l^{-1} of Ca, 100 mg l^{-1} of As, B, Be, Fe, Se and Zn, and 10 mg l^{-1} of Ag, Al, Ba, Bi, Cd, Co, Cr, Cu, Ga, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sr, Te, Tl, U and V) from Merck, all for ICP-MS use, were used to prepare standard solutions for establishment of the calibration graphs for the different elements. For the semi-quantitative mode of analysis both the REEs stock standard solution and the multi-element stock standard solution with 30 elements mentioned earlier were used for a calibration with just one multi-element standard solution. The multi-element standard solution used is recommended for semi-quantitative analysis since it simulates the major isobaric and molecular interferences and allows a suitable update of the pre-calibrated internal response of the software. For internal standardisation in multi-element determinations, a *pro analysis* stock standard solution (1000 mg l^{-1}) of Rh, obtained from Alfa, was used.

For *AAS measurements*, standard solutions of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were prepared from stock solutions obtained from BDH (Spectrosol).

EDTA in the acid form *pro analysis*, from Merck, was used to prepared 0.05 mol l^{-1} EDTA solution for *soil extracts*.

All the other reagents used were *pro analysis* grade or equivalent

Standard solutions were prepared daily from the stocks, in polyethylene tubes, by weighing, with de-ionised water (resistivity > 14 MΩ cm, conductivity < 0.1 μS cm^{-1}) or diluted HNO_3, as necessary.

To prevent contamination, all material used was soaked in 20 % v/v HNO_3 for at least 24 h, rinsed several times with de-ionised water and dried in a Class 100 laminar flow hood.

The sample manipulation was carried in a clean room with Class 100 filtered air.

3.2. COMMERCIAL WINES

The wines used in the study reported in Chapter 4 were genuine Port wines from the Douro Region, Northeast of Portugal, supplied (by the "Instituto do Vinho do Porto", Porto, Portugal) in small glass bottles.

All the wines used in the studies reported in Chapters 6, 7, 9 and 10 were commercially available in glass bottles with cork stoppers. In the study dealt within Chapter 6, ten samples of different table and fortified wines were used. Eight samples were from five different Portuguese regions, Douro, Dão, Bairrada, Borba and Madeira, and two were from one French region, Bordeaux. The same wines, with the exception of two fortified wines from the Douro region, were analysed in the study reported in Chapter 7. Another table and fortified wines both from the Douro region were also used in Chapter 7 for preliminary tests. In Chapter 9 and 10 only three wines were used, being two of them the mentioned Bordeaux French wines and the other one a genuine red Port wine from the Douro region, Portugal.

All wines were sampled by removing the cork, washing the neck of the bottle with 2 % HNO_3 solution, followed by de-ionised water, and pouring the required volume of wine to an appropriate container.

3.3. SAMPLING OF ATMOSPHERIC AEROSOLS, VINEYARDS SOIL, VINE LEAVES, GRAPES AND INTERMEDIARIES PRODUCTS AND PRODUCED WINES

In the studies reported in the Chapters 5, 8 and 12, the different phases of wine production, from the vineyard to the final product ready to be sold to the consumers, were monitored during an annual

cycle of wine production. The sampling strategy is schematically described in Fig. 3.1, which also includes resumed information on sample treatments.

Two vineyards, one with sixty to seventy years of age (forward called old vineyard) and other ten years old raised in a forest area (young vineyard), from the Douro Region, Northeast of Portugal, were selected. In both vineyards, only treatments with copper sulphate solutions were carried out during the annual cycle monitored which took place in the year of 2000. No other pesticides or fertilizers were used. Nevertheless, in previous years, several treatment products (not identified) had been use, particularly in the old vineyard. The vineyards are located in an agricultural area, mainly also vineyards, far from industrial activities, having nearby a road with moderate traffic.

The grapes, from the polyvarietal vines that have been grown in the old vineyard, were used to produce a red fortified wine similar to Port. The vinification process used for that purpose (summarised in Fig. 3.2A) was perform manually, in an old fashion way, without automatic controls, and involved a small number of steps, the wine being pour directly from one container to the next. At the end of the vinification process, the fortified wine has been age in oak barrels for periods between two and twenty years, depending on the desirable wine quality. For the present studies, the sample called "final product" was collect after only one-year ageing by practical reasons. However, its composition could still change after that time, since the oak barrels where wine is aged contains metallic bracelets.

In the young vineyard only "Touriga Nacional" vines have been growing and their grapes were use to produce a monovarietal red table wine. The vinification process, summarised in Fig. 3.2B, was automatically perform and controlled, involving much more steps than those of the fortified wine. Stainless-steel tubes and containers were use in most steps. Plastic containers and tubes (polyethylene, high-density polyethylene and flexible PVC) were also used for the harvest and for transfer of must and wine in same of the steps of the vinification. Ageing of the wine took place in oak barrels. At the end, the wine was stored in glass bottles.

Samples were select in order to follow the entire pathway of wine production: aerosols from the vineyards atmosphere, vineyard soil, vine leaves, grapes, as well as intermediaries and final products collected throughout the two different vinification processes. To collect the samples no metallic instruments were use.

Atmospheric aerosols from the vineyards were collected, in duplicate, whenever vine leaves were collected, in a point representative of the all studied area. For this purpose, it was used a low volume sampler (non-commercial, gently offered by the Lawrence Berkeley Laboratory, Berkeley University, California, USA) provided with a 0.8 μm pore size filter (47 mm of diameter) of nitro-cellulose, from Millipore. Approximately 75 h of sampling with an air flux of approximately 11 l min^{-1} was used.

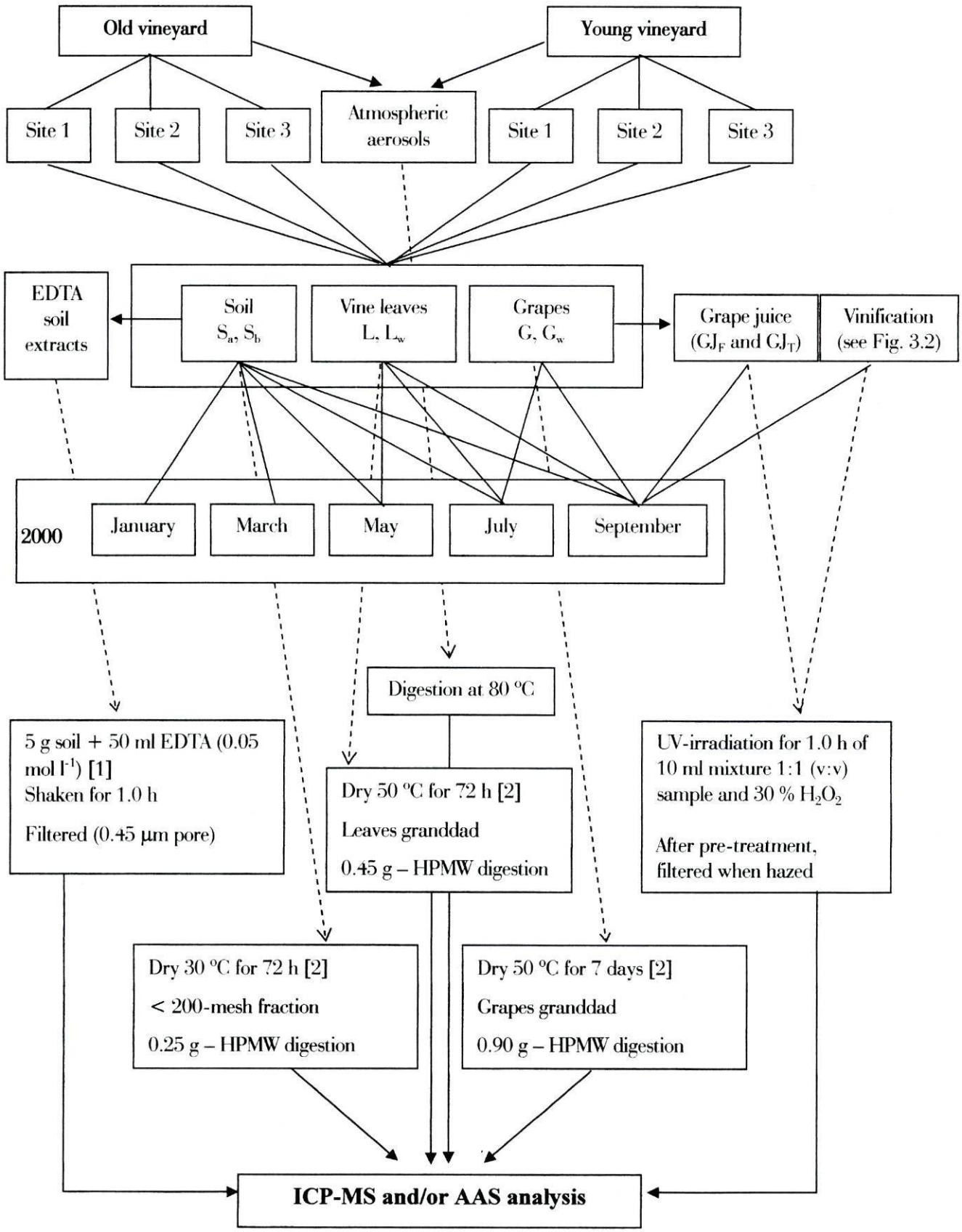

Fig. 3.1. Schematics of the sampling strategy and analytical procedures used in this work. S_a and S_b: soil samples collected at surface and 20 cm depth, respectively; L and G: non-washed vine leaves and grapes, respectively; L_w and G_w: washed vine leaves and grapes, respectively; GJ_F and GJ_T: grape juice prepared in the laboratory by smashing grapes from the old and from the young vineyard, respectively.

The samples of vineyard soil, vine leaves and grapes were collected using plastic shovels (soil samples) and plastic gloves (leaves and grapes) and were stored into individual plastic bags. In each vineyard, samples were collected in three different sites, selected to be representative of the entire area.

In order to differentiate any eventual change in its composition and identify possible contaminations as a result of atmospheric deposition, in each site soil was collected at the surface and at 20 cm depth. With the same purpose, both vine leaves and grapes were divided in two parts: one was washed with de-ionised water while the other remained as collected for analysis.

Throughout the entire vinification processes, samples were collected in triplicate, to polyethylene tubes, at points where a possible change in the wine's composition could be expected, for instance, because of a contamination (see Fig. 3.2A and 3.2B).

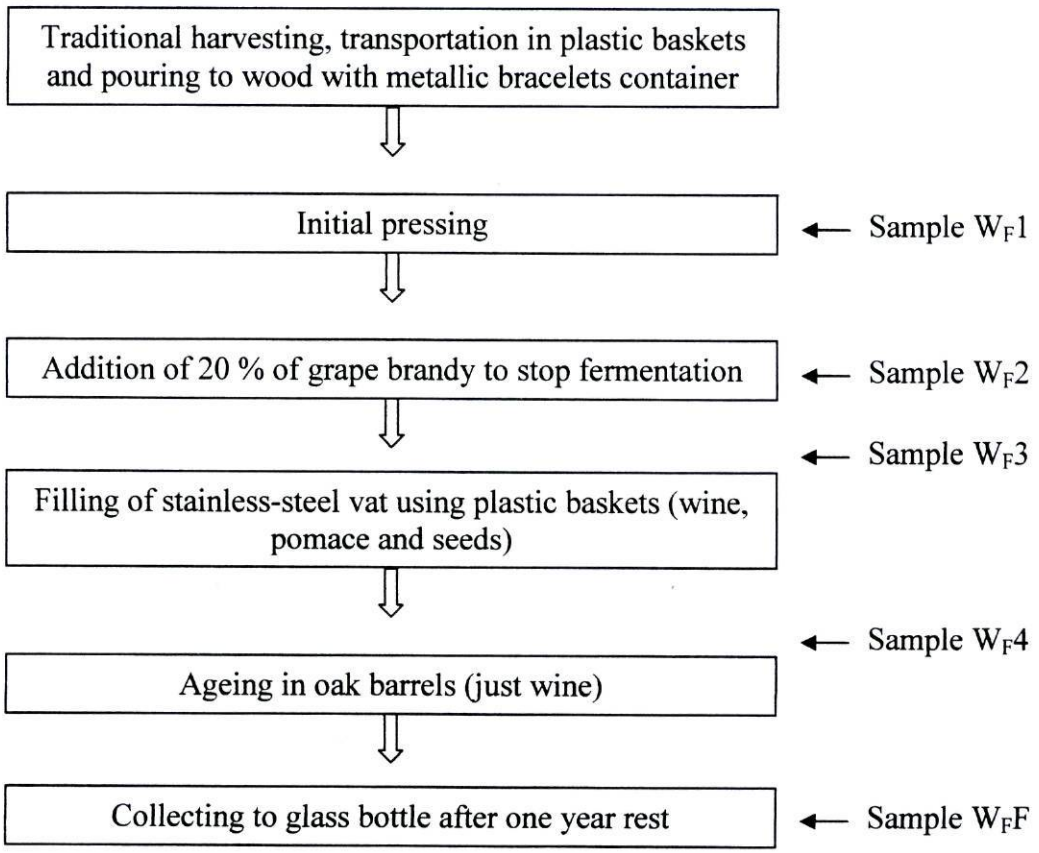

Fig. 3.2A. Schematics of the vinification process used to produced the red fortified wine from grapes of the old vineyard with indication of the points where samples were collected (W_F1 to W_F4 and W_FF).

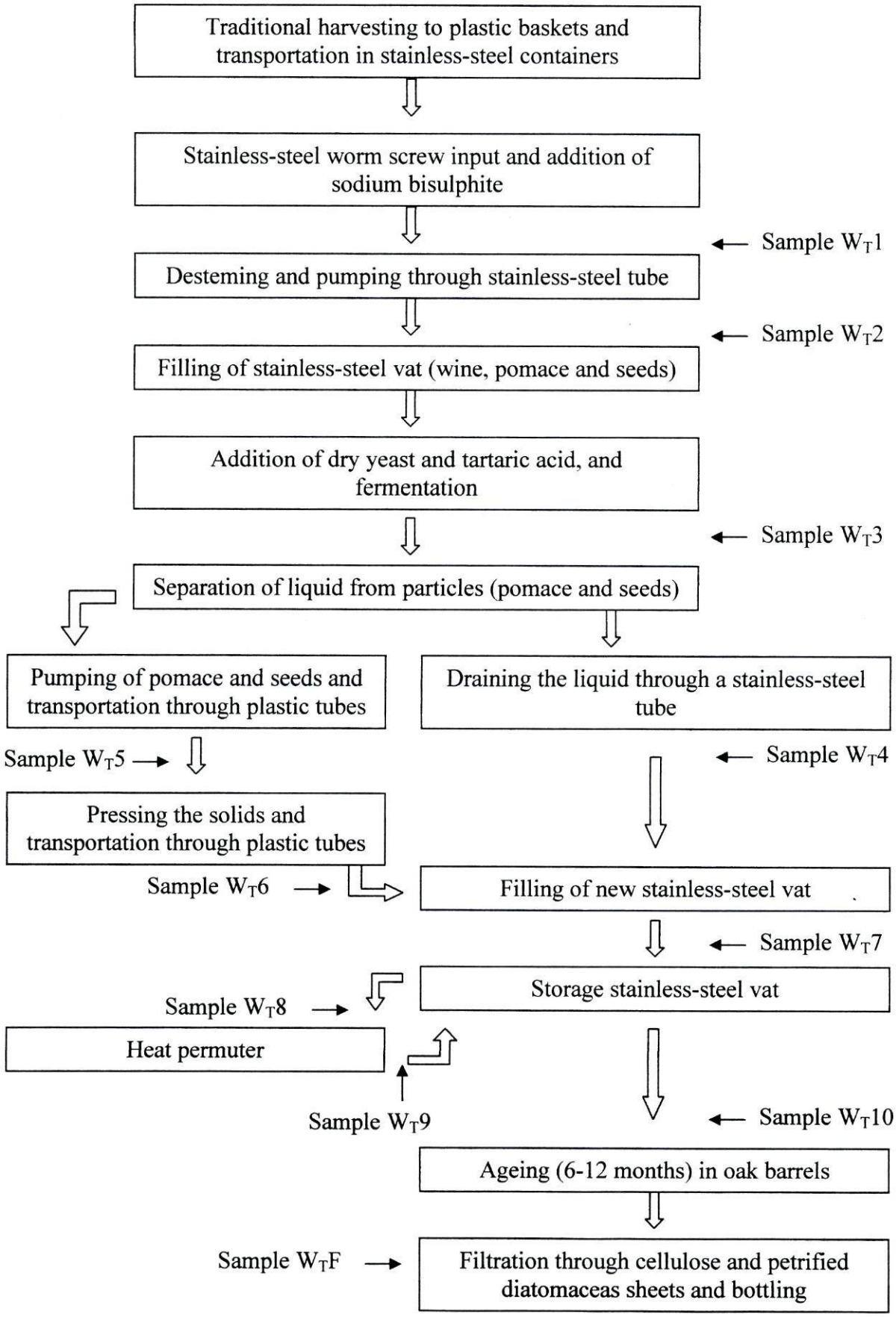

Fig. 3.2B. Schematics of the vinification process used to produced the red table wine from grapes of the young vineyard with indication of the points where samples were collected. (W_T1 to W_T10 and W_TF).

3.4. SAMPLES TREATMENTS

3.4.1. Wines

For *ICP-MS analysis*, all the wine samples were put into to silica tubes of *ca.* 45 ml of capacity and submitted to an UV–irradiation pre–treatment (optimisation described in Chapter 4) by using a home-made apparatus. The pre–treatment was carried in order to remove, by evaporation, the ethanol and to destroy the organic matter of the wines, whose matrix is very complex.

In some studies, for comparison purposes, a high–pressure microwave (HPMW)-digestion (optimisation described in Chapter 4) of the wine samples was also carried out.

In the studies reported in Chapters 6 to 8 a cation-exchange chromatographic separation of Sr and Rb in the wine samples (optimisation described in Chapter 6), was performed to eliminate the isobaric overlap of ^{87}Rb and ^{87}Sr in order to allow the determination of the IR $^{87}Sr/^{86}Sr$.

The three optimised procedures are described in the following sub-sections.

For *AAS analysis*, the wines were only dilute with de-ionized water, the dilution rate depending of the element to be determined.

In all studies, blank solutions treated as samples were always prepared and analysed together with the wine samples in order to subtract the background signal.

3.4.1.1. UV–irradiation pre–treatment of wine

UV-irradiation of the samples was carried out with a 1000 W mercury high pressure vapour lamp (with 3 cm of diameter and 6 cm of length) from Osram, in polytetrafluoroethylene (PTFE) -capped silica tubes (with 2 cm of diameter and 15 cm of length) positioned concentrically around an UV lamp (distance between the lamp and the centre of the tubes was 5 cm). This system was inserted in a close aluminium box, provided with appropriated ventilation, which protects workers against UV-radiation.

The pre-treatment consisted in UV-irradiation for 1.0 h of 10.0 ml mixture of 1:1 (v:v) wine and 30 % H_2O_2, and dilution with different solutions (depending of the purpose of analysis and/or measured element) to a specific volume, which was chosen on the base of the required dilution of the wine.

When the wine samples were hazed after UV-irradiation, they were filtered through a 0.45 μm pore size filter of mixed cellulose esters, from Schleicher & Schuell, using a syringe with an appropriate filter holder.

The treated samples were kept at 4 °C and analysed within 48 h.

3.4.1.2. HPMW-digestion of wine

Aliquots of 1.5 ml of wine were digested with 150 µl concentrated HNO_3 and 1.5 ml of 30 % H_2O_2 in closed PTFE vessels, using a HPMW system MLS-1200 Mega, from Millestone, coupled to an exhaust EM-30 of the same brand. The digestion programme and procedure was base on that developed by Alimoni *et al.* [3] for determination of Ni and Cr in blood by ICP-MS, and consisted in six steps: two steps of 2 min each at 250 and 0 W, respectively, followed by one step of 6 min at 250 W and two steps of 5 min each at 400 and 500 W, respectively, being the last step of 12 min at 600 W.

After cooling to room temperature, the vessels were open, being the obtained solutions quantitatively transferred to polyethylene tubes and diluted to 15.0 ml with de-ionised water. The final solutions were kept at 4 °C and analysed within 48 h.

3.4.1.3. Chromatographic separation of strontium and rubidium in wine

The cation-exchange chromatographic separation of Sr and Rb was carried out in a DOWEX AG50WX-8/400 mesh column (bed length of 11 cm, internal diameter of 1.2 cm, corresponding to a bed volume of 50 ml). The procedures described by Chassery *et al* [4] and Vanhaecke *et al* [5], for geological samples, were used as a starting point. Under optimised conditions, a solution of 2.5 M HCl was used both for regeneration of the column (100 ml) and for elution (flow rate approximately 1 ml min^{-1}). The efficient separation of Sr and Rb required a two-step chromatographic separation. In the first separation step, the sample was introduced into the column without acidification (pH about 4). Most of Rb, which has less affinity to the column than Sr, was removed from the column in the first 120 ml of elution. This volume was rejected and the next 40 ml, which contained most of the Sr, were collected, and introduced again in the column without a previous pH neutralisation (pH about zero). In this second separation step, Rb was eluted in the first 80 ml (and rejected) and the next 40 ml were collected. These retention volumes were markedly lower than those observed during the first step separation, due to the much higher acidity of the sample. The Sr-containing fraction was subsequently evaporated to near-dryness and converted into the nitrate-form by addition of 1.0 ml concentrated HNO_3 and evaporated again to near-dryness. Finally, the residue was taken up in 5 ml of de-ionised water and diluted appropriately, using 0.5 % HNO_3 solution, to a specific volume depending of the required dilution of the wine. The acid evaporations were carried out in a hood with an acid scrubber.

3.4.2. Grape juices and samples from the vinification processes

For each studied wine, grape juice was prepared, in September, in the laboratory, by smashing (with gloved hands) in plastic cups, the respective non-washed grapes. For this purpose, identical quantities of grapes collected in each of the three sampling sites of each vineyard were mixed, being the juice transferred to polyethylene tubes. With this procedure (manual pressing) only the grape pulp was pressed, the skins and seeds remained intact and were rejected.

Aliquots of not filtered grape juices and not filtered samples collected in the different steps of the vinification processes were pre-treated by UV-irradiation (see 3.4.1.1).

For the studies described in Chapter 8 the samples were submitted afterwards to the cation-exchange chromatographic separation of ^{87}Rb and ^{87}Sr (see 3.4.1.3).

Blank solutions treated as the samples were prepared regularly.

3.4.3. Atmospheric aerosols

The atmospheric aerosol samples were attacked with 0.25 ml of concentrate HNO_3 using a previously optimised procedure [6]. After digestion, the obtained solutions were quantitatively transferred to polyethylene tubes being diluted to 10.0 ml with de-ionised water. The final solutions were kept at 4 °C and analysed within 48 h. The solutions were used directly or diluted with de-ionised water. Bank filters were treated as a sample to be analysed in order to subtract the background signal

3.4.4. Vineyards soil, soil extracts, vine leaves and grapes

Vineyards soil, vine leaves and grapes, after being dried in an oven up to a constant weight, were digested by HPMW, carried out in the previously mentioned system. The digestion programme consisted in three steps of 5 min. each, at 250, 400 and 500 W, respectively. For soil samples this programme was run twice, whereas for the remaining samples it was run only once.

For *soil samples* digestion (about 0.25 $g_{dry\ soil}$) 5.0 ml concentrated HNO_3 were used, based on the literature [7,8]. The efficiency of the procedure was tested with a standard soil reference material, which was San Joaquin Soil, SRM 2709, from NIST. The optimised procedure (reported in Chapter 11) was a

strong acid digestion that dissolves almost all elements that could become "environmentally available" [8]. After digestion, the obtained solutions were quantitatively transferred to polyethylene tubes being diluted to 25.0 ml with de-ionised water. The final solutions were kept at room temperature until analysis, which took place within two days. Depending of the purpose of the analysis and/or of the measured element, different dilutions of this soil solution with different solutions were carried out before measurements.

For the *EDTA soil extracts*, the method established by the Measuring and Testing Program of the European Community [1] was used. The extractions were carried out with EDTA 0.05 mol l^{-1} solution at pH 7.0. This solution was prepared by dissolving a suitable amount of EDTA with de-ionised water and adjusting the pH to 7.0 with a few millilitres of concentrated NaHO solution. Before analysis, the extract solutions were used directly or after a five-fold dilution with a 0.2 % HNO_3 solution, depending of the element to be determined.

Vine leaves (about 0.45 $g_{dry\ leaves}$) were attacked with 1.0 ml concentrated HNO_3 and 3.0 ml of 30 % H_2O_2 and *grapes* (about 0.90 $g_{dry\ grapes}$) with 1.5 ml concentrated HNO_3 and 3.5 ml of 30 % H_2O_2, using as starting point literature data [7]. After HPMW digestion, the obtained solutions were quantitatively transferred to polyethylene tubes being diluted with de-ionised water to 10.0 ml and 15.0 ml for vine leaves and grapes, respectively. The final solutions were kept at room temperature until analysis, which took place within two days. Before analysis, depending of its purpose, the different sample solutions were diluted with different solutions at different rates or used directly.

For each type of sample, three independent replicates were prepared for analysis. Blank solutions were treated as samples to be regularly analysed in order to subtract the background signal.

3.5. ICP-MS DETERMINATIONS

The analytical measurements were carried out in a Perkin-Elmer SCIEX Elan 5000 ICP-MS (Perkin-Elmer, Norwalk, CT, USA) apparatus equipped with a crossflow nebulizer, double-pass Scott-type spray chamber made of Ryton and nickel cones. A peristaltic sample delivery pump was used.

The operating conditions for ICP-MS measurements were optimised daily, by using an aqueous solution containing 10 μg l^{-1} of Mg, Rh, Pb, Ce and Ba and monitoring the intensities of the isotopes ^{24}Mg, ^{103}Rh, ^{208}Pb, ^{140}Ce and ^{138}Ba, as well as the intensities at mass 156 (corresponding to $^{140}Ce^{16}O^{+}$)

and mass 69 (corresponding to $^{138}Ba^{2+}$). The chosen conditions were a compromise between the highest ^{103}Rh ion signal and the lowest percentage of doubly charge ions (obtained by the intensities ratio Ba^{2+}/Ba^{+}; always ≤ 2%) and of oxide ions (obtained by the intensities ratio CeO^{+}/Ce^{+}; always ≤ 3%).

When measuring only Pb IRs, since doubly charge ions were not a concern and oxide ions were considered unlikely in the *m/z* 204-208 regions, the operating conditions that maximised the ion intensity for mass 208 were selected.

Operating conditions used were as follows: RF power of 1200 W; sample uptake rate of 0.800 ml min^{-1}; plasma flow rate of 15.00 l min^{-1}, auxiliary flow rate of 0.800 l min^{-1}; nebulizer flow rate between: 0.750 and 0.810 (Chapter 4), 1.000 and 1.050 (Chapter 5), 0.990 and 1.015 (Chapter 6), 1.100 and 1.120 (Chapter 8), 1.020 and 1.035 (Chapter 9 and 10), 1.030 and 1.055 (Chapter 11 and 12) l min^{-1}; ions lens settings (in arbitrary units) were P = 54 (52 for Pb IRs measurements), S2 = 24, B = 70 and E1 = 15. The variations in the nebulizer flow from study to study most of the times coincided with instrumental maintenances, which always involved peaces replacement.

For signal stabilisation, a sample read delay between 75 s and 1.5 min was chosen. In-between the loading of solutions of both samples and standards, the sampling system was rinsed with 2 % HNO_3 solution for 1.5 min in order to prevent contaminations.

3.5.1. Lead isotope ratios measurements

The Pb IRs were measured in the peak hopping mode, at normal resolution, using 1500 sweeps per reading and a dwell time of 10 ms. The data acquisition procedure was optimised with a Pb isotopic standard solution and it is described in Chapter 4. In order to obtain the best precision only two Pb isotopes were measured each time and three replicates for each measurement were carried out. The results of each measurement were the mean of the replicates with the respective standard deviation, after blank subtraction.

Since ^{204}Pb is the least abundant stable isotope of Pb, to obtain similar precision for the four isotopes, this isotope signal was measured twice (by choosing the "time factor" 2 in the parameter file of the ICP-MS software) as long as the other Pb isotopes' signal.

Although wines usually have very low contents of Hg [9], the IR with the Pb isotope ^{204}Pb may be altered if this correction is not performed. Therefore, a mathematical correction of ^{204}Hg interference with ^{204}Pb was systematically carried out (using the software of the equipment) for all the determinations: the net signal at the mass-to-charge ratio (*m/z*) of 202 ($^{202}Hg^{+}$) was multiplied by

0.229 (the $^{204}Hg/^{202}Hg$ natural abundance ratio) and then subtracted from the signal at a *m/z* of 204 (contributions of $^{204}Hg^+$ and $^{204}Pb^+$). The same correction was applied for the ratio $^{204}Pb/^{206}Pb$ when measuring not only wine samples but also all the remaining types of samples.

3.5.2. Strontium isotope ratios measurements

The data acquisition procedure was optimised with the Sr isotopic standard solution, NIST SRM-987, and it is described in Chapter 6. In order to obtain the best precision (lowest RSD), 1500 sweeps per reading and a dwell time of 10 ms were used. Three replicates for each measurement were carried out. The Sr isotopes were measured using the peak hopping mode, at normal resolution.

Some residual Rb can still be present in the pre-treated (UV-irradiated and chromatographic separated) samples. Therefore, a mathematical correction for the interference of $^{87}Rb^+$ on $^{87}Sr^+$ was systematically carried out (using the software of the equipment): the net signal at *m/z* of 85 (^{85}Rb) was multiplied by 0.386 (the $^{87}Rb/^{85}Rb$ natural abundance ratio) and the result obtained subtracted from the signal at a *m/z* of 87 (contributions of $^{87}Rb^+$ and $^{87}Sr^+$).

3.5.3. Mass bias correction for isotope ratio determinations

In order to determine IRs accurately, correction of the obtained data for the phenomena of mass bias is necessary [10,11]. This correction can be either external or internal.

For Pb IRs the mass bias correction can be performed by:

(i) external correction, using Common Pb Isotopic standard NIST SRM-981. Every workday, the standard (Pb concentration similar to that pre-estimated for the samples) was analysed first and then every two or three samples (for IR $^{204}Pb/^{206}Pb$ it was measured only every four or six samples, because it was more time consuming and for this IR the repeatability was higher than for the other two), in order to correct for a possible shift with time;

(ii) internal correction, using the Tl IR (naturally occurring ratio $^{205}Tl/^{203}Tl = 2.3871$). Tl isotopes were measured simultaneous with two of the Pb isotopes, after addition of a chosen amount of Tl to the sample solution. The corrected IRs were obtained by using the power laws [12]:

$$(^{204}Pb/^{206}Pb)_c = (^{204}Pb/^{206}Pb)_m * [(^{205}Tl/^{203}Tl)_m/2.3871]$$

$$(^{207}Pb/^{206}Pb)_c = (^{207}Pb/^{206}Pb)_m * [2.3871/(^{205}Tl/^{203}Tl)_m]^{0.5}$$

$$(^{208}Pb/^{206}Pb)_c = (^{208}Pb/^{206}Pb)_m * [2.3871/(^{205}Tl/^{203}Tl)_m]$$

where *c* means corrected and *m* measured.

For Sr IR $^{87}Sr/^{86}Sr$ also two mass bias corrections can be performed:

(i) external correction, using the $SrCO_3$ (isotopic) standard NIST SRM-987. The concentration of the standard was similar to that pre-estimated for the samples. The standard was analysed before and every two to three samples, in order to correct for a possible shift with time;

(ii) internal correction, using the $^{88}Sr/^{86}Sr$ IR (constant value of 8.37861). The corrected IR was obtained by using the power law [12]:

$$(^{87}Sr/^{86}Sr)_c = (^{87}Sr/^{86}Sr)_m * [8.37861/(^{88}Sr/^{86}Sr)_m]^{0.5}$$

c and *m* having the same meaning as before.

For all the different types of samples analysed, both correction types were applied and, after results comparison, the suitable mass bias correction chosen.

Due to these mass bias corrections the standard deviation was calculated for each measurement according to propagation of errors (resulting from the determination of the IR both in the sample and in the isotopic standard (external correction) or from the determination of both IRs in the samples (internal correction)).

3.5.4. Multi-element determinations

ICP-MS offers different quantification procedures depending on the accuracy and precision required. Two of those procedures are quantitative and semi-quantitative mode of analysis. Both procedures were optimised for wine (Chapter 9) and soil samples (Chapter 11) and the advantages and limitation of each one are then discussed.

The data acquisition procedures for quantitative and semi-quantitative modes of analysis, both for wine and soil samples, are summarised in Table 3.1. In all cases, Rh was used for internal standardisation in order to eliminate the matrix discrepancies and to compensate for any drift occurring during the analysis.

When the *quantitative mode* was used for the analysis of both wine and soil samples, multi-element aqueous standards were prepared in five concentration ranges. The measurements were performed in five runs embracing different elements and/or concentration ranges (see Table 3.1).

External calibration was used and the appropriate interpolation was carried out for each element to determine its concentration in the corresponding calibration line.

Table 3.1. Data acquisition procedure and experimental conditions used for the ICP-MS measurements of both wine and soil samples, in the quantitative and semi-quantitative modes of analysis.

	Quantitative mode					Semi-quantitative mode
Dwell time (ms)	10	100	100	100	100	10
Sweeps/reading	200	10	10	10	10	50
Reading/replicates	1	1	1	1	1	1
Number of replicates	5	5	5	5	5	1
Time per run (s):						
wine samples	231	52	73	42	16	133
soil samples	231	11	83	26	21	
Isotopes monitored:						
wine samples	^{111}Cd, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{151}Eu, ^{160}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{173}Yb, ^{175}Lu	^{7}Li, ^{51}V, ^{59}Co, ^{69}Ga, ^{71}Ga, ^{206}Pb, ^{207}Pb, ^{208}Pb	^{7}Li, ^{51}V, ^{53}Cr, ^{60}Ni, ^{65}Cu, ^{75}As, ^{138}Ba, ^{206}Pb, ^{207}Pb, ^{208}Pb	^{27}Al, ^{55}Mn, ^{64}Zn, ^{65}Cu, ^{85}Rb, ^{88}Sr	^{57}Fe	
soil samples	^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{151}Eu, ^{160}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{173}Yb, ^{175}Lu	^{111}Cd	^{7}Li, ^{51}V, ^{59}Co, ^{53}Cr, ^{60}Ni, ^{64}Zn, ^{65}Cu, ^{69}Ga, ^{71}Ga, ^{75}As, ^{206}Pb, ^{207}Pb, ^{208}Pb	^{55}Mn, ^{85}Rb, ^{88}Sr, ^{138}Ba	^{27}Al, ^{57}Fe	range from 6 to 238
Concentration range (in μg l^{-1}) for external calibration:						
wine samples	0.1 - 1.0	1.0 - 10	10 – 100	20 – 200	60 – 500	
soil samples	0.5 - 100	0.1 – 2.0	10 - 200	25 - 200	50 - 250	Single standard

A careful selection of the isotopes of the elements to be determined was performed, except for those monoisotopic elements like Al, As, Co or V. The selected isotopes mentioned in Table 3.1 were those free from isobaric or important matrix-induced interferences, when that was possible. Otherwise, suitable elemental equations (Table 3.2) were applied (equations introduced and automatically applied by the

software) to correct matrix-induced interferences. The software of the instrument performs automatic corrections of isobaric interferences. For example, as Fe suffers from a severe interference from $^{40}Ca^{16}O$ for its most abundant isotope (^{56}Fe) the isotope ^{57}Fe was chosen. However, as this isotope also suffers interference from $^{40}Ca^{16}O^{1}H$, an elemental equation was applied for correction. In Table 3.2 the equations used for mathematical correction of matrix-induced interferences are presented. Since Pb isotope ratios may change from sample to sample, the three major isotopes, ^{206}Pb, ^{207}Pb and ^{208}Pb, were measured.

Table 3.2. Elemental equations (automatically applied) used for compensation of matrix-induced polyatomic interferences in ICP-MS measurements.

Analyte	Interference Species	Correction equations[a]
^{57}Fe	$^{40}Ca^{16}O^{1}H$	$Fe = I(^{57}Fe) - [0.00015 \times A(^{40}Ca)/A(^{43}Ca) \times I(^{43}Ca)]$
^{59}Co	$^{43}Ca^{16}O$	$Co = I(^{59}Co) - 0.001 \times I(^{43}Ca)$
^{60}Ni	$^{44}Ca^{16}O$	$Ni = I(^{60}Ni) - [0.001 \times A(^{44}Ca)/A(^{43}Ca) \times I(^{43}Ca)]$
^{75}As	$^{40}Ar^{35}Cl$	$As = I(^{75}As) - [A(^{35}Cl)/A(^{37}Cl) \times I(^{77}Mass)] + [2.54 \times I(^{82}Mass)]$
^{151}Eu	$^{135}Ba^{16}O$	$Eu = I(^{151}Eu) - 0.001 \times I(^{135}Ba)$

a: I = intensity; A = abundance.

When the *semi-quantitative mode of analysis* was applied, a full mass spectrum (m/z = 6-238, but omitting the mass ranges of 12-22 and 28-42 and the mass 80 to avoid overloading of the detector) was obtained by full mass range scanning. The software available to perform the analysis in the semi-quantitative mode (Perkin-Elmer TotalQuant II) [13] includes a reference response table of the different elements. Since sensitivity depends on experimental factors, it is essential to update that reference response in order to achieve accurate results. Such update was performed with one multi-element aqueous standard solution, with HNO_3 and Rh concentrations similar to those present in the samples. This solution contained forty-four elements, at major, minor and trace levels, including the REEs: 10 mg l^{-1} of Ca, 1 mg l^{-1} of As, B, Be, Fe, Se and Zn, 100 μg l^{-1} of Ag, Al, Ba, Bi, Cd, Co, Cr, Cu, Ga, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sr, Te, Tl, U and V, and 5 μg l^{-1} of REEs (from La to Lu).

The software also includes the most common isobaric and matrix-induced interferences, the correction being automatically applied. In addition, the elemental equations given in Table 3.2 were also automatically applied, as for the quantitative mode described above. Furthermore, specific isotopes free

or with less interference were chosen for several elements, as follows: ^{43}Ca, ^{49}Ti, ^{53}Cr, ^{65}Cu, ^{82}Se and ^{111}Cd. For Pb the three major isotopes, ^{206}Pb, ^{207}Pb and ^{208}Pb, were also measured.

3.6. AAS DETERMINATIONS

For AAS measurements, either with flame (F) atomisation (PU 9200X, Philips, Cambridge, UK) or with electrothermal atomisation (ET) provided with Zeeman background correction (4100 ZL, Perkin-Elmer, Norwalk, CT, USA, coupled to an AS-70 auto-sampler) apparatus were used.

For wine samples, methods previously optimised [14], which included external calibration with aqueous standards (with 0.2 % HNO_3) were used. For the remaining types of samples, the analytical procedures (see Table 3.3 and 3.4), including the optimised furnace programmes, were based on the ones described in the instruments manual [15,16] and former experiments. For quality control, regular analysis of Pb in blood (PbS) and atmospheric aerosols (MET) has been carried out in our Laboratory integrated in the following inter-calibration programmes: PICC (Programa Interlaboratorios de Control Calidad) – PbS (from "Gabinete de Seguridad e Higiene en le Trabajo", Zaragoza, Spain) and - MET (from "Centro Nacional de Condiciones de Trabajo", Barcelona, Spain).

Table 3.3. FAAS instrumental conditions used for the determination of Al, Co, Cr, Cu, Fe, Mn, Ni and Zn in the different types of samples.

	Al	Co	Cr	Cu	Fe	Mn	Ni	Zn
Wave length (nm)	396.2	240.7	357.9	324.8	248.3	279.5	232.0	213.9
Band pass (nm)	0.5	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Lamp current (mA)	7	7	5	4	7	4	7	4
Background Correction	no	no	no	no	no	no	yes	yes
Flame	N_2O/C_2H_2	Air/C_2H_2	N_2O/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2
Fuel flow ($l\ min^{-1}$)	5	0.8	4.5	0.8	0.8	0.8	0.8	0.8

The concentrations of Cu, Fe, Mn and Zn in wine samples were determined by FAAS, and those of Pb and Cd by ETAAS. For determinations of Pb and Cd a mixture of $Pd(NO_3)_2$ and $Mg(NO_3)_2$ was used as matrix modifier. The accuracy of the results was tested through an inter-comparison with those obtained, in parallel, in a few selected wines, by the laboratory of the "Instituto do Vinho do Porto" in Portugal. These analyses were used for Pb concentration determinations in Chapter 4 and for validation purposes in Chapter 9.

Table 3.4. ETAAS furnace programmes used for the determination of Cd, Co, Cr, Ni and Pb in soils (S), EDTA soil extracts, vine leaves (L), grapes (G) and atmospheric aerosols (A).

	Cd		Cr		Co	Ni		Pb	
	EDTA, L, G	S	EDTA	L, G	L, G	EDTA	L, G	EDTA	S, L, G, A
Step 1:									
Drying									
Temperature (°C)	130	110	80	110	110	130	110	130	110
Ramp time (s)	10	1	1	1	10	10	1	10	1
Hold time (s)	5	20	5	20	5	5	20	5	20
Step 2:									
Drying									
Temperature (°C)	200	130	300	130	130	200	130	200	130
Ramp time (s)	5	5	10	5	5	5	5	5	5
Hold time (s)	5	30	1	30	5	5	30	5	30
Step 3:									
Pyrolysis									
Temperature (°C)	600	500	1600	1550	1400	1100	1100	700	350
Ramp time (s)	1	10	10	10	1	1	10	1	10
Hold time (s)	5	20	5	20	5	5	20	5	20
Step 4:									
Atomisation									
Temperature (°C)	1400	1400	2400	2300	2400	2300	2300	1700	1500
Ramp time (s)	0	0	0	0	0	0	0	0	0
Hold time (s)	3	5	5	5	3	3	5	3	5
Step 5:									
Clean up									
Temperature (°C)	2400	2400	2600	2400	2400	2600	2400	2400	2400
Ramp time (s)	1	1	1	1	1	1	1	1	1
Hold time (s)	2	2	3	2	2	2	2	2	2

For quality control purposes (Chapter 11), several elements were also analysed by AAS in soil samples: FAAS was used for Al, Co, Cr, Cu, Fe Mn, Ni and Zn determinations, whereas ETAAS was used for Pb and Cd. External calibrations for all elements were obtained with aqueous standards containing a HNO_3 concentration similar to that present in the sample solutions.

In the EDTA vineyard soil extracts (Chapter 12), FAAS was used for Cu and Zn, while ETAAS was used for Cd, Cr, Ni and Pb determinations. For all elements, aqueous standards with 2 % HNO_3 (FAAS) or with both 0.2 % HNO_3 and 0.01 mol l^{-1} EDTA (ETAAS) were used for external calibrations. For Cd and Pb determinations $(NH_4)_2HPO_4$ was included as matrix modifier.

In vine leaves and grapes (Chapter 5 and 12), Al, Cu, Fe, Mn, and Zn were determined by FAAS, using procedures similar to those used for EDTA soil extracts, and Cd, Co, Cr, Ni and Pb were measured by ETAAS using the standard addition method. For Cd, $(NH_4)_2HPO_4$ was also used as matrix modifier.

For Pb total content determination in aerosol samples (Chapter 5) ETAAS was used. External calibration with aqueous standards was used and an appropriate interpolation carried out.

3.7. STATISTICAL CALCULATIONS

Through out the studies reported in the different Chapters of this Dissertation, several statistical tests were carried out [17].

For comparison of two mean values, paired t-tests at 95 % level were performed, while for comparison of several mean values, the least significance difference (LSD) test was used. For the LSD test, values of all samples to be compare, were arranged in ascending order and the difference between adjacent values were compared with the calculated LSD value (this value was calculated considering the standard deviation associated to the values of each samples). In Chapter 5 the one-tailed F-test for one-way ANOVA was also used to compare several mean values. This statistical method tests whether the difference between the samples means is too great to be explain by the random error.

Whenever comparison of different procedures was necessary, linear regression, through a linear least-squares adjustment of the global results was performed and the regression line compared with the ideal one.

For the statistical analyse calculations in Chapter 12, one-way ANOVA for comparison of several means and Pearson's correlation tests for correlation evaluation were carried out using the software package SPSS 10.0 for Windows.

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Part III

Results and Discussion

III.A - Studies with Isotope Ratios

III.A.1 - Lead Contamination in Wines

III.A.2 - Suitability of $^{87}Sr/^{86}Sr$ for Wine Provenance Determination

III.B - Multi-Element Composition and Its Suitability for the Characterisation of the Wine Origin Region

Chapter 4

Optimisation of Methods for Determination of Lead Isotope Ratios in Port Wines

4.1. INTRODUCTION AND AIMS

Fortified wines, in which Port wine is included, are much richer than table wines in alcohol (generally between 19 % and 22 % volume), particles in suspension and polymeric organic compounds, particularly sugars. All of these substances strongly interfere with ICP-MS measurements. Alcohol causes suppressing of the signal (which drastically reduces the sensitivity) and signal instability [1]. Although a signal depression not necessarily prevent the isotopic ratio determinations, it may affect the measurement precision if the ion intensities of the different isotopes are low. The polymeric organic matter causes blockage of the injector tube and cones of the ICP, preventing the flux of the ions of the analyte to the MS detector. Such blockage is the result of an incomplete pyrolysis of the sugars in the plasma and formation of residual carbon deposits (carbon build-up). Therefore, a simple dilution of the wine does not solve the problem of interference of the Port wine matrix in ICP-MS measurements, because the maximum dilution allowed on account of sensitivity (ten times) is not sufficient to reduce neither the alcohol nor the sugar to acceptable levels. Such problems could probably be reduced by the addition of a small stream of oxygen to the plasma region, but this procedure requires a specific accessory not available in most of the ICP-MS apparatus. Therefore, a more drastic sample pre-treatment of Port wine is required for ICP-MS measurements but not available at the moment this study was carried out.

In this study, it was looked for a pre-treatment that requires smaller amount of chemical oxidising, to prevent contamination, and provides analytical signal intensity as high as possible in the wine samples.

Goossens *et al.* [1] used classical acid digestion, in open vessels, for determination of total lead in a liqueur red wine by ICP-MS. As such methods are generally very time consuming and require the addition of high concentrations of reagents to the sample, procedures by UV-irradiation that required less chemical addition were implemented. An UV-irradiation pre-treatment has been applied recently by Sanllorente *et al.* [2] for determination of nickel in commercial table wines by differential-pulse adsorptive stripping voltammetry.

For comparison purpose, a HPMW-digestion pre-treatment was also optimised. HPMW-digestion procedures have been used for determination of total metals concentrations in different types of samples using different analytical techniques [3], including determination by ICP-MS of several trace metals in

biological materials [4], chromium and nickel in human blood [5], rare earth elements in tea [6] and arsenic, cadmium and lead in seafood products [7].

The aim of the study reported in this Chapter (which was already published [8,9]) was the development and optimisation of sample pre-treatment procedures and of an analysis method suitable for the determination of lead isotope ratios in Port wine using ICP-MS, with precision enough to warrant the investigation of the provenance of the metal. As complementary information, the total lead concentration in the samples was also determined by ETAAS.

ICP-MS measurements are subject to several mass biases, which include instrumental and sample induced bias. In order to determine isotope ratios accurately, correction of the observed data for the phenomena of mass bias is necessary. Mass bias occurs when an instrument does not have the same sensitivity for different masses due to differences in ion transmission [10]. This bias can be measure and hence correct [11]. For this purpose, both external correction (with an external standard of known isotopic composition) and internal correction (using a fixed constant isotope ratio within the sample) can be used. External mass bias correction is very common in ICP-MS measurements including in determinations of lead isotope ratios in wines [12-14]. As both external and internal mass bias corrections have advantages and disadvantages [11], both were tested in this study in order to obtain the highest accuracy and precision for lead isotope ratios in Port wine samples.

In this Chapter, the selected UV-irradiation pre-treatment and implemented analytical method is described in detail and the quality of the results it provides is discussed. A comparison with the two other implemented pre-treatments requiring higher amounts of chemicals is also carried out.

4.2. EXPERIMENTAL

Twenty–four wine samples of three different types of genuine Port wine were used: three wines Late Bottled Vintage (LBV) harvest of 1988 (forward named LBV 88, LBV 88' and LBV 88"); eighteen Dated wines (DP) from harvests of 1935 (forward named DP 35 and DP 35'), 1940 (DP 40), 1947 (DP 47), 1952 (DP 52 and DP 52'), 1957 (DP 57), 1962 (DP 62), 1963 (DP 63), 1968 (DP 68), 1969 (DP 69), 1974 (DP 74 and DP 74'), 1977 (DP 77), 1978 (DP 78), 1979 (DP 79 and DP 79') and 1984 (DP 84); and three wines with an indication of age of 10 years (forward named IA 10, IA 10' and IA 10"). LBV are wines from a single year of harvest (year of excellent quality) that are bottled between the

fourth and sixth year after they were made; DP are also wines from a single year that aged in wooden barrels for several years and can only be sold after they have attained 7 years of age; IA Port wines are similar in style to DP but, unlike the latter, are blended from wines of different years. All the wines studied were bottled in 1992, except the wine DP 78 that was bottled in 1993.

To verify their suitability, the UV-irradiation pre-treatment procedures implemented in the study reported in this Chapter were also applied to Madeira wine (from the island of Madeira) and Favaios wine (from Douro Region), two other Portuguese fortified wines.

After UV-irradiation, all treated samples were diluted with 0.5 % HNO_3 solution. For comparison purposes, dilution with de-ionised water or 1 % HNO_3 solution were also carried out.

For ETAAS measurements, the wines were only diluted four times with de-ionised water.

Other experimental details are given in the Experimental Section - Chapter 3.

4.3. OPTIMISATION OF A PRE-TREATMENT OF PORT WINE FOR ICP-MS DETERMINATIONS

4.3.1. Development of a UV-irradiation pre-treatment

The optimisation of the pre-treatment of the Port wine included the study of the influence on the analytical results of the following parameters: volume of sample (varied between 10 and 40 ml), time of UV-irradiation (between 0.5 and 2.0 h) and addition of different volumes of 30 % H_2O_2 (wine:H_2O_2 proportions between 150:1 and 1:1 v/v).

In a first stage, volumes of 10 ml of mixtures of Port wine and 30 % H_2O_2, in v:v proportions of 150:1, 20:1, 5:1, 2:1 and 1:1, were UV-irradiated, in parallel, for 0.5 h. The obtained solutions were diluted with a 0.5 % HNO_3 solution, in order to obtain a 10 times dilution of the wine, and the ion intensities of the different Pb isotopes were measured.

That set of experiments was repeated for 1.0, 1.5 and 2.0 h of UV-irradiation. The influence of UV-irradiation time and wine: H_2O_2 proportions on the ion intensities of ^{207}Pb and ^{206}Pb isotopes are illustrated in Table 4.1. For the other Pb isotopes, similar relative intensities and precisions were obtained.

Table 4.1. Influence of UV-irradiation time and wine:H_2O_2 proportions on the ion intensities[a] of ^{206}Pb and ^{207}Pb isotopes and on the respective precision, observed for a Port wine sample.

Wine:H_2O_2	Time[b] / h	Haze after pre-treatment	^{206}Pb ion intensity x 10^{-3}/ion s^{-1}	^{207}Pb ion intensity x 10^{-3}/ion s^{-1}
150:1	0.5	no	8.4 (0.4)	7.34 (0.04)
	1.0	yes[c]	10.6 (0.1)	9.25 (0.03)
	1.5	yes[c]	13.08 (0.04)	11.39 (0.07)
20:1	0.5	no	11.1 (0.2)	9.74 (0.02)
	1.0	no	11.5 (0.1)	10.04 (0.02)
	1.5	yes[c]	13.13 (0.04)	11.45 (0.02)
5:1	0.5	no	12.39 (0.06)	10.85 (0.07)
	1.0	no	12.49 (0.02)	10.97 (0.04)
	1.5	no	11.94 (0.03)	10.39 (0.04)
2:1	0.5	no	11.2 (0.2)	9.79 (0.02)
	1.0	no	11.4 (0.1)	9.95 (0.04)
	1.5	no	11.5 (0.1)	10.07 (0.02)
1:1	0.5	no	13.14 (0.05)	11.54 (0.07)
	1.0	no	13.26 (0.02)	11.57 (0.01)
	1.5	no	13.05 (0.03)	11.41 (0.07)

a: Mean and standard deviation in brackets (n = 3);
b: For 2.0 h of UV-irradiation the results were statically identical to those of 1.5 h;
c: The solutions were filtered before the analysis.

The different results were statistically compared through a test for comparison of several means, the least significance difference (LSD) test [15], as is illustrated in Fig. 4.1 for ^{206}Pb (for the other isotopes the results were comparable). For 0.5 h of UV-irradiation the highest signal was obtained for the 1:1 mixture, indicating that the highest H_2O_2 concentration originated the most efficient elimination of matrix interference. For 1.0 h of irradiation no improvement of sensitivity was observed for 1:1 mixture, but the signal presented slightly higher stability. In few other solutions an improvement of the signals was observed but in all cases they were statistically lower than those obtained for 1:1 mixture. For 1.5 h of irradiation, statistically identical signals were obtained for 1:1, 20:1 and 150:1. For 5:1 and 2:1 mixtures, lower signals were observed. Higher UV exposure time (2.0 h) did not improved neither the magnitude nor the precision of the signals, for any of the wine:H_2O_2 mixtures.

Higher signals for 150:1 and 20:1 than for 5:1 and 2:1 mixtures were probable a result of evaporation of alcohol (an important interference in ICP-MS) during UV-irradiation which occurred only for the less diluted wine. In fact, after irradiation of 150:1 and 20:1 mixtures, their volumes were,

respectively, 80 % and 85 % of the initial ones. The treated solutions where evaporation of the alcohol occurred became hazed and were filtered before analysis.

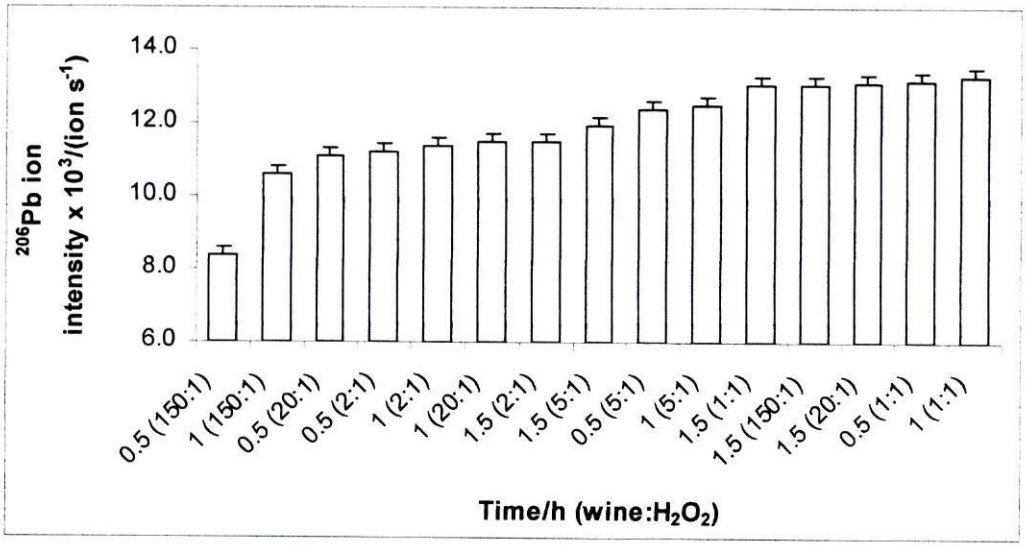

Fig. 4.1. Comparison, through the least significant difference (LSD) test [15], of the mean values of ^{206}Pb ion intensity obtained for the different UV-irradiation times and wine:H_2O_2 proportions. The straight lines indicate the respective LSD value.

In terms of sensitivity, the 150:1 and 20:1 mixtures UV-irradiated for 1.5 h and the 1:1 mixture UV-irradiated for 0.5 h or 1.0 h provided statistically identical results (see Table 4.1). The 150:1 mixture has the advantage of enabling the treatment of a larger volume of wine per batch: up to 20 ml (higher volumes can not be used because solution came out of the silica tube during the UV-irradiation due to the heating) provides about 160 ml of solution for analysis after a 10 times dilution of the treated solution (since about 4 ml evaporate during the UV-irradiation). For 1:1 wine:H_2O_2 mixture more than 10 ml mixture (a quart of the vessel volume) cannot be used because tumultuous release of gas occurs during UV-irradiation. Therefore, after a 10 times dilution of the wine, only 50 ml of final solution is obtain. Larger solution volumes are useful when different analysis by ICP-MS have to be performed. The 150:1 mixture has the additional advantage of requiring much less volume of H_2O_2. One the other hand, the 1:1 mixture has the advantage of being less time consuming, since it requires less time of UV-irradiation, and no filtration is necessary. The 20:1 mixture offers no advantages comparing to the other two mixtures. For comparison purposes, UV-irradiation pre-treatments of 150:1 and 1:1 mixtures were applied, in parallel, to two Port wine samples. The results obtained for the different IRs were statistically identical (paired t-test [15]) (see Table 4.2).

Table 4.2. Comparison of the Pb IRs obtained for two Port wines and two other fortified wines, with two different UV-irradiation pre-treatments: 150:1 and 1:1 (see the text).

	$^{207}Pb/^{206}Pb$[a]		$^{208}Pb/^{206}Pb$[a]		$^{204}Pb/^{206}Pb$[a]	
	150:1	1:1	150:1	1:1	150:1	1:1
DP 52	0.857 (2) 0.858 (2) 0.858 (3) RSD[b]: 0.106 %	0.859 (3) 0.857 (3) 0.858 (3) RSD[b]: 0.129 %	2.101 (4) 2.102 (4) 2.100 (3) RSD[b]: 0.063 %	2.103 (2) 2.100 (7) 2.098 (6) RSD[b]: 0.127 %	0.0553 (3) 0.0552 (1) 0.0551 (3) RSD[b]: 0.105 %	0.0550 (3) 0.0552 (2) 0.0551 (1) RSD[b]: 0.181 %
DP 69	0.866 (4) 0.866 (3) 0.866 (3) RSD[b]: 0.027 %	0.865 (6) 0.864 (7) 0.866 (6) RSD[b]: 0.072 %	2.100 (5) 2.099 (8) 2.094 (8) RSD[b]: 0.165 %	2.109 (3) 2.099 (6) 2.103 (4) RSD[b]: 0.248 %	0.0555 (2) 0.0560 (4) 0.0562 (2) RSD[b]: 0. 645 %	0.0554 (3) 0.0555 (3) 0.0554 (5) RSD[b]: 0.104 %
Madeira	0.843 (6) 0.844 (9) 0.844 (4) RSD[b]: 0.054 %	0.841 (4) 0.842 (3) 0.842 (3) RSD[b]: 0.076 %	2.064 (12) 2.064 (16) 2.068 (17) RSD[b]: 0.100 %	2.064 (17) 2.058 (12) 2.063 (8) RSD[b]: 0.174 %	not determined	not determined
Favaios	0.859 (7) 0.863 (9) 0.862 (4) RSD[b]: 0.267 %	0.859 (3) 0.858 (3) 0.864 (3) RSD[b]: 0.379 %	2.093 (5) 2.091 (15) 2.099 (6) RSD[b]: 0.197 %	2.100 (8) 2.096 (7) 2.097 (6) RSD[b]: 0.083 %	not determined	not determined

a: Mean and standard deviation (value affecting last digit) of each measurement;
b: Relative standard deviation of the mean of the three replicates.

The same pre-treatments were also applied to two other types of fortified wines (Madeira wine and Favaios wine) and the two Pb IRs $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ determined. The obtained results (included in Table 4.2) suggested that both UV-irradiation procedures were also suitable for ICP-MS analysis of these wines.

Since the 150:1 mixture requires lower addition of chemicals and can provide larger sample volume for analysis it was the selected for subsequent studies presented in this Chapter.

4.3.2. Optimisation of a HPMW-digestion pre-treatment

A more classical pre-treatment procedure was also optimised and applied to Port wine. A sample volume of 1.5 ml was chosen, because it was a compromise between the maximum volume suitable for digestion in the closed vessels of the microwave system and the volume required for the ICP-MS determinations (after a 10 times dilution of the wine). The volumes of HNO_3 and H_2O_2 used in the attack varied between 150 µl and 1.5 ml and 150 µl and 3 ml, respectively. Volumes of 150 µl HNO_3

and 1.5 ml H_2O_2 were selected because they provided clear solutions, and higher concentrations of these two reagents did not improved the analytical signals in ICP-MS.

4.3.3. Comparison of HPMW-digestion and UV-irradiation pre-treatments

The HPMW-digestion and the UV-irradiation of 150:1 Port wine:H_2O_2 mixture pre-treatments were applied to five wines (DP 35, DP 74, LBV 88a, LBV 88b and IA 10), in parallel, for comparison. For each sample, two replicates were independently pre-treated by each procedure and the mean of the ICP-MS signals (three per replicate) and the respective relative standard deviation (RSD) (n = 6, calculated according to the propagation of errors) were obtained. The UV-irradiation procedure provided results with RSD between 0.045 and 0.330 %, more precise than those obtained by HPMW digestion whose RSDs were between 0.100 and 1.820 %.

In Fig. 4.2, the results obtained for the various Pb IRs in samples pre-treated by both procedures are compared by linear regression.

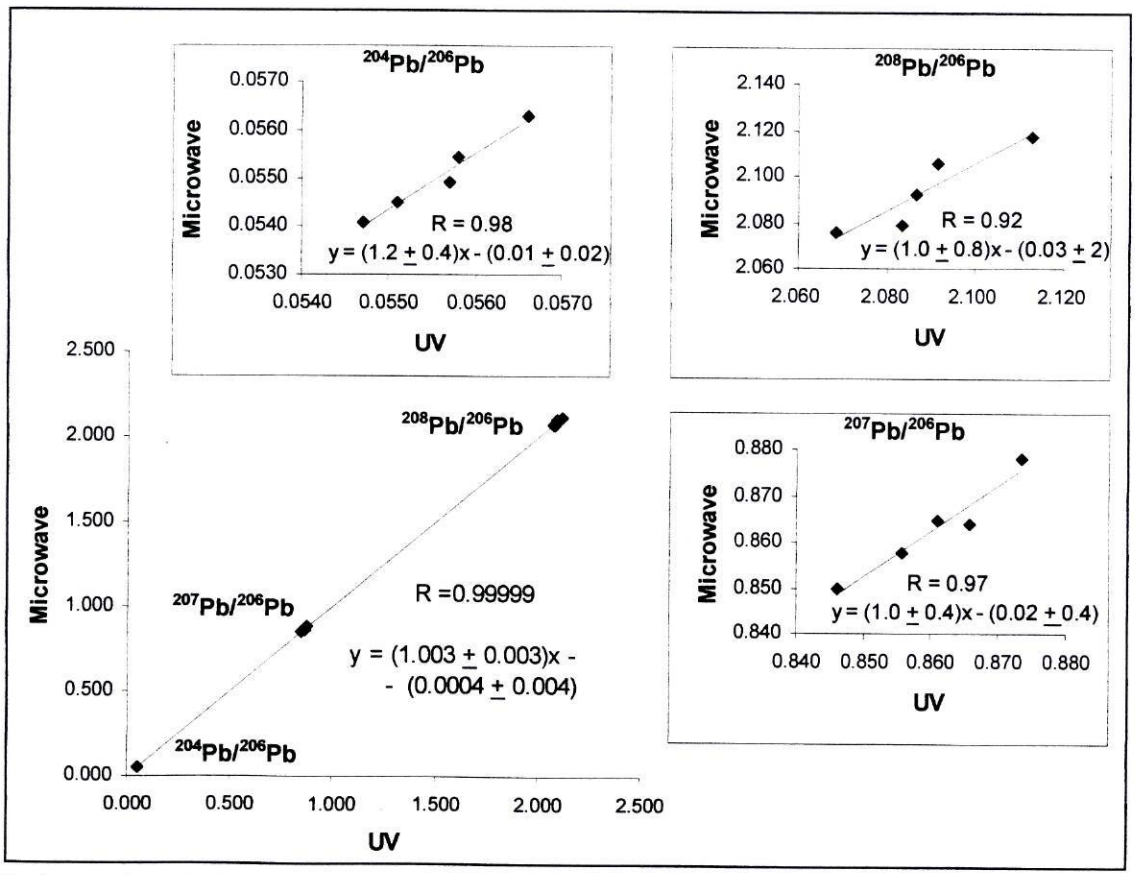

Fig. 4.2. Comparison, by linear regression, of the Pb IRs obtained for Port wine samples pre-treated by 150:1 UV-irradiation and by HPMW-digestion (the ± values are the 95 % confidence limits).

A linear least-squares adjustment of the global results for the different Pb IRs yield the equation: [HPMW] = (1.003 ± 0.003) [UV] - (0.0004 ± 0.004). When each IR was treated separately, the following equations were obtained: [HPMW] = (1.0 ± 0.4) [UV] - (0.02 ± 0.4) for $^{207}Pb/^{206}Pb$; [HPMW] = (1.0 ± 0.8) [UV] - (0.03 ± 2) for $^{208}Pb/^{206}Pb$; and [HPMW] = (1.2 ± 0.4) [UV] - (0.009 ± 0.02) for $^{204}Pb/^{206}Pb$. This statistical analysis showed no evidence of either relative or fixed bias in the measured range. Therefore, both the proposed methods were considered acceptable for the determination of Pb IRs by ICP-MS, being the UV-irradiation pre-treatment chosen.

4.3.4. Analysis of blanks

The influence of the pre-treatment in the blank signals (ion intensities of the various Pb isotopes) is illustrated in Table 4.3. For comparison purposes, besides dilution of the pre-treated samples with 0.5 % solution, dilution with de-ionised water or with 1 % HNO_3 solution were also carried out (see below) and the signals referring to those blanks (not submitted to the pre-treatment) and to a pre-treated wine sample are also shown. Although the ion intensities of Pb isotopes in the blank pre-treated by 150:1 UV-irradiation were significantly higher than those on the blank not UV-irradiated, they were significantly lower than those obtained for the other pre-treatments blanks, which included higher amounts of chemicals. A comparison of the signals for de-ionised water and the HNO_3 solutions indicates that even the suprapure HNO_3 contributes significantly to the blank signal. Nevertheless, Table 4.3 also shows that, in all cases, the blank signals were two to three orders of magnitude lower than the signals obtained for the Port wine samples.

Table 4.3. Comparison of the ion intensities[a] obtained for the different Pb isotopes in the blanks of the different pre-treatment procedures. Results for a Port wine solution were included for comparison.

	^{204}Pb ion intensity/ion s^{-1}	^{206}Pb ion intensity /ion s^{-1}	^{207}Pb ion intensity /ion s^{-1}	^{208}Pb ion intensity /ion s^{-1}
De-ionised water	3.1 (0.2)	7 (1)	8 (2)	10 (3)
0.5 % HNO_3	4.4 (0.7)	46 (1)	40 (1)	82 (3)
1 % HNO_3	6.2 (0.5)	56 (3)	46 (2)	111 (5)
UV-irradiation (150:1)	17.7 (0.5)	143 (2)	134 (2)	259 (4)
UV-irradiation (1:1)	24.1 (0.3)	385 (3)	339 (4)	818 (9)
HPMW-digestion	30.5 (0.5)	537 (1)	465 (4)	1127 (3)
DP 84 wine	7.67 (0.02) x 10^2	141.6 (0.2) x 10^2	123.8 (0.4) x 10^2	301.5 (0.5) x 10^2

a: Mean and standard deviation in brackets (n = 3). Different independent replicates provided statistically identical results.

4.4. PRECISION OF THE LEAD ISOTOPE RATIOS MEASUREMENTS

The data acquisition procedure was optimised with the Pb isotopic standard NIST SRM-981, the Pb IRs of this standard ($^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$, certified values: 0.91464 ± 0.00033, 2.1681 ± 0.0008 and 0.059042 ± 0.000037, respectively) being determined every working day.

For the optimisation of the data acquisition procedure, the influence of the instrument parameters: number of replicates (varied between 3 and 10, with one reading per replicate), dwell time (varied between 5 and 10 ms) and number of sweeps per reading (varied between 200 and 1500), on the three Pb IRs $^{204}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ (determine individually or together) and on the respective RSD, obtained for a Pb isotopic standard solution (50 μg l^{-1} Pb concentration) was studied. The optimised procedure was selected in order to obtain the best precision (lowest RSD), and it is described in the Experimental section.

Considering a period of about two months, the means and respective RSDs of all the determinations (n = 11-13) were calculated (see also Fig. 4.3): $^{207}Pb/^{206}Pb$ = 0.920, RSD: 0.08 %; $^{208}Pb/^{206}Pb$ = 2.208, RSD: 0.17 %; $^{204}Pb/^{206}Pb$ = 0.0582, RSD: 0.38 %. The precisions for long-term measurements were similar to those obtained for short-term measurements (except for $^{204}Pb/^{206}Pb$ ratio, to which short-term precision was lower) and even to those obtained for single determinations of the respective Pb IR. The long-term precision obtained for the $^{207}Pb/^{206}Pb$ ratio was better than that obtain for the same Pb isotopic standard by Dean *et al.* [12] and Campbell *et al.* [16], by using VG PlasmaQuad ICP-MS apparatus, and similar to that obtain by Halicz *et al.* [17] using a Perkin-Elmer SCIEX Elan 6000 ICP-MS. For the $^{204}Pb/^{206}Pb$ ratio worse precision was obtained, comparatively to that obtained for the other two Pb IRs, probably due to poor counting statistic on the ^{204}Pb isotope relatively to the other Pb isotopes, since this is the least abundant isotope.

Stroh *et al.* [14] reported that the addition of acid to table wines increases the stability of the signals for Pb IRs determined by ICP-MS. In order to test the influence of acid in the signals, measurements were carried out, in parallel, in samples pre-treated by the chosen procedure either not acidified or acidified with 0.5 % or 1 % HNO_3. Three different Port wine samples and the Pb isotopic standard solution (50 μg l^{-1} Pb concentration) were analysed. It was observed that the acidification improved markedly the intensity of the ion intensities but not the precision of the Pb IRs. For instance, the ion intensity of the isotope ^{206}Pb in the isotopic standard increased three times between not acidified

and acidified with 0.5 %. Nevertheless, in all the wines that increase was of only *ca.* 15 %. Similar results were observed for the remaining Pb isotopes. No significant differences were observed, through paired t-test [15], among the Pb ion intensities or the Pb IRs precisions obtained in solutions with 0.5 % and 1 % HNO_3. From these results it was decided to acidify to 0.5 % HNO_3 all the subsequent solutions (samples, blanks and standards) before the ICP-MS measurements.

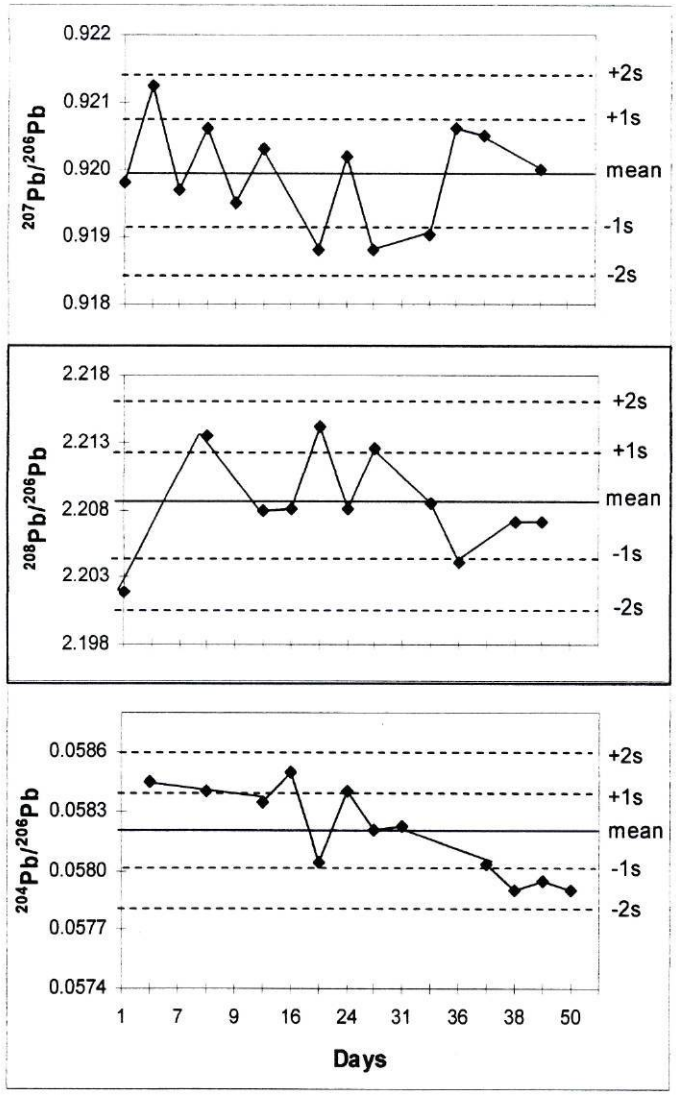

Fig. 4.3. Variations of the IRs of the Pb isotopic standard NIST SRM-981, observed over a period of 50 days (s = standard deviation of the mean). Certified values for $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$ are 0.91464 ± 0.00033, 2.1681 ± 0.0008 and 0.059042 ± 0.000037, respectively.

In order to test the repeatability of the results obtained with the chosen pre-treatment procedure, three replicates of each wine sample were independently pre-treated and analysed. The respective mean and variance were calculated and compared with the mean and variance obtained for a single pre-

treated sample that was analysed three times. All the experimental work was carried out in a single work day. Since the measurement of each Pb IR is affected by an error resulting from its own determination and since three analyses were performed for each wine, the variance associated to the respective mean was calculated according to propagation of errors. Results are presented in Table 4.4.

Table 4.4. Precision of the Pb IRs observed in different Port wine samples.

		Analysis of three aliquots of a single UV-irradiated solution	Analysis of three independently UV-irradiated solutions	σ^2 (UV-irradiation)[a]
			$^{207}Pb/^{206}Pb$	
DP 52	Mean	0.858	0.858	
	σ^2	$1.2x10^{-5}$	$1.8x10^{-5}$	$5.6x10^{-6}$
DP 69	Mean	0.866	0.866	
	σ^2	$2.4x10^{-5}$	$3.4x10^{-5}$	$1.0x10^{-5}$
DP 74	Mean	0.858	0.860	
	σ^2	$3.9x10^{-5}$	$3.6x10^{-5}$	$-2.8x10^{-6}$
DP 84	Mean	0.870	0.871	
	σ^2	$3.7x10^{-5}$	$4.3x10^{-5}$	$6.3x10^{-6}$
LBV 88a	Mean	0.862	0.862	
	σ^2	$1.2x10^{-5}$	$2.4x10^{-5}$	$1.2x10^{-5}$
			$^{208}Pb/^{206}Pb$	
DP 52	Mean	2.100	2.101	
	σ^2	$7.8x10^{-5}$	$9.7x10^{-5}$	$1.9x10^{-5}$
DP 69	Mean	2.099	2.097	
	σ^2	$1.9x10^{-4}$	$1.6x10^{-4}$	$-3.6x10^{-5}$
DP 74	Mean	2.088	2.086	
	σ^2	$1.8x10^{-4}$	$1.7x10^{-4}$	$-1.2x10^{-5}$
DP 84	Mean	2.092	2.093	
	σ^2	$6.4x10^{-5}$	$1.2x10^{-4}$	$5.7x10^{-5}$
LBV 88a	Mean	2.084	2.080	
	σ^2	$9.4x10^{-5}$	$1.3x10^{-4}$	$3.8x10^{-5}$
			$^{204}Pb/^{206}Pb$[b]	
DP 52	Mean	0.0552	0.0552	
	σ^2	$1.1x10^{-7}$	$2.7x10^{-7}$	$1.3x10^{-7}$
DP 74	Mean	0.0557	0.0556	
	σ^2	$2.2x10^{-7}$	$2.5x10^{-7}$	$3.2x10^{-8}$
DP 84	Mean	0.0557	0.0557	
	σ^2	$2.4x10^{-8}$	$2.8x10^{-7}$	$2.6x10^{-7}$

a: Difference between the total variance (analysis of three independently UV-irradiated solutions) and the determine variance (analysis of three aliquots of a single UV-irradiated solution);
b: Not measured in the wines DP 69 and LBV 88a, due to insufficient sample volume.

The obtained results shows that the variance obtained for the three independently pre-treated samples were of the same order of magnitude as that obtained for a single pre-treated one analysed three times. For $^{204}Pb/^{206}Pb$ data referring to the wine samples DP 69 and LBV 88a are not presented due to insufficient sample volume but similar results are expected. These results indicated that the implemented pre-treatment did not contribute markedly for the variance of the overall method (pre-treatment plus determination), being the ICP-MS determinations the decisive factor for the precision of the obtained Pb IRs.

4.5. MASS BIAS CORRECTION FOR LEAD ISOTOPE RATIOS MEASUREMENTS

Two different procedures for mass bias correction: (i) external correction, with a Pb isotopic standard solution and (ii) internal correction, with Tl as internal standard [11] were applied and the results were compared. External correction was used before in the determination of Pb IRs in table wines [12-14]. As far as we know, by the time this study was performed, there was no report of correction of Pb IRs in wines with an internal standard, but this method has been applied previously to aqueous ethanolic solutions [18].

Tl was chosen for internal standard because of its constant natural IR and its proximity in mass to the Pb isotopes. Besides, the natural Tl content in wine can be considered negligible (see, for example, the study reported in Chapter 10). The effectiveness of this internal standard was firstly investigated with a Pb isotopic standard solution (Pb concentration 50 $\mu g\ l^{-1}$). For this purpose, 25 $\mu g\ l^{-1}$ of Tl was added to the solution and three sequential measures of each Pb IR were performed. The Pb ratios were corrected with the Tl IR using the power laws mentioned earlier (see Chapter 3). As shown in Table 4.5, this correction originated an increase in the accuracy of the Pb IRs although there was a decrease in the precision of the results. A significant increase of the accuracy of the Pb IRs measured in Pb isotopic standard solutions by using $^{205}Tl/^{203}Tl$ was also observed before [10,11,19].

Table 4.5. Uncorrected and mass bias corrected results for the Pb IRs in a NIST SRM-981 isotopic standard solution (50 $\mu g\ l^{-1}$ Pb concentration).

	Uncorrected	Corrected	
Measurement	$^{207}Pb/^{206}Pb^{a,b}$		$^{205}Tl/^{203}Tl^{b,c}$
1	0.919 (3)	0.914 (3)	2.410 (4)
2	0.918 (2)	0.914 (2)	2.404 (3)
3	0.917 (1)	0.913 (2)	2.407 (4)
	$^{208}Pb/^{206}Pb^{a,b}$		
1	2.193 (8)	2.168 (11)	2.415 (8)
2	2.193 (4)	2.182 (11)	2.400 (11)
3	2.193 (3)	2.181 (4)	2.400 (3)
	$^{204}Pb/^{206}Pb^{a,b}$		
1	0.0584 (2)	0.0591 (2)	2.416 (6)
2	0.0584 (4)	0.0588 (4)	2.402 (5)
3	0.0585 (3)	0.0591 (3)	2.410 (6)

a: Certified values for $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$ are 0.91464 ± 0.00033, 2.1681 ± 0.0008 and 0.059042 ± 0.000037, respectively;

b: Mean values and standard deviation (calculated according to the propagation of errors, value affecting last digit);

c: Natural occurring ratio: $^{205}Tl/^{203}Tl = 2.3871$.

The results obtained when both external and internal mass bias correction were carried out for Port wine samples are shown in Table 4.6. Two independent replicates of each sample were measured. No significant differences were found (paired t-test [15]) between external and internal correction for $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios. This result indicates that only instrumental bias occurs, since the matrix effects are reduced, owing to the pre-treatment of the Port wine samples. Similar result was obtained by Goossens *et al.* [18] for aqueous ethanolic solutions. For $^{204}Pb/^{206}Pb$ ratio, a significant difference between the two types of mass bias correction was observed. No explanation was found for such behaviour. Nevertheless, the variation between samples of the ratios values was similar for both corrections. Therefore, it can be concluded that any type of mass bias correction is suitable for the present study.

Table 4.6. Comparison of external and internal mass bias correction for Pb IRs.

	$^{207}Pb/^{206}Pb^a$		$^{208}Pb/^{206}Pb^a$		$^{204}Pb/^{206}Pb^a$	
Wine sample	External correction	Internal correction	External correction	Internal correction	External correction	Internal correction
DP 47	0.861 (2)	0.860 (3)	2.110 (7)	2.120 (9)	--	--
	0.862 (2)	0.864 (1)	2.103 (4)	2.116 (8)		
DP 52	0.857 (3)	0.860 (2)	--	--	0.0549 (2)	0.0555 (2)
	0.857 (1)	0.859 (2)			0.0548 (2)	0.0558 (3)
DP 57	0.861 (4)	0.861 (2)	2.087 (3)	2.091 (7)	--	--
	0.859 (2)	0.860 (3)	2.080 (1)	2.101 (4)		
DP 63	0.863 (3)	0.860 (2)	--	--	0.0549 (3)	0.0558 (3)
	0.861 (3)	0.855 (2)			0.0550 (1)	0.0556 (2)
DP 68	0.864 (5)	0.867 (4)	2.093 (5)	2.102 (9)	0.0554 (3)	0.0563 (3)
	0.863 (4)	0.865 (6)	2.095 (6)	2.100 (10)	0.0552 (3)	0.0564 (3)
DP 74	0.865 (3)	0.864 (1)	--	--	0.0554 (3)	0.0564 (3)
	0.865 (4)	0.862 (2)			0.0555 (3)	0.0564 (5)
DP 77	0.866 (2)	0.864 (2)	--	--	0.0557 (2)	0.0569 (3)
	0.866 (2)	0.864 (2)			0.0555 (4)	0.0564 (2)
DP 78	0.867 (4)	0.863 (2)	--	--	--	--
	0.862 (3)	0.865 (2)				

a: Mean and standard deviation (calculated attending to the propagation of errors, value affecting last digit).

Later, Pb IRs were analysed in the IMEP-16 wine [20] (pre-treated by UV-irradiation). The IMEP-16 is a wine with certified values for lead total content and respective IRs from the *Institute for Reference Material and Measurements* from the *European Commission Joint Research Centre* (Geel, Belgium) and was used in an inter-laboratory comparison programme, the *International Measurement Evaluation Programme*. As Table 4.7 shows, the Pb IRs $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$, externally corrected for mass bias, were statistically identical to the certified values (paired t-test [15]). However, for the ratio $^{204}Pb/^{206}Pb$ only the internally corrected value was identical to the certified one. Therefore, although external mass bias correction is suitable for all Pb IRs when the same type of wine samples are being compared, like in this study, in order to compare $^{204}Pb/^{206}Pb$ in samples of different types of wines or in inter-laboratory comparison programmes, internal mass bias correction must be perform.

Table 4.7. Certified values (uncertainties not provided) and values determined[a] using the presented methodology for the Pb IRs $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$ in the IMEP-16 wine [20].

	Certified value	Determined value
$^{207}Pb/^{206}Pb$	0.8582	0.859 (5)[b]
$^{208}Pb/^{206}Pb$	2.093	2.100 (11)[b]
$^{204}Pb/^{206}Pb$	0.0557	0.0545 (6)[b] / 0.0554 (3)[c]

a: Mean and standard deviation (calculated according to the propagation of errors, value affecting last digit, n = 3);
b: Values externally corrected for mass bias with Pb isotopic standard;
c: Values internally corrected for mass bias with Tl IR.

4.6. LEAD ISOTOPE RATIOS MEASUREMENTS IN PORT WINES

The Pb IRs were measured in twenty-four Port wine samples with different ages and designations (Fig. 4.4). For the $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios the relative standard deviations (RSDs) were between 0.05 and 0.5 %, being lower than 0.3 % in most of the cases. Similar precisions were obtained by Dean *et al.* [12] and Augagneur *et al.* [13] in table wines and were considered to be sufficient to differentiate natural variation of the Pb isotope abundances [13]. For $^{204}Pb/^{206}Pb$ ratio the RSDs were between 0.2 and 1 %, being higher than 0.8 % only in three samples. Therefore, the precision of this IR was worse than that obtained for the remainders, presumable entirely due to poor counting statistic on the ^{204}Pb isotope, since this is the least abundant isotope. A similar result was observed by Goossens *et al.* [18] in table wines and is typical of IRs involving an isotope of low abundance, such as ^{204}Pb [21].

With the purpose of testing if among the analysed Port wines significantly differences in the values of the Pb IRs occurred, the LSD test [15] was applied. For this purpose, the Pb IRs were arranged in ascending order and the difference between adjacent values were compared with the LSD calculated. As shown in Fig. 4.5, some significant differences were found. For example, the LBV 88 wine displayed both $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios significantly different from those of all the other wines and a $^{204}Pb/^{206}Pb$ ratio significantly different from that of the DP 35, DP 40, DP 52', DP 57, DP 68, DP 69, DP 74, DP 74', DP 77, DP 78, DP 79', DP 84, LBV 88', LBV 88", IA 10, IA 10' and IA 10" wines. The three LBV 88 wines, although with the same age and designation, displayed different Pb IRs, having one of them (LBV 88) a Pb isotopic composition significantly different ($P < 0.05$ [15]) from the remainders (LBV 88' and LBV 88"). This result indicates that the Pb present in the LBV 88 wine came from a different source. The three IA 10 samples displayed, in general, higher IRs (statistically identical among them) than those of the DP wines. These results indicate that the precision obtained in the

measurements of the Pb IRs was sufficient to distinguish Pb isotopic composition in some Port wines samples.

Significant correlations ($P < 0.05$ [15]) were found between the age of the DP wines and the $^{207}Pb/^{206}Pb$ ratio or the $^{204}Pb/^{206}Pb$ ratio. No significant correlation was observed between the age of the wines and the $^{208}Pb/^{206}Pb$ ratio. As the IA 10 Port wine samples are a mixture of wines of different years, the comparison between age and Pb IRs has lack of significance.

The total Pb concentrations were also determined in all the Port wine samples and the results were included in Fig. 4.3. The Pb concentration in the Port wine samples fluctuated between 47 and 804 $\mu g\ l^{-1}$, but only two Port wines displayed Pb concentrations higher than 250 $\mu g\ l^{-1}$. A significant correlation ($P < 0.05$ [15]) was found between the age of the DP and LBV wines and the total Pb concentration. A decrease of Pb concentration was observed, from the older (DP 35) to the younger (LBV 88) Port wines, although interrupted by high values in DP 47 and DP 52 wines. Similar tendencies have been found in other types of wines [22-24] and are the result of the improved control of the metal levels in the wines. As the IA 10 Port wines are a mixture of wines of different years the comparison between age and Pb concentration has lack of significance.

A significant correlation ($P < 0.05$ [15]) between the total Pb concentration and the $^{208}Pb/^{206}Pb$ ratio was observed. For the remainder IRs significant correlations were not found.

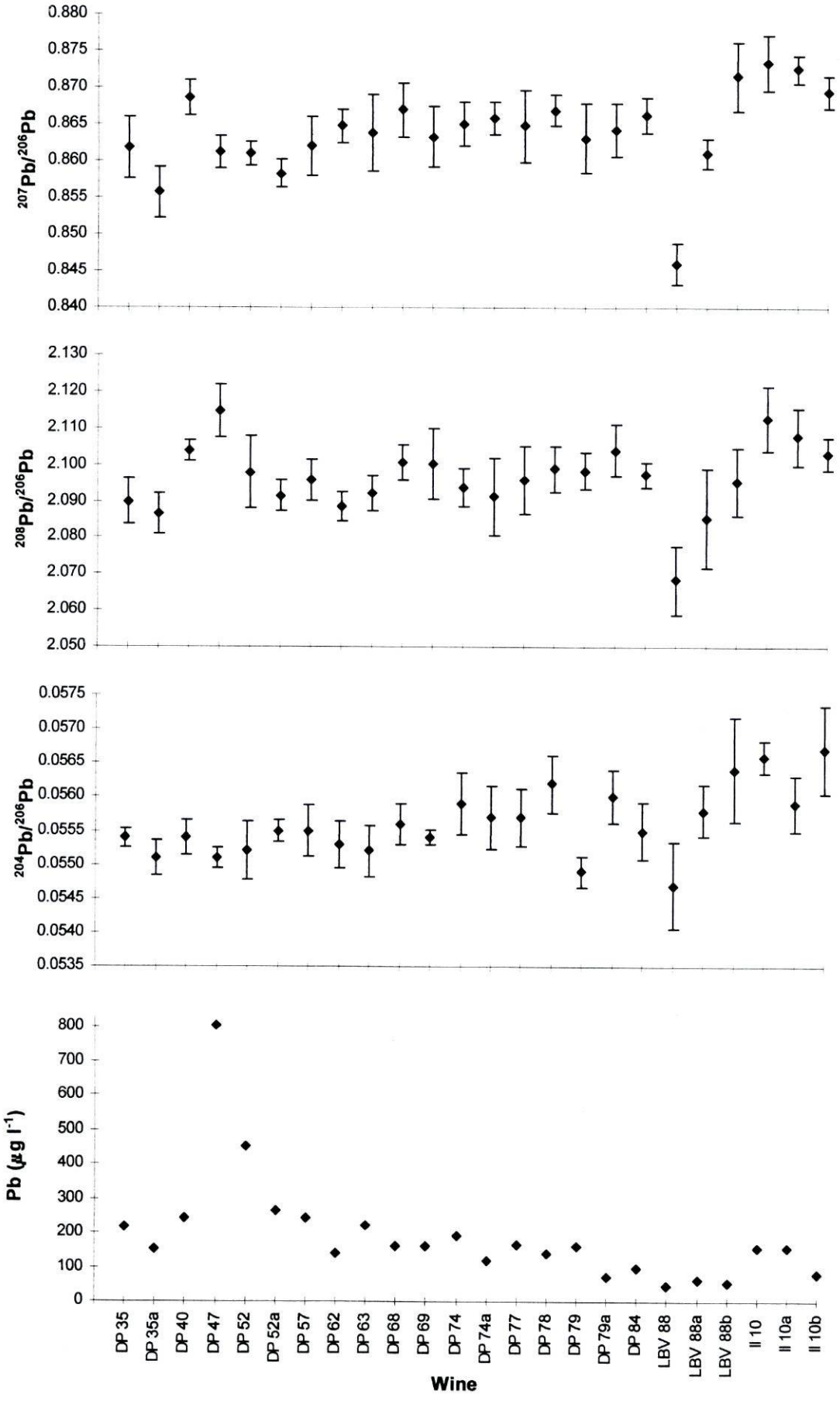

Fig. 4.4. Pb IRs and total Pb concentration (means and standard deviations, n=3), obtained for twenty-four different samples of Port wine.

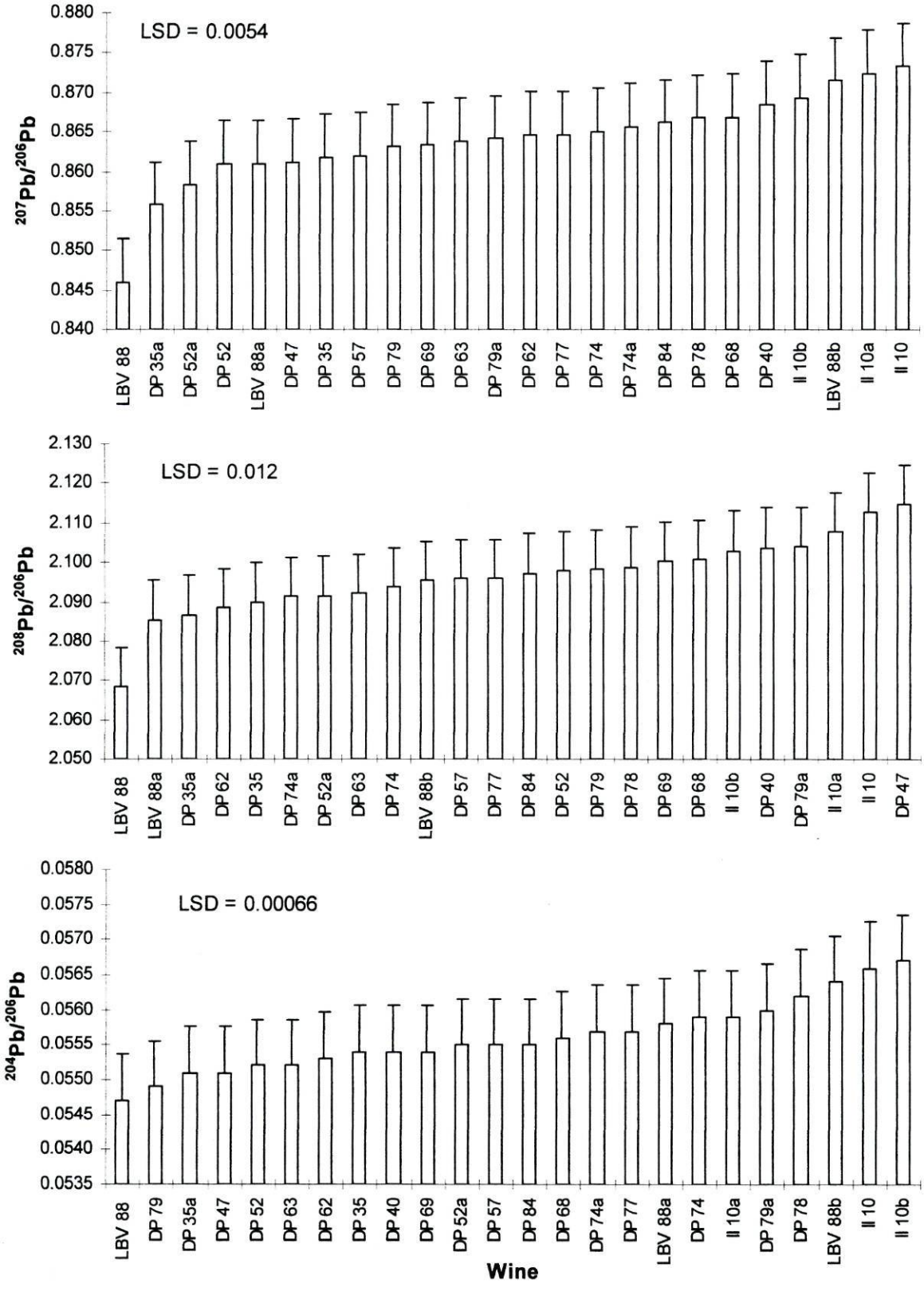

Fig. 4.5. Comparison, through the LSD test [15] of the mean values of Pb IRs in the twenty-four Port wine samples. The straight lines indicate the respective LSD value.

4.7. CONCLUSIONS

In this study, an UV-irradiation pre-treatment for Port wines was developed and showed to be suitable for lead isotope ratio determinations in these wines by ICP-MS. The methodology was tested for eight different samples of Port wine. A precision study showed that the standard deviation associated with the overall procedure (sample pre-treatment and subsequent determination) was mainly due to the ICP-MS determinations, the sample pre-treatment giving only a small contribution.

Two different types of mass bias correction for ICP-MS determinations of isotope ratios were used for comparison purposes: (i) external correction with a lead isotopic standard solution and (ii) internal correction with thallium as internal standard. No significant differences were found between the results obtained with the two types of corrections, both methods being suitable for mass bias correction in the present experimental conditions. Nevertheless, in order to compare $^{204}Pb/^{206}Pb$ in samples of different types of wines or in inter-laboratory comparison programmes, internal mass bias correction must be applied to this ratio.

The developed method was applied to twenty-four Port wine samples of different ages and characteristics. Precisions (relative standard deviation) of about 0.3 % for $^{207}Pb/^{206}Pb$ and for $^{208}Pb/^{206}Pb$ and about 0.8 % for $^{204}Pb/^{206}Pb$ were obtained and showed to be sufficient to differentiate natural variations of the lead isotope abundances in Port wine samples. Significant correlations were found between the $^{207}Pb/^{206}Pb$ or the $^{204}Pb/^{206}Pb$ ratios and the age of the Dated Port wines. A significant decrease ($P < 0.05$) of the lead concentration with the age of the wine was observed, as also as a significant correlation between the total lead concentration and the $^{208}Pb/^{206}Pb$ ratio.

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Chapter 5

Lead Contamination in Wine: From the Vineyard To the Final Product

5.1. INTRODUCTION AND AIMS

As mentioned in the General Introduction Section, the control of the lead concentration in wine is required because of its well-known toxicity.

In order to be able to reduce the level of lead in wine it is important to know the relative relevance of the different sources of this element and to evaluate their contribution to the lead contamination in the final product. The few works found in the literature concerning this subject indicated that the major lead contamination came from anthropogenic sources. Both Kaufmann [1] and Rosman *et al* [2] concluded that brass (a lead alloy that used to be widely utilised in traditional wine cellars) was the main contamination source, while Medina *et al.* [3] concluded that a series of Bordeaux wines followed the evolution of the atmospheric lead (mainly from leaded gasoline) recorded over the last century. The modernisation of wineries leaded to the gradual replacement of brass by stainless-steel that resulted in gradually lower lead levels in wine. In addition, the use of leaded gasoline stopped some years ago in many countries and the atmospheric lead burden has progressively being reduced, which also contributes to reduce lead in wine. Nevertheless, young wines still have significant levels of lead and it is important to know all the sources of this metal, in order to remove or minimise them.

The aim of the present work was to identify possible sources of lead and their relative relevance in the lead content of young wines produced in a modern winery. For comparison, an old fashion wine production process was also studied. For this purpose, the lead levels and the respective isotopic composition were determined in all the phases of wine production procedure, from the vineyard to the final product ready to be sold to the consumers, were monitored during an annual cycle of wine production (year of 2000).

5.2. EXPERIMENTAL

In this study, aerosols from the vineyards atmosphere, vineyard soil, vine leaves and grapes collected in two selected vineyards, characterised in Chapter 3, as well as, grape juices and samples from the respective vinification processes were analysed for their Pb total concentration levels (Pb_{total}) and respective IRs.

For IRs measurements, ICP-MS was used. Regarding Pb_{total}, measurements were carried out either by ICP-MS (vineyard soil, grape juices and samples from the vinification processes) or by ETAAS (atmospheric aerosols, vine leaves and grapes).

For Pb_{total} determinations in atmospheric aerosol samples a four-time dilution of the final solution with de-ionised water was carried out. For the Pb IRs determination the final solutions were analysed undiluted. For vineyard soil samples, after the HPMW-digestion a five-fold dilution of the final solutions with a solution containing Rh (for Pb_{total} determination) or with de-ionised water (for Pb IRs determinations) was carried out before analysis. For leaves and grapes, final solutions were diluted four-times or two-times, respectively, with de-ionised water before Pb_{total} determination. For Pb IRs determination in these samples the solutions were used directly. The grape juices and samples from the vinification processes, after UV-irradiation pre-treatment, were diluted to 12.5 ml (a two and half times dilution of the sample) with a solution containing HNO_3 and Tl (for Pb_{total} determinations) or with 0.5 % HNO_3 solution (for Pb IRs determinations).

For all samples three independent replicates (two for the aerosol samples) were prepared and analysed and, after blank subtraction, the mean and respective standard deviations were calculated.

5.2.1. ICP-MS measurements

5.2.1.1. Lead total concentrations

The Pb_{total} in the vineyard soil samples was measured using the semi-quantitative ICP-MS multi-element procedure described in Chapter 3 – Experimental Section. A sample of standard soil reference material: San Joaquin Soil SRM 2709 from NIST, was analysed together with the vineyard soil samples. Daily instrumental variations between 1.6 and 4.4 %, and long-term instrumental variation around 10 % were observed.

For the measurement of Pb_{total} in the wine samples, including those of grape juices and from the vinification steps, the data acquisition procedure was adapted from the instrument manual [4]. The Pb isotopes (the ones with higher isotopic abundance) were measured using 10 sweeps per reading, a dwell time of 100 ms and five replicates per measurement, in peak hopping mode, at normal resolution. Since changes in the isotopic abundance between samples could occur, the obtained concentration in each sample replicate was the mean of the one obtained for each Pb isotope. External calibration was used and the appropriate interpolation was carried out. In the final samples and standard solutions 0.5 % HNO_3 and 30 $\mu g\ l^{-1}$ Tl were always present. Tl was used for internal standardisation in order to

eliminate the matrix discrepancies and to compensate for any drift occurring during the analysis. The accuracy of the results was tested through an inter-comparison with those obtained, in parallel, for two selected wines, by the laboratory of the "Instituto do Vinho do Porto" in Portugal.

5.2.1.2. Lead isotope ratios

The Pb IRs were measured in the different types of samples using the analytical procedure described in the Experimental Section of this Dissertation.

Mass bias correction for IRs measurements. For the vineyard soil, vine leaves and grapes, mass bias corrections with both Pb isotopic standard and Tl IR were carried out in parallel for the firstly collected samples and the results were compared. No significant differences were obtained for all the Pb IRs (results are presented in the Appendix Section A.1 – Table A.1.1). For wine samples, as described in Chapter 4, no significant differences had been observed either between the results obtained using both types of corrections for the ratios $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$, but for the ratio $^{204}Pb/^{206}Pb$ the correction with Tl IR was the only one suitable. Therefore, the ratio $^{204}Pb/^{206}Pb$ in the grape juices and in all the samples collected in the different steps of the vinification processes was corrected with Tl IR. The remaining Pb IRs in these samples and all the Pb IRs in the other type of samples were corrected with the Pb isotopic standard.

In a first stage, to test the repeatability of the results obtained for the Pb IRs in soil samples (three replicates per sample), and since Pb_{total} did not changed significantly among the different replicates (see below), the mean and variance of the Pb IRs of three independent replicates were calculated and compared with the mean and variance obtained for a single replicate which was analysed three times. The results (presented in the Appendix Section A.1 – Table A.1.2) were similar to those reported in Chapter 4 for wine samples, being the variance observed for the Pb IRs in three independent replicates of each soil sample of the same order of magnitude as that obtained for a single replicate. The obtained results indicated that the ICP-MS determinations were the decisive factor for the precision of the Pb IRs. Therefore, for subsequent studies only one portion of each soil sample was processed for the determination of the respective Pb IRs.

Experimental and sampling conditions, samples treatments and analytical procedures are described in detail in the Experimental Section of this Dissertation.

5.3. LEAD TOTAL CONCENTRATIONS

5.3.1. Atmospheric aerosols

The Pb_{total} contents in the atmospheric aerosols in the vineyards area were relatively low, around 18 ng m_{air}^{-3} in May (during a dry and sunny weather period) and around 7.5 ng m_{air}^{-3} in July and September (during more wet weather with raining periods), being much lower than the threshold limit value fixed in the Portuguese legislation, 2.0 μg m_{air}^{-3}. The observed variations in Pb concentrations were probably related with the weather conditions, since rain facilitates the deposition of the aerosols thus decreasing the concentration of contaminants in the air.

5.3.2. Vineyards soil

The levels of Pb_{total} observed in the vineyard soil (monthly values) are shown in Fig. 5.1. The Pb contents were similar at surface and 20 cm depth, but they were slightly higher in the old vineyard, between 13.1 ± 0.7 and 22 ± 1 μg g_{soil}^{-1}, than in the young vineyard, between 9 ± 1 and 17.1 ± 0.7 μg g_{soil}^{-1} (± standard deviations, n =3).

Significant and systematic variations of the Pb_{total} were not found either among the three different sampling sites per vineyard, or during the year. Nevertheless, in few months the levels of Pb_{total} in one of the three sampling sites were significantly higher (and higher than the equipment experimental long-term variation, 10 %, and daily variation, 1.6 - 4.4 %) than those observed in the other two sites. However, such differences probably only reflected the heterogeneity of the metal in the soil instead of a specific anthropogenic contamination.

5.3.3. Vine leaves

Fig. 5.2 shows that the Pb_{total} content in the vine leaves varied between 0.15 ± 0.05 and 0.62 ± 0.02 μg $g_{dry\ leave}^{-1}$ in the old vineyard and between 0.22 ± 0.04 and 0.7 ± 0.2 μg $g_{dry\ leave}^{-1}$ in the young vineyard.

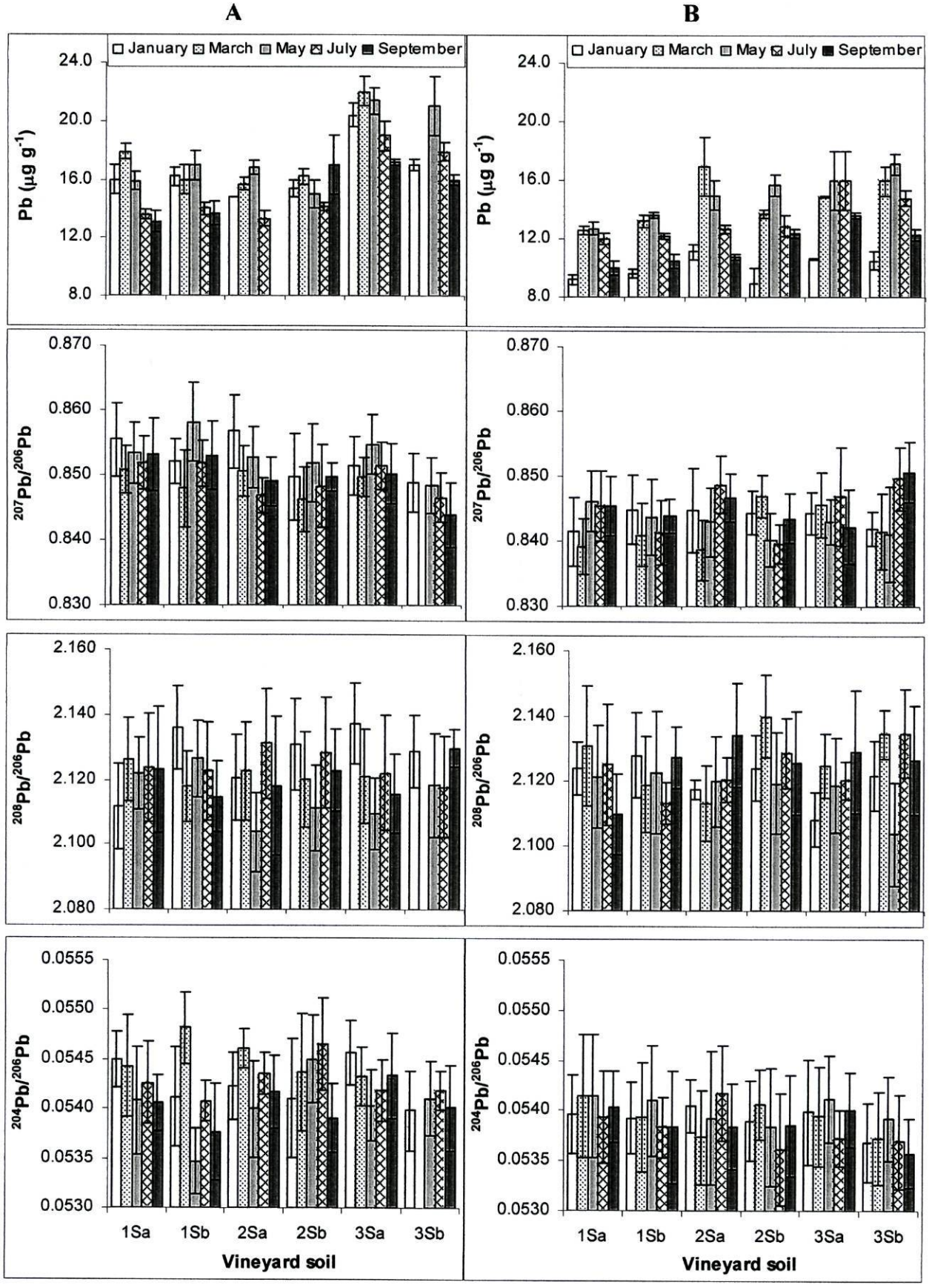

Fig. 5.1. Values of Pb_{total} (in µg per g of dry soil) and respective IRs obtained in the vineyard soil samples collected at surface (S_a) and at 20 cm depth (S_b), in the three selected sites (1S, 2S, 3S), in the old (A) and young (B) vineyards, in five different months. The sample $3S_b$ from the old vineyard and from March was lost.

Therefore, the Pb_{total} levels were similar in both vineyards in spite of Pb_{total} in soil being higher in the old vineyard

In most cases, and in both vineyards, the monthly values of Pb_{total} were lower in the washed leaves than in those non-washed, though the differences were statistically significant only for the samples collected in July and September in the young vineyard. A decrease of Pb_{total} after washing was expected, as water removes the atmospheric particles deposited on the leaves. Therefore, direct atmospheric deposition showed not to be an important source of Pb in the leaves.

The relatively poor reproducibility observed among the three independent replicates (high standard deviations) may be a result of leaves heterogeneity (for instance, different stages of growth, with some leaves new and small and other ones older and bigger).

In many cases, the Pb_{total} levels were higher in September than in May or July. This increase occurred mainly in the old vineyard (both in washed and non-washed leaves) whose soil was richer in Pb. This fact indicates that the Pb concentration in the leaves slightly increased with age.

5.3.4. Grapes

The results obtained for grapes are shown in Fig. 5.3. The Pb_{total} contents were similar in both vineyards, between 26 ± 14 and 52 ± 21 ng $g_{dry\ grape}^{-1}$ in the old vineyard and between 17 ± 8 and 57 ± 20 ng $g_{dry\ grape}^{-1}$ in the young vineyard. These levels were about one order of magnitude lower than those found in the leaves. It is known that the different organs of a plant show different abilities to accumulate metals, seeds and fruits accumulating less metal than leaves and roots in most species [5].

In contrast with the vine leaves, the Pb_{total} in the grapes did not increased from July to September. However, these data may be only apparent, resulting of an incomplete drying of the mature grapes collected in September (in spite of they had been dried in an oven up to a constant weight) due to the presence of a very high sugar content.

Statistically significant differences in the Pb_{total} levels in grapes collected in different sites of both vineyards, as well as between washed and non-washed grapes were not found. The reproducibility of the data was poor (RSDs of 50 % in some cases) probably due to the either low Pb concentrations or variation in the degree of hydration of the grapes.

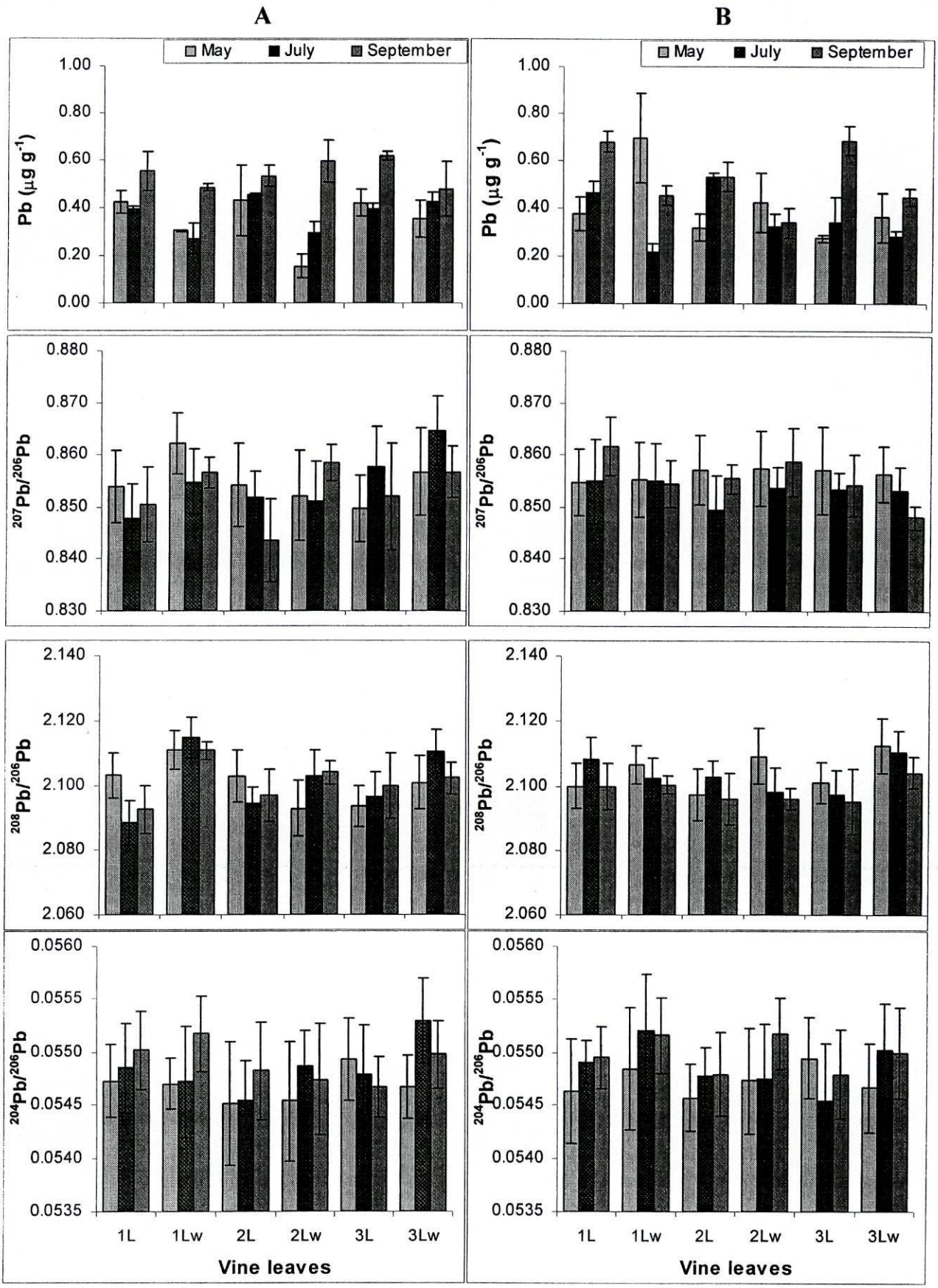

Fig. 5.2. Values of Pb_{total} (in µg per g of dry vine leave) and respective IRs obtained in the vine leave samples washed (L_w) and non-washed (L) collected in the three selected sites (1L to 3L), in the old (A) and young (B) vineyards, in three different months.

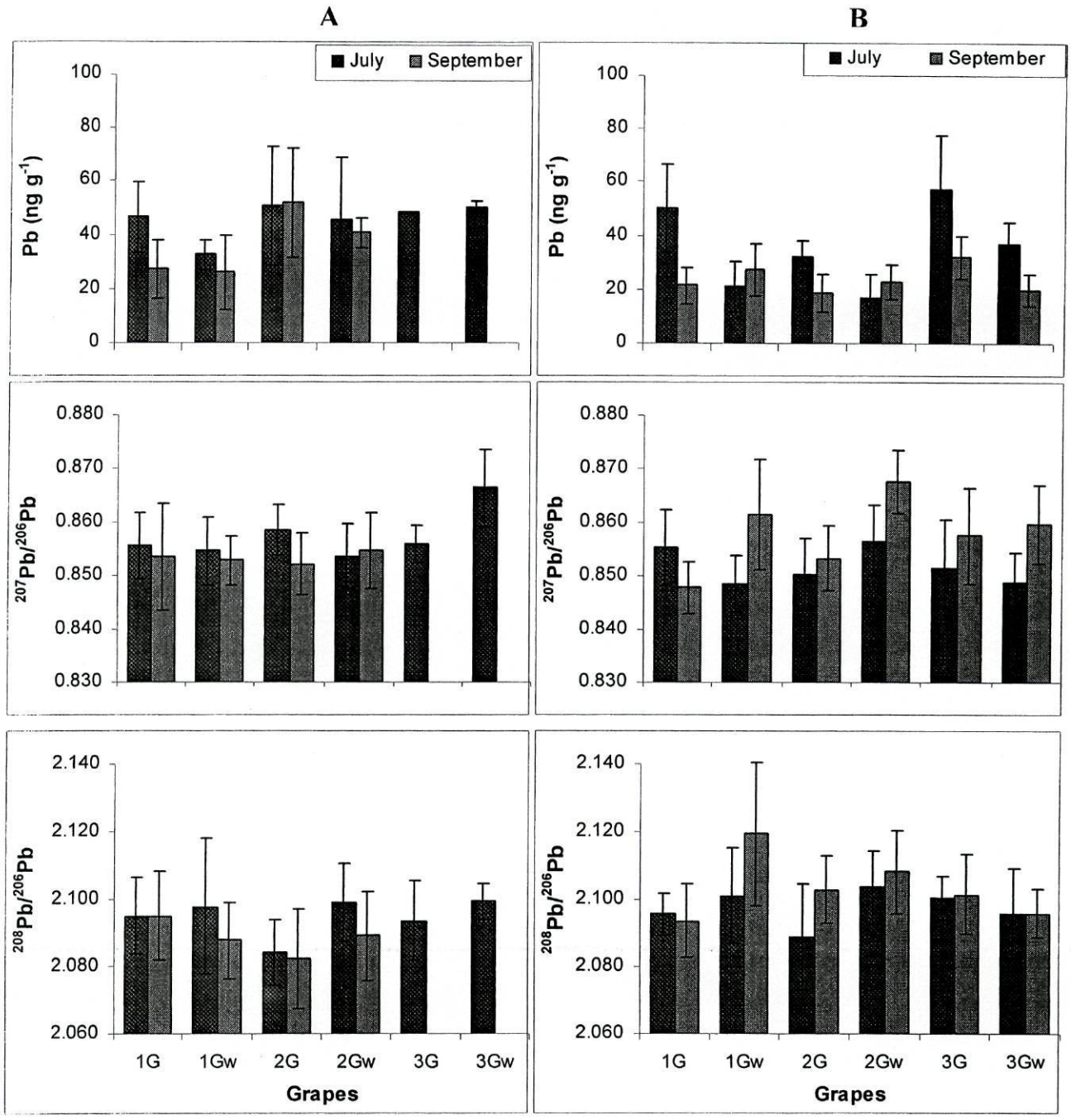

Fig. 5.3. Values of Pb_{total} (in ng per g of dry grape) and respective IRs obtained in the grape samples washed (G_w) and non-washed (G) collected in the three selected sites (1G to 3G), in the old (A) and young (B) vineyards, in two different months. The samples 3G and $3G_w$ from the old vineyard and from September were lost.

5.3.5. Grape juices and samples from the vinification processes

The levels of Pb_{total} determined in the grape juices (GJ_F and GJ_T for the fortified and table wine, respectively) and in the different samples collected during and at the end of the vinification processes (see Fig. 3.2, A – fortified wine and B – table wine, in Chapter 3) are presented in Fig. 5.4. For the

fortified wine (from the old vineyard), the concentration of Pb increased about 265 %, from 4.7 ± 0.3 $\mu g\ l^{-1}$ in the GJ_F to 17.2 ± 0.3 $\mu g\ l^{-1}$ in the final product (W_FF). For the table wine (from the young vineyard) the Pb concentration increased about 220 %, from 4.1 ± 0.5 $\mu g\ l^{-1}$ in the GJ_T to 13.1 ± 0.1 $\mu g\ l^{-1}$ in the final product (W_TF). These marked increases of the Pb levels during the vinification procedures indicated that, at least in the studied area (Portuguese Douro region) environmental contamination was not the major source of the Pb found in the wines. Even the very modern vinification system used to produce the table wine introduced significant amounts of Pb in the final product, although lower than those introduced by the old fashion process used to produce the fortified wine.

It must be stressed that both studied wines presented Pb concentrations much lower than the threshold limit value established by the OIV (200 $\mu g\ l^{-1}$). In addition, recent studies indicated that only a fraction less than 20 % of Pb_{total} is in assimilable forms after ingestion [6,7].

A more detailed analysis of the data (Fig. 5.4) shows that the increase in Pb_{total} throughout the vinification processes was very regular, with only a few exceptions. The sources of Pb were probably the alloys used to weld pieces of the different containers and tubes used in the vinification system (see Fig. 3.2 in Chapter 3), as well as, same fittings like traps.

In the case of the fortified wine a slightly but significant decrease in the Pb concentration occurred between samples W_F1 and W_F2, which was due to the addition of grape brandy (practically free of Pb) to stop the fermentation, resulting in a dilution of the product. In the following steps of the vinification process the Pb levels increased again, indicating that probably the metallic bracelets of the container were a Pb source, attaining a maximum in sample W_F4 due to the rest in the stainless-steel vat, which, therefore, should have some sources of Pb, possibly welding strings. In the next step the particles (skins and seeds) were removed and the liquid was transferred to oak barrels where it aged for one year, after which the last sample was collected (W_FF) for analysis. The Pb_{total} in W_FF was markedly lower than in W_F4 probably because a large fraction of Pb was chemically bounded with some colloidal polymeric organic compounds and was removed with the solid phase or co-precipitated with it.

During the vinification of the table wine it was observed a progressive increase in Pb_{total}, which was probably related with the presence of Pb sources in the stainless-steel of the tubes and vats. In sample W_T3 a significant and marked increase of Pb content occurred. However, from W_T3 to W_T4 there was a decrease of Pb, indicating that some metal was removed with the particles (skins and seeds), since the liquid extracted from the pressing of the solids (W_T5) was slightly richer in Pb than the previous separated liquid phase (W_T4).

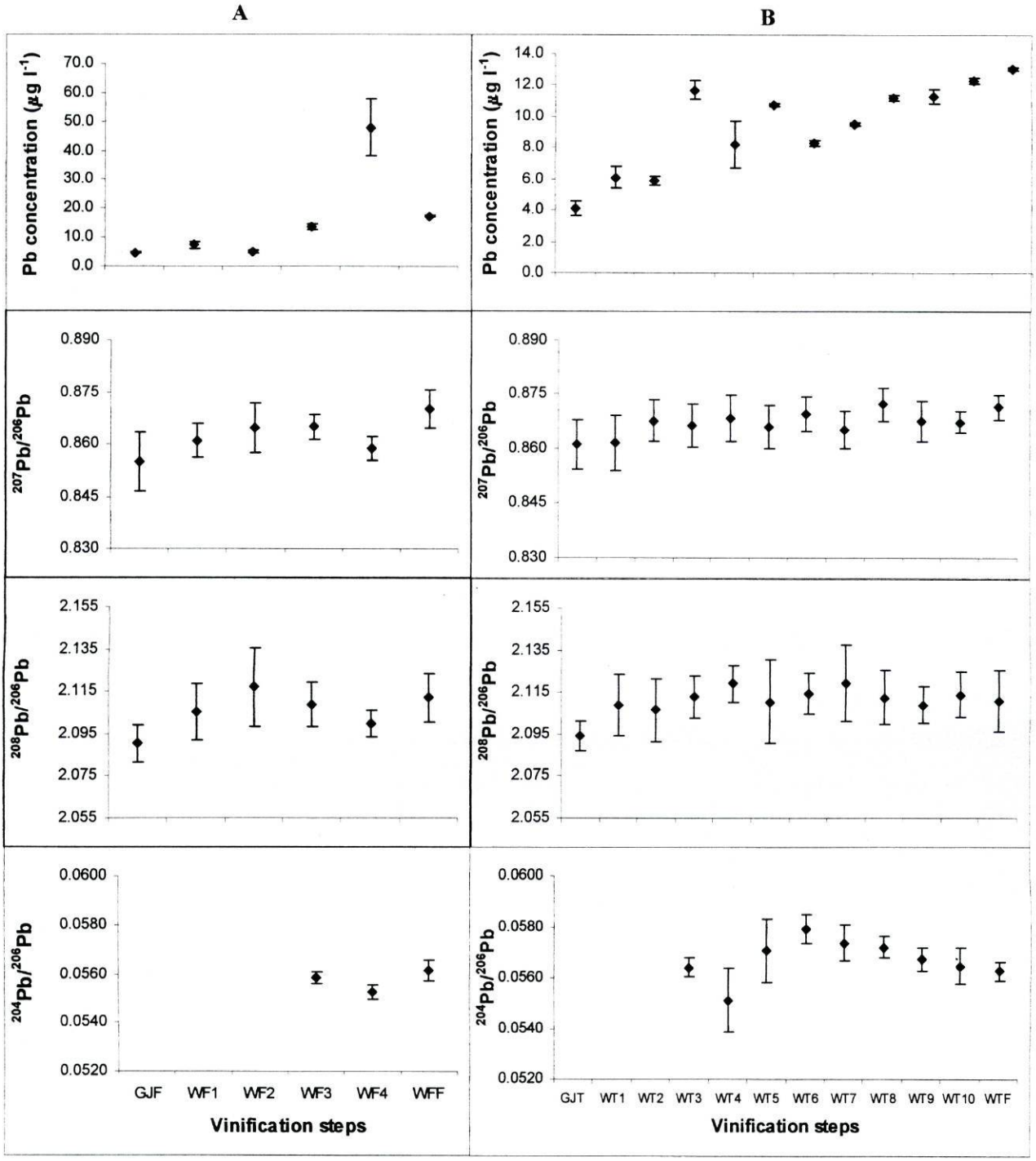

Fig. 5.4. Values of Pb_{total} (in µg l^{-1}) and respective IRs obtained for the grape juices (GJ_F and GJ_T for the fortified and table wine, respectively), for all the samples collected throughout the vinification processes (W_F1 to W_F4 for the fortified and W_T1 to W_T10 for the table wine) and for the final products (W_FF and W_TF for the fortified and table wine, respectively). (A) red fortified wine produced with grapes from the old vineyard; (B) red table wine produced with grapes from the young vineyard. The ratio $^{204}Pb/^{206}Pb$ could not be determined in the samples GJ, W1 and W2 of both wines.

In summary, the available data on Pb_{total} clearly demonstrated that the major contribution for the Pb levels in the studied wines came from the vinification processes, the traditional process introducing more Pb in the final product than the modern one. Concerning the Pb fraction that was already present in the grapes, based only on the Pb levels found in the soil and in the atmospheric aerosols from the vineyard it is impossible to conclude which of these two Pb sources were more relevant. As the Pb IRs may vary with the origin of the element, they were also measured in all the samples.

5.4. LEAD ISOTOPE RATIOS

5.4.1. Atmospheric aerosols

The values of the ratios $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$ were measured in the aerosols collected from the atmosphere of the vineyards in May, July and September. As Table 5.1 shows, the Pb isotopic composition was identical in the three months, suggesting that the atmospheric Pb sources were similar in those sampling dates.

Table 5.1. Values of $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$ obtained for the atmospheric aerosol samples collected in three different months.

	$^{207}Pb/^{206}Pb$	$^{208}Pb/^{206}Pb$	$^{204}Pb/^{206}Pb$
May	0.859 (4)	2.100 (8)	0.0551 (4)
July	0.865 (5)	2.101 (10)	0.0558 (3)
September	0.863 (4)	2.106 (11)	0.0554 (6)

a: Mean and standard deviation (calculated according to errors propagation, value affecting last digit)

5.4.2. Vineyards soil

The values of the three Pb IRs observed in the vineyard soil are included in Fig. 5.1. For $^{204}Pb/^{206}Pb$ ratio the RSDs (n = 3) associated to the means, between 0.35 and 1.2 %, were worse than those obtained for the remainders IRs (≤0.85 % for $^{207}Pb/^{206}Pb$ and ≤ 1.0 % for $^{208}Pb/^{206}Pb$), presumable entirely due to poor counting statistic on the ^{204}Pb isotope, since this is the least abundant isotope.

Similar results were observed before in wine samples (see Chapter 4)) and for the other types of samples analysed.

Results of statistical tests indicated that, for each vineyard, no significant variations of the Pb IRs occurred either throughout the months or between the two soil layers: surface (S_a) and 20 cm depth (S_b). Therefore, an eventual anthropogenic contamination, resulting, for instance, of the vine treatment, could not be identified. These results are consistent with the constant level of Pb_{total} observed in the vineyards soil.

5.4.3. Vine leaves

For each vineyard, significant changes did not occur in the Pb IRs measured either in the vine leaves throughout the vineyard, or during the period of study (see Fig. 5.2). These results corroborated the previous conclusions that the differences observed in the levels of Pb_{total} were probably related to sample heterogeneity due to, for instance, different degrees of leaves growth.

Regarding washed and non-washed vine leaves, also no statistically significant differences between them were found. Therefore, eventual contamination by atmospheric deposition could not be clearly identified through the Pb isotopic signature. Combining these results with the ones provided by Pb_{total}, it could be concluded that external contamination of the leaves during growth was very small.

5.4.4. Grapes

The very low content of Pb present in grapes of both vineyards prevented the determination of the IR $^{204}Pb/^{206}Pb$. Therefore, only $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ were measured (Fig. 5.3). Similarly to that observed for vine leaves, no significant change occurred for the two Pb IRs in the grapes throughout either each vineyard or the period of study. As for the leaves, no statistically significant differences were obtained between washed and non-washed grapes, which corroborate previous conclusions about absence of significant atmospheric Pb contamination.

5.4.5. Grape juices and samples from the vinification processes

The values of $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ were determined in all the samples collected throughout the vinification processes, where eventual Pb contamination could be introduced (see Fig. 3.2 in Chapter 3). The ratio $^{204}Pb/^{206}Pb$ was only determined in the latest points (from W3 to WF for both types of wines) (Fig. 5.4), owing to the too low Pb_{total} of the remaining sampling points.

No significant differences in the Pb IRs values were observed throughout the vinification steps in both monitored processes. As discussed before, the Pb_{total} increased during these vinification processes, indicating the presence of Pb sources. Unfortunately, the variations of the Pb IRs values throughout the vinification were within the errors associated to the analytical measurements, preventing the identification of the Pb sources. This fact resulted of a combination of relatively low level of Pb contamination with the presence of different Pb sources probably with distinct Pb isotopic signatures, which masked each other.

5.4.6. Global comparison of lead isotope ratios in the samples of different origins

When the values of the different IRs of an element measured in various samples are plotted one against the other, a simple source of the element will form a cluster on the graph, while linear arrays tend to appear if several disparate sources are mixed [8]. With the purpose of trying to extract some valuable information about the Pb sources, the global mean values of each Pb IR in the different types of samples from each vineyard (see Table 5.2) and from the respective vinification process (Fig. 5.4) were compared by plotting (y versus x): $^{208}Pb/^{206}Pb$ *vs.* $^{207}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$ *vs.* $^{207}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$ *vs.* $^{208}Pb/^{206}Pb$. The results are shown in Fig. 5.5 (A - fortified and B - table wines).

For the fortified wine it was observed that the old vineyard soil and the atmospheric aerosols displayed Pb of different isotopic compositions. Vine leaves presented not very different Pb isotopic composition from that of grapes, being this composition a mixture of those present in soil, in aerosols and probably in a third component not analysed in this work, since it got out of the linear array formed by soil and aerosol samples formed. Such third component may be related with pesticides and/or fertilizers used in previous years in this vineyard, which have been accumulating in the rhizosphere of the vine (the rhizosphere composition was not determined in this study). Regarding the washed and non-washed leaves, Fig. 5.5A shows that these two set of samples displayed some differences. Similarly,

some changes in the Pb IRs occurred during the vinification process. For instance, GJ_F and grapes had similar IRs, which differed from those of W_F2 and W_F3, while W_F1 presented a Pb isotopic composition between GJ_F and W_F2 or W_F3. These results are compatible with the fact of during the first steps of the vinification, the must with pomace and seeds had been in a wood container with metallic bracelets, while GJ_F, produced at the laboratory, had no contact with metallic devices. Therefore, some Pb from metallic allows with Pb isotopic composition different from that of GJ_F contaminated the samples. This result is in agreement with the increase in Pb_{total} observed in those samples, as discussed previously. The sample W_F4, which was collected after the wine (together with pomace and seeds) had rested in a stainless-steel vat, presented different Pb IRs than the previous samples. This suggests that the container had also some sources of Pb different from the previous ones, which is corroborated by the high increase observed in Pb_{total} at this step. The Pb IRs of the final product, W_FF, were also different from all the previous ones, suggesting the presence of a different Pb source of contamination during the one-year rest in an oak barrel.

Table 5.2. Global average values of Pb IRs, with respective standard deviation (value in brackets affecting last digit) calculated for the different types of samples.

	Old vineyard			Young vineyard		
	$^{207}Pb/^{206}Pb$	$^{208}Pb/^{206}Pb$	$^{204}Pb/^{206}Pb$	$^{207}Pb/^{206}Pb$	$^{208}Pb/^{206}Pb$	$^{204}Pb/^{206}Pb$
Soil						
S_a [a]	0.852 (3)	2.121 (8)	0.0543 (2)	0.844 (3)	2.121 (7)	0.0540 (1)
S_b [a]	0.850 (3)	2.123 (7)	0.0541 (1)	0.844 (3)	2.125 (9)	0.0538 (2)
Leaves						
L [a]	0.851 (1)	2.097 (4)	0.0548 (1)	0.855 (2)	2.100 (4)	0.0548 (1)
L_w [a]	0.857 (2)	2.106 (3)	0.0549 (1)	0.855 (2)	2.104 (4)	0.0550 (1)
Grapes						
G [a]	0.855 (3)	2.090 (1)	ND [b]	0.851 (1)	2.096 (4)	ND [b]
G_w [a]	0.856 (1)	2.095 (5)	ND [b]	0.857 (2)	2.106 (4)	ND [b]
Aerosol [c]	0.862 (3)	2.102 (6)	0.0554 (4)			

a: S_a and S_b: soil samples collected at surface and 20 cm depth, respectively; L and G: non-washed vine leaves and grapes, respectively; L_w and G_w: washed vine leaves and grapes, respectively;
b: ND – not determined;
c: Collected in a point representative of both vineyards.

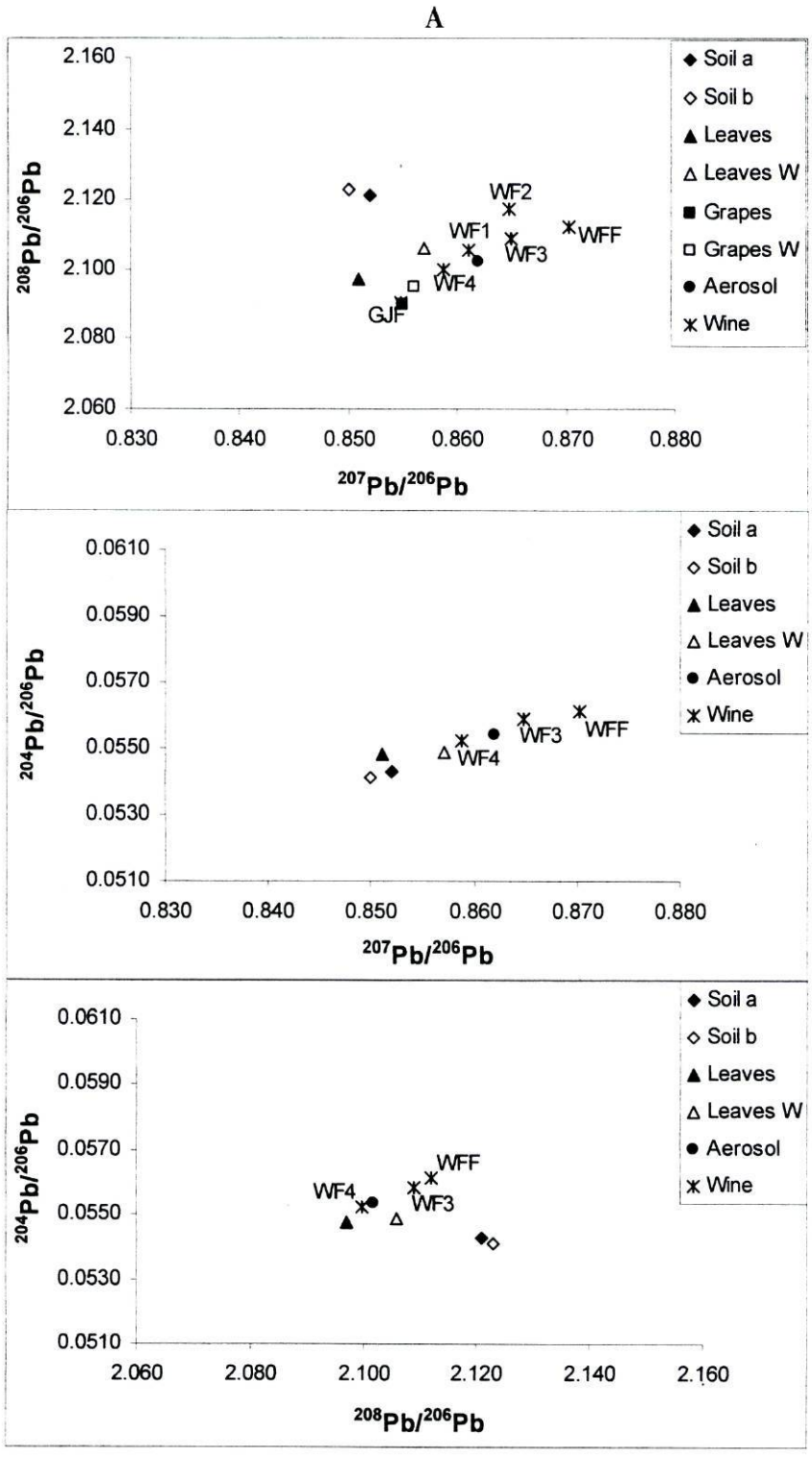
A
2.160
2.140
2.120
2.100
2.080
2.060
208Pb/206Pb
0.830
0.840
0.850
0.860
0.870
0.880
207Pb/206Pb
Soil a
Soil b
Leaves
Leaves W
Grapes
Grapes W
Aerosol
Wine
WF2
WF1
WFF
WF3
WF4
GJF
0.0610
0.0590
0.0570
0.0550
0.0530
0.0510
204Pb/206Pb
207Pb/206Pb
Soil a
Soil b
Leaves
Leaves W
Aerosol
Wine
WF3
WFF
WF4
0.0610
0.0590
0.0570
0.0550
0.0530
0.0510
204Pb/206Pb
2.060
2.080
2.100
2.120
2.140
2.160
208Pb/206Pb
Soil a
Soil b
Leaves
Leaves W
Aerosol
Wine
WFF
WF4
WF3

Fig. 5.5.

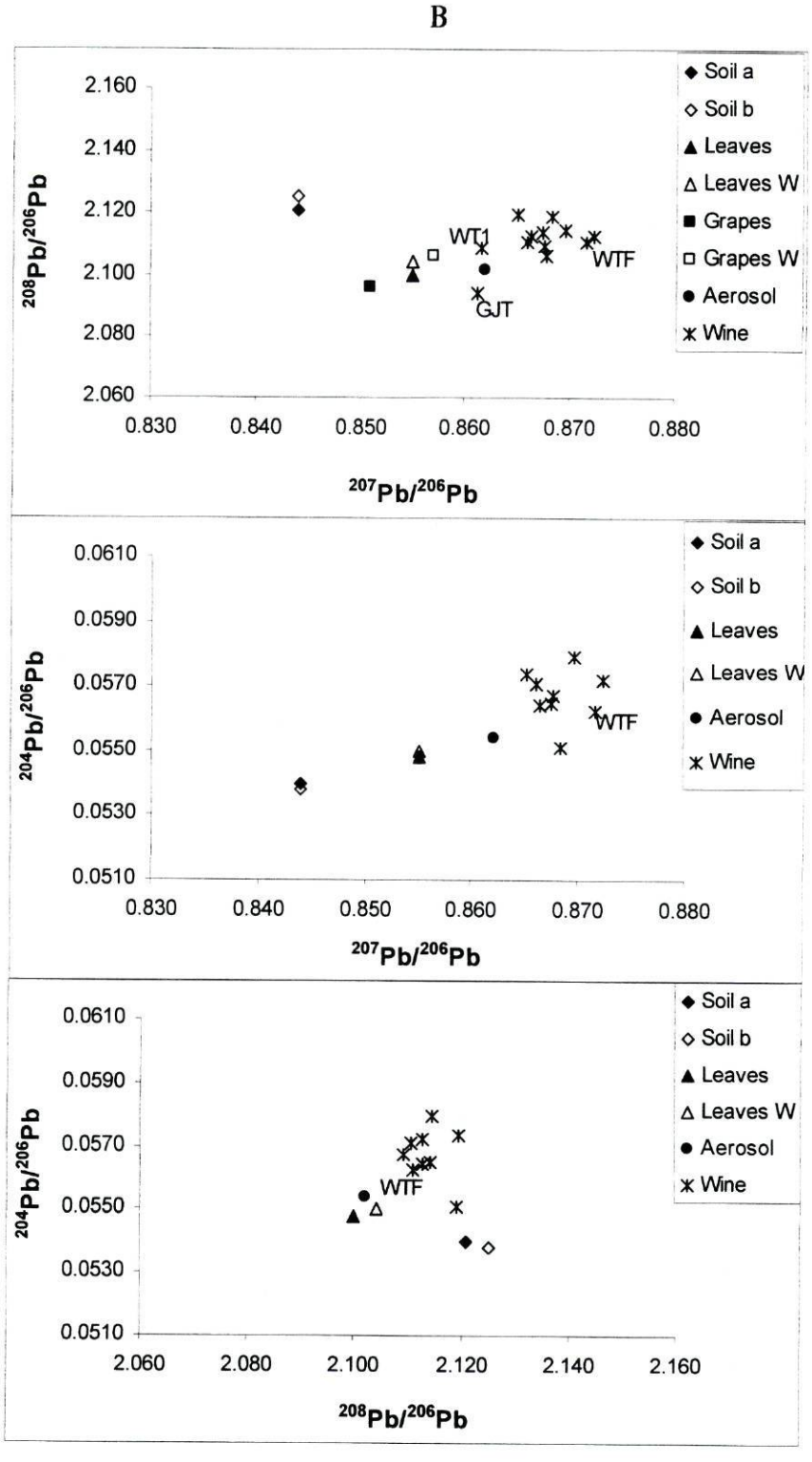

Fig. 5.5. ***continuation.*** Plots of $^{208}Pb/^{206}Pb$ *vs.* $^{207}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$ *vs.* $^{207}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$ *vs.* $^{208}Pb/^{206}Pb$ for comparison between the mean values of the Pb IRs of the different samples: surface (◆) and 20 cm depth (◊) vineyard soil; non-washed (▲) and washed (Δ) vine leaves; non-washed (■) and washed (□) grapes; atmospheric aerosols (●); grape juices (GJ_F and GJ_T for the fortified and table wine, respectively), all samples collected throughout the vinification processes (W_F1 to W_F4 for the fortified and W_T1 to W_T10 for the table wine) and final products (W_FF and W_TF for the fortified and table wine, respectively) (*). (A) red fortified wine produced from grapes of the old vineyard; (B) red table wine produced from grapes of the young vineyard.

Regarding the table wine/young vineyard, it was observed that soil and aerosols presented markedly different Pb isotopic compositions. Vine leaves presented Pb isotopic composition similar to that of the grapes, which seemed to be mostly a mixture of those present in soil and in aerosols. The influence of a third component, probably related with pesticides and/or fertilizers used in previous years and accumulated in the vine rhizosphere, was much less evident in this case than in the old vineyard, as both leaves and grapes fitted more or less in the linear array formed by soil and aerosol samples. This is compatible with the fact of the young vineyard has been submitted to much less treatments in the past than the old vineyard. Some differences were observed between washed and non-washed grapes and between grapes and GJ_T. However, these differences resulted probably of instrumental errors (related with low Pb content of the samples), since GJ_T had no contact with metallic devices and, therefore, isotopic composition identical to that of the grapes would be expected as it was observed for the fortified wine. Concerning the vinification process, the sample W_T1 presented Pb isotopic composition different from GJ_T and from the subsequent samples. Considering the significant increase in Pb_{total} that was observed between GJ and W_T1, it could be concluded that there was already some Pb contamination in this step, probably from sources present in the stainless-steel containers used to transport the grapes after the harvest. The Pb isotopic signature observed in that sample (W_T1) resulted probably of a non-homogenise mixture of different isotopic compositions. This is compatible with the fact of different isotopic compositions being observed in W_T1 and W_T2, despite they have Pb_{total} similar and having the must in these steps only a small contact with stainless-steel containers. The samples collected during the remaining steps of vinification, including W_T2, formed a cluster. This suggested that only one type of source of Pb contamination was present in the stainless-steel tubes and vats used in most of the steps of the modern vinification system. This is in agreement with the fact the Pb concentration increase proportionally to the vinification steps, as discussed previously.

5.5. CONCLUSIONS

With the aim of studying lead contamination in wine (both levels and sources) the levels of this element and those of the isotopes ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb were monitored in soil and air of two vineyards, as well as in vine leaves, grapes and in samples collected throughout two different vinification processes, during an annual cycle of wine production.

Both studied wines contained relatively low levels of lead. However, the lead levels in the fortified wine, which was produced by a traditional vinification process, were higher, 17.2 $\mu g\ l^{-1}$, than in the table wine, 13.1 $\mu g\ l^{-1}$, which was produced accordingly to very modern technologies. In both cases the lead levels were much lower than the threshold limit value established by the International Office of Vine and Wine (200 $\mu g\ l^{-1}$).

The major sources of the contamination were found in the vinification processes. Soil and direct atmospheric deposition only contributed with about 1/4 (fortified wine) or 1/3 (table wine) to the lead content of the wines. Some of the lead present in the musts seems to be removed by precipitation or co-precipitation with particles during the vinification processes. No significant differences were observed among the lead isotope ratios obtained in the different types of samples due to the relatively high standard deviations associated with the measurements. However, when the values of the different lead isotope ratios measured in the several samples were plotted one against the other, different sources of contamination became visible, corroborating the conclusions obtained from lead total concentration measurements. Therefore, those plots showed to be valuable tools for differentiating lead isotopic composition, giving, in this case, interesting information about lead sources of contamination in wines.

This study indicates that a drastic reduction of the lead level in the wine would be possible by a very strict control of the lead sources in the devices used in the vinification system, particularly in the alloys used in welding processes and in small fittings, like taps.

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Chapter 6

ICP-MS Determination of $^{87}Sr/^{86}Sr$ in Wines in Order to Be Used as a Fingerprint of Its Regional Origin

6.1. INTRODUCTION AND AIMS

As referred in the Chapter 1, elements with variable isotopic composition, such as strontium, are excellent provenance indicators or tracers in the biosphere. For this element, the relative abundance of ^{87}Sr is established with geological age and the rubidium/strontium ratio and consequently, with the geographical location.

The aim of this work (already published [3]) was the development of a methodology suitable for the determination, by ICP-MS, of $^{87}Sr/^{86}Sr$ ratio in both table and fortified wines, with precision enough to allow distinguishing of wines of different origins.

The application of quadrupole ICP-MS to the determination of the $^{87}Sr/^{86}Sr$ strontium isotope ratio in wines is not an easy task. Due to the isobaric overlap of the $^{87}Sr^+$ and $^{87}Rb^+$, strontium and rubidium have to be separated from each other before the analysis. For geological samples, a similar problem has been successfully overcome through cation-exchange chromatography [1,2]. Based on these previous works, a cation-exchange chromatographic procedure was optimised for the wines. However, as wines have a very complex matrix (rich in organic matter and alcohol content), destruction/removal of organic matter was required prior to the chromatographic separation.

6.2. EXPERIMENTAL

Prior to the chromatographic separation, all wine samples were UV-irradiated after which a dilution to 15.0 ml with de-ionised water (a three times dilution of the wines) was carried out. After the chromatographic separation and acid evaporation, the Sr-containing fraction was diluted to 15.0 ml with 0.5 % HNO_3 solution.

For each wine sample, three independent replicates were pre-treated, chromatographically separated and analysed. After blank subtraction, the mean and respective standard deviation were calculated.

Experimental details and the analytical procedures are presented in Chapter 3.

6.3. OPTIMISATION OF THE CHROMATOGRAPHIC SEPARATION OF STRONTIUM

In a first stage, for a preliminary optimisation of the chromatographic separation step, tests were conducted, using 15 ml of blank (de-ionised water) and standard solutions containing different concentrations of Rb and Sr (Sr between 100 and 500 μg l^{-1} and Rb between 50 and 750 μg l^{-1}), in order to determine the concentration and volume of HCl suitable for separated elution of the metals and for the regeneration of the column. The pH of the solutions introduced into the column was about 6. For this purposes, 2.5, 4 and 6 M HCl solutions were successively used, the eluant being collected in sequential fractions of 20 ml. In each fraction the ion signals of the isotopes ^{85}Rb, ^{87}Sr and ^{86}Sr were measured by ICP-MS. It was observed that 2.5 M HCl was enough and efficient for both the elution and the regeneration of the column. Most of Rb was eluted in the firsts 120 ml and Sr in the following 40 ml.

To study the efficiency of the chromatographic separation and elimination of Rb, standard solutions containing different concentrations of Rb and Sr (isotopic standard) were treated by chromatography and the $^{87}Sr/^{86}Sr$ determined by ICP-MS. Table 6.1 illustrates the obtained results. It was observed that the Rb present in the Sr-containing fraction after a single extraction step could still influence the $^{87}Sr/^{86}Sr$ IR, causing values higher than the real, particularly when the Rb concentration was higher than Sr concentration.

Table 6.1. Ion intensities[a] obtained for the Rb and Sr isotopes and respective $^{87}Sr/^{86}Sr$ IR (corrected with $^{88}Sr/^{86}Sr$ IR after blank subtraction) in standard solutions after chromatographic separation to remove Rb.

Standard solution	^{85}Rb ion intensity /ion s^{-1}	^{87}Sr ion intensity /ion s^{-1}	$^{87}Sr/^{86}Sr$[b]
100 μg l^{-1} Sr without extraction	2.13 (0.09) x 10^2	128 (1) x 10^2	0.715 (RSD[c]: 0.272 %)
50 μg l^{-1} Rb + 100 μg l^{-1} Sr, single extraction	35.2 (0.3) x 10^2	39.9 (0.1) x 10^2	0.721 (RSD[c]: 0.659 %)
100 μg l^{-1} Rb + 100 μg l^{-1} Sr, single extraction	49.7 (0.6) x 10^2	51.9 (0.8) x 10^2	0.721 (RSD[c]: 0.871 %)
150 μg l^{-1} Rb + 100 μg l^{-1} Sr, single extraction	146.2 (0.4) x 10^2	34.9 (0.2) x 10^2	0.754 (RSD[c]: 0.424 %)
750 μg l^{-1} Rb + 500 μg l^{-1} Sr, single extraction	584 (8) x 10^2	153 (1) x 10^2	0.751 (RSD[c]: 0.995 %)
750 μg l^{-1} Rb + 500 μg l^{-1} Sr, double extraction	8.8 (0.3) x 10^2	102 (3) x 10^2	0.718 (RSD[c]: 0.111 %)

a: Mean and standard deviation (in brackets, n = 3). Different replicates provided statistically identical results;
b: Certified value for $^{87}Sr/^{86}Sr$ is 0.71034 ± 0.00026;
c: Relative standard deviation.

Therefore, a second separation step was required. Thus, the 40 ml Sr-containing fraction obtained from the first extraction was passed again through the column (without pH neutralisation) and eluted. In this case, only the first 80 ml of eluant were rejected (the Rb-containing fraction), the next 40 ml being used for Sr analysis. Table 6.1 shows that, even for solutions with initial Rb concentration higher than that of Sr, after a second extraction the value of Sr IR obtained was statistically identical to that observed for a pure solution of Sr.

Similar tests were conducted on wine samples. After the UV-irradiation pre-treatment of the wine samples (implemented in Chapter 4), the 15.0 ml of the solution were passed through the chromatographic column, eluted with 2.5 M HCl, and the eluant was collected in fractions of 20 ml. In each fraction, the ion signals of the isotopes ^{85}Rb, ^{88}Sr, ^{87}Sr and ^{86}Sr were measured by ICP-MS. It was observed that Rb and Sr in the pre-treated wine presented retention volumes similar to those obtained for the standard solutions. Double cation-exchange chromatographic separation was also required for the treated wine samples.

6.3.1. Analysis of blanks

The influence of the UV-irradiation pre-treatment plus chromatographic separations on the blank signals (ion intensities of the various Sr isotopes) is illustrated in Table 6.2. For comparison, the signal obtained for a wine sample is also shown. The ion intensities of Sr and Rb increased with reagent addition and solution manipulation. Therefore, the blank signal should be subtracted from the sample signals. Nevertheless, in all cases the signals were two to three orders of magnitude lower than those obtained for the wine samples (see Table 6.2).

Table 6.2. Comparison of the ion intensities[a] obtained for the different Sr isotopes and for the ^{85}Rb isotope in untreated and pre-treated as the samples blanks solutions, and in a wine sample.

	^{86}Sr ion intensity/ion s^{-1}	^{87}Sr ion intensity /ion s^{-1}	^{88}Sr ion intensity /ion s^{-1}	^{85}Rb ion intensity /ion s^{-1}
Blank	30 (3)	33 (2)	64 (2)	226 (6)
Pre-treated blank (single CS[b])	90.6 (0.6)	103 (8)	558 (9)	289 (3)
Pre-treated blank (double CS[b])	128 (9)	88 (3)	752 (7)	212 (5)
T_1R_1 wine[c] (double CS[b])	288.6 (0.9) x 10^2	212 (1) x 10^2	245 (1) x 10^3	658 (5)

a: Mean and standard deviation (in brackets, n = 3). Different replicates provided statistically identical results;
b: CS: cation-exchange Rb separation;
c: T_1R_1 wine: a table wine from the Douro region.

6.4. ISOTOPE RATIO MEASUREMENTS

The data acquisition procedure was optimised with the Sr isotopic standard NIST SRM-987, and the Sr IR $^{87}Sr/^{86}Sr$ of this standard (certified value: 0.71034 ± 0.00026) was determined every working day.

For the optimisation of the data acquisition procedure, the influence of the instrumental parameters: number of replicates (varied between 3 and 10, with one reading per replicate), dwell time (varied between 5 and 10 ms) and number of sweeps per reading (varied between 200 and 1500), on the $^{87}Sr/^{86}Sr$ and $^{88}Sr/^{86}Sr$ ratios and on the respective RSD, obtained for a Sr isotopic standard solution (100 μg l^{-1} Sr concentration), was studied. The optimised procedure was selected in order to obtain the best precision (lowest RSD), and it is described in the Experimental section.

The means and RSD of all the determinations, from a six month period (n = 12) were calculated: $^{87}Sr/^{86}Sr$ = 0.721, RSD: 0.41 % (see also Fig. 6.1). The difference in the $^{87}Sr/^{86}Sr$ values observed in Fig. 6.1, between the 6th and 7th working days coincided with instrumental maintenance. However, that technical problem did not affect the accuracy of the results presented here, since all the data obtained between the 6th and 9th working days were rejected.

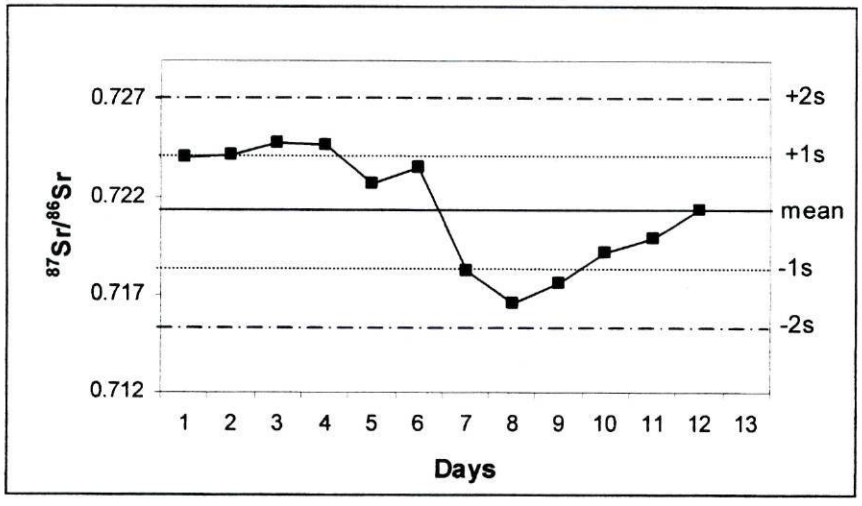

Fig. 6.1. Variation of the IR of the Sr isotopic standard NIST SRM-987, observed over a period of six months (s = standard deviation of the mean). The certified value for $^{87}Sr/^{86}Sr$ is 0.71034 ± 0.00026. The difference in the $^{87}Sr/^{86}Sr$ values observed between the 6th and 7th working days coincided with an equipment technical assistance (with some pieces replacement).

The precision for long-term measurements was slightly higher than that obtained for short-term (a single working day) measurements (RSD: 0.3 %). The short-term precision was similar to that obtained by Chassery *et al.* [1] for the same IR in the same Sr standard using a similar apparatus (a quadrupole ICP-MS). Although such precision is "poor" compared to that provided by TIMS, it was considered sufficient to satisfactorily differentiate natural variations of the Sr isotopic abundance in geological samples [1,2].

6.4.1. Mass bias correction for strontium isotope ratio measurements

ICP-MS measurements are subject to several mass biases, which includes instrumental and sample induced bias. As mentioned in Chapter 3 and 4, in order to determine IRs accurately, correction of the observed data for the phenomena of mass bias is necessary [4,5]. In previous works on Sr determination by ICP-MS [1,2,6-8] only external correction has been applied.

In the present work, for comparison purposes, two different procedures for mass bias correction were applied: (i) external correction, with a Sr isotopic standard solution, and (ii) internal correction, using the $^{88}Sr/^{86}Sr$ IR.

The effectiveness of the internal mass bias correction was firstly investigated with a 100 $\mu g\ l^{-1}$ of Sr isotopic standard solution. For this purpose, three sequential measures of each Sr IR were performed and the $^{87}Sr/^{86}Sr$ ratio was corrected with the $^{88}Sr/^{86}Sr$ ratio using the power law mentioned earlier (see Chapter 3). As Table 6.3 shows, the correction originated an increase in the accuracy of the Sr IRs (and a decrease in the precision). The present results corroborate those obtained before by Latkoczy *et al* [8].

Table 6.3. Uncorrected and mass bias corrected (using the $^{88}Sr/^{86}Sr$ ratio) results[a] obtained for the Sr IR in a NIST SRM-987 Sr isotopic standard solution[b] (100 $\mu g\ l^{-1}$ Sr concentration).

	$^{87}Sr/^{86}Sr$		$^{88}Sr/^{86}Sr$
Measurement	**Uncorrected**	**Corrected**	
1	0.727 (1)	0.717 (3)	8.60 (3)
2	0.728 (3)	0.717 (4)	8.63 (3)
3	0.724 (2)	0.713 (5)	8.63 (1)

a: Mean values and standard deviation (calculated according to the propagation of errors, value affecting last digit);
b: Certified values: 0.71034 ±0.00026 for $^{87}Sr/^{86}Sr$ and 8.37861 ±0.00325 for $^{88}Sr/^{86}Sr$.

External and internal mass bias correction were then carried out, in parallel, for seven wine samples (after blank subtraction) and the results for the $^{87}Sr/^{86}Sr$ ratio compared. As shown in Table 6.4, no significant differences (pair-t test [9]) were found. Therefore, it could be concluded that both type of mass bias corrections were suitable in the present case. Internal mass bias correction has the disadvantage of requiring longer acquisition time (since it is necessary to measure the ^{88}Sr isotope). In addition, the high count rates of ^{88}Sr isotope measurements can cause problems on the detector when the Sr concentration markedly changes among the samples. However, the standard deviations associated with the mean value are higher with external mass bias correction than with internal correction (see Table 6.4). This limitation of the external mass bias correction was considered decisive to select the internal mass bias correction for the subsequent ICP-MS analysis of the wines.

Table 6.4. Comparison of the $^{87}Sr/^{86}Sr$ values obtained when external and internal mass bias correction were applied.

	$^{87}Sr/^{86}Sr$[a]	
Wine sample[b]	**External correction**	**Internal correction**
F_1R_1	0.721 (4)	0.725 (3)
F_3R_1	0.716 (4)	0.720 (1)
F_4R_5	0.710 (1)	0.710 (2)
T_2R_2	0.728 (4)	0.726 (2)
T_3R_3	0.710 (1)	0.712 (2)
T_4R_4	0.708 (4)	0.712 (2)
T_5R_6	0.702 (7)	0.703 (2)

a: Mean and standard deviation (value affecting last digit, n =3);
b: See the text for letters meaning.

6.5. STRONTIUM ISOTOPE RATIOS IN THE WINE SAMPLES

The $^{87}Sr/^{86}Sr$ ratio was determined in ten samples of different table (T_1-T_6) and fortified (F_1-F_4) wines. Eight samples were from five different Portuguese regions, Douro (R_1), Dão (R_2), Bairrada (R_3), Borba (R_4) and Madeira (R_5), and two were from one French region, Bordeaux (R_6).

The RSDs associated to the mean values (three independent replicates) of the $^{87}Sr/^{86}Sr$ ratio varied between 0.15 and 0.50 %, being lower than 0.3 % in most of the cases.

With the purpose of testing if among the analysed wines significantly differences in the values of the $^{87}Sr/^{86}Sr$ ratio would occur, the LSD statistical test [9] was applied. Thus, the Sr IRs were arranged in ascending order and the difference between adjacent values were compared with the calculated LSD

value. As shown in Fig. 6.2, some significant differences were found between wines of different regions. For example, the T_5R_6 and T_6R_6 wines, both from the Bordeaux region, displayed the lowest Sr IRs, which were significantly different from those observed for all the Portuguese wines. In addition, the wines from northeast of Portugal (Douro and Dão regions) displayed statistically different and higher Sr IRs than all the other Portuguese wines tested (from the centre/southern of Portugal and from Madeira Island). These results indicate that the precision of the Sr IR obtained with the proposed method was sufficient to distinguish Sr isotopic composition in wines. They also suggest that Sr IR $^{87}Sr/^{86}Sr$ is a promising fingerprint of the wine origin.

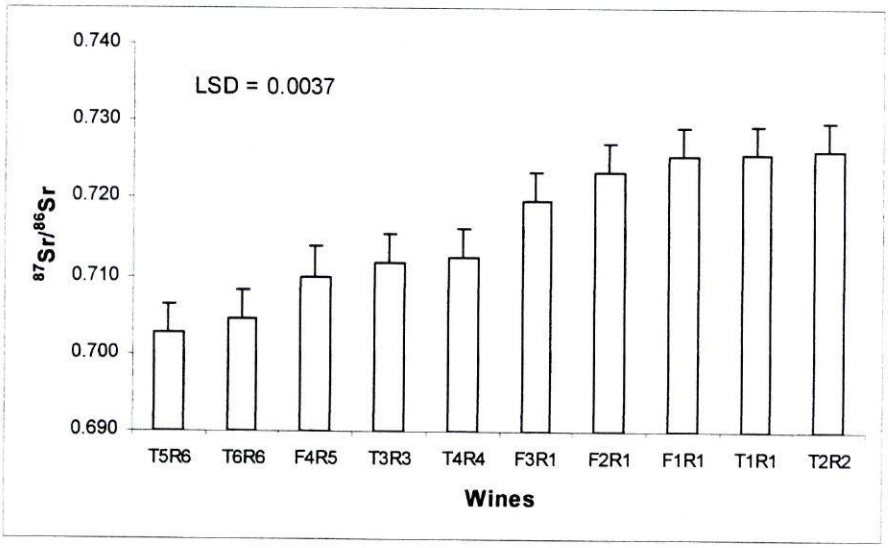

Fig. 6.2. Comparison of the mean Sr IRs through the LSD test [9]. The straight lines indicate the respective LSD value.

6.6. CONCLUSIONS

A suitable methodology for determination of the strontium isotope ratio $^{87}Sr/^{86}Sr$ in wines by ICP-MS was developed and applied to ten samples of table and fortified wines, from five different Portuguese regions and one French region.

After a sample pre-treatment by UV-irradiation, the solution was submitted to a two-step cation-exchange chromatographic separation for efficient elimination of rubidium interference.

Both external mass bias correction with a strontium isotopic standard solution and internal correction using the value of the $^{88}Sr/^{86}Sr$ ratio provided similar results, both showing to be suitable in the present experimental conditions.

The precision of the strontium isotope ratio obtained in wines with the proposed method (relative standard deviations < 0.3 %) was sufficient to distinguish strontium isotopic composition in wines from different regions. Therefore, the results suggest that $^{87}Sr/^{86}Sr$ ratio is a promising fingerprint of the wine origin.

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Chapter 7

Determination of $^{87}Sr/^{86}Sr$ in Wines by DRC-ICP-MS. A Comparison with Q and MC-ICP-MS

7.1. INTRODUCTION AND AIMS

The application of ICP-MS to the determination of the $^{87}Sr/^{86}Sr$ isotope ratio is, as mentioned before, not an easy task, due to the isobaric overlap of the signals of $^{87}Sr^+$ and $^{87}Rb^+$. Even when equipped with a double-focusing mass spectrometer, ICP-MS instruments are incapable of resolving spectroscopic interferences caused by isobaric nuclides [1]. So, strontium and rubidium have to be separated from each other before the analysis, which is normally accomplished by a rather tedious and time-consuming procedure, like cation-exchange chromatography, as described in the previous Chapter.

In a recent work, Moens and collaborators [2] showed that the $^{87}Sr^+/^{87}Rb^+$ isobaric interference could be efficiently eliminated by selective ion-molecule reactions in a dynamic cell situated in-between the interface and the mass analyser of an ICP-MS instrument. In that work, the strontium isotopic analysis of geological samples without previous rubidium/strontium separation was reported. The isobaric overlap of the signals from $^{87}Rb^+$ and $^{87}Sr^+$ was avoided by selectively converting the Sr^+ ions into the corresponding SrF^+ species by use of methylfluoride gas.

The aim of the work described in this Chapter, was the development of a methodology suitable for strontium isotope ratio determinations in wines using such a dynamic reaction cell (DRC) ICP-MS instrument. The procedure was based on that developed by Moens and collaborators [2] for geological material and methylfluoride gas was also used to avoid the isobaric overlap.

As far as we know, wine with a certified isotopic composition is only available for lead: *i.e.*, the wine IMEP-16, certified by the *Institute for Reference Materials and Measurements* from the *European Commission Joint Research Centre* (Geel, Belgium) (used in an inter-laboratory comparison programme, the *International Measurement Evaluation Programme*) [3]. The lack of wine reference materials with a certified strontium isotopic composition makes an evaluation of the accuracy of the data obtained not self-evident. To overcome this limitation, the same wines used in the previous Chapter were analysed according to the DRC-ICP-MS methodology developed. In the work reported in Chapter 6, strontium isotope ratio determinations were carried out using a quadrupole-based (Q) ICP-MS instrument (after chemical rubidium/strontium separation). Although the precision obtained (0.3 % relative standard deviation) was sufficient to distinguish the strontium isotopic composition of a few wines, the lack of a better precision can be a limiting factor for the use of $^{87}Sr/^{86}Sr$ as a tool for wine provenance determination. Therefore, highly precise strontium isotope ratio measurements were also carried out by multi-collector double-focusing sector field (MC) ICP-MS in the same wines (also after chemical rubidium/strontium separation) and the three sets of results were compared.

7.2. EXPERIMENTAL

The work described in this Chapter was carried out at the Laboratory of Analytical Chemistry of Ghent University, Belgium, under the supervision of Prof. Dr. Frank Vanhaecke. In this section, all the experimental procedures used in that lab are described.

The determinations by MC-ICP-MS were carried out by Dr. Jürgen Diemer at the Institute for Reference Materials and Measurements, Geel, Belgium. Experimental conditions and details are described elsewhere [4]. For these analyses, a separation of Sr from Rb had to be performed (similar to that preceding the Q-ICP-MS analysis, which is described in Chapter 6). This was carried out at the LAQUIPAI lab and was achieved by the double cation-exchange chromatographic separation procedure described in Chapter 3. Experimental details and conditions are described in Chapters 3 and 6.

7.2.1. Materials and reagents

Milli-Q water (18 MΩ cm, purified by means of a Millipore system) was used for preparing the standard solutions. 14 M HNO_3 was purified in-house by sub-boiling distillation in quartz equipment. For mass discrimination correction and for quality control, the isotopic reference material NIST SRM-987 $SrCO_3$ was used. All other reagents used were *pro analysis* or equivalent.

Methylfluoride, CH_3F, gas from Air Liquide, was used as a reaction gas in the dynamic cell.

7.2.2. Wine samples

In this work, eight of the wine samples used in the study reported in Chapter 6, were analysed. The samples F1R1 and F2R1, analysed in the work described in Chapter 6, were excluded from this study. The symbols are the same as presented in Chapter 6.

Two test wines of two different types, one red table wine (alcohol content ≈ 10 %) and one fortified wine (alcohol content ≈ 20 %), both from the Douro region (region R1), were used for optimisation purposes.

7.2.3. Analytical procedures

For the Sr IR determination in the wine samples by DRC-ICP-MS, a pre-treatment by either UV-irradiation or HPMW-digestion was carried out prior to analysis. No separation of Rb from Sr was performed. Details of both pre-treatment approaches can be found in Chapter 3.

For the analysis by Q-ICP-MS (described in Chapter 6) and by MC-ICP-MS, a separation of Sr from Rb had to be performed. This was achieved by the double cation-exchange chromatographic separation described in Chapter 3.

7.2.4. DRC-ICP-MS analysis

Measurements were carried out on a Perkin-Elmer Sciex Elan 6100 DRC^{plus} ICP-MS instrument (Perkin-Elmer, Concord, Ontario, Canada) equipped with a dynamic reaction cell [5]. The sample introduction system comprised a peristaltic pump, a Meinhard concentric nebulizer and a cyclonic spray chamber. Experimental conditions are summarised in Table 7.1.

Table 7.1. Instrumental settings and data acquisition parameters for DRC-ICP-MS.

RF power (W)	1200
Gas flow rates (l min^{-1}): Nebulizer	between 0.98 and 1.00
Auxiliary	1.20
Plasma	17
CH_3F gas flow rate (ml min^{-1})	between 0.20 and 0.25
Ar gas flow rate (ml min^{-1})	between 0.04 and 0.05
CPV (V)	-18.0
Lens setting (V)	between 6.5 and 8.5
CRO and QRO (V)	+3.00 and -4.00
RPa and RPq	0.00 and 0.35
Measured isotopes (m/z)	105, 106, 107
Dwell time (ms)	2
Number of sweeps	100
Number of readings	70
Number of replicates per sample	5
Settling time (μs)	200
Total measurement time per sample (min)	*ca.* 5

Since the reaction of Rb with the CH_3F gas is practically inexistent, no correction for isobaric interferences was carried out.

As previously mentioned, in order to determine IRs accurately, correction of the observed data for the detector dead time and the mass bias is necessary. For the latter, the two mass bias correction methods (external and internal) described in Chapter 3 were used and the results obtained will be discuss.

7.3. DRC-ICP-MS STRONTIUM ISOTOPE RATIO MEASUREMENTS

For the Sr IR measurements in the wines by DRC-ICP-MS, the procedure described by Moens and collaborators [2] was followed. CH_3F was used to selectively convert the Sr^+ ions into the corresponding SrF^+ species in order to avoid the isobaric overlap of the signals of $^{87}Rb^+$ and $^{87}Sr^+$.

7.3.1. Optimisation of instrument settings and data acquisition parameters

In a first stage, the instrument settings were optimised for the measurement of the $^{88}Sr^{19}F^+$ specie at m/z 107 with a Sr standard solution. The settings selected are presented in Table 7.1 and were checked regularly. The voltage values selected for cell path voltage (CPV), cell rod offset (CRO), quadrupole rod offset (QRO) and Lens were different from those used by Moens *et al* [2] despite the fact that similar equipment was used. Besides, during the time this study took place, optimisation of the lens settings had to be performed on a regular basis. At the moment no explanation for these differences is available and further studies will have to be carried out for a better understating of the DRC technology.

Data acquisition parameters were selected such as to obtain good precision and short measurement time (see Table 7.1).

7.3.2. Gas flow optimisation

After the optimisation of the instrument settings and data acquisition parameters, the CH_3F gas flow rate was optimised, using a standard solution containing 100 $\mu g\ l^{-1}$ of both Sr and Rb, to give the

maximum intensity at m/z = 107 (corresponding to $^{88}Sr^{19}F^+$). It was observed that the best flow rate was around 0.25 ml min^{-1} (see Fig. 7.1). At that flow rate, the signal intensity of the SrF^+ monitored attained a maximum and as expected, no significant formation of RbF^+ was established. Working at higher flow rates was not desirable since a decrease in the SrF^+ intensity was observed, probably due to collisional losses. Similar results were obtained by Moens and collaborators [2]. The increase observed in the signal intensity of $^{85}Rb^+$ was probably due to collisional focusing as a result of non-reactive collisions.

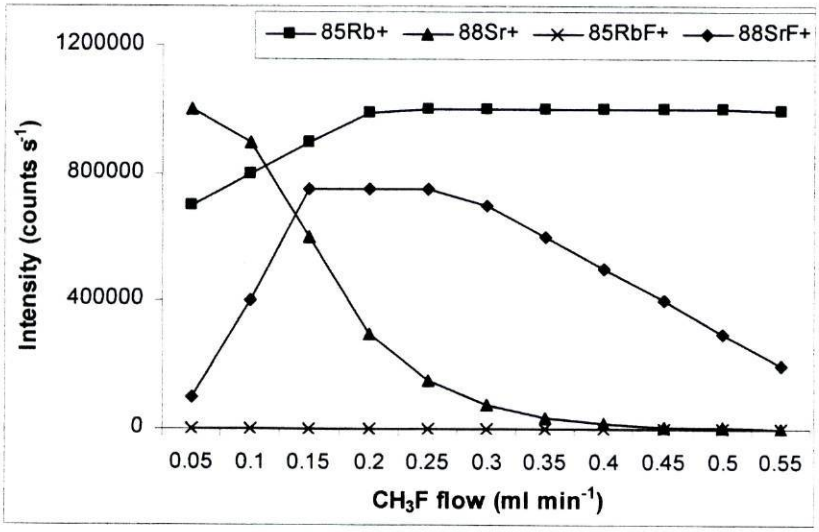

Fig. 7.1. CH_3F gas flow rate optimisation.

7.3.3. RPq parameter optimisation

In a second stage, the RPq parameter was optimised. Normally, when working in DRC mode, this parameter should be set at 0.45 to obtain the maximum ion transmission efficiency for plasma-derived ions. In this case, its value was optimised for the maximum intensity of the three SrF^+ signals of interest at m/z values of 105, 106 and 107. As observed in Fig. 7.2, for $^{88}Sr^{19}F^+$ (m/z 107), two maxima occurred around 0.25 and around 0.35 instead of 0.45. Similar results were obtained for $^{86}Sr^{19}F^+$ and $^{87}Sr^{19}F^+$. At the moment no explanation is available for the observed RPq influence and further studies will be necessary to give more insight into the origin of the two maxima. For the following analyses, a value of 0.35 was chosen.

A re-optimisation of the CH_3F flow rate was performed after adapting the RPq parameter setting, but no significant differences were observed due to the change in this parameter and the optimal value was still around 0.25 ml min^{-1}.

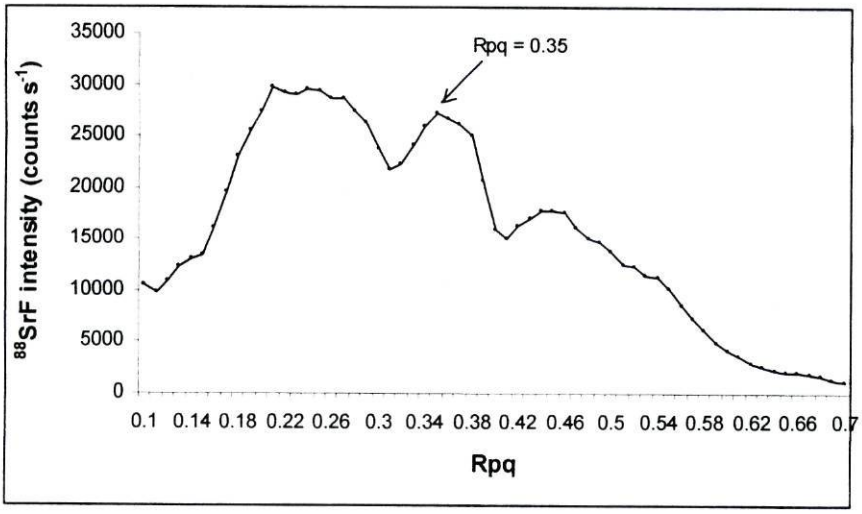

Fig. 7.2. Influence of the RPq parameter on the intensity of ^{88}SrF (m/z 107).

7.3.4. Buffer gas

The use of a buffer gas could, as reported by Moens and collaborators [2], improve the precision of the IR measurements since it would promote the temporal homogenisation of the ion population inside the cell. In this case, Ar was chosen as a buffer gas and its flow rate optimised at the optimal CH_3F flow rate of 0.25 ml min^{-1}. As observed in Fig 7.3, the optimal Ar flow rate that gives the maximum intensity for $^{88}Sr^{19}F^+$ would be 0.04 ml min^{-1}. A slight increase of this flow already brings about a drop in signal intensity, probably as a result of collisional losses.

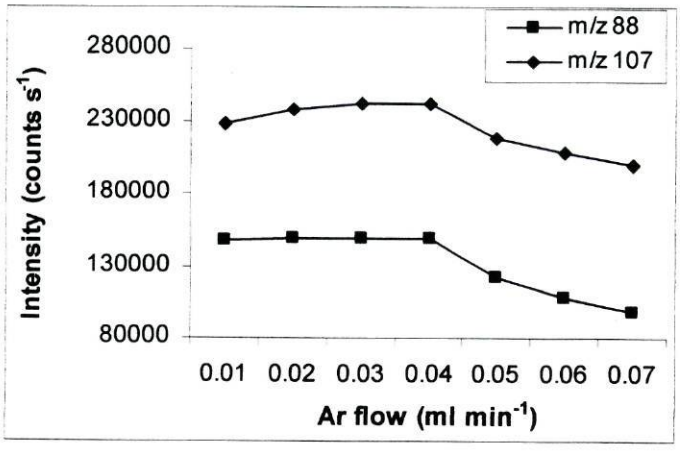

Fig. 7.3. Influence of Ar flow rate on the intensities of ^{88}Sr (m/z 88) and ^{88}SrF (m/z 107).

A re-optimisation of the CH_3F flow rate was performed since a new gas was being introduced in the DRC and the efficiency of the reaction could be affected. This time, a slight decrease in the optimum CH_3F flow rate was observed as the maximum intensity for $^{88}Sr^{19}F^+$ was attained at 0.20 ml min^{-1}. Nevertheless, both flow rates (CH_3F and Ar) were checked regularly during the experiments and slight changes in these values were observed to occur (see Table 7.1).

In order to test if there was really an improvement in the IR precision by using a buffer gas, a 50 $\mu g\ l^{-1}$ standard solution of Sr was measured five times using only the CH_3F at the optimum flow rate and five times using a mixture of CH_3F as a reaction gas and Ar as a buffer gas at their optimum flow rates. As can be seen in Table 7.2, there was a slight decrease of the Sr IRs, ($^{87}SrF/^{86}SrF$ and $^{88}SrF/^{86}SrF$ – species measured at m/z 105, 106 and 107) when using the Ar/CH_3F mixture but the standard deviations were lower. A statistical F-test was applied to the results and, at a 95 % confidence limit, it was observe that the use of the Ar/CH_3F mixture significantly improved the precision of the measurements. As a result, Ar was used as a buffer gas for all following analyses.

Table 7.2. $^{87}SrF/^{86}SrF$ and $^{88}SrF/^{86}SrF$ determined in a 50 $\mu g\ l^{-1}$ Sr standard solution measured five times using only CH_3F at optimum flow rate and five times using a mixture of CH_3F as a reaction gas and Ar as a buffer gas at their optimum flow rates.

	CH_3F				CH_3F + Ar			
Analysis	$^{87}SrF/^{86}SrF$	RSD (%)	$^{88}SrF/^{86}SrF$	RSD (%)	$^{87}SrF/^{86}SrF$	RSD (%)	$^{88}SrF/^{86}SrF$	RSD (%)
1	0.7282	0.27	8.7568	0.26	0.7248	0.16	8.6560	0.11
2	0.7271	0.12	8.7546	0.18	0.7242	0.17	8.6578	0.074
3	0.7274	0.22	8.7415	0.18	0.7247	0.17	8.6578	0.15
4	0.7282	0.26	8.7380	0.29	0.7250	0.21	8.6384	0.24
5	0.7264	0.25	8.7358	0.24	0.7251	0.20	8.6547	0.19

7.3.5. Kinetic effects

Preliminary tests with standard solutions and wine samples pointed out for the possibility of different reactions rates between the Sr isotopes and the CH_3F. Apparently, the lighter isotope (^{86}Sr) was reacting faster than the other two isotopes, ^{87}Sr and ^{88}Sr, indicating that kinetic effects seemed to be taking place in the reaction of Sr with the CH_3F. In order to prove the existence of such kinetic effects and to evaluate their extent, tests were carried out with standard solutions of different concentrations.

Standard solutions containing between 25 and 200 μg l^{-1} of Sr (isotopic standard) were analysed in standard mode (no reaction or buffer gas), in DRC mode using only Ar as a buffer gas (at its optimum flow rate) and in DRC mode using CH_3F as a reaction gas (at its optimum flow rate) and the ratios $^{87}Sr/^{86}Sr$, $^{88}Sr/^{86}Sr$, $^{87}SrF/^{86}SrF$ and $^{88}SrF/^{86}SrF$ were determined under all conditions. Since the reaction of Sr with the CH_3F gas is not complete [2], the ratios $^{87}Sr/^{86}Sr$ and $^{88}Sr/^{86}Sr$ could also be measured when using the DRC mode with the reaction gas.

As observed in Table 7.3, for each mode of analysis, the concentration of Sr did not influence the IRs. Nevertheless, the Sr IR results were significantly different (paired t-test [6]) from one mode of analysis to the other. As observed in Table 7.3 the results obtained in vented mode for the ratios $^{87}Sr/^{86}Sr$ and $^{88}Sr/^{86}Sr$ were higher than those obtained in the DRC mode with reaction gas for the ratios $^{87}SrF/^{86}SrF$ and $^{88}SrF/^{86}SrF$. These results pointed to the possible existence of kinetic effects: lighter isotopes, like ^{86}Sr, react faster with the CH_3F and the values of the ratios $^{87}SrF/^{86}SrF$ and $^{88}SrF/^{86}SrF$ become lower than the ones obtained in the vented mode. Besides, the ratios obtained in the vented mode were lower than the ratios $^{87}Sr/^{86}Sr$ and $^{88}Sr/^{86}Sr$ also measured in the DRC mode with reaction gas, which is additional evidence of a faster reaction of ^{86}Sr with CH_3F compare to that of the other two isotopes, ^{87}Sr and ^{88}Sr.

When comparing the vented mode with the DRC mode with buffer gas some significant differences were also observed among the values of $^{87}Sr/^{86}Sr$ and of $^{88}Sr/^{86}Sr$, which could be related either with scattering losses.

Table 7.3. Sr IRs[a] $^{87}Sr/^{86}Sr$, $^{88}Sr/^{86}Sr$, $^{87}SrF/^{86}SrF$ and $^{88}SrF/^{86}SrF$ determined in Sr standard solutions of different concentrations using the standard mode (no reaction or buffer gas), the DRC mode with only Ar as buffer gas and the DRC mode with CH_3F as reaction gas.

	Standard mode	DRC mode with buffer gas	DRC mode with reaction gas	
Solution	$^{87}Sr/^{86}Sr$	$^{87}Sr/^{86}Sr$	$^{87}Sr/^{86}Sr$	$^{87}SrF/^{86}SrF$
25 μg l^{-1}	0.730 (0.001)	0.719 (0.002)	0.749 (0.002)	0.698 (0.002)
50 μg l^{-1}	0.730 (0.001)	0.719 (0.001)	0.7574 (0.0007)	0.698 (0.002)
100 μg l^{-1}	0.731 (0.001)	0.717 (0.001)	0.744 (0.003)	0.699 (0.001)
200 μg l^{-1}	---	0.7189 (0.0008)	0.736 (0.001)	0.6982 (0.0004)
	$^{88}Sr/^{86}Sr$	$^{88}Sr/^{86}Sr$	$^{88}Sr/^{86}Sr$	$^{88}SrF/^{86}SrF$
25 μg l^{-1}	8.554 (0.001)	8.8084 (0.0005)	8.774 (0.001)	8.085 (0.002)
50 μg l^{-1}	8.566 (0.001)	8.802 (0.002)	8.758 (0.003)	8.107 (0.001)
100 μg l^{-1}	8.564 (0.001)	8.8043 (0.0007)	8.784 (0.003)	8.1189 (0.0006)
200 μg l^{-1}	---	8.7971(0.0009)	8.7803 (0.0006)	8.1314 (0.0007)

a: Mean of five measured replicates and standard deviation (in brackets).

7.3.6. Test wine samples

After optimisation of the instrument operating conditions, several tests were carried out first with Sr isotopic standard solutions with different alcohol contents (ranging from 0.5 % to 3 % ethanol percentage) and afterwards with the two test wines. The wines were ten-fold diluted with 1 % HNO_3 solution before the SrF IRs measurements.

Significant differences were observed in the SrF IRs measured in Sr isotopic standard solutions with different alcohol contents. For instance, for $^{87}SrF/^{86}SrF$ the values (internally corrected for mass bias with $^{88}SrF/^{86}SrF$) 0.715 ± 0.002, 0.702 ± 0.002, 0.692 ± 0.002 and 0.687 ± 0.001 were obtained for a 50 μg l^{-1} Sr standard solution without alcohol and with 0.5 %, 1 % and 3 % of ethanol, respectively. Therefore, alcohol-matched standard solutions should be used.

When analysing the wine test samples several problems were observed, like instability of the equipment and irreproducibility of the results. It was assumed that the cause was the alcohol content and the wine matrix, which is very complex. Higher dilution of the wine samples did not alleviate this problem to a sufficient extent. So, it was decided to carry out a pre-treatment of the wine samples that would remove the alcohol and simplify the wine matrix, in order to try to avoid the instability and irreproducibility characterising the results obtained for the diluted samples.

Therefore, an UV-irradiation of the wine samples was performed as a pre-treatment. This procedure was optimised previously for the determination of Pb IRs in fortified wines by Q-ICP-MS (see Chapter 4). This was also the sample pre-treatment used prior to the chromatographic separation of Rb from Sr performed to allow the measurement of the Sr IRs by Q-ICP-MS and by MC-ICP-MS. The pre-treated wine samples were used for the following analyses.

As mentioned earlier, in order to determine IRs accurately, correction of the observed data for mass bias is necessary. Two different procedures for mass bias correction, as described in Chapter 3, can be applied: (i) external correction, with a Sr isotopic standard solution, and (ii) internal correction, using the $^{88}Sr/^{86}Sr$ IR.

In this case, due to the kinetic effects observed, internal correction with $^{88}SrF/^{86}SrF$ IR should not be applied since this ratio will show a negative bias because $^{86}Sr^+$ reacts faster with CH_3F than $^{88}Sr^+$. So, external correction should be used.

In order to determine whether the matrix of the wines has an influence on the kinetic effects, in the case of which an aqueous isotopic standard of Sr could no longer be used for external mass bias

correction, also the Sr IR of one of the wine samples selected for this study was used as a "reference" value to correct the measured $^{87}SrF/^{86}SrF$ IR in the two test wines. The wine selected was F4R5 and the "reference" value used for its $^{87}Sr/^{86}Sr$ IR was the one determined by MC-ICP-MS (see below). As observed in Table 7.4, significant differences were found (paired t-test [6]) between the results corrected for mass discrimination with an aqueous standard and those corrected using another wine, indicating that probably matrix effects are present. So, IRs corrected with another wine (acting as a "reference" wine) should be considered. Nevertheless, the $^{87}SrF/^{86}SrF$ IRs obtained after this correction were still not in agreement with the MC-ICP-MS and Q-ICP-MS values. The two test wines used were from region R1, and based on the results obtained by both Q-ICP-MS and MC-ICP-MS, a value for the Sr IR $^{87}SrF/^{86}SrF$ around 0.725 should be obtained. The obtained value of around 0.736 (see Table 7.4) indicated that probably matrix and kinetic effects are related and influence one another.

Another pre-treatment of the two test wine samples, by HPMW-digestion, was performed and the same comparisons were made. The results obtained were similar to the ones obtained for the UV-irradiated samples, indicating that matrix effects were still present.

Table 7.4. Sr IR $^{87}SrF/^{86}SrF^{a}$ obtained in the two test wines (table and fortified) after mass bias correction by different procedures.

	Internal correction	External correction with Sr standard	External correction with F4R5 wine	Double correction (internal plus external with F4R5 wine)
Table wine				
1	0.7364 (0.0004)	0.7248 (0.0009)	0.736 (0.002)	0.722 (0.001)
2	0.737 (0.002)	0.726 (0.002)	0.737 (0.002)	0.723 (0.002)
3	0.7387 (0.0009)	0.725 (0.001)	0.736 (0.001)	0.726 (0.001)
Fortified wine				
1	0.7407 (0.0006)	0.724 (0.001)	0.734 (0.001)	0.729 (0.002)
2	0.7387 (0.0007)	0.723 (0.001)	0.733 (0.001)	0.727 (0.002)
3	0.741 (0.001)	0.726 (0.001)	0.739 (0.001)	0.728 (0.001)

a: Mean of five measured replicates and standard deviation (in brackets).

As presented in Table 7.4, the internally corrected IRs measured in the test wines were obviously wrong as they provided Sr IRs higher than expected and not encountered in terrestrial materials. But it was observed that the use of one of the wines as an external standard could compensate for this offset. This can be achieved by calculating a correction factor on the basis of "true $^{87}Sr/^{86}Sr$ value" and the experimental internally corrected $^{87}SrF/^{86}SrF$ result in a "reference" wine ("double correction"). As

before, the wine used as a "reference" was F4R5 and the reference value used for its $^{87}Sr/^{86}Sr$ IR was the one determined by MC-ICP-MS (see below).

Results obtained using this approach for the two test wines are also shown in Table 7.4 and were near the value of 0.725 expected for these two wines. Therefore, this approach seems promising to overcome the problems encountered (kinetic and matrix effects) and allow the determination of $^{87}Sr/^{86}Sr$ in wine samples without Rb/Sr separation. These kinetic and matrix effects are not understood yet and these issues are now the subject of a systematic study at Ghent University.

As observed in Table 7.4, no significant differences were found between three independent (including pre-treatment) replicates, therefore, for the analysis of the SrF IRs in the wine samples selected for this study, only one replicate of each type of wine was analysed.

7.4. COMPARISON BETWEEN THE RESULTS OBTAINED BY DRC, Q AND MC-ICP-MS

The $^{87}Sr/^{86}Sr$ IRs ($^{87}SrF/^{86}SrF$ in case of DRC-ICP-MS) measured in all eight wine samples selected for this study by the three different equipments are presented in Table 7.5. For the DRC-ICP-MS, the IRs presented are corrected by the four different procedures mentioned early: internal correction with $^{88}Sr/^{86}Sr$, external correction with $^{87}Sr/^{86}Sr$ measured in an aqueous standard solution of NIST SRM 987, external correction with the wine F4R5 and double correction (internal plus external with wine F4R5). For both Q and MC-ICP-MS, Sr IRs results were internally corrected for mass discrimination using the (constant) $^{88}Sr/^{86}Sr$ IR. The Q-ICP-MS results presented in Table 7.5 are those obtained in the study reported in the previous Chapter. For the MC-ICP-MS, three independent (including pre-treatment and chromatographic separation) replicates of each wine were analysed. Since no significant differences were observed, the Sr IRs presented in Table 7.5 are those obtained for a single replicate.

The estimated relative combined uncertainties (also given in Table 7.5) associated with the values of the $^{87}Sr/^{86}Sr$ IR determined by DRC-ICP-MS varied between 0.15 and 0.25 %. For the Q-ICP-MS values these uncertainties were between 0.15 and 0.50 %, being lower than 0.3 % in most of the cases, while for the Sr IRs determined by MC-ICP-MS ($k = 2$) the relative combined uncertainties were between 0.02 and 0.07 %. As expected, the uncertainties associated to the MC-ICP-MS results were lower (by approximately an order of magnitude), making it more suitable for wine origin discrimination.

Table 7.5. $^{87}Sr/^{86}Sr$ IRs ($^{87}SrF/^{86}SrF$ in the DRC-ICP-MS) measured in all the wine samples selected for this study by DRC-ICP-MS, Q-ICP-MS and MC-ICP-MS. For the DRC-ICP-MS the IRs presented are corrected by different procedures.

	DRC-ICP-MS[b]				Q-ICP-MS[c]	MC-ICP-MS[d]
Wine[a]	Internal correction	External correction with Sr standard	External correction with F4R5 wine	Double correction		
T1R1	0.7445 (0.0007)	0.728 (0.001)	0.736 (0.001)	0.726 (0.001)	0.726 (0.001)	0.7209 (0.0001)
T2R2	0.7511 (0.0009)	0.7289 (0.001)	0.736 (0.001)	0.732 (0.001)	0.726 (0.002)	0.7264 (0.0004)
T3R3	0.725 (0.001)	0.686 (0.001)	0.697 (0.002)	0.707 (0.002)	0.712 (0.002)	0.7104 (0.0003)
T4R4	0.7248 (0.0007)	0.702 (0.001)	0.717 (0.001)	0.709 (0.001)	0.712 (0.002)	0.7092 (0.0005)
T5R6	0.723 (0.001)	0.692 (0.001)	0.707 (0.001)	0.708 (0.001)	0.702 (0.002)	0.7086 (0.0005)
T6R6	0.7187 (0.0006)	0.6749 (0.0008)	0.690 (0.001)	0.705 (0.002)	0.704 (0.004)	0.7096 (0.0004)
F3R1	0.737 (0.001)	0.717 (0.001)	0.733 (0.001)	0.724 (0.001)	0.720 (0.001)	0.7188 (0.0002)
F4R5	0.7247 (0.0009)	0.699 (0.001)			0.710 (0.002)	0.7067 (0.0004)

a: For symbols meaning see text;
b: Mean of five replicates measured in one analysis and standard deviation (calculated according to errors propagation, in brackets;
c: Mean of three independent (pre-treated and chromatographic separated) replicates and standard deviation (in brackets);
d: Combined uncertainties (k =2) in brackets.

The uncertainty associated with the Sr IRs obtained by DRC-ICP-MS was slightly lower than that obtained using Q-ICP-MS, but still deviated substantially from that obtained using MC-ICP-MS.

As observed in Table 7.5, the Q-ICP-MS and the MC-ICP-MS data, from two different laboratories, were in good agreement with each other within the experimental uncertainties. A linear least-squares adjustment [6] of the Sr IRs values obtained by Q-ICP-MS (y-axis) for the eight wines against those obtained by MC-ICP-MS (x-axis) is shown in Fig. 7.4. No significant differences could be established between the adjustment curve ($y = (1.1 \pm 0.5)x - (0.1 \pm 0.4)$) and the ideal one ($y = x$), at the 95 % confidence level.

Comparatively, the internally corrected DRC-ICP-MS data appeared to be systematically biased. In fact, if any set of DRC-ICP-MS values corrected by a different procedure other than the "double correction" is considered several discrepancies are observed, indicating that those values cannot be used. For instance, T1R1 and F3R1 should be characterised by similar $^{87}Sr/^{86}Sr$ values since they come from the same region. This assumption is supported by the results obtained by Q-ICP-MS and MC-ICP-MS. But, as observed in Table 7.5, comparable results are only obtained if the measured IRs are externally corrected with the "reference" wine or "doubly corrected". For the wines T5R6 and T6R6 an identical situation is observed but in this case, only the "doubly corrected" IRs are similar. These results corroborates the previous ones obtained for the test wines, indicating that valid Sr IRs are only obtained if the internally corrected values were corrected by a "reference" wine ("double correction"). This correction points, once again, at both kinetic and wine matrix effects taking place during the DRC-ICP-MS measurements and causing biases. It is worth notice that in the work reported in [2] it was also not possible to use an aqueous standard, being used a matrix-matched isotopic standard in order to obtain reliable results.

Nevertheless, using this DRC-ICP-MS approach ("double correction") some differences were observed between the DRC results and those obtained by the other two equipments. For instance, the $^{87}Sr/^{86}Sr$ ratios obtained by DRC-ICP-MS for the T2R2 and F3R1 wines were significantly higher than the ratio obtained by the other two types of equipment, while the $^{87}Sr/^{86}Sr$ ratio obtained for the T3R3 was significantly lower. This may be an indication that some sources of error are still not identified and that further investigation is required. For comparison purpose, correlation plots are also presented in Fig. 7.4. The least-square adjustment [6] with the respective equation and correlation coefficient are included. As observed, between the Q-ICP-MS and DRC-ICP-MS values, the agreement was good with a high correlation coefficient ($R = 0.93$) and both the slope and intercept were not significantly different from one and zero, respectively, at the 95 % confidence level. Nevertheless, the DRC-ICP-MS Sr IRs

values showed not such a good agreement with the MC-ICP-MS results. Despite the high correlation coefficient (R = 0.99), the least-square adjust curve was statistically different from the ideal one, at the 95 % confidence level. This corroborates the fact that some sources of error may still exist.

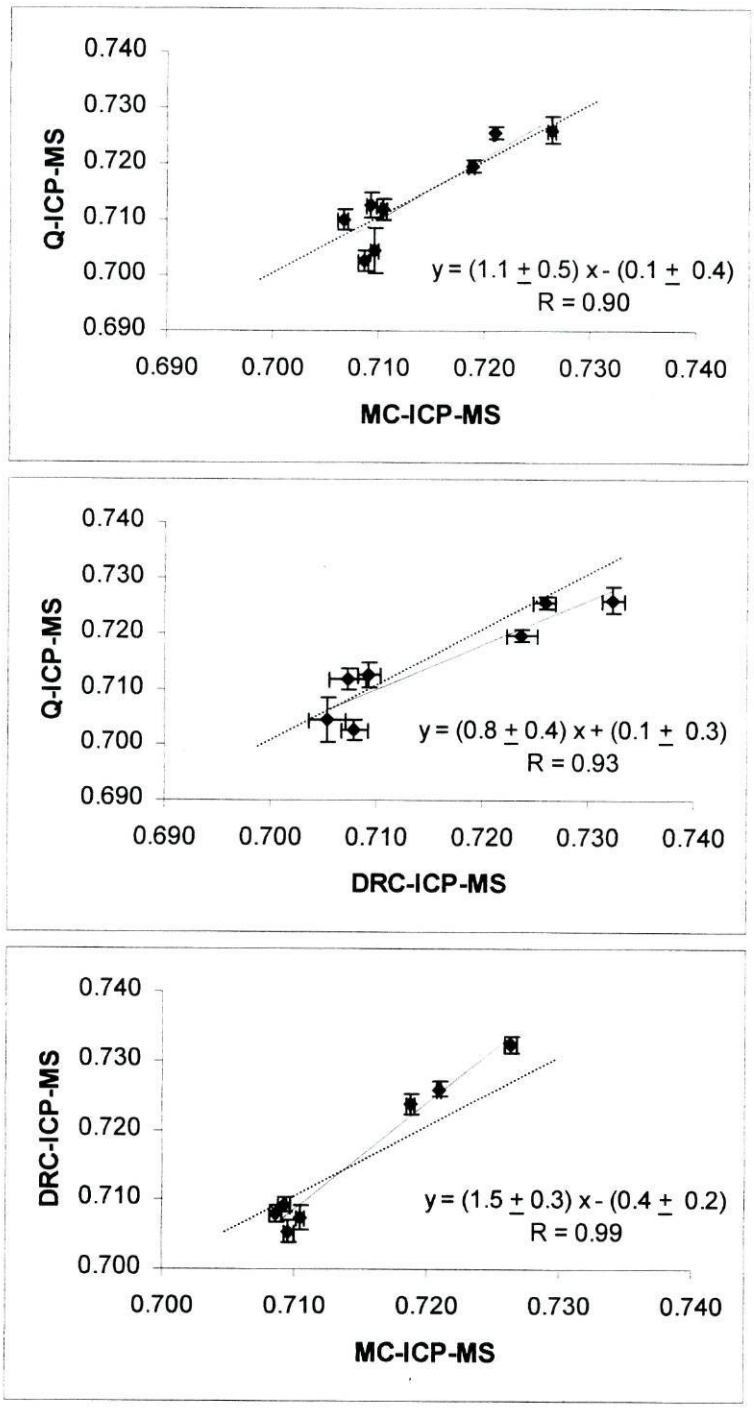

Fig. 7.4. Comparison of the results of the Sr IRs determined in the eight selected wine samples by the three ICP-MS equipments. Solid line: least square approximation. Dashed line: y = x curve. Least square adjustment equations and respective correlation coefficient are also presented (± values are the 95 % confidence limits).

Despite these differences, the trend in Sr isotopic composition of the wines obtained by the DRC-ICP-MS analysis was similar to that observed with the other two equipments, as can be seen in Fig. 7.5. So, with this DRC approach it was possible to differentiate between wines from different regions. The chosen "double correction" approach did not really interfere with the trend, but just shifted the entire set of results. However, the nature of the matrix and kinetic effects observed remain to be understood and the magnitude of the uncertainties associated with the various corrections implemented need to be assessed.

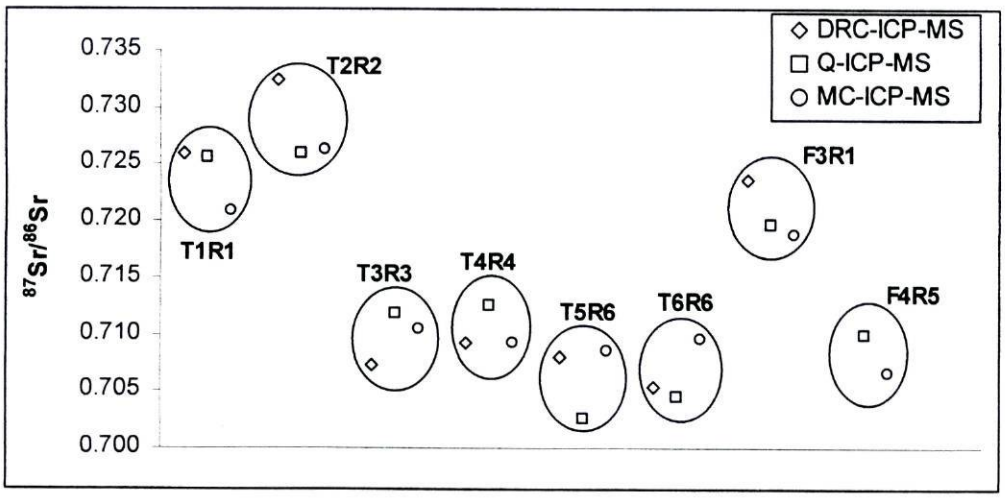

Fig. 7.5. $^{87}Sr/^{86}Sr$ ratios determine by DRC-ICP-MS (◊), by Q-ICP-MS (□) and by MC-ICP-MS (○) in eight samples of table (T1-T6) and fortified (F3 and F4) wines, from different Portuguese regions, Douro (R_1), Dão (R_2), Bairrada (R_3), Borba (R_4) and Madeira (R_5), and from one French region, Bordeaux (R_6).

7.5. CONCLUSIONS

The results obtained for the selected wine samples indicated that the DRC-ICP-MS methodology developed provide adequate strontium isotope ratio results for wine provenance determination as long as a "reference" wine is available. With this approach it was possible to differentiate between wines from different regions. Despite minor differences in some instances, the trend in strontium isotopic composition of the wines was similar to that observed with the other two equipments. The most important advantage of the DRC-approach is the possibility of measuring strontium isotope ratios without having to carry out the long and tedious separation procedures to remove rubidium previously. But, a careful evaluation of the measured strontium isotope ratios, as well as of the associated

uncertainties, must be performed and adequate correction for matrix and kinetic effects, which still remain to be understood, should be carried out in order to obtain valid results.

Although the precisions obtained with DRC-ICP-MS were better than the ones of the used Q-ICP-MS they were still far from the ones characteristic for MC-ICP-MS, what was expected since it is still a quadrupole-based ICP-mass spectrometer. Nevertheless, the procedure described here for strontium isotope ratios determination in wines by DRC-ICP-MS can be used for preliminary analysis in order to reduce the number of samples to be analysed by more accurate and precise equipment, such as MC-ICP-MS or TIMS.

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Chapter 8

Does Winemaking Process Influence the $^{87}Sr/^{86}Sr$ Ratio of Wines? A Case Study

8.1. INTRODUCTION AND AIMS

Trace elements concentrations in wines depend, among others factors, on geographical origin. Elements are taken up by the roots of plants, passing to the grapes with the same isotopic proportions in which they occur in the soil. Thus, the $^{87}Sr/^{86}Sr$ ratio can be used as a tracer of wine origin if a statistically significant correlation between this ratio in the soil and in the wine is observed. Therefore, significant influence of the winemaking process in the strontium isotope ratio of the wine must not take place. Horn *et al* [1] and Lancelot *et al* [2] demonstrated the existence of the required correlation for German and French wines, respectively. Nevertheless, studies like those are scarce. As wine processing changes from wine to wine, from winery to winery and from one country to another, more studies are required before one be able to generalise the suitability of $^{87}Sr/^{86}Sr$ as tracer of wine origin.

The aim of the study reported in this Chapter was to determine if the winemaking processes used for producing one red table and one fortified wine significantly changed the $^{87}Sr/^{86}Sr$ ratio present in the wines. Two vineyards and two wineries of the Douro region, Northeast of Portugal, were selected for this work and the strontium isotope ratio was determined in each wine and in the corresponding grape juice and vineyard soil. The strontium total concentration was also determined in grape juice and samples collected throughout the vinification processes in order to detect any eventual change in the strontium total concentration during the winemaking process.

8.2. EXPERIMENTAL

The vineyards and the winemaking processes were described in Chapter 3 – Experimental section.

The total concentration of Sr (Sr_{total}) was determined by ICP-MS using the semi-quantitative mode of analysis. Prior to the analysis of Sr_{total} in grape juices and samples collected in the different steps of the vinification processes, the samples were pre-treated by UV-irradiation and diluted two and half times with a solution containing HNO_3 and Rh. Final solutions always contained 20 $\mu g\ l^{-1}$ Rh and 1 % HNO_3. The same solutions were used for Sr IR determination, after removal of most of the Rb from the samples. A cation-exchange chromatographic procedure, described in the Experimental Section, was used for this purpose. After the chromatographic separation and acid evaporation, the Sr-containing fraction was diluted to 15.0 ml with 0.5 % HNO_3 solution, proving a three times dilution of the samples.

Soil composed samples, collected at 20 cm depth in three different sampling sites selected in the old vineyard, six month before the vintage (when the first grapes appeared), were used for determinations of $^{87}Sr/^{86}Sr$ in the soil. The samples were HPMW digested as described in Chapter 3 and diluted to 20.0 ml with de-ionised water. For removing Rb from the sample solutions a procedure similar to that carried for the remaining samples was used after optimisation. The chromatographic column and eluant were the same. The procedure consisted in a single extraction step, in which the 20.0 ml digested soil solution was introduced in the column. Most of Rb was eliminated in the first 80 ml of eluant and the Sr-containing fraction collected in the following 40 ml. The acid was removed by evaporation as described in Chapter 3 for wine samples, being the final solution diluted to 20.0 ml with 0.5 % HNO_3 solution. A blank solution was chromatographically separated in each run.

The $^{87}Sr/^{86}Sr$ values measured were internally corrected for mass bias with the constant $^{88}Sr/^{86}Sr$ ratio, using the power law described in Chapter 3.

Three independent replicates of each sample were prepared and analysed and, after blank subtraction, the mean and respective standard deviation were calculated.

Experimental conditions as well as analytical procedures are described in detail in the Experimental Section.

8.3. STRONTIUM TOTAL CONCENTRATION

The levels of Sr_{total} determined in GJ (GJ_F and GJ_T for the fortified and table wine, respectively) and in the samples collected throughout and at the end of the vinification processes (see Fig. 3.2A – fortified wine and 3.2B – table wine, in Chapter 3) are presented in Fig. 8.1. For the fortified wine (from grapes of the old vineyard), Sr_{total} increased about 215 % during the vinification, from 0.49 ± 0.04 mg l^{-1} in GJ_F to 1.56 ± 0.01 mg l^{-1} in W_FF. For the table wine (from grapes of the young vineyard) Sr_{total} increased only about 70 %, from 0.85 ± 0.01 mg l^{-1} in GJ_T to 1.45 ± 0.02 mg l^{-1} in W_TF.

A more detailed analysis of the data (Fig. 8.1) showed that the increase in Sr_{total} throughout both vinification processes was not regular. For the fortified wine a significant and marked increase in the Sr_{total} level was only observed at the beginning of the vinification (concentration higher in W_F1 than in GJ_F). This increase (about 190 %) may result of natural sources or of anthropogenic contamination introduced by the components of the vinification process (containers). The natural sources of Sr could be the seeds and skins of the grapes, which would release Sr into the must contributing for the Sr_{total} content

of the wine. It must be notice that for preparing the grape juice in the lab, manual pressing was performed and only the pomace was pressed. In contrast, W_F1 was obtained by mechanical pressing of the grapes in the winery, where not only pulp but also skin and some seeds are pressed together. It is know that some elements, like K, Ca, Fe, Mg, Na and Pb, have a heterogeneous distribution within the grape berry, being most of them in higher concentration in the seeds or skin of the berry than in the pulp [3,4]. Therefore, it can be assumed that other elements, including Sr, have different concentrations in the different parts of the grape berry. During the remaining steps of the vinification process the Sr_{total} variation was very little.

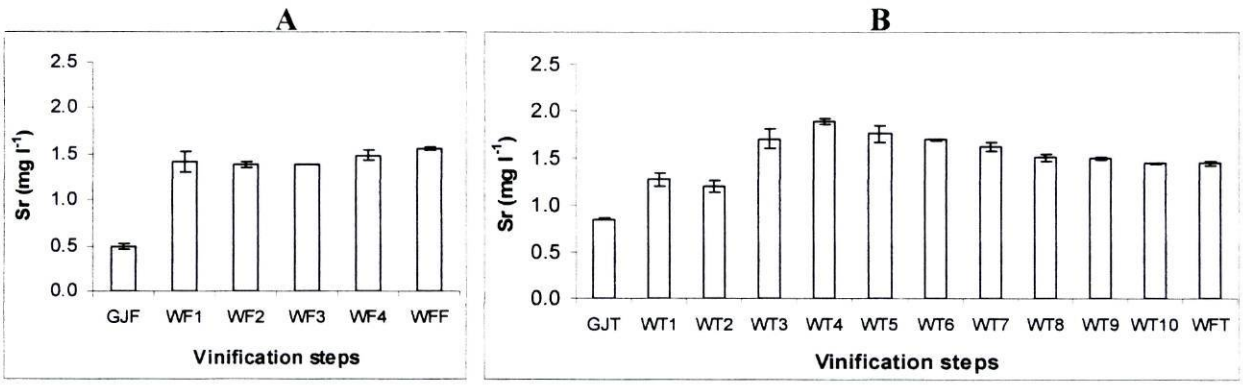

Fig. 8.1. Values of Sr_{total} obtained in the grape juices (GJ), in the samples collected throughout the vinification processes (from W_F1 to W_F4 for fortified and from W_T1 to W_T10 for table wine) and in the final products (WF). (A) red fortified wine produced with grapes from the old vineyard; (B) red table wine produced with grapes from the young vineyard.

As concerns the table wine, also a significant increase in Sr_{total} (about 110 %) was observed in the first steps of the vinification process, between W_T1 to W_T4 when compared with the Sr_{total} level in GJ_T. In the following steps, in which only a liquid phase was present (no further contact with seeds and skins) it was observed a slight but significant decrease in the Sr_{total} level, which could be related with the precipitation or co-precipitation of Sr with suspended colloidal particles during ageing. Decreasing of the concentration of other elements during ageing of wines has been observed, as discuss in Chapters 5 and 12.

These results suggest that, in both vinifications, the relevant sources of Sr were in the first steps of the processes when the must was in contact with seeds and skins. Therefore, the increase of Sr_{total} observed in WF relatively to GJ possibly resulted of the relatively high content of this element in seeds and skins, which passed to the must mostly at the initial mechanical pressing and further contact

between pulp and those solid components during the fermentation. Nevertheless, the hypothesis of anthropogenic contamination during the first vinification steps should not be rejected.

8.4. STRONTIUM ISOTOPE RATIO

As previously mentioned, $^{87}Sr/^{86}Sr$ may vary with the origin of the element. If anthropogenic contamination did not occur during the vinification, the Sr IR will be the same in GJ and in the respective WF. To test this hypothesis, that Sr IR was determined in GJ and WF, as well as in the vineyard soil.

The measurements took place in a simple working day and the short-term precision of the $^{87}Sr/^{86}Sr$ ratio was measured in the $SrCO_3$ (isotopic) standard NIST SRM-987 (that was run regularly for quality control), being of 0.4 %. This value was similar to that previously obtained (study reported in Chapter 6). Nevertheless, the RSDs associated with the Sr IR in the standard solutions (that varied between 0.2 and 0.7 %) were slightly higher than those obtained previously, indicating some instability of the instrument, which was not possible to compensate.

The mean and the standard deviation of the Sr IR determined in GJ, WF and soil are presented in Table 8.1. The RSDs were around 0.7 %, therefore higher than that previously obtained in wine samples (Chapter 6), probably due to the already mentioned instability of the instrument. Table 8.1 shows that the values of $^{87}Sr/^{86}Sr$ were similar in GJ and respective produced wines. Therefore, the vinification processes did not influence significantly the Sr IR, which is compatible with no significant Sr anthropogenic contamination. This result corroborates the hypothesis above formulated, that the increase of Sr_{total} in the first steps of the vinification processes probably resulted of release into the must of Sr from skins and seeds of grapes instead of anthropogenic contamination.

Table 8.1. Sr IR[a] values obtained in grape juices, wines and soil.

	Old vineyard			Young vineyard	
	Grape juice	Wine	Soil	Grape juice	Wine
$^{87}Sr/^{86}Sr$	0.727	0.729	0.732	0.731	0.729
SD	0.006	0.005	0.006	0.004	0.006

a: Mean and standard deviation (SD) calculated attending to errors propagation (n = 3). Results corrected for mass bias discrimination with $^{88}Sr/^{86}Sr$.

In addition, a statistical comparison through t-paired test [5] between Sr IR in wines and soil indicated that they did not differ significantly, which makes $^{87}Sr/^{86}Sr$ suitable to be used as a tracer of the origin of the studied wines.

8.5. CONCLUSIONS

Two Portuguese wines, one fortified and another table wine, produced by two different winemaking processes with grapes of two distinct vineyards, were selected to study the influence of the vinification on the strontium isotope ratio $^{87}Sr/^{86}Sr$ and the possible existence of a correlation between this isotope ratio in wine and respective soil.

The strontium total concentration was significantly higher in the produced wine than in the respective grape juice, being the increase higher in the fortified than in the table wine. The enrichment in Sr was attributed to the releasing of the element into the must from the seeds and skins of the grapes instead of anthropogenic contamination. This hypothesis was compatible with the statistically identical $^{87}Sr/^{86}Sr$ values observed in grape juices and in the respective wines.

The $^{87}Sr/^{86}Sr$ values were also statistically identical in the wines and soil.

The strontium isotope ratio obtained for both studied wines (as well as for the soil and the grape juices) was identical to that observed in other wines from the same region – Douro region, Northeast of Portugal, reported in Chapters 6 and 7.

These results indicate that $^{87}Sr/^{86}Sr$ is suitable to be used as a tracer of these wines' origin.

Although a higher number of wine samples and winemaking processes need to be study to validate the obtained results, they are promissory as concerns the suitability of this strontium ratio to be use for wine fingerprinting.

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Chapter 9

Advantages and Limitations of the ICP-MS Semi-Quantitative Operation Mode for Multi-Element Analysis of Wines

9.1. INTRODUCTION AND AIMS

ICP-MS offers different quantification procedures depending on the accuracy and precision required. When the highest quality of results is needed, isotope dilution mode of analysis is the choice, whereas quantitative mode of analysis is the default strategy [1]. The quantitative mode of analysis in ICP-MS requires external calibration with standards of each element to be determined. This strategy is time consuming and it is not easy to have a complete set of the multi-element standards required for the calibration. A third option, the semi-quantitative mode of analysis, is a versatile application of ICP-MS that it is claimed of allowing the determination of about 80 elements with accuracy errors lower than 20 % for most elements [1-3]. This methodology has been successively applied to samples of different nature and origin, like biological [3-5], environmental [5-7], industrial [8], food [9-12] and plastics [13]. The semi-quantitative analysis software available for commercial ICP-MS instrumentation (*e.g.*, TotalQuant II from Perkin-Elmer) has facilitated the rapid acquisition of analytical data, by correcting automatically isobaric and molecular interferences as well as relative isotope abundances. This type of analysis is based on a pre-calibrated internal response (defined as ions per second per concentration unit) for all elements, which can be update with a single-point calibration [3,14].

As previously referred in the Introduction Section, ICP-MS can be an import tool for elemental characterisation of wines and several publications can be found in the literature, reporting its use for multi-elemental determinations in wines, *e.g.* [15-18]. In all these works, the quantitative mode of analysis has been used.

Results of the application of ICP-MS semi-quantitative mode of analysis to wine samples are still scarce in the literature. Jakubowski *et al* [10] carried out (using a VG Elemental (quadrupole) ICP-MS equipment with a cooled nebulizer) a comparative study of the concentrations of REEs in young and finished product wines. Using similar equipment, Castiñeira *et al* [12] developed a procedure for determination of trace elements in wines, by studying the matrix effects of ethanol and by comparing the results obtained by ICP-MS with those obtained by total reflection X-ray fluorescence spectrometry. Pérez-Jordán *et al* [11] determined (using a Perkin-Elmer (quadrupole) ICP-MS equipment) major, minor and trace components by using both quantitative and semi-quantitative ICP-MS modes of analysis. Those authors concluded that the semi-quantitative mode of analysis represented a fast way of obtaining information about as many elements as possible in wine samples in a short analysis time. In the three mentioned works only table wines were studied. Castiñeira *et al* [12] studied just red wine,

while in the two other works [10,11] both red and white wine samples were tested. In all cases, the wine pre-treatment consisted only of 1:1 dilution of the wine samples, which is not suitable for ICP-MS analysis of fortified wines.

The first aim of the present work (already published [19]) was the optimisation of methodologies for multi-element analysis in wines using a Perkin-Elmer ICP-MS apparatus. Both the quantitative and semi-quantitative modes of analysis were used, and the results were compared. The evaluation of the advantages and limitations of the semi-quantitative mode of analysis comparatively to the respective quantitative mode, for multi-element (major, minor and trace elements) characterization of both table (red and white) and fortified wines constituted a second aim of this work. Particularly, one was intended to evaluate the suitability of the semi-quantitative mode of ICP-MS analysis for provenance testing of different wines.

9.2. EXPERIMENTAL

The studied wines were one red and one white table wines and one Port wine. The samples were chosen to represent three different types of wines. For each wine sample three independent replicates were analysed.

For ICP-MS analysis, all wine samples were pre-treated by UV-irradiation followed by dilution to 11.0 ml (providing a 2.2 final dilution of the wine) with a solution containing HNO_3 and Rh. These solutions were used for both quantitative and semi-quantitative modes of analysis. For determination of Al, Cu, Fe, Mn, Rb, Sr and Zn in the quantitative mode, higher dilutions (ten or twenty (only Fe) times) of the wine samples were necessary and the proper solutions were prepared accordingly. In all cases, the final sample solutions, as well as the standard solutions, contained 20 $\mu g\ l^{-1}$ Rh and 1 % HNO_3.

For validation of the ICP-MS results obtained using the quantitative mode of analysis, a few elements (Cd, Cu, Fe, Mn, Pb and Zn) were also determined by AAS in the three wine samples and the results compared. For this analysis the wine samples were diluted four times with de-ionised water.

Experimental conditions and details are presented in Chapter 3.

In an early step of the work, the limits of detection (LODs) and quantification (LOQs) of the elements of interest were determined. The LODs were determined as being the concentrations

corresponding to signals equal to three times the standard deviation of a blank solution signal (ten replicates), while the LOQs were those corresponding to ten times the same standard deviation.

In addition, recovery tests were carried out for wine samples spiked with 1 to 200 $\mu g\ l^{-1}$ standard solution (depending of the element). Three different spikes of each element for each wine sample were performed and the solutions obtained were analysed using the ICP-MS quantitative mode. Then, the mean and the respective standard deviation were calculated from the three recovery values obtained in the three spiked samples.

9.3. QUANTITATIVE MODE OF ANALYSIS

Thirty-one elements (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Ga, Li, Mn, Ni, Pb, Rb, Sr, V, Zn and the REEs La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu), for which standards are easily available, were selected for the present study.

The LODs were determined and presented in Table 9.1. The LODs were lower than those reported by Baxter *et al.* [18] and similar to those published by Pérez-Jordán *et al* [11]. For the REEs, the values were in agreement with those obtained by Stroh *et al.* [16] using a similar ICP-MS equipment.

Recovery tests were carried out for the three wine samples under study. As Table 9.2 shows, with the exception of As, recovery percentages between 80 % and 120 % were obtained for all the wines, with most of the values around 100 %. These percentages were in agreement with those obtained by Baxter *et al.* [18] and Pérez-Jordán *et al.* [11], for the same elements measured in table wines by ICP-MS.

For V, Cr and Fe the recovery percentages were higher than 110 % probably because the matrix-induced interference was not properly corrected in the case of Fe or not corrected at all in the case of Cr and V (interference from Cl species).

For Li the high recovery percentages (around 115 %) may be due to the fact of Rh not being an appropriate internal standard for such low mass element. Rh may also not be an appropriate internal standard for very high mass elements, which may explain the low recovery percentages observed for Pb (83-84 %). Additional tests were carried out using Tl as an internal standard, but only a slight improvement was observed for Pb (around 86 %).

Table 9.1. Limits of detection (LODs) and concentrations of the several elements (μg l^{-1} or mg l^{-1}, see below) obtained by ICP-MS[a], using both the quantitative (Q) and the semi-quantitative (SQ) modes of analysis, for the three wine samples. Values in bold were below or near the limit of quantification For a few elements, the results obtained by AAS are also included.

Element	LODs (Q)	LODs (SQ)	Red wine			White wine			Port wine		
			AAS	ICP-MS (Q)	ICP-MS (SQ)	AAS	ICP-MS (Q)	ICP-MS (SQ)	AAS	ICP-MS (Q)	ICP-MS (SQ)
Li	0.01	0.08		8.8 (0.2)	5.22 (0.03)		7.09 (0.06)	3.8 (0.3)		42.7 (0.9)	33.7 (0.8)
Be		0.2			4 (1)			4.4 (0.2)			7.5 (0.7)
B[b]		15			5.8 (0.2)			3.5 (0.2)			5.2 (0.2)
Al	1	0.3		696 (18)	607 (23)		871 (6)	758 (9)		1008 (22)	832 (27)
Ca[b]		34			62 (3)			87 (4)			59 (1)
Sc		ND[c]			23.3 (0.6)			21 (2)			36 (2)
Ti		ND[c]			216 (16)			223 (7)			171 (12)
V	0.005	0.002		52 (1)	43.2 (0.3)		100 (5)	97 (2)		13.4 (0.4)	11.2 (0.1)
Cr	0.2	0.1		123 (3)	109 (4)		62 (3)	54 (2)		75 (4)	65 (3)
Mn	0.03	0.08	1029 (16)	1180 (23)	1061 (7)	691 (3)	799 (16)	728 (5)	2321 (16)	2676 (25)	2522 (88)
Fe	0.7	2	4118 (50)	3539 (333)	3219 (245)	1556 (31)	1387 (31)	1337 (174)	1092 (10)	1358 (78)	1277 (116)
Co	0.02	0.04		3.86 (0.08)	3.01 (0.07)		2.9 (0.1)	2.3 (0.1)		11.2 (0.2)	9.4 (0.1)
Ni	0.2	0.1		81.7 (0.7)	76 (2)		16.1 (0.5)	20.0 (0.3)		36 (1)	35 (1)
Cu	0.07	0.2	126 (1)	127 (1)	115 (1)	[d]	37.5 (0.4)	34 (2)	446 (2)	442 (4)	445 (1)
Zn	0.2	1	1046 (11)	1211 (13)	1154 (27)	1256 (11)	1382 (18)	1343 (6)	760 (29)	1039 (18)	999 (4)
Ga	0.005	0.002		3.5 (0.1)	1.00 (0.07)		3.0 (0.1)	0.41 (0.01)		3.59 (0.01)	1.2 (0.2)
Ge		ND[c]			NDT[c]			NDT[c]			NDT[c]
As	0.07	0.4		ND[c]	ND[c]		ND[c]	ND[c]		ND[c]	ND[c]
Se		3			< LOD			< LOD			< LOD
Rb	0.01	0.04		1917 (41)	1799 (4)		921 (12)	863 (9)		1903 (23)	1797 (20)
Sr	0.01	0.02		247 (4)	254 (5)		238 (4)	241 (2)		1269 (20)	1218 (30)
Y		ND[c]			2.8 (0.4)			1.32 (0.06)			6.3 (0.1)
Zr		ND[c]			2.7 (0.8)			3.6 (0.7)			10.5 (0.6)
Nb		ND[c]			0.36 (0.01)			1.20 (0.06)			0.64 (0.02)
Mo		2			5.7 (0.4)			21 (2)			< LOD
Ru		ND[c]			NDT[c]			NDT[c]			NDT[c]
Pd		ND[c]			NDT[c]			NDT[c]			NDT[c]
Ag		0.1			< LOD			< LOD			< LOD
Cd	0.09	0.07	[d]	**0.16 (0.03)**	< LOD	[d]	**0.29 (0.06)**	< LOD	0.93 (0.08)	0.98 (0.06)	0.42 (0.06)
In		ND[c]			NDT[c]			NDT[c]			NDT[c]
Sn		ND[c]			NDT[c]			NDT[c]			NDT[c]
Sb		ND[c]			NDT[c]			NDT[c]			0.73 (0.09)

Table 9.1. *continuation*

Te		0.02			< LOD			< LOD			< LOD
Cs		ND[c]			3.47 (0.01)			1.04 (0.05)			19.8 (0.4)
Ba	0.01	0.04		103.5 (0.2)	94.9 (0.2)		75 (3)	70 (2)		61 (2)	54 (1)
La	0.006	0.003		0.366 (0.002)	0.21 (0.01)		0.20 (0.02)	0.12 (0.01)		0.532 (0.005)	0.29 (0.01)
Ce	0.003	0.004		0.62 (0.02)	0.37 (0.04)		0.35 (0.03)	0.246 (0.006)		1.04 (0.01)	0.73 (0.08)
Pr	0.0009	0.003		0.099 (0.002)	0.059 (0.013)		0.057 (0.005)	<LOD		0.169 (0.002)	0.114 (0.006)
Nd	0.004	0.006		0.363 (0.005)	0.18 (0.03)		0.18 (0.01)	0.081 (0.013)		0.66 (0.01)	0.356 (0.006)
Sm	0.004	0.005		0.068 (0.006)	< LOD		0.033 (0.002)	< LOD		0.163 (0.009)	< LOD
Eu	0.001	0.003		0.032 (0.004)	< LOD		0.022 (0.002)	< LOD		0.049 (0.002)	< LOD
Gd	0.004	0.007		0.087 (0.005)	< LOD		0.051 (0.004)	< LOD		0.197 (0.003)	< LOD
Tb	0.0004	0.002		0.025 (0.003)	< LOD		0.0173 (0.0006)	< LOD		0.038 (0.001)	< LOD
Dy	0.003	0.004		0.066 (0.003)	< LOD		0.039 (0.003)	< LOD		0.164 (0.003)	< LOD
Ho	0.0007	0.004		0.031 (0.002)	< LOD		0.0229 (0.0004)	< LOD		0.0458 (0.0006)	< LOD
Er	0.001	0.003		0.057 (0.002)	< LOD		0.041 (0.004)	< LOD		0.105 (0.003)	< LOD
Tm	0.0008	0.002		0.020 (0.002)	< LOD		0.0165 (0.0003)	< LOD		0.024 (0.001)	< LOD
Yb	0.005	0.003		**0.032 (0.002)**	< LOD		**0.018 (0.006)**	< LOD		0.070 (0.003)	< LOD
Lu	0.0006	0.004		0.024 (0.002)	< LOD		0.0205 (0.0008)	< LOD		0.029 (0.003)	< LOD
Hf		ND[c]			NDT[c]			NDT[c]			NDT[c]
Ta		ND[c]			NDT[c]			NDT[c]			NDT[c]
W		ND[c]			1.24 (0.01)			0.32 (0.03)			NDT[c]
Re		ND[c]			NDT[c]			NDT[c]			NDT[c]
Os		ND[c]			NDT[c]			NDT[c]			NDT[c]
Ir		ND[c]			NDT[c]			NDT[c]			NDT[c]
Pt		ND[c]			NDT[c]			NDT[c]			NDT[c]
Au		ND[c]			NDT[c]			NDT[c]			NDT[c]
Hg		ND[c]			NDT[c]			NDT[c]			NDT[c]
Tl		0.03			< LOD			< LOD			0.21 (0.01)
Pb	0.02	0.05	13.2 (0.8)	12.9 (0.2)	11.5 (0.2)	15 (1)	13.57 (0.08)	12.7 (0.2)	80 (3)	81 (3)	76 (3)
Bi		2.1			< LOD			< LOD			< LOD
Th		ND[c]			NDT[c]			NDT[c]			NDT[c]
U		0.03			< LOD			0.21 (0.01)			0.41 (0.01)

a: Concentrations values (after a correction for the dilution factors) correspond to the mean of three replicates and the corresponding standard deviation (in brackets);
b: Concentrations values in mg l^{-1};
c: ND = not determined; NDT = not detected;
d: Below the limit of detection of the method (0.70 and 40 μg l^{-1} for Cd and Cu, respectively);
ICP-MS: Inductively coupled plasma mass spectrometry; AAS: Atomic absorption spectroscopy;

For As, average recovery percentages of 267 ± 35 % were obtained. This indicates that the correction of the matrix-induced polyatomic interference of Cl ($^{40}Ar^{35}Cl$) was not effective for this element. Efforts were made in order to solve the problem without success. Therefore, it was concluded that As could not be measured in the wine samples with the present ICP-MS equipment and methodology.

Table 9.2. Recovery percentages (%)[a] obtained by ICP-MS using the quantitative mode of analysis. The wine samples were spiked with known amounts of elements from 1 to 200 μg l^{-1} (depending of the element).

Element	Red wine	White wine	Port wine
Li	117 ± 8	114 ± 3	120 ± 5
Al	102 ± 3	101 ± 3	105 ± 2
V	115 ± 5	115 ± 4	118 ± 2
Cr	106 ± 6	111 ± 6	127 ± 14
Mn	97 ± 2	102 ± 1	99 ± 4
Fe	126 ± 11	128 ± 7	116 ± 4
Co	104 ± 4	102 ± 2	102 ± 1
Ni	97 ± 2	101 ± 2	106 ± 4
Cu	94 ± 2	97 ± 1	99 ± 3
Zn	95 ± 3	95 ± 2	98 ± 2
Ga	96 ± 3	100 ± 1	102 ± 4
As	235 ± 15	262 ± 61	304 ± 16
Rb	97 ± 3	97 ± 1	93 ± 5
Sr	103 ± 2	104 ± 1	99 ± 6
Cd	103 ± 3	105 ± 4	98 ± 4
Ba	83 ± 1	89 ± 4	88.0 ± 0.4
La	95.8 ± 0.6	97.5 ± 0.8	ND[b]
Ce	98 ± 2	97 ± 6	ND[b]
Pr	97 ± 1	96.7 ± 0.3	ND[b]
Nd	100 ± 2	98 ± 1	ND[b]
Sm	104 ± 4	96.0 ± 0.4	ND[b]
Eu	102 ± 1	99.0 ± 0.3	ND[b]
Gd	101.1 ± 0.2	98 ± 1	ND[b]
Tb	102.3 ± 0.5	99 ± 2	ND[b]
Dy	101 ± 1	99 ± 1	ND[b]
Ho	101.4 ± 0.4	99.4 ± 0.1	ND[b]
Er	100 ± 3	98.8 ± 0.3	ND[b]
Tm	99 ± 1	99 ± 1	ND[b]
Yb	102 ± 3	101 ± 2	ND[b]
Lu	101.7 ± 0.7	101 ± 1	ND[b]
Pb	84 ± 2	83 ± 1	84 ± 5

a: Mean of recovery values of three different spikes ± standard deviation;
b: ND = not determined.

The quantitative mode of analysis was then applied to multi-element analysis in the three wine samples. For each wine sample three independent replicates were analysed and the mean and the respective RSD calculated for each of the thirty selected elements (As was excluded). The obtained results are in Table 9.1. The RSDs obtained were between 0.5 and 5 %, being lower than 3 % in most of the cases. These precisions were slightly better than those obtained by Pérez-Jordán *et al.* [11] and similar to those reported by Stroh *et al.* [16], both for table wines analysed with a similar ICP-MS equipment and just a 1:1 sample dilution as pre-treatment.

Higher RSDs were observed only for Fe in the red table wine, Cd and most of the REEs in the table wines. The concentrations of Cd and of the few REEs in the mentioned samples were relatively low, in most cases close or below the respective LOQ, which explains the relatively high RSDs (between 6 % and 30 %) observed for these elements. For Fe, an RSD of 9.4 % was obtained in the red table wine sample probably due to experimental errors.

No significant differences were observed among the three types of wines in terms of LODs, recovery percentages or precisions, which indicate that the ICP-MS results were not influenced by the matrix of the wine samples after pre-treatment.

The results obtained by *AAS analysis*, for the elements Cd, Cu, Fe, Mn, Pb and Zn, were included in Table 9.1.

A linear least squares adjustment was applied to all elements determined, in parallel, by both AAS (x-axis) and quantitative mode ICP-MS (y-axis) (results in Fig. 9.1). The equation ICP-MS = (0.9 ± 0.1) AAS - (129 ± 157), with a correlation coefficient of R = 0.98 (n = 15), was obtained. Evidence of either relative or fixed bias in the measured range was not observed.

A statistical comparison through t-paired test [20] was also performed. Significant differences were not found between the results obtained by the different analytical methodologies for Cu, Pb and Cd. For Fe and Mn in the three tested wines and Zn in the two table wines, the differences were around 15 %, but statistically significant. For Zn in the Port wine sample a significant difference around 30 % was observed.

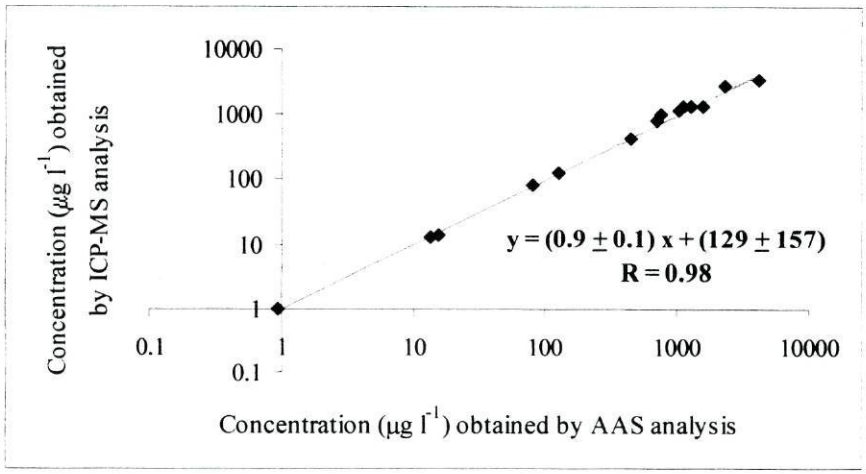

Fig. 9.1. Comparison, by linear regression, between AAS and ICP-MS results obtained for the elements Cd, Cu, Fe, Mn, Pb and Zn measured in common in the three wine samples (the 95 % confidence limits of the linear regression parameters are given). In both axis is represented the logarithm of concentrations (concentrations expressed in $\mu g\ l^{-1}$) for a larger scattering of the points.

9.4. SEMI-QUANTITATIVE MODE OF ANALYSIS

The semi-quantitative mode of analysis was applied to sixty-three elements at major, minor and trace concentration levels, including those measured in quantitative mode, in the three wine samples. The analysis took little more than two minutes per sample, in contrast with the time required for the quantitative mode, which was about seven minutes per sample (see Experimental section). Three independent replicates were carried out and the mean and respective RSD were calculated for each element. The obtained results were included in Table 9.1.

The LODs and the LOQs of the elements were determined only for forty-one elements (for the remaining twenty-two elements standard solutions were not available) and the LODs were included in Table 9.1. For most of the studied elements, the LODs were slightly higher (two to six times) than those obtained by quantitative mode of analysis.

Fourteen of the tested elements were not detected in any of the studied wine samples while another fourteen elements, including most of the REEs, were below or near the LODs (see Table 9.1), which prevented the respective determination.

In terms of precision, for most of the determined elements, the RSDs were similar to those observed by using the quantitative mode of analysis, between 1 and 5 %. For Fe, Ga, Mo and Sc the RSDs were slightly higher, but lower than 10 %. For Zr and a few other elements, like some REEs,

RSDs up to 30 % were found, probably due to the relatively low concentrations present in the wines, which were close to the LOQ or lower.

Again, significant differences were not observed among the three types of wines in terms of either precisions or LODs.

9.5. COMPARISON BETWEEN SEMI-QUANTITTATIVE AND QUANTITTATIVE MODES OF ANALYSIS

Thirty elements (Al, Ba, Cd, Co, Cr, Cu, Fe, Ga, Li, Mn, Ni, Pb, Rb, Sr, V, Zn and the REEs La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) were measured, in parallel, by using the quantitative and the semi-quantitative modes. Comparable results (differences lower than 20 %) were obtained, except for Cd, Ga and the REEs in all the tested wines and Li in the table wines. In most cases the differences were lower than 10 %. Differences between 10 and 20 % were obtained only for Al and Co in all the wines, Li in the Port wine and V in the red table and Port wines. The present results corroborates previous ones, since accuracy errors lower than 20 % for most elements have been reported in literature [1-3] for semi-quantitative analysis, including for table wines [12].

Higher differences were observed for Li in the table wines (41 % and 46 % for red and white table wines, respectively). A probable reason for so large differences was the unsuitability of the semi-quantitative mode of analysis for such a low mass element.

For the REEs in the three wine samples, differences up to 50 % were obtained. For Cd, a difference of 57 % was obtained for the Port wine sample (for the other two wine samples the Cd concentrations were below the LOD in the semi-quantitative mode of analysis). The main reason for the large observed differences may be the fact that the respective concentrations were below or near the LOQ of both quantitative and semi-quantitative modes of analysis, which unable their accurate determination.

Differences between 65 and 85 % were observed for Ga. The isotope ^{69}Ga can suffer matrix-induced interferences of $^{138}Ba^{2+}$, $^{53}Cr^{16}O$ or $^{37}Cl^{16}O^{16}O$, which were not corrected in the quantitative mode of analysis. However, the software for semi-quantitative includes the most common isobaric and matrix-induced interferences and automatically corrects this interference. This fact is a probable explanation for the large differences observed for Ga.

The potential risk of error of a semi-quantitative mode of analysis should not be overlooked. Therefore, a linear least-squares adjustment of the two sets of results (semi-quantitative mode, SQ (y-axis) *versus* quantitative mode, Q (x-axis)) was performed and the results are shown in Fig. 9.2. Among the measured elements, only Cd, Ga, the REEs in all the wines and Li in the table wines for which non-comparable results were found (differences higher than 20 %), were not considered in the adjustment.

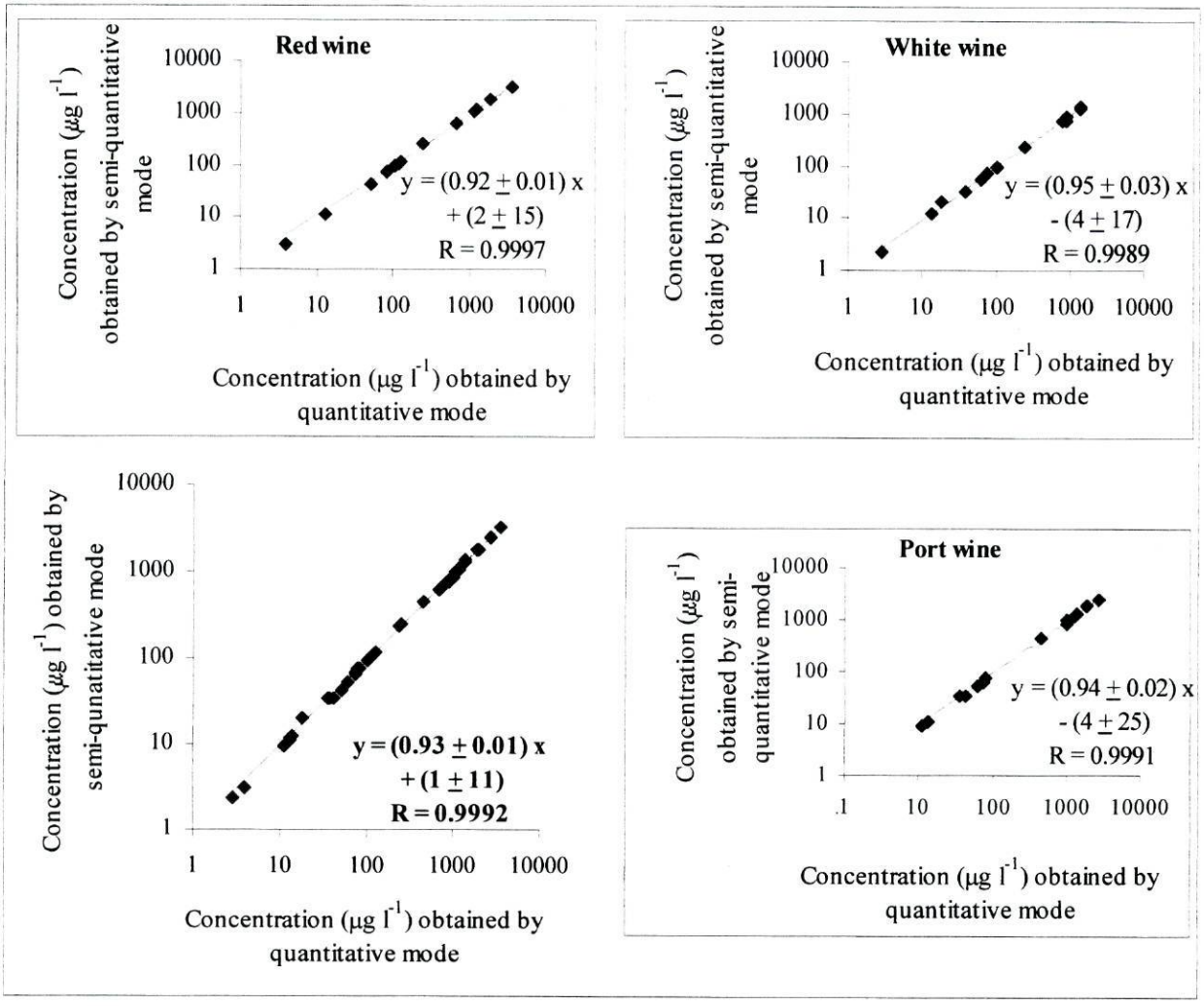

Fig. 9.2. Comparison, by linear regression, of the results obtained for the three wine samples by ICP-MS using quantitative and semi-quantitative modes of analysis (the 95 % confidence limits of the linear regression parameters are given). In both axis is represented the logarithm of concentrations (concentrations expressed in μg l^{-1}) for a larger scattering of the points.

The regression line of the global results (including the red and white table and Port wine samples), yielded the equation: SQ = (0.93 ± 0.01) Q + (1 ± 11) with a correlation coefficient (R) of 0.9992 (n = 40). When each wine was treated separately, the following equations were obtained: SQ= (0.92 ± 0.01) Q + (2 ± 15), R = 0.9997 (n = 13), for table red wine; SQ = (0.95 ± 0.03) Q - (4 ± 17), R =

0.9989 (n = 13), for table white wine; and SQ = (0.94 ± 0.02) Q - (4 ± 25), with R = 0.9991 (n = 14), for Port wine. High correlations, the values of R being close to one, and intercepts statistically identical to zero (95 % confidence level) were found in all cases. However, evidence of a slight fixed bias (lower values for the semi-quantitative mode of analysis, that is, a slope significantly lower than one) was observed. The bias was practically independent of the type of wine. Similar results were obtained when only the elements with differences (between quantitative and semi-quantitative modes) ≤ 10 % were considered in the adjustment.

9.6. CONCLUSIONS

It was demonstrated that besides the quantitative mode of analysis of the ICP-MS Perkin-Elmer SCIEX Elan 5000, the semi-quantitative one also could provide information about the concentration levels of a large number of elements in wine samples. In this work, comparable results, in terms of precision (relative standard deviations between 0.5 and 5 % in many cases) and accuracy (relative differences inferior to 20 %), were obtained by the quantitative and semi-quantitative modes of analysis for Al, Ba, Co, Cr, Cu, Fe, Mn, Ni, Pb, Rb, Sr, V and Zn.

The semi-quantitative mode of analysis can be an important tool for comparison of elemental compositions of different wines, for instance for provenance testing, having some economic advantages relatively to the quantitative mode: it is much faster and requires fewer reagents.

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Chapter 10

ICP-MS Multi-Element Analysis of Wines. A Comparative Study of the Methodologies Used In Two Laboratories

10.1. INTRODUCTION AND AIMS

As already mentioned, multi-element analyses have be considered promising for the establishment of wines origin [1-5].

Reference wines with certified concentrations of only major elements such as K, Na, Ca and Fe are available, like the Tritivin wines, prepared, certified and commercialised by the *Chambre d'Agriculture de la Gironde* (Blanquefort, France), the reference values being established by the *Union des Oenologues de France*. Wines with certified total lead concentration can also be found, like the BCR wines (B, C and E), *Standards, Measurement and Testing Programme* from the *Community Bureau of Reference* (Brussels, Belgium) now out of stock, or the IMEP-16, *Institute for Reference Material and Measurements* from the *European Commission Joint Research Centre* (Geel, Belgium) (used in an inter-laboratory comparison programme, the *International Measurement Evaluation Programme*) [6]. With the exception of lead, as far as we know, there are no available wines with minor, trace or ultra-trace element certified concentrations. Therefore, the accuracy of ICP-MS multi-element determinations in wines has been evaluated through recovery tests (*e.g.* the study reported in Chapter 9 and [3,4,7]) or by comparison of the results obtained with different analytical techniques (*e.g.* the study reported in Chapter 9 and [7,8]).

In order to be able to use multi-elemental analysis of wines as a tool for detecting/preventing wine fraud, comparable results must be obtained between different Laboratories notwithstanding they have been using different instruments or methodologies. To our knowledge, ICP-MS inter-instrumental comparison of results obtained for wine samples have been performed only for REEs [2] and for lead isotope ratios [9,10].

The aim of this study (already published [11]) was to make a comparison of the performance of the methodology used in the lab of LAQUIPAI (here named Lab A) and in the lab of the "Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes (DGCCRF)", France, supervised by Dr. Bernard Medina (here named Lab B), for multi-element analysis in different wines. These Laboratories have been using ICP-MS apparatus of different brands as well as different methodologies of wine preparation for analysis. Both ICP-MS apparatus were quadrupole mass analysers. At Lab A, a pre-treatment by UV-irradiation (which optimisation is described in Chapter 4), was used. At Lab B, a micro-concentric nebulizer was used with direct analysis of the wine. With the mentioned purpose, the results obtained for different elements (Li, V, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu and Pb) in three selected wines, one red and

one white table wines and one red Port wine (the same wines used in the study reported in the previous Chapter), were compared. The samples were chosen in order to represent three different types of wine matrix - the most commonly found.

10.2. EXPERIMENTAL

Experimental conditions and procedures used at Lab A for the multi-element determinations by ICP-MS in the quantitative mode are described in detail in the Experimental Section. The optimised methodology is described in the previous Chapter 9. The experimental conditions and methodology used at Lab B are reported in [11].

In spite of only twenty-six elements (enumerated above) have been determined in common by both Labs, at each Lab over thirty elements (for which standards are easily available) were measured simultaneously in the selected wines. Therefore, some standard solutions used for calibration included elements other than those object of present study.

10.3. MEASURED ELEMENTS

For each element in each wine sample, three independent replicates were measured and the mean and RSD calculated. The results for each of the twenty-six elements measured in common by the two Labs, including the LODs, are presented in Table 10.1.

10.4. LIMITS OF DETECTION

At Lab A the LODs of each element were calculated from the signal corresponding to three times the standard deviation of a blank solution, which was measured ten times for this purpose.

Table 10.1. Comparison of the limits of detection (LODs) and of the elemental concentrations[a] (μg l^{-1}) obtained for the three wines at Lab A and at Lab B using different ICP-MS apparatus and methodologies. The concentrations in bold are below or near the quantification limit.

Element	LODs (Lab A)	LODs (Lab B)	Red wine		White wine		Port wine	
			Lab A	Lab B	Lab A	Lab B	Lab A	Lab B
Li	0.014	0.1	8.8 (0.2)	8.99 (0.09)	7.09 (0.06)	7.70 (0.06)	42.7 (0.9)	31 (17)
V	0.0047	0.05	52 (1)	49.4 (0.4)	100 (5)	105.3 (0.5)	13.4 (0.4)	12.3 (0.3)
Co	0.019	0.0009	3.86 (0.08)	3.71 (0.02)	2.9 (0.1)	2.67 (0.01)	11.2 (0.2)	10.1 (0.1)
Ni	0.25	0.1	81.7 (0.7)	82.8 (0.8)	16.1 (0.5)	26.6 (0.1)	36 (1)	41.2 (0.7)
Cu	0.073	0.0003	127 (1)	210 (3)	37.5 (0.4)	ND[b]	442 (4)	445 (7)
Zn	0.18	2	1211 (13)	1139 (1)	1382 (18)	1404 (4)	1039 (18)	914 (6)
Ga	0.0053	0.01	3.5 (0.1)	5.04 (0.08)	3.0 (0.1)	3.37 (0.06)	3.59 (0.01)	17 (4)
As	0.067	0.07	15 (1)	8.51 (0.05)	56 (2)	25.3 (2)	14 (3)	4.4 (0.2)
Rb	0.010	0.02	1917 (41)	1657 (28)	921 (12)	930 (4)	1903 (23)	1829 (4)
Sr	0.011	0.01	247 (4)	249 (3)	238 (4)	249 (3)	1269 (20)	1251.0 (0.6)
Ba	0.011	0.03	103.5 (0.2)	119 (3)	75 (3)	86 (2)	61 (2)	66.6 (0.6)
La	0.0058	0.0003	0.366 (0.002)	0.57 (0.01)	0.20 (0.02)	0.240 (0.008)	0.532 (0.005)	0.73 (0.04)
Ce	0.0033	0.00001	0.62 (0.02)	0.92 (0.01)	0.35 (0.03)	0.439 (0.007)	1.04 (0.01)	1.33 (0.03)
Pr	0.00091	0.005	0.099 (0.002)	0.12 (0.02)	0.057 (0.005)	0.063 (0.003)	0.169 (0.002)	0.19 (0.01)
Nd	0.0038	0.0004	0.363 (0.005)	0.50 (0.02)	0.18 (0.01)	0.25 (0.03)	0.66 (0.01)	0.82 (0.08)
Sm	0.0040	0.008	0.068 (0.006)	0.13 (0.01)	0.033 (0.002)	0.08 (0.02)	0.163 (0.009)	0.24 (0.01)
Eu	0.0014	0.007	0.032 (0.004)	0.045 (0.005)	0.022 (0.002)	0.025 (0.003)	0.049 (0.002)	0.06 (0.01)
Gd	0.0037	0.009	0.087 (0.005)	0.10 (0.01)	0.051 (0.004)	0.051 (0.006)	0.197 (0.003)	0.230 (0.004)
Tb	0.00043	0.005	0.025 (0.003)	**0.015 (0.002)**	0.0173 (0.0006)	**0.0091 (0.0003)**	0.038 (0.001)	0.048 (0.007)
Dy	0.0028	0.009	0.066 (0.003)	0.088 (0.007)	0.039 (0.003)	0.047 (0.005)	0.164 (0.003)	0.195 (0.009)
Ho	0.00067	0.007	0.031 (0.002)	**0.0181 (0.0005)**	0.0229 (0.0004)	**0.013 (0.002)**	0.0458 (0.0006)	0.040 (0.008)
Er	0.0012	0.004	0.057 (0.002)	0.068 (0.009)	0.041 (0.004)	0.045 (0.004)	0.105 (0.003)	0.126 (0.007)
Tm	0.00085	0.007	0.020 (0.002)	**0.0076 (0.0004)**	0.0165 (0.0003)	< LOD	0.024 (0.001)	0.016 (0.003)
Yb	0.0053	0.008	**0.032 (0.002)**	0.06 (0.01)	**0.018 (0.006)**	0.053 (0.008)	0.070 (0.003)	0.14 (0.01)
Lu	0.0065	0.009	0.024 (0.002)	**0.010 (0.001)**	0.0205 (0.0008)	< LOD	0.029 (0.003)	0.021 (0.006)
Pb	0.016	0.2	12.9 (0.2)	15.0 (0.2)	13.57 (0.08)	16.2 (0.2)	81 (3)	92 (4)

a: Concentrations values correspond to the mean of three replicates and the corresponding standard deviation (in brackets);
b: ND = not determined.

At Lab B, LODs were calculated using the linear regression method. Three standard solutions (*e.g.*, 0.2 ng l^{-1}, 0.6 ng l^{-1} and 1 ng l^{-1} of each of the elements analysed in the study) were analysed. If acceptable regression coefficient was obtained for an element (> 0.999), the measurements were repeated five times and the LOD for the element calculated with the regression equation [12]. If not, a new set of three standard solutions more concentrated (*e.g.*, 2 ng l^{-1}, 6 ng l^{-1} and 10 ng l^{-1}) were prepared and tested for linearity. This operation was repeated until linearity was good and allowed the calculation of a LOD.

The obtained values (see Table 10.1) were of similar magnitude at both Lab A and Lab B. The LODs were similar or even lower than those reported in the literature [4,7], as discussed in the previous Chapter. For the REEs, the values were in agreement with those obtained by Stroh *et al.* [3] using an ICP-MS equipment similar to that used at Lab A

10.5. RECOVERY ESTIMATES

As referred earlier in the introduction, the accuracy of ICP-MS multi-element determinations in wines has been evaluated from recovery tests or by comparison of the results obtained by different analytical techniques because there are no reference wines with certified concentrations for minor, trace or ultra-trace elements, except for Pb.

At Lab A, the recovery tests were performed (see Chapter 9) for the same three wine samples and the obtained recovery percentages were included in Table 10.2, to allow a comparison with those obtained at Lab B. The recovery percentages (mean of recovery values obtained for three independent spikes of each element for each wine sample) varied between 85 % and 120 % for Ba, Co, Cu, Ga, Li, Ni, Pb, Rb, Sr, V and Zn in all the wines, with an average value of 102 ± 12 %. For the REEs, recovery percentages (only determined in the table wines) presented an average value of 99 ± 2 %. For As, recovery percentages around 250 % were obtained which indicates that the ICP-MS equipment/ methodology used at Lab A was not suitable for As determination in wines. No significant differences were observed among the three types of wines indicating that the matrix of the wines did not influenced the ICP-MS results.

At Lab B, only the white and red table wines were chosen to measure recovery percentages. Element concentrations of the spiked wines were measured. Each measurement was repeated three times and a standard deviation was calculated. As Table 10.2 shows, recovery values ranged from 78 %

to 119 %, with an average value of 100 ± 10 %. These percentages were comparable with those obtained at Lab A, being similar to those reported in the literature [4,7] for the same elements measured in table wines by ICP-MS.

Table 10.2. Recovery percentages (%)[a] obtained for the three wines in Lab A and in Lab B using different ICP-MS methodologies.

	Lab A			Lab B	
Element	Red wine	White wine	Port wine	White wine	Red wine
Li	117 ± 8	114 ± 3	120 ± 5	108 ± 6	118 ± 7
V	115 ± 5	115 ± 4	118 ± 2	96 ± 4	93 ± 4
Co	104 ± 4	102 ± 2	102 ± 1	100 ± 3	104 ± 3
Ni	97 ± 2	101 ± 2	106 ± 4	101 ± 3	105 ± 3
Cu	94 ± 2	97 ± 1	99 ± 3	90 ± 1	93 ± 1
Zn	95 ± 3	95 ± 2	98 ± 2	80 ± 2	98 ± 1
Ga	96 ± 3	100 ± 1	102 ± 4	97 ± 4	101 ± 4
As	235 ± 15	262 ± 61	304 ± 16	97 ± 4	105 ± 2
Rb	97 ± 3	97 ± 1	93 ± 5	98.9 ± 0.4	95.1 ± 0.3
Sr	103 ± 2	104 ± 1	99 ± 6	91.8 ± 0.2	96.1 ± 0.01
Ba	83 ± 1	89 ± 4	88.0 ± 0.4	92.7 ± 0.3	95.6 ± 0.4
La	95.8 ± 0.6	97.5 ± 0.8	ND[b]	106 ± 4	100 ± 2
Ce	98 ± 2	97 ± 6	ND[b]	105 ± 2	99 ± 2
Pr	97 ± 1	96.7 ± 0.3	ND[b]	112 ± 7	101 ± 3
Nd	100 ± 2	98 ± 1	ND[b]	110 ± 6	95 ± 5
Sm	104 ± 4	96.0 ± 0.4	ND[b]	113 ± 14	95 ± 8
Eu	102 ± 1	99.0 ± 0.3	ND[b]	118 ± 14	99 ± 7
Gd	101.1 ± 0.2	98 ± 1	ND[b]	103 ± 9	94 ± 7
Tb	102.3 ± 0.5	99 ± 2	ND[b]	119 ± 6	99 ± 6
Dy	101 ± 1	99 ± 1	ND[b]	115 ± 7	92 ± 7
Ho	101.4 ± 0.4	99.4 ± 0.1	ND[b]	111 ± 6	93 ± 7
Er	100 ± 3	98.8 ± 0.3	ND[b]	113 ± 8	98 ± 10
Tm	99 ± 1	99 ± 1	ND[b]	108 ± 7	85 ± 9
Yb	102 ± 3	101 ± 2	ND[b]	110 ± 5	86 ± 11
Lu	101.7 ± 0.7	101 ± 1	ND[b]	112 ± 7	88 ± 10
Pb	84 ± 2	83 ± 1	84 ± 5	112 ± 4	90 ± 2

a: Mean ± standard deviation (n = 3);
b: ND = not determined.

At Lab B, a matrix effect was noticed for ultra trace elements such as lanthanides: the obtained recoveries indicated that these elements were slightly overestimated in table white wines and slightly underestimated in table red wines. Differences of quality and quantity of organic matter in those two types of wine (*e.g.* organic acids, tannins, etc...) may justify such behaviour. It was also noticed that ^{48}Ti values were largely overestimated due to the direct ^{48}Ca overlap.

10.6. PRECISION OF THE MEASUREMENTS

Table 10.1 shows that the precision values observed in Lab A and in Lab B were similar, between 0.5 and 5 %, being lower than 3 % in most cases. These precisions were similar or slightly better than those reported in the literature [3,7], both for table wines analysed with an ICP-MS equipment similar to that used at Lab A and a pre-treatment methodology (1:1 dilution of the wine samples) similar to that used at Lab B.

At Lab A, higher RSDs were observed only for most of the REEs in the table wines. The concentrations of the few REEs in the mentioned samples were relatively low, in most cases close or below the limit of quantification, which explains the relatively high RSDs (between 6 and 30 %) observed for these elements.

At Lab B, Li (RSD = 17 %) and Ga (RSD = 22 %) in Port wine displayed relatively high RSDs. This behaviour was probably due to the large difference in ethanol concentration and sugar content between Port wine and standard solutions or tune solution. The variation in ethanol concentration during acquisition can induce instability in the plasma. The elements Li and Ga seemed to be the most affected by those changes. As observed at Lab A, the REEs also presented higher RSD values than those observed for other elements when measured at Lab B. Those RSDs ranged between 0.8 and 28 %, sixteen values (in forty-two) being over 10 %, and four over 20 %.

In terms of the precision, at Lab A, no significant differences were observed among the three types of wines, which indicate that the matrix of the wine samples after pre-treatment did not influenced the precision of the ICP-MS analysis. At Lab B, differences were noticed for Port wine as discussed above.

10.7. LAB A VERSUS LAB B RESULTS

As shown in Table 10.1, with the exception of As, Ga (red table and Port wines) and most of the REEs, comparable results were obtained, despite of the different ICP-MS equipment and methodologies used.

A statistical comparison through t-paired test [12] indicated that statistically identical results were obtained for Li, Ni and Sr in the red table wine; V, Zn and Rb in the white table wine; and Li, Cu and Sr in the Port wine, with the results for the remaining elements being statistically different. Nevertheless,

the differences were lower than 10 % in most cases. Relative differences between 10 and 20 % (Pb in all the wines, Ba in the red and white table wines, Zn and Ni in the Port wine, and Rb in the red table wine) and above 20 % (Cu in red table wine, Li in Port wine and Ni in white table wine) occurred only in a few cases.

For Pb, differences around 15 % were obtained for the three wine samples, Lab A obtaining lower concentrations than Lab B. This element was also analysed in the IMEP-16 wine [6] by both Labs, using the present methodologies. For a certified value of 27.18 ± 0.25 μg l^{-1} for Pb, a concentration of 23 ± 1 μg l^{-1} was obtained by Lab A and of 25.0 ± 0.4 μg l^{-1} by Lab B, therefore, both significantly lower than the certified value. Besides, as Table 10.2 shows, recovery percentages of 83-84 % were obtained for Pb in the three samples at Lab A. Lab B obtained 90 ± 2 % for red table wine and 112 ± 4 % for white table wine. Therefore, for this element, Lab A obtained lower concentrations than Lab B and that was a probable reason to the differences observed between the two Labs results.

For Li in the Port wine sample, the large difference may be related to the low precision (high RSD) obtained in this case at Lab B, but both results overlaps taking into account the uncertainty. Nevertheless, at Lab B no recovery tests were performed for Port wine, which prevents a deeper discussion of the differences observed for Li in Port wine between the two Labs. Regarding Cu in the red wine and Ni in the white wine, the differences could be related with matrix-induced interferences (that affect differently the performance of different ICP-MS equipment), since ^{65}Cu suffers interference from $^{40}Ar^{25}Mg$ and ^{60}Ni from $^{44}Ca^{16}O$.

For the REEs, differences between 10 % and 200 % were observed. This probably resulted of most of the REEs elements being below or near the limit of quantification of the method used in both Labs, which unable their accurate determination. Nevertheless, very consistent values were obtained considering the ultra-trace (sub ppb) values measured for those elements.

For As, the differences were around 55 %, but since the methodology used at Lab A was unsuitable for this element (see above) there is no point in making a comparison between the results obtained at the two Labs.

For Ga the results differed markedly for the red table wine (44 %) and particularly for the Port wine (370 %). The isotope ^{69}Ga suffers interference from $^{138}Ba^{2+}$, $^{53}Cr^{16}O$ and $^{37}Cl^{16}O^{16}O$ (matrix-induced interferences), which can affect ICP-MS equipment differently. This may be the cause of the observed results for Ga. However, the alcohol interference that occurred at Lab B, resulting in high RSD for this element in the Port wine (see above), may also contributed for the large difference observed for the fortified wine. At Lab A good recovery percentages were obtained for this element (between 96 % and 102 %) in the different wines, including Port wine.

A linear least-squares adjustment of the mean concentration values (in $\mu g\ l^{-1}$) obtained at Lab B (y axis), for all the elements in the three wine samples (global data), against those obtained at Lab A (x axis), is shown in Fig. 10.1 (As, Ga and the REEs were not included in the adjustment).

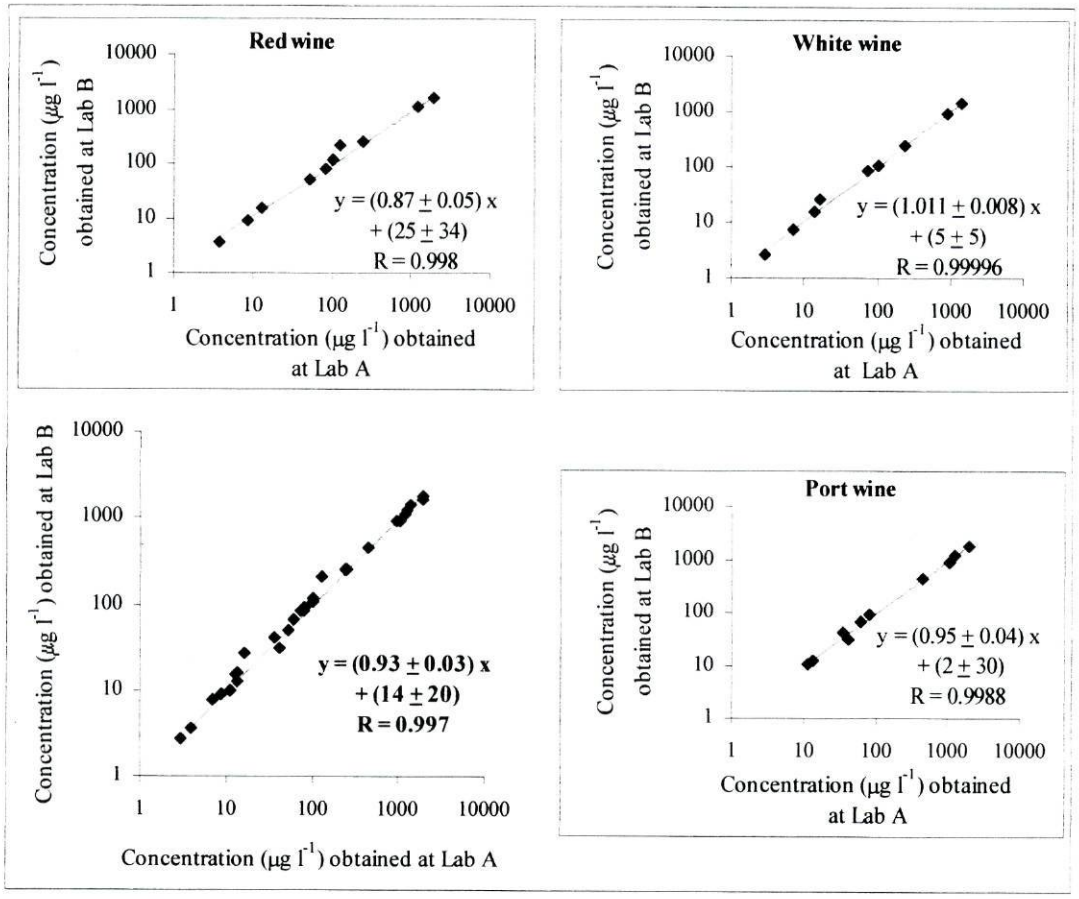

Fig. 10.1. Comparison, by linear regression, of the results obtained at Lab A and those obtained at Lab B for the elements measured, in common, in the three wine samples (the 95 % confidence limits of the linear regression parameters are given). In both axis is represented the logarithm of concentrations (concentrations expressed in $\mu g\ l^{-1}$) for a larger scattering of the points.

The adjustment yielded the equation: Lab B = (0.93 ± 0.03) Lab A + (14 ± 20) with a correlation coefficient (R) of 0.997 (n = 29). The ± values are the slope and intercept at 95 % confidence limits. When the data for each wine sample was treated separately, the following equations were obtained: Lab B = (0.87 ± 0.05) Lab A + (25 ± 34), R = 0.998 (n = 10), red table wine; Lab B = (1.011 ± 0.008) Lab A + (5 ± 5), R = 0.99996 (n = 9), white table wine; and Lab B = (0.95 ± 0.04) Lab A + (2 ± 30), R = 09988 (n = 10), Port wine. Despite the two Labs have been using different equipments and methodologies, high correlation coefficients and intercepts statistically

identical to zero (95 % confidence level) were observed in all cases. However, slopes statistically different from one were found for all lines. Though the results obtained at the two Labs were significantly different, the differences were very low, particularly for the white table and Port wines.

10.8. CONCLUSIONS

Most of the twenty-six elements that were measured in common by Lab A and Lab B in the same wines (one white and one red table wines and a Port wine) using different ICP-MS equipment and methodologies were accurately (assuming that no systematic error exists) and precisely measured.

Comparable results were found for Li, V, Co, Ni, Cu, Zn, Rb, Sr, Ba, and Pb, in the three different wines studied, while for the REEs, As and Ga some discrepancies were observed. Nevertheless, for the REEs, very consistent values were obtained considering the ultra-trace (sub-ppb) values measured for those elements. For As and Ga, the methodologies influenced differently the results. A comparison through linear least-squares adjustment indicated that the results obtained by the two Labs were linearly correlated (correlation coefficient ≥ 0.997) but statistically different as the slope was slightly, but significantly different from one, for a confidence level of 95 % (the intercept was statistically identical to zero in any case).

Therefore, very different sample processing used in the two Labs (UV treatment at Lab A and direct analysis using micro-concentric nebulizer at Lab B) led to similar results for ten elements. When taken into account, few interactions existed, either with higher ethanol content (12 - 20 %) or other components such as sugar (0 - 100 g l^{-1}) or even minerals such as potassium (600 - 1500 mg l^{-1}) [13].

Due to these encouraging results, an inter-laboratory trial should be planned to evaluate the performance of ICP-MS methodologies (repeatability and reproducibility) according to the International Office of Vine and Wine protocol.

By using a reference wine sample, strictly more identical results can be achieved. Therefore, it can be concluded that a systematic information exchange, by different Labs involved in detecting/preventing wine fraud, on wines multi-element compositions will be advantageous in terms of quality control for the final decisions on authenticity of a wine.

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Chapter 11

Optimisation of an ICP-MS Semi-Quantitative Mode of Analysis for Multi-Element Determination in Soils

11.1. INTRODUCTION AND AIMS

As discussed in Chapter 9, ICP-MS offers different quantification procedures, depending on the accuracy and precision required: isotope dilution, quantitative and semi-quantitative modes of analysis.

The objective of the present work, was the optimisation of a semi-quantitative procedure, similar to that optimised before for wine samples, suitable for routine multi-element determinations in soil samples, in order to improve the speed and reduce the cost of analysis relatively to those of quantitative mode of analysis. The study included the optimisation of both ICP-MS quantitative and semi-quantitative modes of analysis for soil samples, and a comparison with results provided by AAS.

The semi-quantitative mode of analysis has been successively applied to different types of samples including those of environmental origin [1-4]. Although some of the published studies have reported the application of this mode of analysis to sediment samples [2,4], application to soil samples was not mentioned in any case.

In a first stage, a HPMW-digestion procedure was optimised for soil. HPMW-digestion procedures have been extensively used for determination of metals concentrations in different types of samples, including diverse of environmental origin, by using analytical techniques like ICP-MS and AAS [5].

11.2. EXPERIMENTAL

Two soil samples (one from the old and another from the young vineyards described in the Experimental Section) and a soil standard reference material, which was San Joaquin Soil SRM 2709, from NIST, were used. For each soil sample three independent replicates were prepared.

Before ICP-MS quantitative mode of analysis, all soil solutions, obtained after HPMW-digestion (see Chapter 3), were diluted with a solution containing Rh to different rates, depending of the element to be determined: two-thousand times for Al and Fe, twenty times for Ba, Mn, Rb and Sr, and five times for the remaining elements. For the semi-quantitative mode, one-hundred (only for Mn, Fe and Cr) and five (for the remaining elements) times dilutions were carried out. In all cases, the final sample solutions, as well as the standard solutions, contained 20 $\mu g\ l^{-1}$ Rh.

Several elements were also analysed by AAS in two of the soil samples (reference soil and one vineyard soil), in order to compare the results obtained by ICP-MS with those obtained by a different analytical technique. For this purpose, the soil solutions were used directly (Co, Cr, Cu and Ni determinations) or diluted four (Cd and Pb), ten (Mn and Zn), one-hundred (Al) or five-hundred (Fe) times with de-ionised water.

Experimental conditions and procedures are described in detail in Chapter 3.

In a first stage of the work, LODs and LOQs were also determined for each of the measured elements, as described in Chapter 9 for the wine samples. Similarly, recovery test were also carried for soil sample solutions spiked with 0.20 to 75 $\mu g\ l^{-1}$ standard solution (depending of the element).

11.3. QUANTITTATIVE MODE OF ANALYSIS

For the present study, thirty-one elements (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Ga, Li, Mn, Ni, Pb, Rb, Sr, V, Zn and the REEs La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) were selected.

The respective LODs were determined and presented in Table 11.1. The obtained LODs were lower than those reported by Reimann *et al* [6], Falciani *et al* [7] and Wu *et al* [8] for ICP-MS analysis of HPMW digested soils. The first one [6] used only HNO_3 for the soils digestion, while the others authors used a mixture of different acids.

Recovery tests were carried for the three selected soil samples, since both isobaric and matrix-induced interferences could affect each sample differently, due to slightly differences in composition. As Table 11.2 shows, recovery percentages between 83 % and 118 % were obtained for all the soil samples, being most of these percentages around 100 % (average value of 99 ± 6 %). For Al, in two samples, recovery percentages of 110 % were observed, which may be due to the fact of Rh not being an appropriate internal standard for such low mass element.

For Cr and Fe in the reference soil sample the recovery percentages were higher than 110 % probably because the matrix-induced interferences were not properly corrected, in the case of Fe, or not corrected at all, in the case of Cr (interference from Cl species), being the interfering species in higher concentration in this sample than in the vineyard soil samples.

Table 11.1. Limits of detection (LODs) and concentrations of the several elements (μg g^{-1} or mg g^{-1}, see below) obtained by ICP-MS[a], using both the quantitative (Q) and the semi-quantitative (SQ) modes of analysis, for reference soil sample and two vineyard soil samples. Values in bold are below or near the limit of quantification. For a few elements, the results obtained by AAS are also included.

			NIST Reference Soil (SRM 2709)				Old vineyard soil			Young vineyard soil	
Element	**LODs (Q)**	**LODs (S)**	**AAS**	**ICP-MS (Q)**	**ICP-MS (SQ)**	**Certified value[d] (*leach range*)**	**AAS**	**ICP-MS (Q)**	**ICP-MS (SQ)**	**ICP-MS (Q)**	**ICP-MS (SQ)**
Li	0.009	0.02		46.9 (0.2)	43.4(0.4)			39.1 (0.6)	36.3 (0.9)	35 (1)	33 (1)
Be		0.05			0.94 (0.09)				**0.71 (0.04)**		0.99 (0.06)
B[b]		1.3			49 (4)				ND[c]		ND[c]
Al[b]	0.04	ND[c]	40 (1)	37 (2)	ND[c]	75.0 (0.6) (*20-31*)	27.0 (0.5)	29 (2)	ND[c]	25.5 (0.9)	ND[c]
Ca[b]		2.8			16.0 (0.4)	18.9 (0.5) (*14-17*)			0.76 (0.03)		0.49 (0.02)
Sc		ND[c]			74 (1)	*12*			18.27 (0.08)		24 (2)
Ti[b]		ND[c]			0.85 (0.01)	3.42 (0.24) (*0.3-0.4*)			0.22 (0.02)		0.60 (0.02)
V	0.004	0.007		79 (3)	84 (4)	112 (5) (*51-70*)		20 (1)	22.2 (0.8)	25.8 (0.8)	25.2 (0.8)
Cr	0.01	0.02	115 (4)	131 (6)	140 (3)	130 (4) (*60-115*)	39.3 (0.7)	49.4 (0.3)	58.9 (0.9)	54.8 (0.2)	73 (1)
Mn	0.008	0.04	380 (10)	517 (10)	590 (15)	538 (17) (*306-600*)	124 (2)	192 (6)	219 (6)	ND[c]	ND[c]
Fe[b]	0.06	0.6	31 (2)	34 (1)	34 (1)	35.0 (1.1) (*25-33*)	24.2 (0.7)	24 (1)	26.8 (0.6)	27.5 (0.2)	25.0 (0.5)
Co	0.005	0.003	14.4 (0.2)	13.9 (0.3)	13.6 (0.2)	13.4 (0.7) (*10-15*)	10 (2)	9.9 (0.2)	9.9 (0.1)	13.6 (0.4)	13.1 (0.1)
Ni	0.01	0.06	71 (2)	80 (2)	90 (6)	88 (5) (*65-90*)	22 (1)	19.1 (0.3)	21.0 (0.3)	28.1 (0.5)	25.6 (0.6)
Cu	0.1	0.3	35 (1)	33.6 (0.6)	36 (1)	34.6 (0.7) (*26-40*)	35 (1)	38.4 (0.6)	43.9 (0.4)	17.4 (0.5)	16.0 (0.2)
Zn	0.08	0.08	102 (8)	115 (2)	115 (7)	106 (3) (*87-120*)	79 (6)	74 (2)	78 (1)	90 (2)	79 (3)
Ga	0.001	0.006		18.0 (0.2)	9.37 (0.08)	*14*		6.9 (0.1)	6.8 (0.2)	7.9 (0.5)	7.5 (0.5)
Ge		ND[c]			0.07 (0.03)				0.09 (0.01)		0.14 (0.02)
As	0.07	0.1		16.4 (0.5)	16.7 (0.5)	17.7 (0.8) (*<20*)		5.0 (0.1)	4.6 (0.1)	1.62 (0.09)	1.07 (0.06)
Se		0.2			< LOD	1.57 (0.08) (*0.014*)			< LOD		< LOD
Rb	0.005	0.01		49 (1)	45.1 (0.6)	*96*		30 (2)	25 (2)	34.9 (0.5)	30.6 (0.7)
Sr	0.002	0.005		119 (3)	124 (2)	231 (2) (*100-112*)		15.1 (0.4)	9.4 (0.3)	11.6 (0.2)	7.8 (0.3)
Y		ND[c]			104 (2)	*18*			74 (2)		107 (6)
Zr		ND[c]			32 (1)	*160*			21.9 (0.9)		29 (3)
Nb		ND[c]			0.21 (0.05)				0.09 (0.01)		0.107 (0.003)
Mo		0.03			**0.54 (0.07)**	*2.0*			< LOD		< LOD
Ru		ND[c]			NDT[c]				NDT[c]		NDT[c]
Pd		ND[c]			0.075 (0.006)				NDT[c]		NDT[c]
Ag		0.006			0.38 (0.03)	0.41 (0.03)			< LOD		< LOD
Cd	0.04	0.004	0.45 (0.05)	**0.47 (0.01)**	0.23 (0.05)	0.38 (0.01) (*<1*)	ND	< LOD	< LOD	< LOD	< LOD
In		ND[c]			NDT[c]				NDT[c]		NDT[c]
Sn		ND[c]			NDT[c]				NDT[c]		NDT[c]
Sb		ND[c]			0.18 (0.02)	7.9 (0.6) (*<10*)			NDT[c]		NDT[c]
Te		0.004			< LOD				< LOD		< LOD
Cs		ND[c]			2.86 (0.07)	*5.3*			1.26 (0.04)		2.04 (0.09)

Table 11.1. ***continuation***

Ba	0.005	0.01		425 (6)	455 (10)	968 (40) (*392-400*)		44 (6)	41 (6)	54.5 (0.9)	55 (2)
La	0.007	0.003		18.7 (0.6)	18.6 (0.4)	*23*		42 (1)	42.2 (0.8)	45.0 (0.7)	45.4 (0.1)
Ce	0.003	0.002		39.7 (0.9)	44.3 (0.5)	*42*		85 (2)	96 (3)	91 (1)	105 (1)
Pr	0.0006	0.0008		4.6 (0.1)	4.69 (0.07)			8.9 (0.3)	9.4 (0.3)	9.9 (0.1)	10.7 (0.2)
Nd	0.0008	0.001		17.1 (0.4)	17.5 (0.4)	*19*		33 (1)	34 (1)	37.5 (0.6)	39 (1)
Sm	0.0008	0.001		3.47 (0.05)	3.4 (0.2)	*3.8*		5.6 (0.2)	5.7 (0.2)	6.46 (0.09)	6.61 (0.05)
Eu	0.0006	0.001		0.72 (0.02)	0.74 (0.01)	*0.9*		1.05 (0.03)	1.02 (0.04)	1.23 (0.01)	1.233 (0.008)
Gd	0.0006	0.001		3.1 (0.1)	3.2 (0.1)			4.2 (0.1)	4.57 (0.06)	4.97 (0.09)	5.55 (0.08)
Tb	0.0006	0.0007		0.46 (0.02)	0.458 (0.005)			0.54 (0.02)	0.56 (0.02)	0.66 (0.01)	0.69 (0.02)
Dy	0.0007	0.002		2.45 (0.08)	2.473 (0.005)	*3.5*		2.31 (0.07)	2.36 (0.09)	2.91 (0.09)	2.98 (0.06)
Ho	0.0005	0.0006		0.43 (0.02)	0.44 (0.02)	*0.54*		0.35 (0.01)	0.36 (0.001)	0.46 (0.02)	0.49 (0.02)
Er	0.0006	0.0005		1.28 (0.04)	1.23 (0.02)			1.01 (0.02)	0.92 (0.01)	1.34 (0.06)	1.21 (0.05)
Tm	0.0005	0.0006		0.150 (0.007)	0.167 (0.007)			0.095 (0.001)	0.113 (0.006)	0.136 (0.008)	0.159 (0.008)
Yb	0.0006	0.001		1.06 (0.03)	1.01 (0.05)	*1.6*		0.68 (0.02)	0.652 (0.007)	0.94 (0.05)	0.90 (0.02)
Lu	0.0004	0.0009		0.132 (0.005)	0.154 (0.007)			0.069 (0.003)	0.093 (0.003)	0.102 (0.008)	0.121 (0.003)
Hf		ND[c]			0.17 (0.02)	*3.7*			0.23 (0.02)		0.36 (0.03)
Ta		ND[c]			NDT[c]				NDT[c]		NDT[c]
W		ND[c]			NDT[c]	*2*			NDT[c]		NDT[c]
Re		ND[c]			NDT[c]				NDT[c]		NDT[c]
Os		ND[c]			NDT[c]				NDT[c]		NDT[c]
Ir		ND[c]			NDT[c]				NDT[c]		NDT[c]
Pt		ND[c]			NDT[c]				NDT[c]		NDT[c]
Au		ND[c]			0.084 (0.005)	*0.3*			NDT[c]		NDT[c]
Hg		ND[c]			NDT[c]	1.40 (0.08)			NDT[c]		NDT[c]
Tl		0.004			0.7 (0.3)	0.74 (0.05)			0.2 (0.1)		0.3 (0.2)
Pb	0.01	0.03	14.9 (0.3)	16 (1)	16.0 (0.7)	18.9 (0.5) (*12-18*)	ND	16.3 (0.7)	14 (1)	11.5 (0.5)	9.4 (0.4)
Bi		0.03			< LOD				< LOD		< LOD
Th		ND[c]			12.1 (0.2)	*11*			11.7 (0.3)		14.1 (0.8)
U		0.004			2.05 (0.06)	*3*			1.45 (0.03)		1.34 (0.08)

a: Concentrations values (after a correction for the dilution and mass factors) correspond to the mean of three replicates and the corresponding standard deviation (in brackets);
b: Concentrations values in mg g^{-1};
c: ND = not determined; NDT = not detected;
d: Concentrations in italic are only informative, in brackets and italics are informative range values for leachable concentrations using only HNO_3 digestion.
ICP-MS: Inductively coupled plasma mass spectrometry; AAS: Atomic absorption spectroscopy

For Zn and Ga recovery percentages around 110 % were also observed in the reference soil and in one of the vineyard soil samples, being the reason probably related with matrix-induced interferences (for instance, $^{32}S^{16}O^{16}O$ or $^{48}Ti^{16}O$ in the case of ^{64}Zn and $^{37}Cl^{16}O^{16}O$, $^{138}Ba^{2+}$ or $^{53}Cr^{16}O$ in the case of ^{69}Ga).

Table 11.2. Recovery percentages (%)[a] obtained by using the quantitative mode of analysis by ICP-MS. The soil sample solutions were spiked with known amounts of elements from 0.2 to 75 $\mu g\ l^{-1}$ (depending of the element).

Element	Reference Soil	Old vineyard soil	Young vineyard soil
Li	101 ± 14	87 ± 5	94 ± 8
Al	111 ± 8	110 ± 8	101 ± 2
V	103 ± 2	100 ± 9	94 ± 3
Cr	118 ± 18	90 ± 7	109 ± 23
Mn	ND[b]	104 ± 2	97 ± 5
Fe	112 ± 13	108 ± 13	99 ± 2
Co	98 ± 6	95 ± 5	98 ± 21
Ni	99 ± 3	94 ± 10	84 ± 2
Cu	91 ± 4	ND[b]	96 ± 27
Zn	110 ± 23	97 ± 8	113 ± 14
Ga	110 ± 15	95 ± 10	107 ± 5
As	105 ± 8	91 ± 4	88 ± 1
Rb	95 ± 5	103 ± 7	89 ± 3
Sr	92 ± 2	103 ± 2	92 ± 5
Cd	100 ± 5	95.2 ± 0.3	91 ± 6
Ba	83 ± 1	107 ± 5	94 ± 6
La	100 ± 2	93 ± 2	92 ± 4
Ce	111 ± 6	ND[b]	ND[b]
Pr	104 ± 2	105 ± 4	106 ± 5
Nd	107 ± 4	97 ± 1	96 ± 3
Sm	101 ± 4	100 ± 3	100 ± 3
Eu	101 ± 6	99 ± 1	99 ± 1
Gd	100 ± 1	95.9 ± 0.4	98 ± 1
Tb	99 ± 8	97 ± 1	96.7 ± 0.8
Dy	99 ± 2	95.7 ± 0.9	95 ± 1
Ho	100 ± 3	97 ± 2	97 ± 2
Er	100 ± 2	97 ± 1	97 ± 1
Tm	99 ± 7	99 ± 2	98 ± 1
Yb	100 ± 6	100.3 ± 0.4	100 ± 1
Lu	100 ± 10	103.1 ± 0.8	103 ± 1
Pb	98 ± 4	105 ± 5	97 ± 4

a: Mean of recovery values of three different spikes ± standard deviation;
b: ND = not determined.

The quantitative mode was then applied for the multi-element analysis of the three mentioned soil samples. Per sample, three independent replicates were analysed and the mean and the respective RSD calculated for each of the thirty-one selected elements. The obtained results are included in Table 11.1. The observed RSDs were between 0.5 and 6 %, being lower than 3 % in most of the cases. These precisions are similar to those reported by Wu *et al* [8] and slightly better than those presented by Falciani *et al* [7], both for certified soil samples. The exception was Ba in the old vineyard soil (RSD 14 %), probably due to experimental errors.

The HPMW-digestion procedure was carried out only with concentrated HNO_3. This procedure is a strong acid digestion that dissolves almost all elements that could become "environmentally available" [9] and it was chosen because only the total-recoverable fraction was of interest, and not the total decomposition of the soil sample. This fraction is frequently called "total-recoverable metal".

The concentrations of the elements in the total-recoverable fraction are generally lower than the total ones. The NIST reference soil San Joaquin Soil SRM 2709 is an agricultural soil that, besides several certified and informative elemental concentrations for total sample decomposition, presents informative data for several elemental concentrations (ranges of concentrations) in the total-recoverable fraction (leach ranges) (see Table 11.1). These ranges were used as guideline for the evaluation of both the HPMW-digestion procedure and the results provided by the ICP-MS quantitative mode of analysis.

As can be observed in Table 11.1, Mn, Co, Cu, Ni, Zn, As, Cd, and Pb were within the leach concentration range provided by NIST, while Al, V, Cr, Fe, Sr and Ba were slightly above the upper limit of the range. For Al, V and Cr the differences were between 13 and 19 %, which can be related with the reasons pointed out before for the high recovery percentages observed for theses elements, which were inappropriate internal standard and/or matrix-induced interferences. For Fe, Sr and Ba the differences were small, between 3 and 6 %, and were probably related with the HPMW-digestion procedure. Most elements were below the certified total concentration, as expected, while a few, like Cr, Fe, Co or Zn, were very near that value. The concentration of Cd obtained in the reference soil was higher than the certified value, probably because this concentration was below the respective LOQ, which unable its accurate determination.

As refereed above, the San Joaquin Soil SRM 2709 also provides informative (non-certified) on the concentrations of several other elements. For those elements, the concentrations obtained in this work (total-recoverable fraction) were also below the informative value. The only exception was Ga, for which

a concentration 30 % higher was obtained, possibly as a result of matrix-induced interferences, as pointed out before with regard to the high recovery percentage observed in the reference soil sample.

AAS analysis. The results obtained by AAS for Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in both the reference soil and one vineyard soil are also included in Table 11.1.

A statistical comparison (through t-paired test) [10] showed no significant differences between the results obtained by the two analytical techniques (ICP-MS and AAS) for Al, Cd, Co, Cu, Fe, Pb and Zn. Therefore, the differences observed before between the values obtained for these elements in the reference soil and the NIST informative leach range were probably related not only with ICP-MS analysis but also with the HPMW-digestion procedure. For Cr and Ni, although the values obtained by AAS and by ICP-MS were statistically different, such differences were between 11 and 16 %. For Mn the values were not comparable, as differences around 30 % were observed for both soil samples. This element can suffer a matrix-induced interference from $^{40}Ar^{14}N^{1}H$ in ICP-MS measurements and this can be a possible reason for such high difference. Nevertheless, both soil samples present more or less the same differences, so, this element can still be measured at least for comparison of similar samples.

A linear least square adjustment was applied to all elements determined, in parallel, by both AAS (x-axis) and quantitative mode ICP-MS (y-axis) (Fig. 11.1). Mn was excluded from the adjustment since the values were non comparable. The comparison brought to the equation ICP-MS = (1.0 ± 0.1) AAS + (1 ± 6), with a correlation coefficient of R = 0.98 (n = 16). Evidence of either relative or fixed bias in the measured range was not observed.

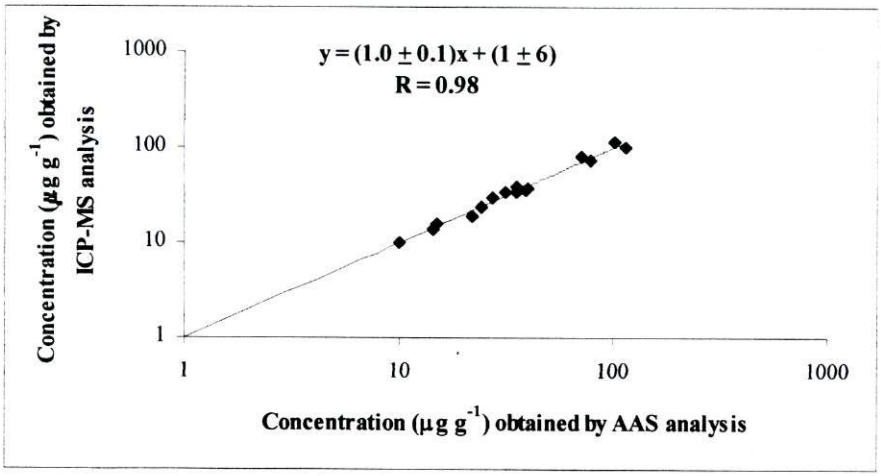

Fig. 11.1. Comparison, by linear regression, between AAS and ICP-MS results obtained for the elements Al, Cd, Co, Cr, Cu, Fe, Ni, Pb and Zn measured in common in the three soil samples (the 95 % confidence limits of the linear regression parameters are given). In both axis is represented the logarithm of concentrations (concentrations expressed in $\mu g\ g^{-1}$) for a larger scattering of the points.

11.4. SEMI-QUANTITTATIVE MODE OF ANALYSIS

The semi-quantitative mode of analysis was applied to sixty-three elements at major, minor and trace concentration levels, including those measured in quantitative mode, in the three selected soil samples. Three independent replicates were analysed and the mean and respective RSD were calculated for each element. The obtained results were included in Table 11.1.

For the measurement of Mn and Fe a higher dilution was required (one-hundred times) due to the high concentration of these elements in the soil samples. Therefore, two analyses in the semi-quantitative mode were performed per sample (one for five-fold and another for one-hundred-fold dilution), which globally took about eight minutes per sample. In contrast, the quantitative mode of analysis required much more time, about thirty-five minutes per sample (see Experimental Section).

In order to analyse Al in the soil samples by ICP-MS semi-quantitative mode, a two-thousand-fold dilution would be necessary which would increase substantially both the preparation and the analysis time per sample, since another diluted solution was required. So, this element was excluded from the analysis in the semi-quantitative mode.

The LODs of the measured elements (calculated as before for the quantitative mode) were also determined and included in Table 11.1. For most of the studied elements, the LODs were higher (between two and six times) than those obtained by using the quantitative mode of analysis. For a set of twenty-two measured elements, the LODs were not determined (see Table 11.1) because no standard solutions were available.

Ten of the measured elements (Ru, In, Sn, Ta, W, Re, Os, Ir, Pt and Hg) were not detected in any of the studied soil samples while another three elements (Se, Te and Bi) were below or near the LODs, which prevented the respective determination. The vineyard soil samples presented lower concentrations of most elements than the reference soil sample and in these samples three more elements (Pt, Sb and Au) were not detected and another three more (Mo, Ag and Cd) were below or near the respective LODs (see Table 11.1.).

In terms of precision, for most of the determined elements, the RSDs were similar to those observed by using the quantitative mode of analysis, between 0.5 and 6 %. For Be, Ge, Nb, Mo, Pd, Ag, Cd, Sb, Hf and Tl the RSDs were higher, between 6 and 40 %, probably due to the relatively low concentrations, in most cases close or below the respective LOQ. For few elements, like B in the reference soil sample and Ti in the vineyard soil samples, the RSDs were slightly higher, but lower than

10 %. The element B in the vineyard soil samples could not measured by the equipment (after the required automatic corrections no signal was detected), being the reasons for it not clear yet.

For the elements determined in the reference soil using the semi-quantitative mode of analysis, the leach range provided for the NIST reference soil San Joaquin Soil SRM 2709 was used also as guideline. As can be observed in Table 11.1, and similarly to what was observed with the quantitative mode of analysis, Ca, Mn, Co, Ni, Cu, Zn, As, Cd, Sb and Pb were within the leach concentration range provided, while V, Cr, Fe, Sr and Ba were slightly above and Ti was much above (twice as maximum) the upper limit of the range. The isotope ^{49}Ti may suffer from matrix-induced interferences from $^{33}S^{16}O$ or $^{35}Cl^{14}N$ and this may be the reason for such high concentration of Ti. Therefore, it was concluded that Ti could not be measured in the soil samples with the present ICP-MS equipment and methodology.

As refereed earlier, Cr may suffer matrix-induced interferences from Cl species and that could be a probable reason for the Cr concentration measured in the reference soil (by both modes of analysis) to be slightly above the leach range. Therefore, using the semi-quantitative mode, Cr was determined in the one-hundred-fold diluted solution used for the measurements of Mn and Fe concentrations, since in this case the matrix-induced interference could be reduced. It was observed that, in this situation, the concentration of Cr would be within the leach range (99 ± 2 $\mu g\ g^{-1}$). Therefore, for further measurement of Cr in soil samples by semi-quantitative mode, the one-hundred-fold dilution of the sample solution was chosen.

Most of the measured elements for which there were only informative total concentrations, presented total-recoverable concentrations below or near those values. The exceptions were Sc and Y whose concentrations were higher (about six times) than the respective informative values. Therefore, similarly to Ti, it was concluded that these two elements could not be measured in the soil samples with the present ICP-MS equipment and methodology.

11.5. COMPARISON BETWEEN QUANTITATIVE AND SEMI-QUANTITATIVE MODES OF ANALYSIS

Thirty elements (As, Ba, Cd, Co, Cr, Cu, Fe, Ga, Li, Mn, Ni, Pb, Rb, Sr, V, Zn and the REEs La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) were measured, in parallel, by using the quantitative and the semi-quantitative analytical modes. Comparable results (differences lower than 20

%) were observed, except for Ga and Cd in the reference soil, Sr and Lu in the old vineyard soil, and Cr, As and Sr in the young vineyard soil. In most cases, the differences were lower than 10 %. Differences between 10 and 20 % were obtained only for a few elements, like Mn, Ni and Lu in the reference soil; Cr, Mn, Cu, Rb, Ce, Tm and Pb in the old vineyard soil; and Zn, Rb, Ce, Tm, Lu and Pb in the young vineyard soil. The present results corroborate previous ones, since accuracy errors lower than 20 % for most elements have been reported in literature for semi-quantitative mode of analysis, including in the analysis of sediments [4,6].

For the elements with non-comparable results, the differences observed between the two modes of analysis were between 30 % and 50 %. The reasons were probably related with either low concentration levels, as observed for Cd and Lu, or matrix-induced interferences not corrected in the quantitative mode but automatically corrected in the semi-quantitative mode.

A linear least-squares adjustment of the two sets of results (semi-quantitative mode, SQ (y-axis) *versus* quantitative mode, Q (x-axis)) was performed and the results are shown in Fig. 11.2. The regression line of the global results (including the three soil samples), yielded the equation: $SQ = (1.12 \pm 0.02)\ Q - (2 \pm 2)$ with a correlation coefficient (R) of 0.998 ($n = 87$). So, high correlation (the value of R being close to one) and intercepts statistically identical to zero (95 % confidence level) were found. However, slightly higher values for the semi-quantitative mode of analysis, that is, a slope significantly higher than one ,were observed, evidencing a slight fixed bias. Similar results were obtained either when only the elements with differences (between quantitative and semi-quantitative modes) ≤ 20 % were considered in the adjustment or when each soil sample was considered separately.

The present results indicated that the semi-quantitative mode of analysis, despite the slight bias, could be used for comparison of elemental compositions of soils, having some economic advantages relatively to the quantitative mode: it is faster and requires fewer reagents.

In conclusion, the ICP-MS semi-quantitative procedure developed is suitable for routine multi-element analysis (at major, minor and trace concentration levels), in soils, allowing the determination of As, Ba, Be, Ca, Cd, Co, Cr, Cs Cu, Fe, Ga, Hf, Li, Mn, Nb, Ni, Pb, Rb, Sr, Th, Tl, U, V, Zn, Zr and the REEs La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, with accuracy errors lower than 20 %.

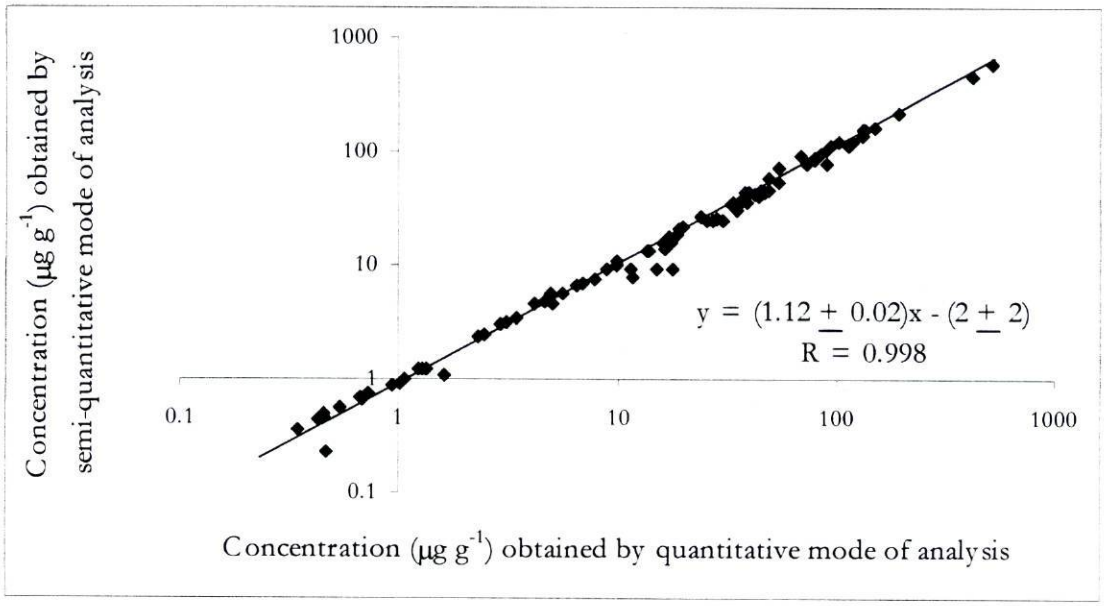

Fig. 11.2. Comparison, by linear regression, of the results obtained for the three soil samples by ICP-MS using quantitative and semi-quantitative modes of analysis (the 95 % confidence limits of the linear regression parameters are given). In both axis is represented the logarithm of concentrations (concentrations expressed in $\mu g\ g^{-1}$) for a larger scattering of the points.

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Chapter 12

Multi-Element Composition of Wines and Their Precursors Including Provenance Soil as Potential Fingerprint of Wine Origin

12.1. INTRODUCTION AND AIMS

Multi-element composition of wines has been used to obtained information about the origin and the authenticity of a wine. This is based on the assumption that the element composition of the soil of provenance is represented in the wine. Therefore, the winemaking process would not change the elemental composition of the wine and only the movement of elements from rock to soil and from soil to grape would influence the elements concentrations in it. Nevertheless, Eschnauer *et al* [1] showed that the concentration of several elements (Al, Cd, Co, Cr, Cu, Fe, Mn, Pb, V and Zn) decreased during fermentation and fining of wines. The same phenomenon was observed by Angelova *et al* [2] for Cd, Cu, Pb and Zn. On the other hand, Jakubowski *et al* [3] reported that the concentrations of REEs increased from young to finished product wines, due to the use of bentonites. In addition, Kristl *et al* [4] concluded that there was a slightly contamination of cadmium, chromium and lead during the maturation of wine released from wine cellars equipment (brass and stainless-steel). As reported in Chapter 5, a lead increase in wine during the vinification process was also observed. Therefore, as the vinification procedure can influence the concentration of several elements in the wine, element fingerprints of the wines should be relict soil signatures that survived metabolic and winery processing.

Wine processing changes from wine to wine, from winery to winery and from one country to another. It is important to determine which elements go unchanged through each vinification process and, in a future stage, to determine if those elements are common or not to all winery processing, in order to be able to use them as tracers for the authenticity of wines.

The aim of the present study was to follow the production of two different Portuguese wines in order to determine the influence of the vinification process in the final product elemental composition, and investigate whether there were significant correlations between the multi-element composition of the wines and that of the provenance soil. Information on the elemental composition of the wines may be of interest by itself, for nutritional and toxicological purposes.

Two different vineyards from the Douro region – Northeast of Portugal - were selected for the present work. The grapes from one vineyard were used to produce a red table wine in a modern winery and the grapes from the other one were used to produce a fortified wine by using an old fashion process. The multi-element composition of the different interveners of each winemaking process was followed (from the vineyard soil to the final wine products) during an annual cycle of wine production (year of 2000). More concretely, multi-element analysis of soil, grape juice and samples collected in the different

steps of the vinification process were performed. In all those samples Al, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Li, Mn, Nb, Ni, Pb, Rb, Sr, Th, Tl, U, V, Zn, Zr and the REEs (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) were determined. Vine leaves and grapes were also collected and analysed for some elements (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) with the purpose of obtaining additional information of the pathway of elements throughout the wine production.

Soils are complex materials comprising of weathered rock, humus, water and air. In addition to providing physical anchorage and support for growth, soil provides nutrients for plants. However, plants cannot extract all the ionic materials within the soil, as some are too strongly bond within the soil structure. The eco-toxicity and mobility of metals in the soil depend strongly on specific chemical forms in which they are present. Therefore, in this work, only the total-recoverable metals, extracted by nitric acid digestion [5], were measured rather than the total-total metal contents. EDTA soil extractions at pH 7 [6] were also carried out in order to estimate the fraction of the element that is available for plant uptake and to compare it with the total-recoverable fraction. However, only Cd, Cr, Cu, Ni, Pb and Zn, for which there were certified extraction procedure [6], were measured in the EDTA extracts.

12.2. EXPERIMENTAL

The origin and characteristic of the studied samples as well as the procedures of sampling, sample treatment and analysis were described in Chapter 3.

For vineyard soil samples, after the HPMW-digestion, five or one hundred-fold (only for Al, Mn, Fe and Cr determinations) dilution with a solution containing Rh was carried out. In the final solutions a 20 $\mu g\ l^{-1}$ Rh concentration was always present. All elements were determined by ICP-MS, with exception of Al, which was determined by AAS. As it was already mentioned discussed in Chapter 11, for analysing Al in the soil samples by ICP-MS semi-quantitative mode of analysis a two thousand-fold dilution would be necessary. Such procedure would increase substantially both the preparation and the analysis time per sample, since another diluted solution would be required. Therefore, it was decided to determine Al by AAS, in the one-hundred-fold diluted solution.

The grape juices and samples from the vinification processes, after UV-irradiation pre-treatment, were diluted to 12.5 ml (a two and half times dilution of the sample) with a solution containing HNO_3 and Rh. Final solutions always contained 20 $\mu g\ l^{-1}$ Rh and 1 % HNO_3.

For analysis in the EDTA soil extracts, the solutions were used directly, for Cu and Zn determinations, and after a five-fold dilution with a 0.2 % HNO_3 solution, for Cd, Cr, Ni and Pb determinations.

For vine leaves and grapes, different dilution ratios, with de-ionised water, of the final solutions were carried out, depending on the element to be analysed. For Al determinations the solutions were used undiluted. For the remaining elements in the vine leaves, two (Cd and Co), four (Cr, Ni, Pb), ten (Cu, Fe and Zn) or twenty (Mn and Zn) times dilution were carried out. For determination in the grapes, the final solutions were analysed directly (Cu, Mn and Zn) or diluted two (Cd, Co, Cr, Ni and Pb) or five (Fe) times.

12.2.1. ICP-MS measurements

The multi-element composition of vineyards soil, grape juice and samples from the vinification processes were measured using the semi-quantitative ICP-MS multi-element mode of analysis. The experimental details are given in Chapter 3. The measured elements were Al, B, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Li, Mn, Mo, Nb, Ni, Pb, Rb, Sb, Sc, Sr, Ti, Th, Tl, U, V, W, Y, Zn, Zr and the REEs (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) in grape juices and samples from the different steps of the vinification processes and As, Ba, Be, Ca, Cd, Co, Cr, Cs Cu, Fe, Ga, Hf, Li, Mn, Nb; Ni, Pb, Rb, Sr, Th, Tl, U, V, Zn, Zr and the REEs in soils samples.

The soil reference material (San Joaquin Soil 2709) was analysed together with the samples of the vineyards soil every working day and several times a day (between three and eight, depending of the number of samples analysed), with the purpose of controlling/detecting possible ICP-MS instrumental bias over time. Daily variations (short-term precision) between 2 % and 12 % were observed for all elements, being lower than 5 % in most of the cases. Exceptions were only observed for Nb with variations between 9 % and 100 %, probably as a result of the relatively low concentration of this element in the reference soil. Since also very low concentrations of Nb were observed in the vineyard soil samples, this element was excluded from the set of elements to be quantified. Long-term variations between 3 % and 20 %, being in most of the cases around 10 %, were observed for all elements measured in the reference soil, as well as similar control graphics, indicating that the ICP-MS instrument exhibited the same performance for low and high masses. Both short-term and long-term precision were within those expected for the ICP-MS semi-quantitative mode of analysis, since accuracy errors up to

20 % for most elements have been reported for this type of measurements, as discussed in Chapters 9 and 11.

Each working day, two red wines (one table and one Port), with previously (Chapter 9) determined multi-elements concentrations were analysed together with the grape juices and the samples from the vinification processes, also for checking possible instrument bias. Daily variations (short-term precision) between 2 % and 40 % were observed for all of the elements measured in both "reference" wines, being lower than 10 % for most of them. Variations higher than 20 % were only observed for Ga (40 %) and Sb (25 %) in the table wine and for Dy, Ga and Sm (25 %) in the Port wine, probably as a result of their relatively low concentrations in the wines.

For all samples three independent replicates were pre-treated and analysed and, after blank subtraction, the mean concentration and respective standard deviations were calculated.

12.3. SUITABILITY OF THE PROVENANCE SOIL COMPOSITION AS FINGERPRINT OF WINE ORIGIN

12.3.1. Vineyards soil

12.3.1.1 Soil content

The total-recoverable metal levels in the soil samples, collected once per month in different vineyard sites and at different depths, are illustrated in Fig. 12.1 for Cd, Ni, Cu, Ca and Fe. Similar results were observed for the remaining measured elements. Results obtained for all measured elements in all soil samples are presented in the Appendix Section at the end of this Dissertation (Fig. A.2.1).

Discussing the depth influence, Cu in the old vineyard soil was the only element whose levels were systematic and significantly higher at surface of soil (S_a) than at 20 cm depth (S_b). This result indicates contamination of the soil surface layer with Cu, which is compatible with the periodical application of copper sulphate in the vine, for several decades. Therefore, for this element only the concentrations obtained at 20 cm depth were considered for calculation of a mean Cu concentration value in the soil, as it seemed that it would be more directly related with plant uptake.

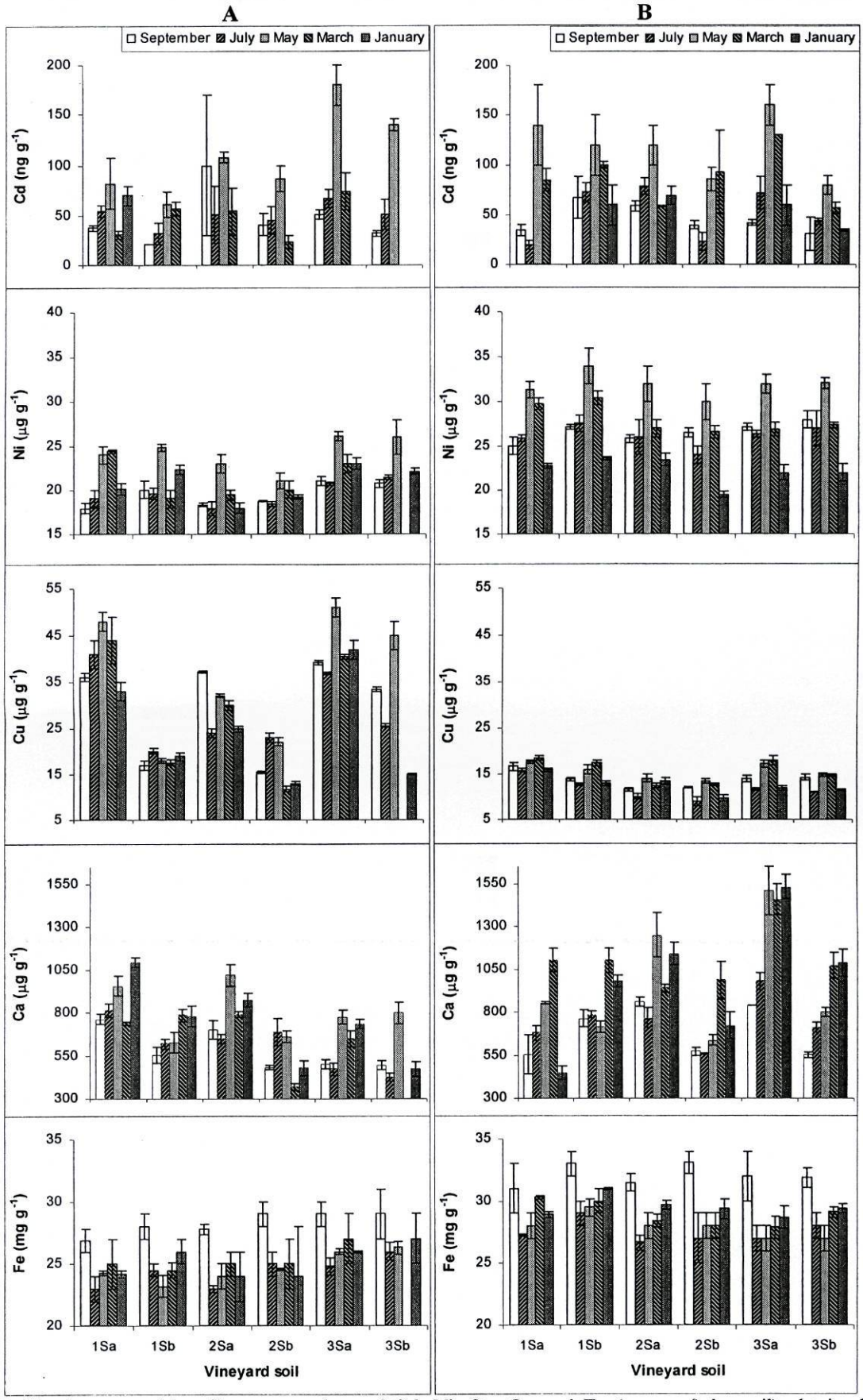

Fig. 12.1. Total-recoverable soil concentrations of Cd, Ni, Cu, Ca and Fe (per g of dry soil) obtained in the vineyard soil samples collected at surface (S_a) and at 20 cm depth (S_b), in the three selected sites (1S, 2S, 3S), of the old (A) and young (B) vineyards, in five different months. The sample $3S_b$ from the old vineyard and from March was lost.

All the other elements did not displayed systematic significant differences in their concentrations at the two levels of soil depth and, therefore, all the available data were accounted when a global concentration mean was calculated.

Considering the three sampling sites per vineyard (1S, 2S, 3S) no systematic significant differences were found among them. The observed differences (for instance, for Cd and Ni in May in the old vineyard) seemed to be mainly a result of a natural heterogeneity of the soil.

As Fig. 12.1 illustrates for both vineyards, significant differences in the concentrations of most elements were observed among the different months. In most cases, an apparent increase in the elements concentrations occurred up to May, decreasing afterwards. Nevertheless, such differences were within the long-term precision of the ICP-MS analysis of the respective element, and the variations observed throughout the different months were similar to that observed in control graphics obtained by the regular analysis of the reference soil sample. It follows therefrom that for most elements, if any concentration variation occurred throughout the period of study, it was small and impossible to distinguish from the instrument bias. Besides, the mean concentration levels of the elements observed in the different months were linear correlated (correlation coefficients, $R \geq 0.985$, $n = 39$, $P < 0.01$), indicating that the concentrations obtained in any of the months were equally suitable to establish any possible relationship between wine and soil multi-element compositions. Therefore, global average concentration values from the month of September were chosen for further discussion, being presented in Fig. 12.2, except those of REEs (discussed below).

It must be noticed that the concentrations of the elements observed in the soils of both vineyards were within typical contents of uncontaminated soils [7]. Even for Cu, the concentration observed in the old vineyard soil, although relatively high was still within the concentration range normally found for unpolluted soils [7].

The REEs patterns are also potential indicators or tracers of samples' origin, and were also measured in the vineyards soil. Similarly to the remaining elements, no statistically significant differences were observed either among the three sampling sites of each vineyard or between the two soil layers. Identical REEs distribution patterns were obtained for all soil samples analysed. The REEs concentrations are often reported relative to levels in chondritic meteorites, facilitating the comparison of different REEs distribution patterns. A plot of such chondrite-normalised concentrations against atomic number should produce a smooth graph, with the possible exceptions of Ce and Eu [8]. In Fig. 12.3 the concentrations obtained in September normalised to chondrite values are presented as an example, being the results obtained for the remaining months presented in the Appendix Section (Fig. A.2.2).

Identical patterns were observed for the remaining months and, therefore, the average global concentration values measured in September were chosen for further discussion.

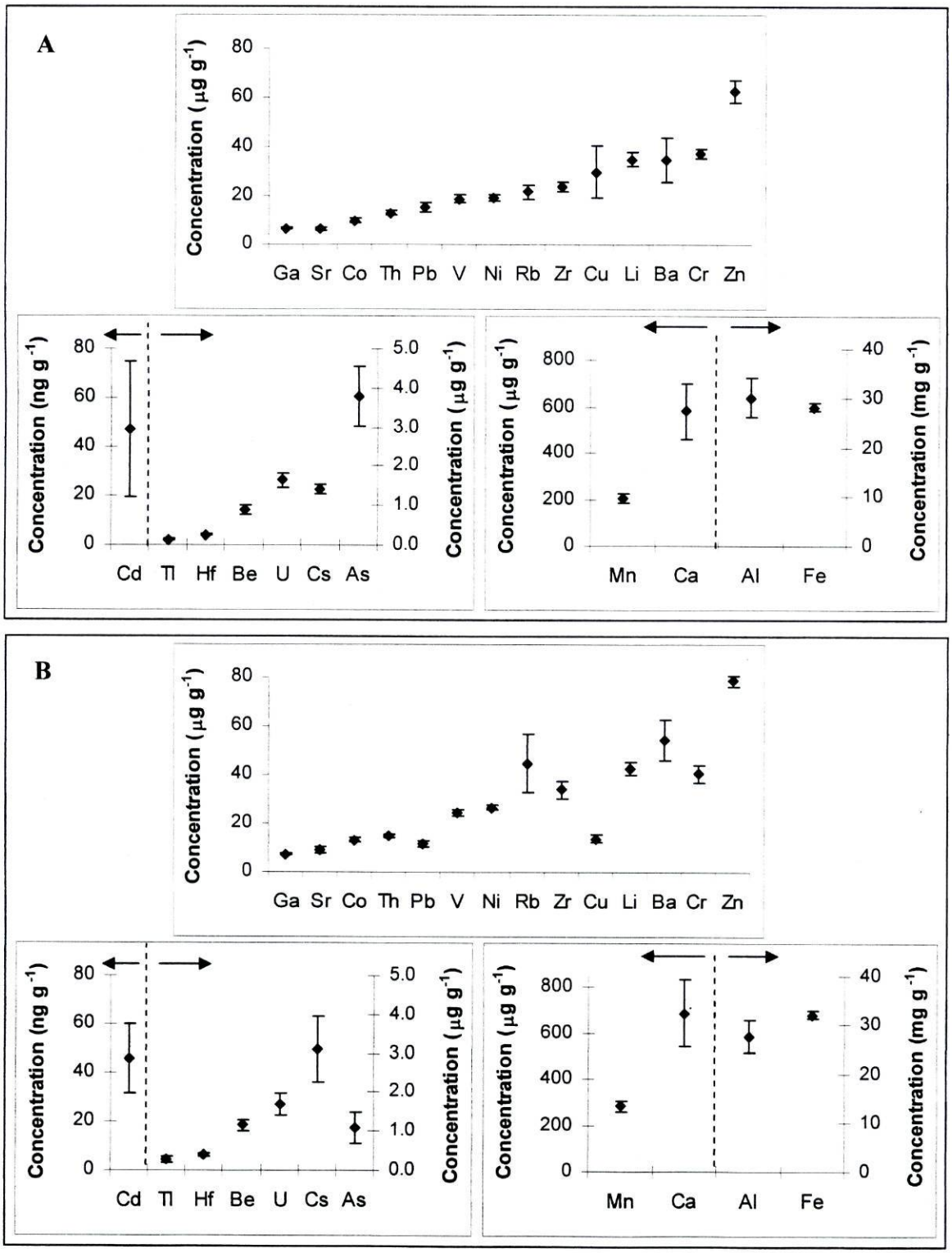

Fig. 12.2. Total-recoverable soil concentrations (global averages) of Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs Cu, Fe, Ga, Hf, Li, Mn, Ni, Pb, Rb, Sr, Th, Tl, U, V, Zn and Zr (per g of dry soil) obtained in September in the old (A) and young (B) vineyards.

As Fig. 12.3 (and Fig. A.2.2 in Appendix Section) shows, similar REEs distribution patterns between the soils of the two vineyards were found.

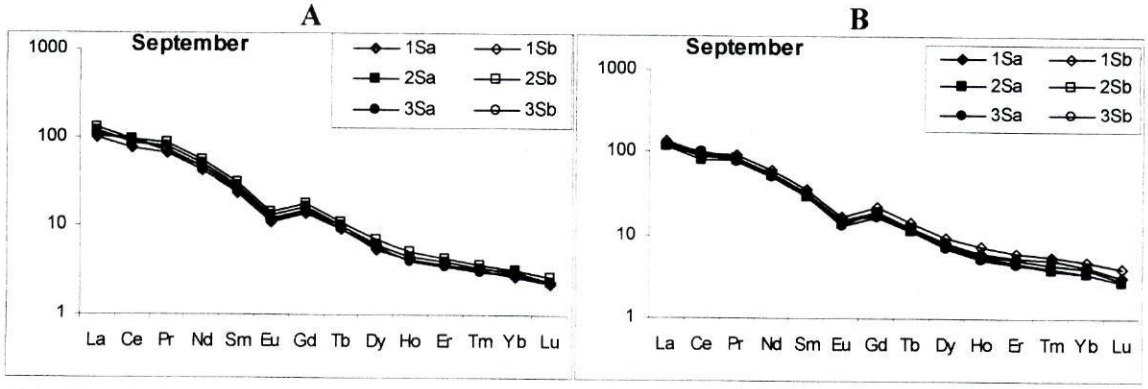

Fig. 12.3. Plot of chondrite-normalised concentrations of the REEs against atomic number (y-axis in logarithm) obtained for the vineyard soil samples collected at surface (S_a) and at 20 cm depth (S_b), in the three selected sites (1S, 2S, 3S), in the old (A) and young (B) vineyards, in September.

When the multi-element concentrations of the two vineyard soils were compared, significant differences were observed for most elements, having the young vineyard soil, in general, slightly higher elemental concentrations (see Fig. 12.2). Nevertheless, a significant linear correlation ($R = 0.995$, $n = 39$, $P < 0.01$) was observed between the two vineyard soil compositions, which was expected as the two vineyards belong to the same region.

12.3.1.2. EDTA soil extracts

For comparison purposes, EDTA soil extractions were also carried out, since this fraction has been considered more directly correlated than the soil total-recoverable fraction with vine leaves or grapes multi-element composition and, therefore, with that of the wine [6]. The obtained results are shown in the Appendix Section (Fig. A.2.3).

Considering the three sampling sites and the two soil layers, the percentages of EDTA extracted metals relatively to the total-recoverable metal contents varied between: 0.10 – 0.30 for Cr, 0.80 –1.6 for Ni, 1.0 – 5.0 for Zn, 9.0 – 48 for Cu, 15 – 21 for Pb and 14 – 45 for Cd in the old vineyard soil. In the young vineyard soil the percentages were of similar magnitude, though slightly lower: 0.03 – 0.15 for Cr, 0.40 – 0.80 for Ni, 0.40 – 3.0 for Zn, 3.0 –12 for Cu, 8.0 – 16 for Pb and 6.0 – 40 for Cd. These results indicated that the available metal varied with the nature of the element, as expected since it conditions both the chemical forms and the binding strengths. Among the measured metals, Cd was the most available and Cr the less available. It is know that Cr is quite immobile in soils [7]. The slightly

lower percentages of extractable metals from the young vineyard soil resulted probably of the different soil utilisation of this vineyard in the past, which was a forest soil, for that very reason richer in organic matter, particularly humic substances. It is known that soil organic matter has an essential function in the accumulation and transport of metals as well as in delaying their circulation in the soil [7].

Also in the EDTA soil extracts, for a few elements, some statistically significant differences were observed both among the three sampling sites per vineyard and between surface and 20 cm depth soil. However, they were not systematic and resulted probably of heterogeneity of the soil or of a heterogeneous distribution of the different chemical forms of the metals. Therefore, in order to have a global characterisation of the soil, average concentrations determined monthly in the three different sites per vineyard were calculated. Among the different months significant differences were only observed for Cd in the old vineyard and for Cr in the young vineyard. Significant correlations ($R \geq 0.90$, $n = 6$, $P < 0.05$) were also observed between EDTA extracted metals obtained in the different months, indicating that any particular month is equally suitable to establish a possible relationship between soil and wine multi-element composition. It follows therefrom, that for further discussion the global results of September for the EDTA soil extracts were chosen, being presented in Fig. 12.4.

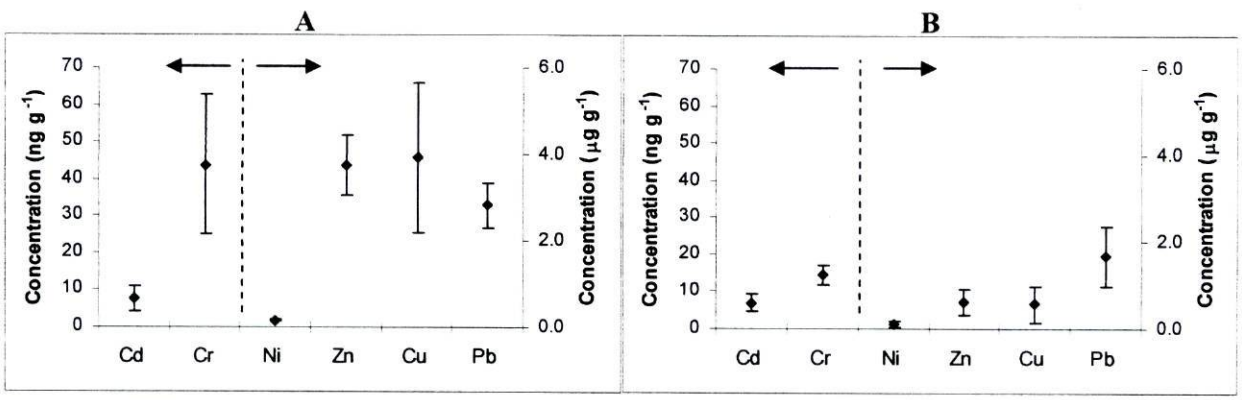

Fig. 12.4. EDTA soil extract concentrations (global averages) of Cd, Cr, Cu, Ni, Pb and Zn (per g of dry soil) obtained in September, in the old (A) and young (B) vineyards.

12.3.2. Grape juices and samples from the vinification processes

Fig. 12.5 (A - fortified and B - table wine) illustrates the results obtained in GJ, prepared in the laboratory, and in the different samples collected during and at the end of the vinification processes of both wines.

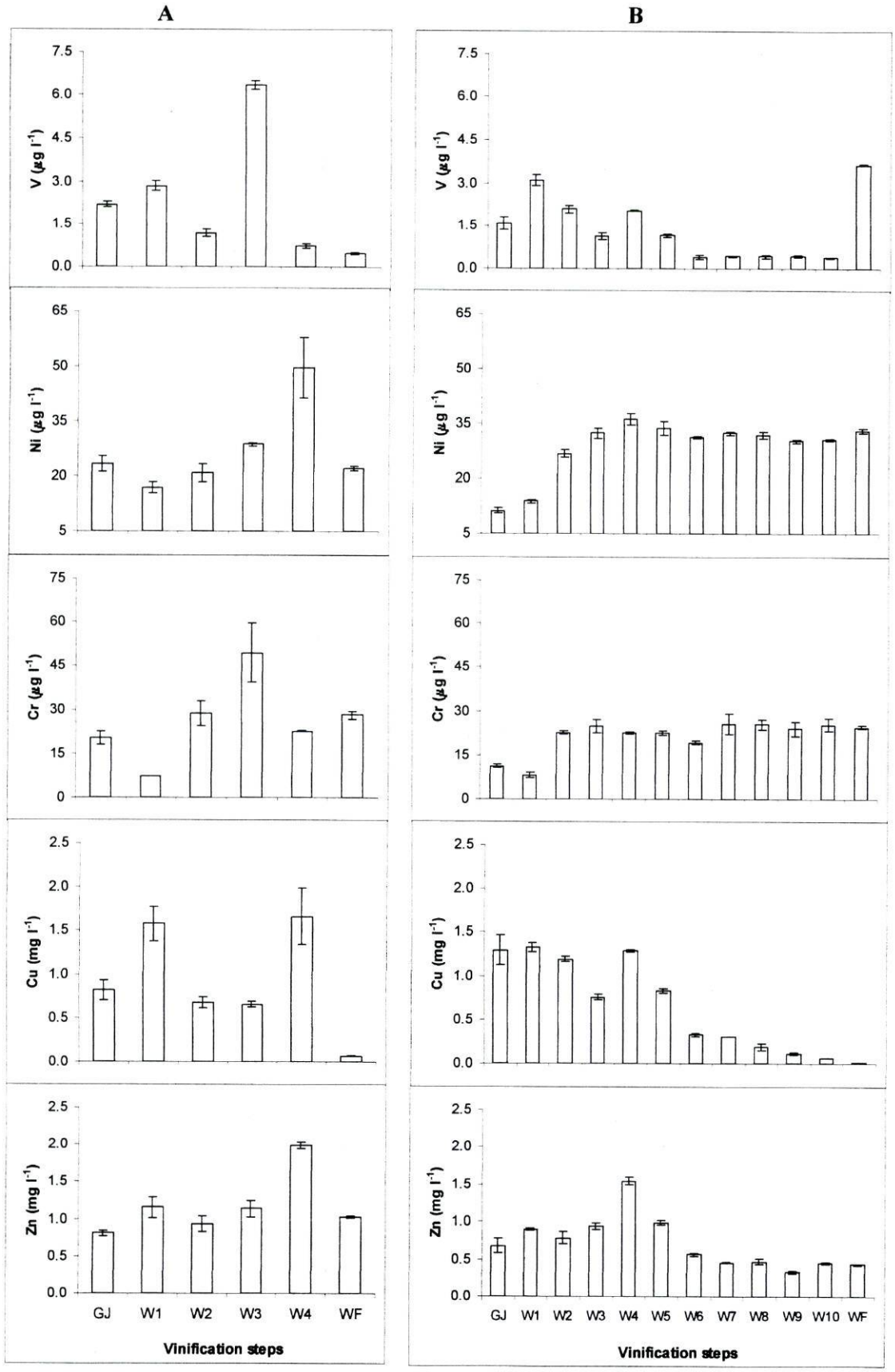

Fig. 12.5. Total concentration of V, Ni, Cr, Cu and Zn (in µg l^{-1}) (mean and standard deviation, n = 3) observed in the grape juices (GJ_F and GJ_T for the fortified and table wine, respectively), and in the samples collected throughout the vinification processes (from W_F1 to W_F4 in the fortified wine and from W_T1 to W_T10 in the table wine) and in the final products (W_FF and W_TF). (A) red fortified wine produced with grapes from the old vineyard; (B) red table wine produced with grapes from the young vineyard.

For the sake of simplicity only illustrative results, which were those obtained for V, Ni, Cr, Cu and Zn, are shown. The results obtained for all measured elements in these samples are presented in the Appendix Section (Fig. A.2.4).

For both wines the concentration of most elements significantly changed during the vinification processes. The only exceptions were Ga and Sb, for which the observed changes were compensated by the instrument daily variations. The concentrations of most of the REEs in both wines decreased down to levels below the limit of detection in WF preventing the establishment of their distribution pattern (for detection limits values see Chapter 10). These results are compatible with the fact of no bentonites had been added to the must during the vinification. Therefore, contamination of finished wine products by REEs, as reported by Jakubowski *et al* for wines to which bentonites had been added [3] was not observed in the present cases.

For the fortified wine, the concentrations of Al, Cu, Nb, Tl, Th, U, V, Zr and REEs in W_FF were lower than in the GJ_F, probably as a result of dilution due to the addition of 20 % grape brandy to stop fermentation and eventually also precipitation or co-precipitation with suspended particles during fermentation and/or ageing. This last phenomenon has been reported in the literature for other wines [1,2]. If contamination by these elements occurred in same steps of the vinification it was compensated by the phenomena described before. For B, Ba, Be, Co, Mo, Ni, Rb, Sc, Ti, Y and W, the respective concentration in W_FF was similar to that found in the GJ_F and for Li, Ca, Cd, Cr, Cs, Fe, Pb, Sr and Zn the levels were even higher in W_FF than in GJ_F. As in the step W_F2 there were a 20 % dilution of the wine with brandy, for all these cases releasing of the elements from the vinification system (contamination) has occurred in some steps of the vinification process. Alternatively or in addition to contamination, liberation of the elements from grapes skins and seeds may have happen, as they were pressed together with pomace and hung together during and after fermentation (W_F2 and W_F3). In contrast, in the preparation of GJ in the laboratory skins and seeds remained intact and were rejected. The alcohol content of the brandy facilitates the solubilisation of polyphenolic compounds present in high concentration in grape skins. These compounds are strong complexing agents of heavy metals. It is known that the distribution of Ca, Fe, K, Mg and Na throughout the berry is not homogeneous [9]. For instance, the berry pulp is richer in K and Na than the seeds or skin, while seeds and skin are richer than pulp in Ca and Mg. Berry skin is particularly rich in Fe. Teissedre *et al* [10] also found significant differences in the levels of Pb in the different parts of the grape berries, having the seeds the highest content in Pb and the pulp the lowest one. In the present case, the enrichment observed in the Sr levels from GJ_F to W_FF was attributed to the releasing of the element into the must from seeds and skins of the grapes instead of anthropogenic contamination, as discussed in detail in Chapter 8. In contrast, the

increase of the concentrations of Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn in different steps of the vinification process was mainly attributed to contamination. For instance, from W_F2 to W_F3 a significant increase of Cr, Ni, Pb and V concentrations was observed. Metallic bracelets of the wood container and its tap may be the main sources of these elements. From W_F3 to W_F4 the Cr, Fe and V concentrations decreased significantly, suggesting that a large fraction of those elements precipitated or co-precipitated with colloidal polymeric organic compounds, such as polyphenols and tannins. In contrast, the levels of Cd, Cu, Ni, Pb and Zn increased from W_F3 to W_F4, which indicated a predominance of the effect of contamination during the rest in the stainless-steel container. The levels of Cr and Fe increased again from W_F4 to W_FF, suggesting the presence of sources of these elements in the oak barrel where the wine rested for one-year. Contamination of wines with Cd, Cr and Pb released from winemaking equipment during its maturation has also been reported in the literature [4]. For Pb in the present wine, the analysis of the respective IRs corroborated the evidence of contamination introduced by the different components of the vinification, as discussed in Chapter 5. As concerns Cu, it is worthwhile to stress that the major sources of the metal were found in W_F1 (grape pressing) and W_F4 (resting in stainless-steel vat) steps of the winemaking process. After addition of grape brandy to stop fermentation in W_F2 the Cu levels markedly decreased, probably due to a dilution factor. Therefore, the present results did not corroborate the hypothesis frequently formulated that relatively high levels of Cu usually found in fortified wines (in comparison with those found in table wines) are a result of the addition to the must of grape brandy, which used to be produced in copper distillation devices. The present grape brandy was produced in stainless-steel distiller, being thereby poor in Cu.

For the table wine (Fig. 12.5B and Fig. A.2.4 – Appendix Section) the results were quite similar, with a few differences, for instance, in the sets of elements not common to the two studied vinification processes whose concentration decreased (Co, Ni and Zn instead of V) or increased during the vinification (V instead of Fe, Cd, Cs and Zn). Evidence of contamination from the material of the devices used in the winemaking process (containers, pumps and tubes) was only observed for Al, Cr, Fe, Ni, Pb and V. For Al, Fe and V the concentrations increased only during the ageing period (levels higher in W_TF than in W_T10). For Pb a more or less regular increase in its concentration throughout the vinification process was observed, which was probably related with the presence of Pb sources in the devices used. Similarly as for the fortified wine, this hypothesis was corroborated by the results obtained for the Pb IR, as discussed in detail in Chapter 5. For Cr and Ni some contamination from the containers probably also occurred, since their concentrations remained approximately constant after the solids removal and did not decreased during ageing (as a result of precipitation or co-precipitation with suspended colloidal matter, as observed in the fortified wine). Increase of Cr and Ni concentrations

during the ageing processes after long contact with stainless-steel utensils have been reported in the literature for other wines [2,11].

In summary, both monitored vinification processes influenced the multi-element composition of the produced wine. Most of the elements presented similar or even lower concentration in the WF compared to that observed in GJ, in which the contribution of skins and seeds did not account and contact with the winemaking system did not occurred. Just for the fortified wine a 20 % dilution of the must with grape brandy constitutes a relevant additional factor. Evidence of effective contamination of intermediary products induced by the vinification system, was observed for a few elements: Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in the fortified and Al, Cr, Fe, Ni, Pb and V in the table wine. Nevertheless, only the levels of Cd, Cr, Fe, Pb and Zn in the fortified wine and of Cr, Ni, Pb and V in the table wine were higher in WF than in GJ.

It is worth mentioning that, in spite of the elemental concentrations variations observed throughout the vinification process, for both wines statistically significant and even linear correlations were observed between the concentrations of the different elements measured in the GJ and in the respective WF (see Table 12.1). In Fig. 12.6 such correlation is illustrated for the fortified wine, being the plot obtained for the table wine similar to that. Therefore, those concentration variations were not a preventive of the usefulness of wine multi-element composition as fingerprint of wine origin.

A linear correlation ($R = 0.999$, $n = 19$, $P < 0.01$) was also observed between the multi-element concentrations of the two wines.

12.3.3. Relationships between soil, grape juice and wine

Pearson's correlations between different types of samples were carried out in order to find eventual significant relationships. The obtained correlation coefficients are shown in Table 12.1. For the set of elements Ba, Be, Cd, Co, Cr, Cs, Cu, Ga, Li, Mn, Ni, Pb, Rb, Sr, V, Zn, Zr and the REEs La, Ce and Nd, statistically significant correlations between their mean concentrations soil and produced wine were found (Al, Fe and Ca, the three more abundant elements from the soil were excluded). Fig. 12.6 illustrates for the fortified wine the obtained results, being identical for the table wine. Similar results were obtained for soil and GJ.

The median of the multi-element concentrations (excluding Al, Ca and Fe) observed in the two vineyards soils was also linear correlated with those observed in the two wines ($R = 0.993$, $n = 19$, $P < 0.01$).

Table 12.1. Pearson's correlations observed for average multi-element concentrations obtained in the different types of samples.

		Soil		Leaves				
		Total-recoverable	EDTA extract	Non-washed	Washed	Grapes	Grape juice	Wine
		Old vineyard / Fortified wine						
Soil	Total-recoverable	1						
	EDTA extract	0.988[a] (n=6)	1					
Leaves	Non-washed	0.038 / 0.992[a,b] (n=7)	0.622 / 0.991[a,c] (n=4)	1				
	Washed	0.002 / 0.988[a,b] (n=7)	0.654 / 0.988[a,f] (n=4)	0.997[a] (n=10)	1			
	Grapes	-0.014 / 0.972[a,b] (n=7)	0.427 / 0.910[a,f] (n=4)	0.988[a] (n=10)	0.993[a] (n=10)	1		
	Grape juice	0.282 / 0.986[a,d] (n=29)	0.998[a] (n=6)	0.294 / 0.992[a,e] (n=9)	0.271 / 0.992[a,e] (n=9)	0.273 / 0.999[a,e] (n=9)	1	
	Wine	0.238 / 0.994[a,d] (n=23)	0.989[a] (n=6)	0.245 / 0.970[a,e] (n=9)	0.220 / 0.975[a,e] (n=9)	0.221 / 0.986[a,e] (n=9)	0.997[a] (n=31)	1
		Young vineyard / Table wine						
Soil	Total-recoverable	1						
	EDTA extract	0.896[f] (n=6)	1					
Leaves	Non-washed	0.201 / 0.979[a,b] (n=7)	0.728 / 0.934[a,f] (n=4)	1				
	Washed	0.148 / 0.985[a,b] (n=7)	0.864[f] (n=6)	0.997[a] (n=10)	1			
	Grapes	0.103 / 0.939[a,b] (n=7)	0.186 / 0.994[a,c] (n=4)	0.947[a] (n=10)	0.949[a] (n=10)	1		
	Grape juice	0.225 / 0.904[a,d] (n=27)	0.877[f] (n=6)	0.635 / 0.969[a,e] (n=9)	0.624 / 0.977[a,e] (n=9)	0.580 / 0.964[a,e] (n=9)	1	
	Wine	0.193 / 0.986[a,d] (n=19)	0.898[f] (n=6)	0.208 / 0.971[a,e] (n=9)	0.187 / 0.977[a,e] (n=9)	0.147 / 0.968[a,e] (n=9)	0.979[a] (n=31)	1

a: Correlation significant at $P < 0.01$;
b: Al, Cr and Fe excluded;
c: Cu and Zn excluded;
d: Al, Ca and Fe excluded;
e: Cr excluded;
f: Correlation significant at $P < 0.05$.

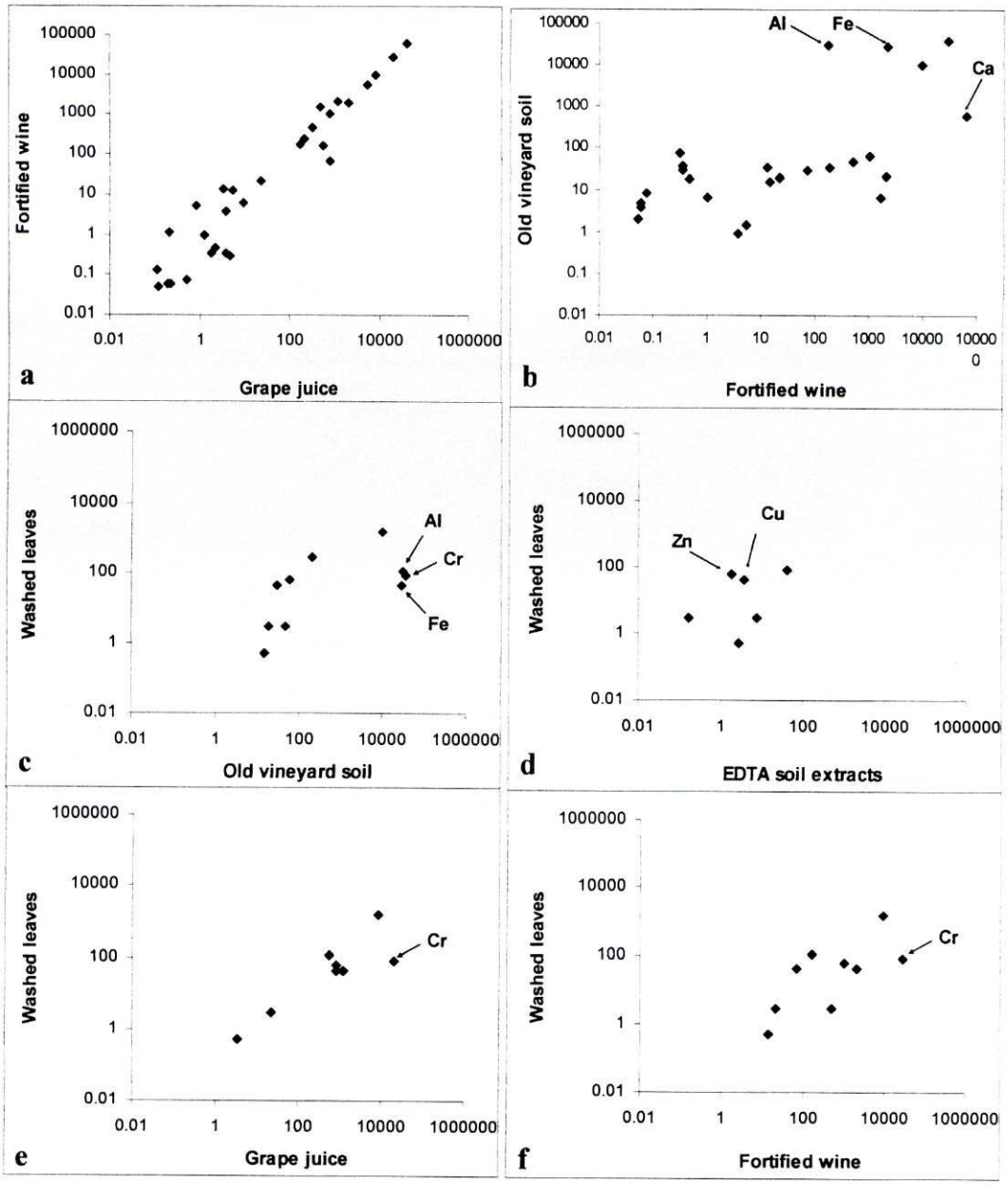

Fig. 12.6. Examples of correlation plots, for the fortified wine/old vineyard obtained between pairs of the different types of samples. In each plot, elements excluded in order to obtain significant correlations are marked. In both axis is represented the logarithm of concentrations in order to obtain a larger scattering of the points.

It is worth mentioning that when the median of the soils multi-element composition was compared with that of a previously studied French red table wine, from the region of Bordeaux (see Chapter 9), no significant correlation was obtained even when Al, Fe and Ca were not included.

These results are promissory as to the usefulness of soil multi-element as fingerprint of the wine origin of the studied wines.

When the multi-element compositions measured in the EDTA soil extracts were used instead of the total-recoverable metals, significant correlations were also observed (see Table 12.1). Nevertheless,

these results have a much more limited reach, as only six elements were measured in the EDTA soil extracts.

12.3.4. Vine leaves and grapes

As complementary information, few elements (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were also measured in vine leaves and grapes. Results are presented in the Appendix Section (Fig. A.2.5 for vine leaves and A.2.6 for grapes).

In general, no significant differences were observed among the vine leaves collected in the different sampling sites of each vineyard, indicating homogeneous distribution of the elements under study.

For most of the measured elements, washed vine leaves presented lower concentrations than the non-washed ones, indicating that atmospheric deposition influenced the results. However, statistically significant differences were only observed in a few cases: in the old vineyard for Al, Cd and Fe in May, Al and Cr in July, and Al, Cr, Fe and Ni in September and in the young vineyard for Cd in May, Al, Cr, Fe and Pb in July, and Al, Fe and Pb in September. The fact of Al and Fe being major constituents of the superficial deposition found on the leaves indicated that the major contamination source was the soil. Data of washed and non-washed vine leaves were treated separated and average values were calculated per vineyard and per month, considering the set of data obtained in the three sampling sites. Despite the presence of some external contamination, the multi-elemental concentrations in washed and non-washed leaves were significantly correlated (see Table 12.1).

It was found that the concentrations of most elements in the washed leaves (expressed in mass of the element per mass of leaves) increased during the vine cycle, that is, during the leaves growth. For instance, from May to September the concentrations in the leaves increased in the old vineyard 85 % for Al, 250 % for Cr, 360 % for Cu and 1000 % for Co, and in the young vineyard 45 %, 130 %, 200 % and 400 %, for Al, Cr, Cu, and Co respectively. The global average concentrations observed in September for washed leaves are shown in Fig. 12.7.

Significant Person's correlations were found between the levels in washed leaves in May and July ($R = 0.662$ and 0.808, for the old and young vineyard, respectively, $n = 10$, $P < 0.05$) and between those observed in July and September ($R = 0.962$ and 0.884, for the old and young vineyard, respectively, $n = 10$, $P < 0.01$), but not between the levels measured in May and September. Therefore, to establish eventually significant relationships between multi-element composition of leaves and soil data from July or later seemed to be more suitable.

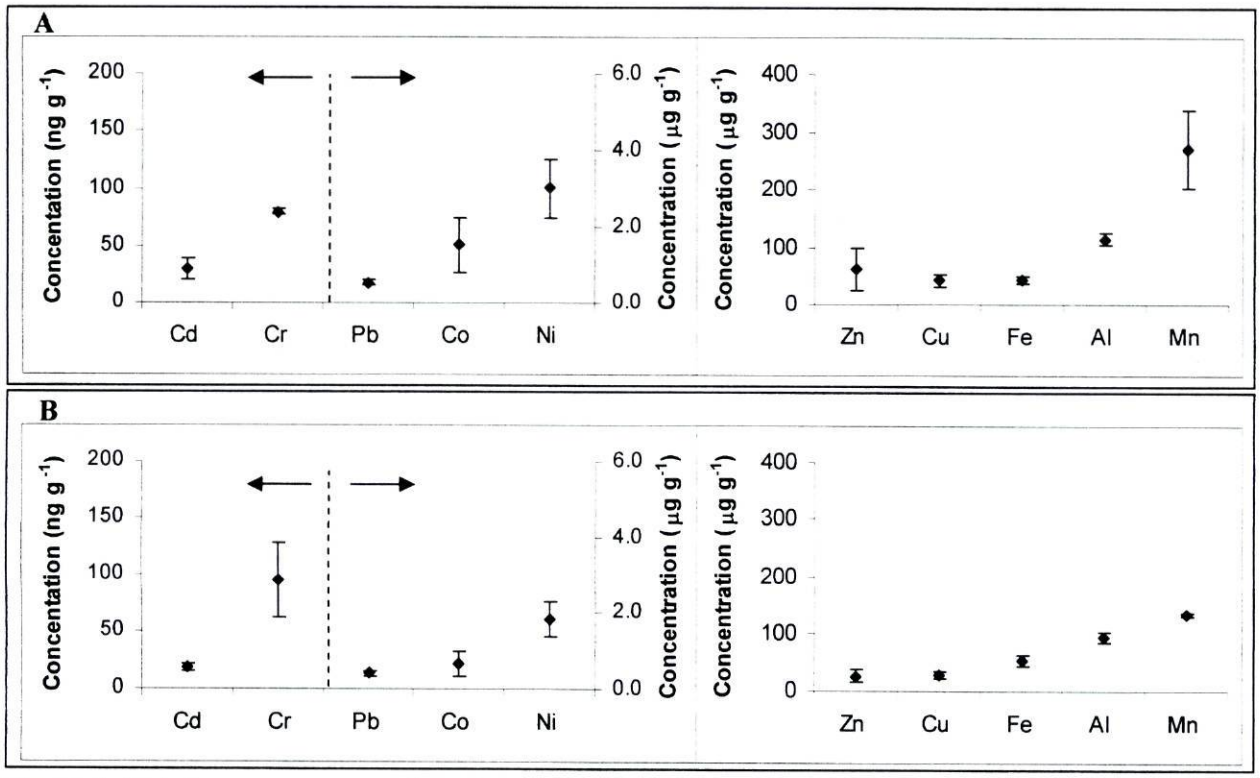

Fig. 12.7. Global average concentration values (per g of dry leave) of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn obtained in the washed vine leave, from the old (A) and young (B) vineyards, in September.

The concentration of most elements in the grapes did not increased between July and September as occurred for the leaves (see Fig. A.2.6 in the Appendix Section). However, this observation may be only apparent, resulting of an incomplete drying of the mature grapes (collected in September), due to the presence of a very high sugar content (in spite of the grapes collected in both months had been dried in an oven up to a constant weight). Therefore, the results obtained for the grapes collected in September were not considered, for comparison purposes, in this work, the discussion being focussed on the data obtained in July. No significant differences were observed either throughout the vineyard (different sampling sites), or between washed and non-washed grapes. The relatively low surface area of the grapes and the fact they are partially covered by leaves explained the absence of significant external contamination. Therefore, only global mean values were calculated (considering the three sampling sites and washed and non-washed) and the results are presented in Fig. 12.8.

The levels of the different metals were lower in the grapes than in leaves, as it is usual in plants [7]. For some elements, like Cd and Fe, the concentration were three times lower, while for other ones, like Al, Cu, Ni or Pb, they were almost ten times lower.

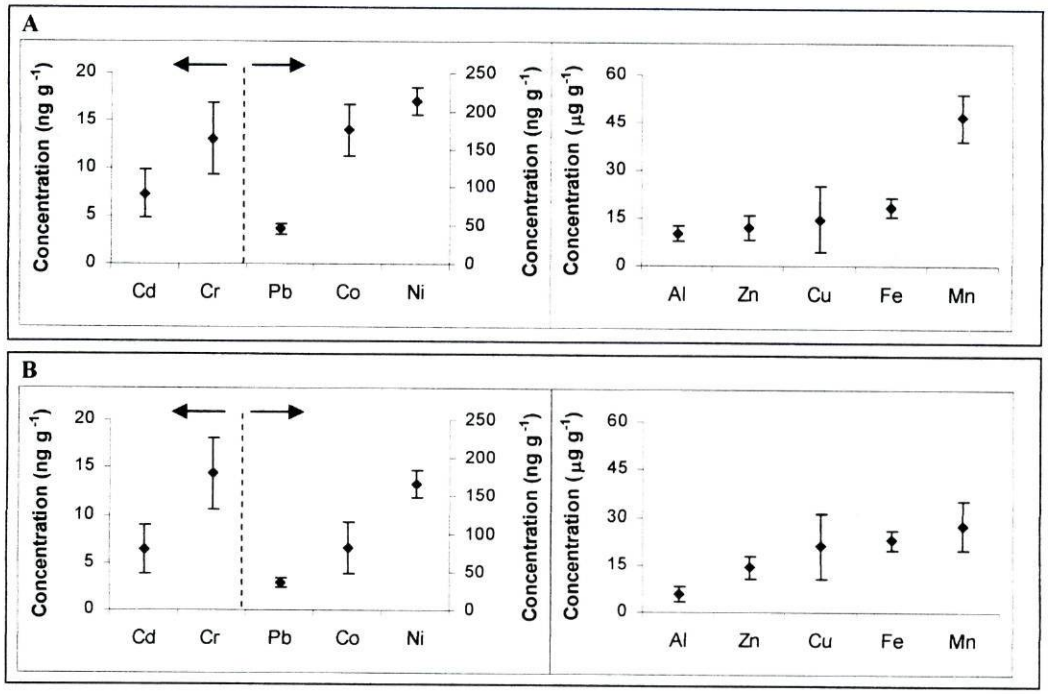

Fig. 12.8. Global average concentration values (per g of dry grape) of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn obtained in July in the grape samples, from the old (A) and young (B) vineyards.

The multi-elemental concentrations in grapes were significantly correlated with those obtained in the leaves (see Table 12.1). This result indicates that leaves and grapes had the same source of the elements measured in this study, which was probably the vineyard soil, atmospheric contamination being negligible.

As concerns correlations between the multi-element compositions of soil and leaves or soil and grapes, significant correlations were observed only for a set of seven elements (Cd, Co, Cu, Mn, Ni, Pb and Zn) (see Table 12.1). Thus, besides the elements Al and Fe, whose levels did not significantly correlated between soil and grape juice or wine (Ca was not measured in leaves and grapes), also Cr prevented significant correlations. The obtained correlation plot for washed leaves *versus* soil of the old vineyard is shown in Fig. 12.6. Similar plots were obtained for non-washed leaves or grapes *versus* soil of the old vineyard and for washed or non-washed leaves or grapes *versus* soil of the young vineyard.

Significant correlations were obtained between leaves or grapes and EDTA soil extracts, only if Cu and Zn were excluded, except for washed leaves from the young vineyard (see Table 12.1). In Fig. 12.6 an example is presented, being the correlations plots similar in the remaining cases. Many authors have reported good correlation between soil-extractable metals and metal uptake by plants [12]. Nevertheless, in this work such correlations were only observed for Cd, Cr, Ni and Pb.

Grape juices, as well as wine, were significantly correlated with the respective leaves and grapes only when Cr was excluded (see Table 12.1 and an example in Fig. 12.6 for washed leaves), which may result of a heterogeneous distribution of Cr in the different parts of the grape berries.

However, the importance of all these correlations is very limited, as the number of the elements considered was very low and, in addition, some elements have to be excluded in order to get significant correlations.

12.4. MULTI-ELEMENT COMPOSITION OF WINES FROM THE PORTUGUESE DOURO REGION

Multi-element concentrations found in the two produced wines are presented in Fig. 12.9 (A - fortified and B - table wines) as these data may have some nutritional and toxicological interest. Concerning the REEs, only results for La, Ce, Nd, Sm, Gd and Dy in the fortified wine and La, Ce and Nd in the table wine were found in measurable and quite similar concentrations, between 0.1 and 0.3 $\mu g\ l^{-1}$.

The multi-element compositions of the fortified and table red wines were globally very similar despite the winemaking processes have been markedly different. In fact, the largest differences were found for V (*ca.* eight times higher in the table wine), for Cu (*ca.* five times higher in the fortified wine) and for Cs (*ca.* three times higher in the table wine).

The concentration of most of the measured elements in the two wines fell within the range of those found in wines produced in several countries as reported in [13] (no information was provided about which countries and how many wines were included in this range). However, the concentrations of Pb in both wines and Al and Cu only in the table wine were below the respective reported range [13] (two, three and four times lower than the range lower limits, which were 500 $\mu g\ l^{-1}$, 30 $\mu g\ l^{-1}$ and 60 $\mu g\ l^{-1}$, for Al, Pb, and Cu, respectively). The concentrations of Cs (only in the table wine) and Be were higher (both *ca.* three times higher than the range upper limit [13], 1.3 and 5.1 $\mu g\ l^{-1}$, respectively).

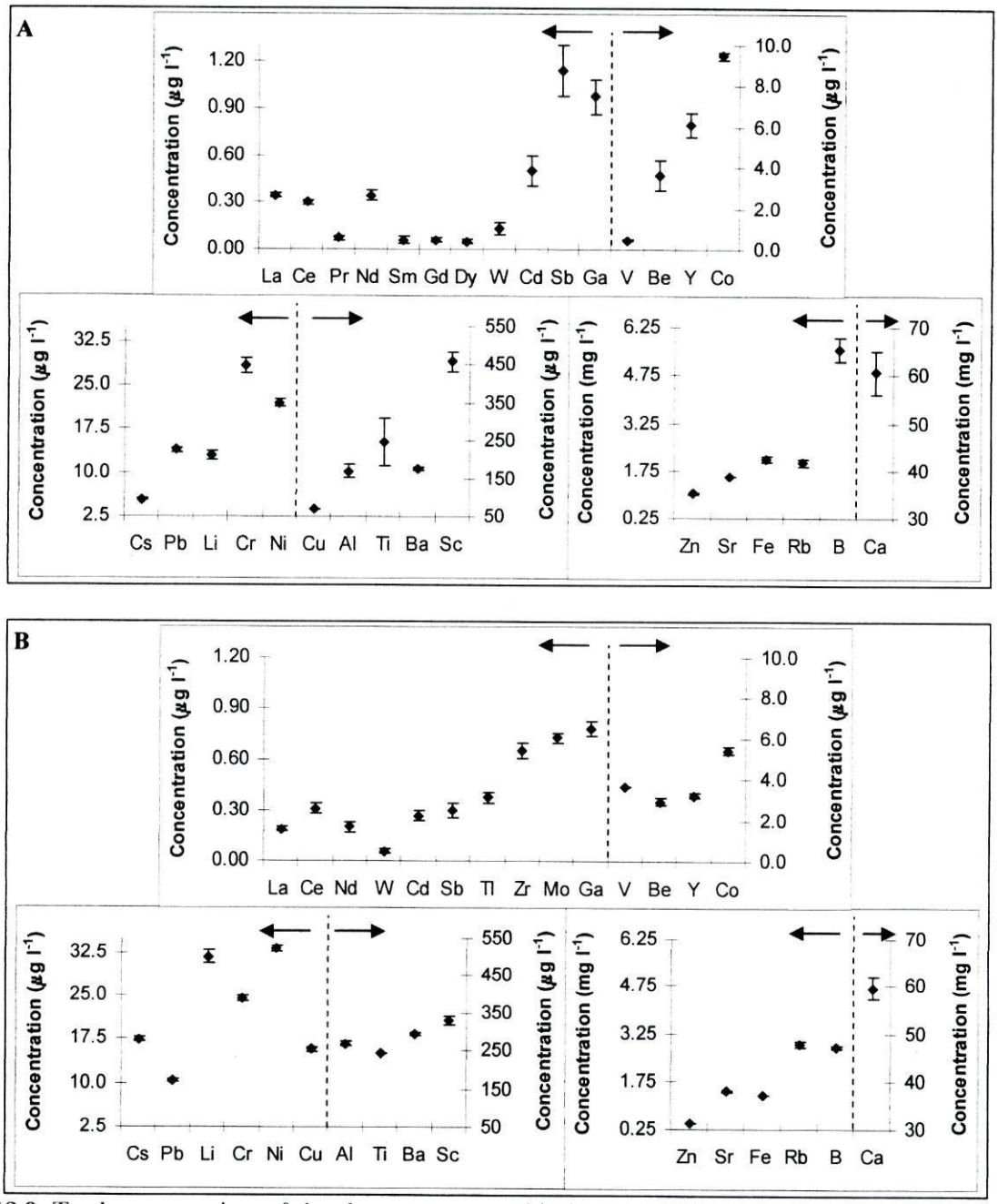

Fig. 12.9. Total concentrations of the elements measured in both studied wine. (A) red fortified wine produced with grapes from the old vineyard; (B) red table wine produced with grapes from the young vineyard. In same cases, as follows, the concentration of Cd, Pr, Sm, Gd, Dy (only table wine), Mo, Tl, Zr (only fortified wine) and Nb, Th, U, Eu, Tb, Ho, Er, Tm, Yb and Lu (both wines) were below the respective limit of quantification (for limit of quantification values see Chapter 10).

As concerns the elements considered of special interest due to either their toxicity in case of excess, like Cd, Cr, Ni, Pb or even Cu (an essential element but toxic when consumed in excess) or the effect they seem to have on the organoleptic properties of wine, like Al, Fe, Zn and Cu [11,13], relatively low concentrations were found. For instances, the Pb concentrations were of 14 µg l^{-1} in the fortified wine

and of 11 μg l^{-1} in the table wine, both well below the threshold limit value established by the OIV, which is presently 200 μg l^{-1}. For Cu, the concentrations were higher in the fortified wine, 70 μg l^{-1}, than in the table wine, 16 μg l^{-1}, but in both cases much lower than the respective OIV threshold limit of 1 mg l^{-1} [11]. The upper limit allowed for Al in wine is 10 mg l^{-1} [11] whereas the values found were only 0.17 mg l^{-1} and 0.27 mg l^{-1} in the fortified and table wines, respectively. For Cd and Zn the levels in both wines were, respectively, 0.51 μg l^{-1} and 1.0 mg l^{-1} in the fortified wine and 0.26 μg l^{-1} and 0.43 mg l^{-1} in the table wine. These values fall in the ranges frequently find in different wines [13], which have been between 0.25 and 0.7 μg l^{-1} for Cd and between 0.5 and 3.5 mg l^{-1} for Zn. The levels of Fe, 2.1 mg l^{-1} and 1.3 mg l^{-1} in the fortified and table wines, respectively, were within the typical range of 0.9 - 10 mg l^{-1} [13], but much lower than the value considered problematic, 10 mg l^{-1} [11]. The levels of Cr and Ni, respectively 28 μg l^{-1} and 22 μg l^{-1} in the fortified wine and 25 μg l^{-1} and 33 μg l^{-1} in the table wine, were also within the range 30 - 60 μg l^{-1} reported for these elements in different wines [13].

12.5. CONCLUSIONS

In this work the multi-element composition of two different Portuguese wines of the Douro region (one table and one fortified) and their precursors including the respective provenance soil were studied, in order to evaluate the suitability of those data as a tool for establishing wine origin.

The two monitored vinification systems (one modern, used for producing the table wine and an old fashion one used for the fortified wine) influenced the multi-element composition of the wine. Evidence of effective contamination was observed for a few elements: Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in the fortified wine and Al, Cr, Fe, Ni, Pb and V in the table wine. Most of the elements presented similar or even lower concentration in the final product compared to that observed in the respective grape juice, prepared in the laboratory. Precipitation or co-precipitation of a fraction of the metal with organic complexing agents present in the must, like polyphenols and tannins, as well as 20 % dilution with grape brandy just in the case of the fortified wine, could be the responsible for the metal levels decrease.

Despite the elemental concentration variations observed throughout the vinification process, significant correlations ($P < 0.01$) were observed between the concentrations of the different elements

($n = 31$) measured in the grape juice and in the final product for both studied wines. Therefore, those concentrations variations were not a preventive of the usefulness of wine multi-element composition for wine provenance determination.

Significant correlations between the wine and the respective provenance soil were also observed when Al, Ca and Fe, the most abundant elements in the soil, were excluded from the set of elements ($n = 19, P < 0.01$).

The present results suggest that the multi-element composition of the provenance soil has potentialities as fingerprint of the wine origin. Nevertheless, much more wines and respective provenance soils from different regions must be analysed in order to be possible to substantiate this methodology.

The concentration of most of the elements measured in the two Portuguese wines fell within the range of those found in other wines of different characteristics and origins. As concerns the elements considered of special interest due to either their toxicity in case of excess, like Cd, Cr, Ni, Pb or even Cu, or the effect they seem to have on the organoleptic properties of wine, like Al, Fe, Zn and Cu, relatively low concentrations (much lower than the respective threshold limit values when available) were found.

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Part IV

Overall Conclusions and Final Remarks

Chapter 13

Overall Conclusions and Final Remarks

13.1. Lead contamination in wines

13.2. Suitability of $^{87}Sr/^{86}Sr$ for wine provenance determination

13.3. Multi-element composition and its suitability for the characterisation of the wine origin region

13.4. Multi-element composition of wines from the Portuguese Douro region

13.5. Future research needs

13.1 LEAD CONTAMINATION IN WINES

Two winemaking processes used to produce two Portuguese wines (one table and one fortified, like Port) were followed from the vineyard to the final product and the lead contents and respective isotope ratios determined.

It was concluded that the major sources of lead were in the vinification system, the more traditional one, used to produce the fortified wine, introducing more lead than the modern one. Only about 1/4 (fortified wine) and 1/3 (table wine) of the lead total content of the wine came from soil and atmospheric deposition. The lead sources were probably welding strings in the containers and tubes used throughout the vinification system and small fittings, like taps.

Therefore, it is expected that marked reductions of the lead content in the wines will occur if the sources of lead are removed from the tubes and containers used in the vinification system, particularly by using welding alloys and small fittings free of lead.

Despite the contamination of the wines with lead, low levels of this metal were found in both cases, only 17.2 $\mu g\ l^{-1}$ and 13.1 $\mu g\ l^{-1}$ in the fortified and table wine, respectively, well below the threshold limit value established by the International Office of Vine and Wine (200 $\mu g\ l^{-1}$).

13.2. SUITABILITY OF $^{87}Sr/^{86}Sr$ FOR WINE PROVENANCE DETERMINATION

The potential of the strontium isotope ratio $^{87}Sr/^{86}Sr$ for wine provenance determination was evaluated by two complementary approaches.

For one hand, ten wines from five different Portuguese regions (Douro, Dão, Bairrada, Borba and Madeira) and two from a French region, Bordeaux, were analysed for their $^{87}Sr/^{86}Sr$ after establishing suitable methodologies. Significant differences among the strontium isotope ratio of the wines of different regions were found. These results point out to the usefulness of the ratio $^{87}Sr/^{86}Sr$ for wine provenance determination. Nevertheless, it was not possible to differentiate all the wines regions with this parameter, indicating that it should be used together with other discriminating parameters, like, for instance, wine or provenance soil multi-element composition.

The possible influence of the winemaking process on $^{87}Sr/^{86}Sr$ was also evaluated for two Portuguese wines, for which vinification processes were detailed followed in the 2000 year. It was found that the produced wines had a $^{87}Sr/^{86}Sr$ similar to that in the respective grape juice (that was prepared in the laboratory and, for which, contact with the winemaking system did not occurred). Thus, it was concluded that the two winemaking systems tested did not significantly changed the ratio $^{87}Sr/^{86}Sr$ in the samples. Identical $^{87}Sr/^{86}Sr$ were also found for wine and the provenance soil. Therefore, it seems that the element is being taken up by the roots of plants, passing to the grapes with the same isotopic proportions in which it occurred in the soil.

These results corroborated the potentiality of $^{87}Sr/^{86}Sr$ as fingerprint of wine region of origin.

13.3. MULTI-ELEMENT COMPOSITION AND ITS SUITABILITY FOR THE CHARACTERISATION OF THE WINE ORIGIN REGION

The suitability of multi-element composition for the determination of the provenance of wines was investigated, used as case studies the two wines from the Douro Portuguese region mentioned above. The table wine was produced in a very modern winery with grapes from a new vineyard, which was raised ten years ago in a forest area. In contrast, the fortified wine was produced with grapes from a sixty to seventy years old vineyard and was made by a traditional vinification process. The multi-element compositions of the wines and their precursors, including the provenance soils were determined.

It was concluded that both monitored vinification processes influenced the multi-element composition of the produced wines.

Most of the elements presented similar or even lower concentration in the produced compared to that observed in the respective grape juice, probably as a result of precipitation or co-precipitation with suspended particles during fermentation and/or wine ageing. Just for the fortified wine a 20 % dilution of the must with grape brandy probably gave a relevant contribution for the observed results. On the other hand, releasing of elements from the vinification system (contamination) was observed during grape pressing, fermentation and/or fining of the wines (depending of the element). Alternatively or in addition to contamination, liberation of the elements from grapes skins and seeds may have happen, as they were pressed together with pomace and hung together during and after fermentation. Evidence of effective contamination in some steps of the vinification process was observed for Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in the fortified wine and Al, Cr, Fe, Ni, Pb and V in the table wine. Nevertheless, only the levels of

Cd, Cr, Fe, Pb and Zn in the fortified wine and of Cr, Ni, Pb and V in the table wine were higher in produced wine than in grape juice.

In spite of the elemental concentrations variations observed throughout the vinification processes, for both wines significant correlations ($P < 0.0.1$) were obtained between the multi-element composition of the produced wine and the respective grape juice, as well as between those in wines and the provenance soil.

These results are promissory as concerns the usefulness of soil multi-element as fingerprint of the studied wines origin.

13.4. MULTI-ELEMENT COMPOSITION OF WINES FROM THE PORTUGUESE DOURO REGION

The multi-element compositions of the studied fortified and table red wines were globally very similar, despite the winemaking processes have been markedly different.

The concentration of most of the measured elements fell within the range of those found in wines of different characteristics and origins. As concerns the elements considered of special interest, due to either their toxicity in case of excess, like Cd, Cr, Ni, Pb or even Cu, or the effect they seem to have on the organoleptic properties of wine, like Al, Fe, Zn and Cu, relatively low concentrations (much lower than the respective threshold limit when available) were found.

13.5. FUTURE RESEARCH NEEDS

Future research work is necessary to complement and substantiate the information provided by the present study.

As concerns lead contamination, wines from other regions of Portugal and respective precursors should be monitored in terms of lead isotope ratios and total metal content, in order to access the sources of this element. This need result of several different winemaking processes and equipments have been used and the knowledge of the main lead sources is required to enable an efficient control of the lead levels in wines.

The results on the suitability of $^{87}Sr/^{86}Sr$ to be used as a tool for wine provenance determination were pioneer at international level and very promissory, but insufficient for permitting to draw more general conclusions. It follows therefrom that this topic deserves further research. Thus, a representative number of wines from different Portuguese regions as well as from other countries should be studied in order to establish regions patterns and permit the distinction of the origin of a specific wine. Probably, the ratio $^{87}Sr/^{86}Sr$ should be combined with other proper information, like multi-element composition of the wines and provenance soils. As the data available on these topics are still incipient, extensive studies are also required.

It can be expected that a data base of these parameters permits the establishment of the origin of wines and help in the detection/prevention of wine fraud.

Appendix

A.1. Additional data on lead isotope ratios measurements
  A.1.1. Mass bias correction
  A.1.2. Precision of the measured lead isotope ratios

A.2. Multi-element concentrations obtained in the vineyards soil, EDTA soil extracts, samples collected throughout the vinification processes, vine leaves and grapes

A.1. ADDITIONAL DATA ON LEAD ISOTOPE RATIOS MEASUREMENTS

A.1.1. Mass bias correction

In order to determine IRs accurately, correction of the observed data for the phenomena of mass bias was necessary. For Pb, two different procedures for mass bias correction were tested: (i) external correction, with a Pb isotopic standard solution and (ii) internal correction, with Tl as internal standard. Both procedures were applied to the different types of samples analysed and the results were compared.

The results obtained for wine samples were presented and discussed in Chapter 4.

For soils and leaves the studies were carried out on two samples of each type (S_1 and S_2) and (L_1 and L_2) and the obtained results are shown in Table A.1.1. Only one replicate of each sample was measured.

Table A.1.1. Comparison of external and internal mass bias correction for soil and leave samples.

	$^{207}Pb/^{206}Pb^a$		$^{208}Pb/^{206}Pb^a$		$^{204}Pb/^{206}Pb^a$	
Sample	External correction	Internal correction	External correction	Internal correction	External correction	Internal correction
S_1	0.849 (5)	0.851 (4)	2.134 (11)	2.129 (8)	0.0544 (9)	0.0538 (3)
S_2	0.843 (5)	0.851 (3)	2.145 (10)	2.151 (9)	0.0540 (9)	0.0532 (3)
L_1	0.856 (3)	0.859 (4)	2.111 (12)	2.097 (10)	0.0549 (3)	0.0546 (3)
L_2	0.860 (5)	0.853 (3)	2.106 (15)	2.087 (10)	0.0549 (2)	0.0547 (4)

a: Mean and standard deviation (in brackets affecting last digit) (calculated according to errors propagation).

For both types of samples, no significant differences were found (pair t-test) between external and internal correction for all the Pb ratios, $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$. Therefore, it can be concluded that any type of mass bias correction is suitable in the present case. For grape samples, as similar results were expected, tests were not carried out.

A.1.2. Precision of the measured lead isotope ratios

In order to test the repeatability of the results obtained for the Pb IRs in soil samples, the mean and variance of the Pb IRs of three independent replicates were calculated and compared with the mean and variance obtained for a single replicate, which was analysed three times. The results obtained for twelve soil samples are shown in Table A.1.2., being similar to those reported in Chapter 4 for wine samples

Table A.1.2. Precision of the Pb IRs observed in twelve different soil samples.

		Analysis of three aliquots of a single replicate	Analysis of three independent replicates	σ^2 [a]
			$^{207}Pb/^{206}Pb$	
S_1	Mean	0.853	0.854	
	σ^2	$3.1x10^{-5}$	$4.8x10^{-5}$	$1.7x10^{-5}$
S_2	Mean	0.853	0.850	
	σ^2	$2.7x10^{-5}$	$2.2x10^{-5}$	$-5.6x10^{-6}$
S_3	Mean	0.849	0.846	
	σ^2	$1.4x10^{-5}$	$1.4x10^{-5}$	$4.3x10^{-7}$
S_4	Mean	0.850	0.852	
	σ^2	$4.1x10^{-5}$	$3.4x10^{-5}$	$2.9x10^{-5}$
S_5	Mean	0.850	0.848	
	σ^2	$2.1x10^{-5}$	$2.1x10^{-5}$	$2.7x10^{-7}$
S_6	Mean	0.844	0.844	
	σ^2	$2.4x10^{-5}$	$2.5x10^{-5}$	$1.3x10^{-6}$
S_7	Mean	0.845	0.844	
	σ^2	$2.0x10^{-5}$	$1.5x10^{-5}$	$-4.7x10^{-6}$
S_8	Mean	0.844	0.845	
	σ^2	$6.9x10^{-6}$	$1.5x10^{-5}$	$8.1x10^{-6}$
S_9	Mean	0.847	0.849	
	σ^2	$1.4x10^{-5}$	$2.5x10^{-5}$	$1.2x10^{-5}$
S_{10}	Mean	0.844	0.841	
	σ^2	$1.4x10^{-5}$	$2.4x10^{-5}$	$9.9x10^{-6}$
S_{11}	Mean	0.840	0.841	
	σ^2	$2.9x10^{-5}$	$4.1x10^{-5}$	$1.2x10^{-5}$
S_{12}	Mean	0.851	0.850	
	σ^2	$2.1x10^{-5}$	$1.7x10^{-5}$	$-4.2x10^{-6}$
			$^{208}Pb/^{206}Pb$	
S_1	Mean	2.123	2.120	
	σ^2	$3.8x10^{-4}$	$3.4x10^{-4}$	$-4.1x10^{-5}$
S_2	Mean	2.115	2.118	
	σ^2	$1.2x10^{-4}$	$1.2x10^{-4}$	$-1.2x10^{-5}$
S_3	Mean	2.118	2.119	
	σ^2	$4.6x10^{-4}$	$4.6x10^{-4}$	$-9.0x10^{-7}$

Table A.1.2. *continuation.*

S_4	Mean	2.123	2.111	
	σ^2	$1.6x10^{-4}$	$1.2x10^{-4}$	$-3.9x10^{-5}$
S_5	Mean	2.116	2.124	
	σ^2	$1.5x10^{-4}$	$1.5x10^{-4}$	$6.9x10^{-7}$
S_6	Mean	2.130	2.125	
	σ^2	$3.4x10^{-5}$	$5.1x10^{-5}$	$1.6x10^{-5}$
S_7	Mean	2.110	2.113	
	σ^2	$71.6x10^{-4}$	$1.7x10^{-4}$	$1.1x10^{-5}$
S_8	Mean	2.127	2.128	
	σ^2	$9.1x10^{-5}$	$7.6x10^{-5}$	$-1.4x10^{-5}$
S_9	Mean	2.134	2.128	
	σ^2	$2.5x10^{-4}$	$4.1x10^{-4}$	$1.5x10^{-4}$
S_{10}	Mean	2.126	2.129	
	σ^2	$2.5x10^{-4}$	$2.9x10^{-4}$	$4.3x10^{-5}$
S_{11}	Mean	2.129	2.121	
	σ^2	$3.6x10^{-4}$	$2.7x10^{-4}$	$-9.2x10^{-5}$
S_{12}	Mean	2.126	2.126	
	σ^2	$2.8x10^{-4}$	$2.8x10^{-4}$	$1.8x10^{-6}$
			$^{204}Pb/^{206}Pb$	
S_1	Mean	0.0541	0.0541	
	σ^2	$7.7x10^{-8}$	$8.1x10^{-8}$	$3.7x10^{-9}$
S_2	Mean	0.0538	0.0540	
	σ^2	$2.3x10^{-7}$	$1.9x10^{-7}$	$-4.2x10^{-8}$
S_3	Mean	0.0542	0.0540	
	σ^2	$1.4x10^{-7}$	$1.9x10^{-7}$	$-5.5x10^{-8}$
S_4	Mean	0.0539	0.0540	
	σ^2	$1.2x10^{-7}$	$1.2x10^{-7}$	$2.8x10^{-9}$
S_5	Mean	0.0543	0.0542	
	σ^2	$1.9x10^{-7}$	$2.0x10^{-7}$	$1.2x10^{-8}$
S_6	Mean	0.0540	0.0539	
	σ^2	$1.9x10^{-7}$	$1.7x10^{-7}$	$1.5x10^{-8}$
S_7	Mean	0.0540	0.0541	
	σ^2	$1.3x10^{-7}$	$1.3x10^{-7}$	$4.4x10^{-9}$
S_8	Mean	0.0538	0.0539	
	σ^2	$3.1x10^{-7}$	$2.5x10^{-7}$	$-5.5x10^{-8}$
S_9	Mean	0.0538	0.0539	
	σ^2	$1.8x10^{-7}$	$2.1x10^{-7}$	$3.1x10^{-8}$
S_{10}	Mean	0.0538	0.0539	
	σ^2	$2.5x10^{-7}$	$2.9x10^{-7}$	$4.0x10^{-8}$
S_{11}	Mean	0.0540	0.0539	
	σ^2	$1.4x10^{-7}$	$1.3x10^{-7}$	$-8.3x10^{-9}$
S_{12}	Mean	0.0536	0.0534	
	σ^2	$1.2x10^{-7}$	$1.2x10^{-7}$	$1.6x10^{-9}$

a: Difference between the total variance (analysis of three independent replicates) and the determine variance (analysis of three aliquots of a single replicate).

A.2. MULTI-ELEMENT CONCENTRATIONS OBTAINED IN THE VINEYARDS SOIL, EDTA SOIL EXTRACTS, SAMPLES COLLECTED THROUGHOUT THE VINIFICATION PROCESSES, VINE LEAVES AND GRAPES

As a complement of the data present in Chapter 12, here are presented the results obtained for the multi-element analysis of the samples of vineyard soils (Fig. A.2.1 and A.2.2), EDTA soil extracts (Fig. A.2.3), samples collected throughout the vinification processes (Fig. A.2.4), vine leaves (Fig. A.2.5) and grapes (Fig. A.2.6) carried out during the study entitled "Multi-Element Composition of Wines and Their Precursors Including Provenance Soil as Potential Fingerprint of Wine Origin".

The presented results are the mean of three independent replicates pre-treated and analysed with the respective standard deviation.

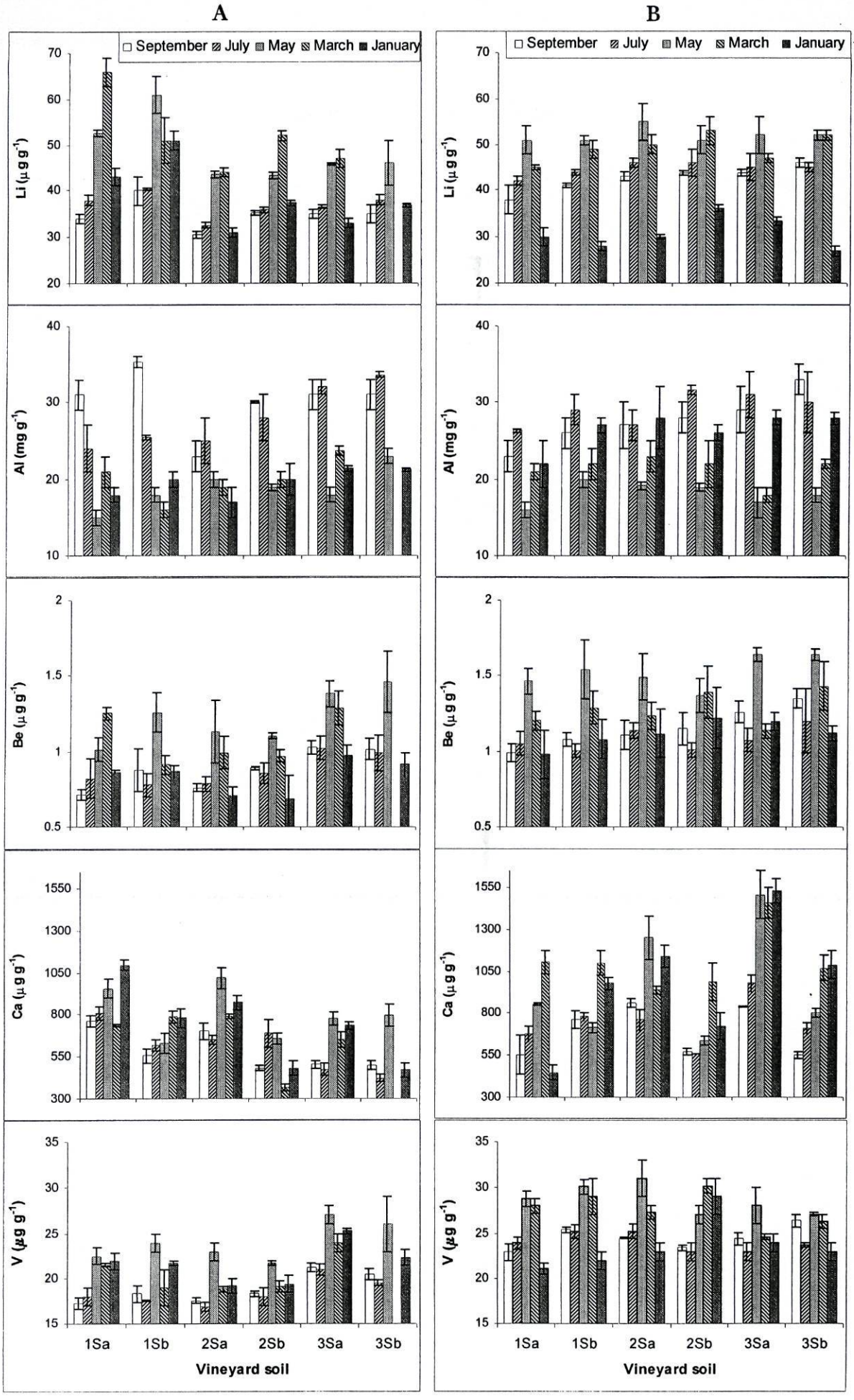
A
B
September
July
May
March
January
Li (µg g-1)
Al (mg g-1)
Be (µg g-1)
Ca (µg g-1)
V (µg g-1)
1Sa
1Sb
2Sa
2Sb
3Sa
3Sb
Vineyard soil

Fig. A.2.1.

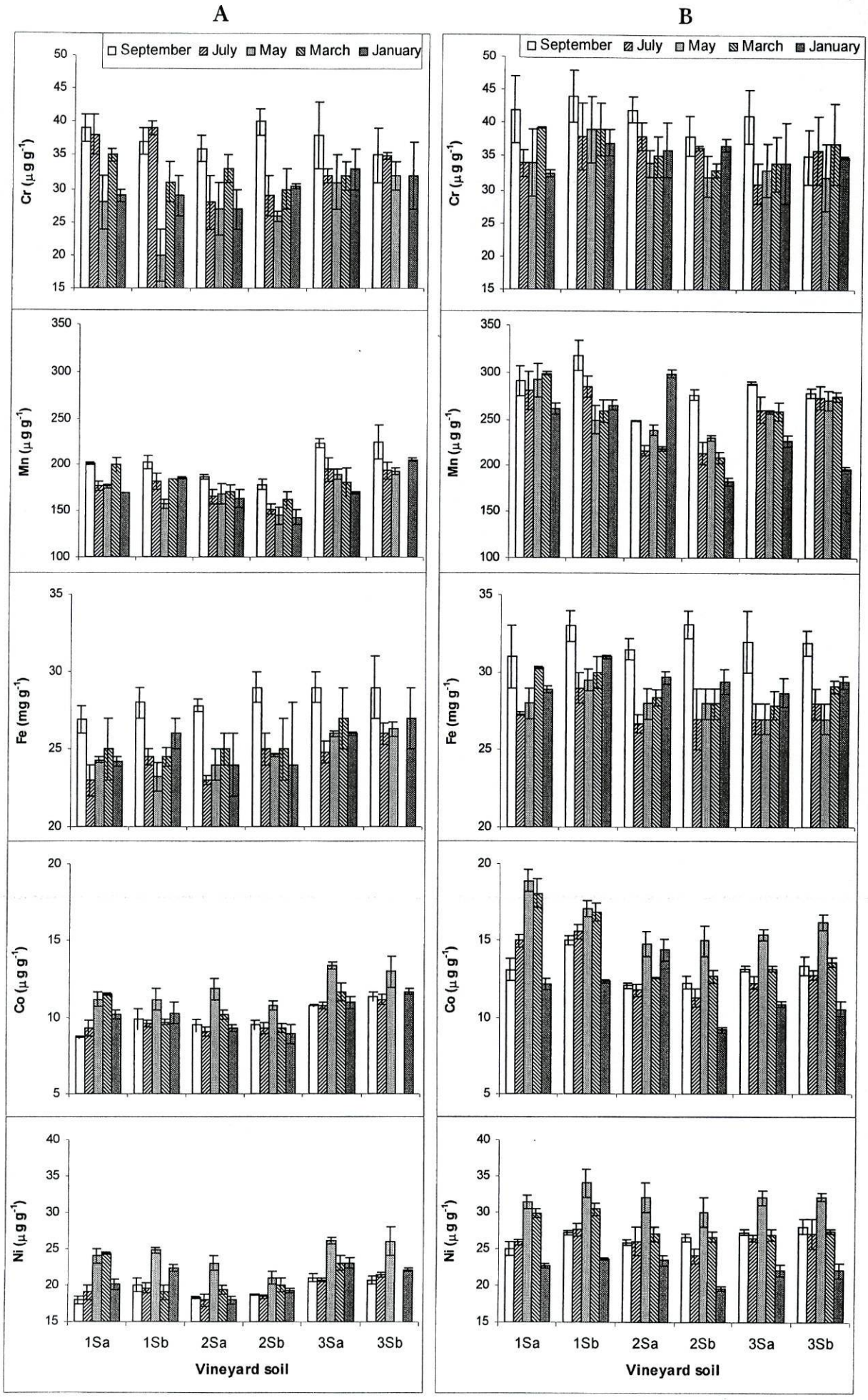

Fig. A.2.1. *continuation*

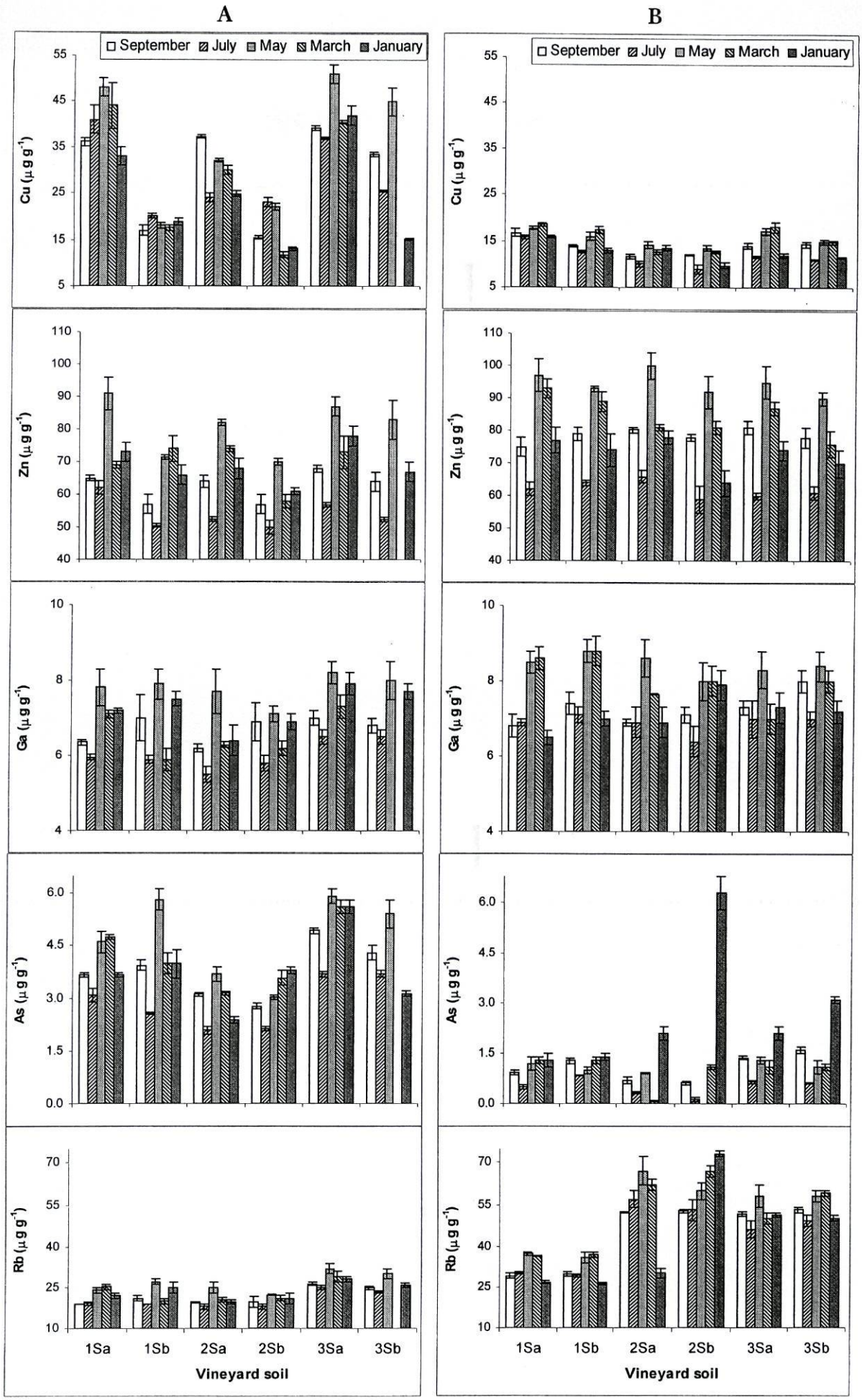

Fig. A.2.1. *continuation*

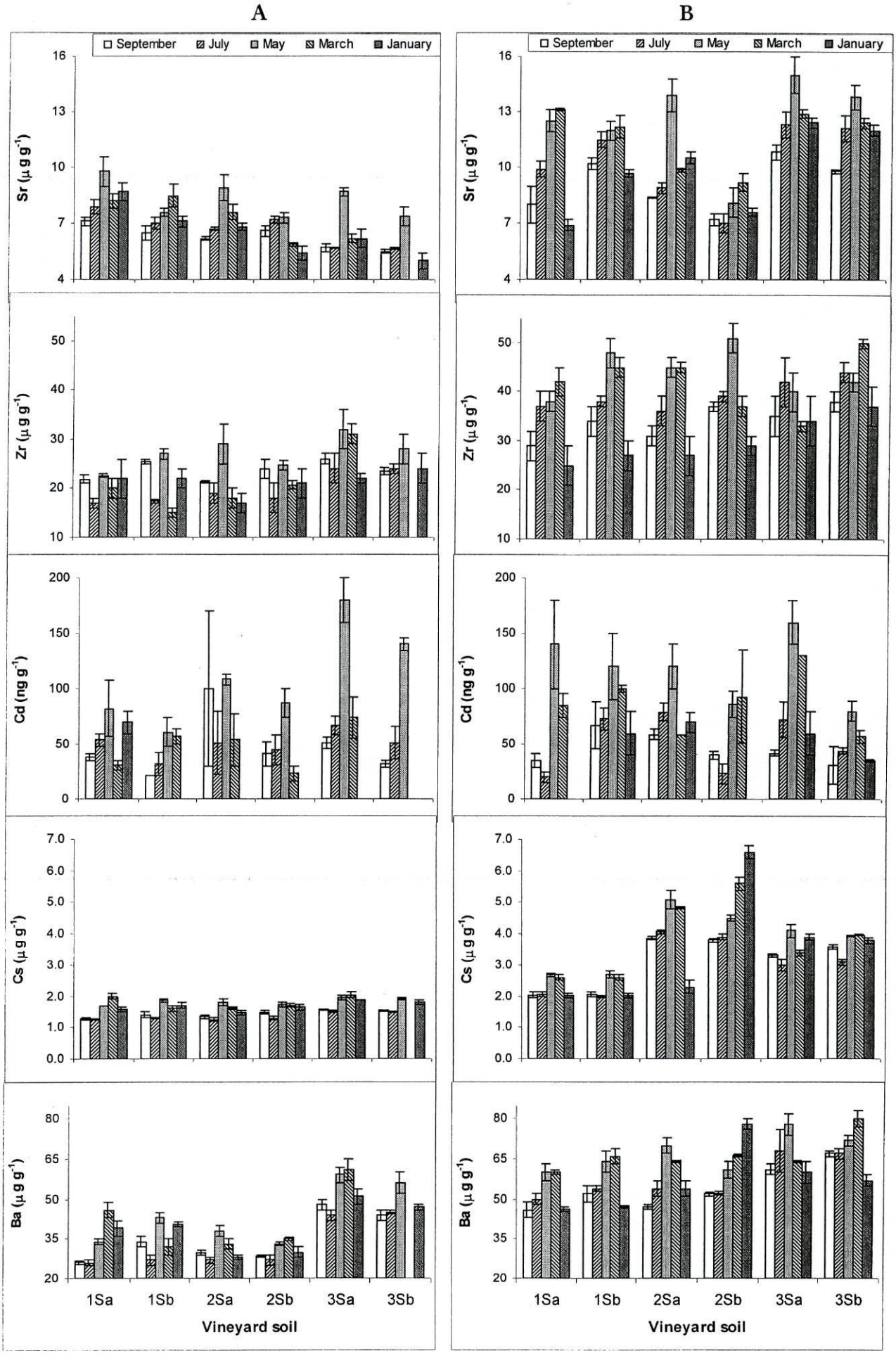

Fig. A.2.1. *continuation*

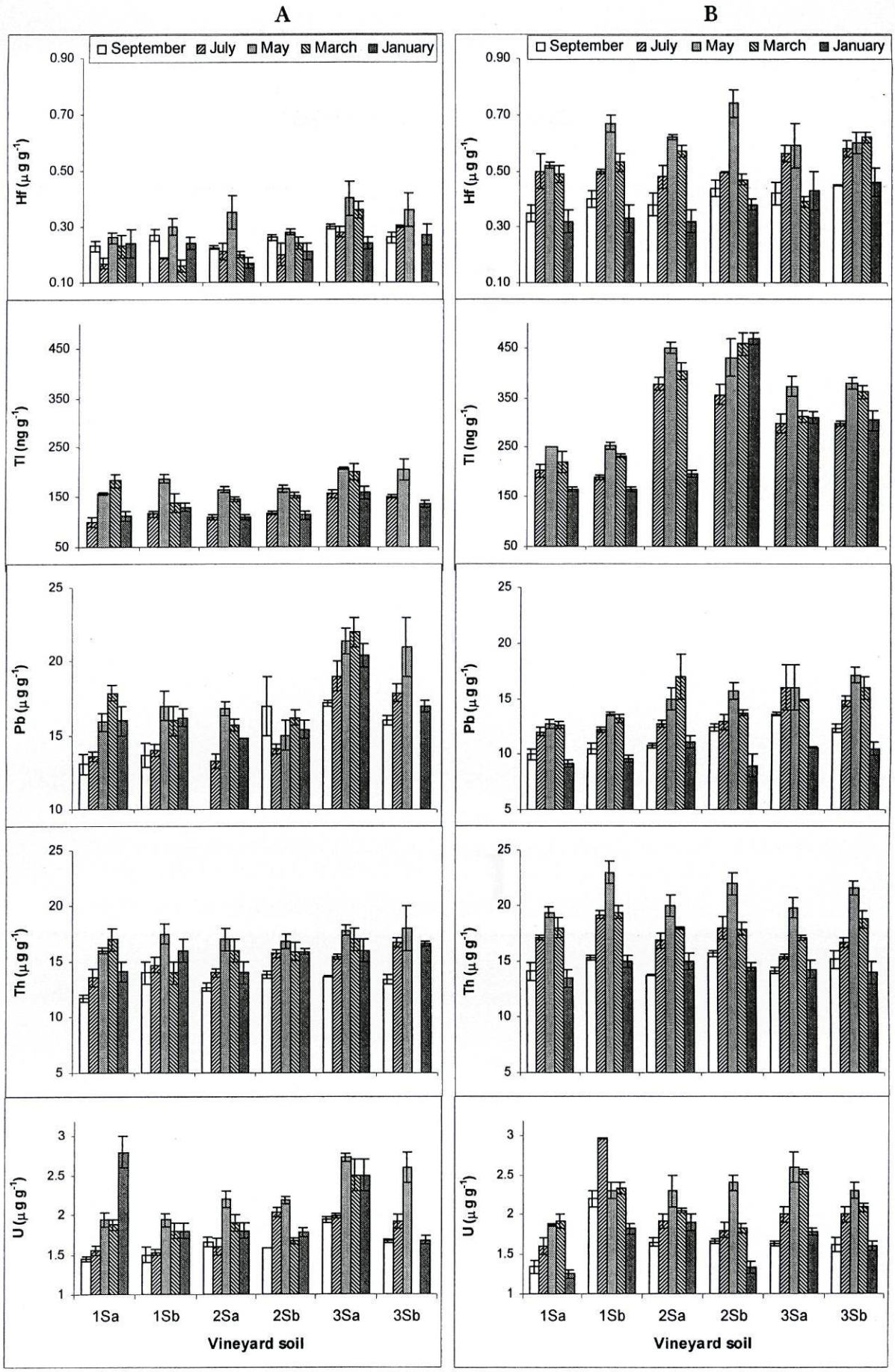

Fig. A.2.1. *continuation.* Total-recoverable concentration of the measured elements, Li, Al, Be, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Zr, Cd, Cs, Ba, Hf, Tl, Pb, Th and U (per g of dry soil) obtained in the vineyard soil samples collected at surface (S_a) and at 20 cm depth (S_b), in the three selected sites (1S, 2S, 3S), in the old (A) and young (B) vineyards, in five different months. Sample $3S_b$ of the old vineyard of the month of March was lost.

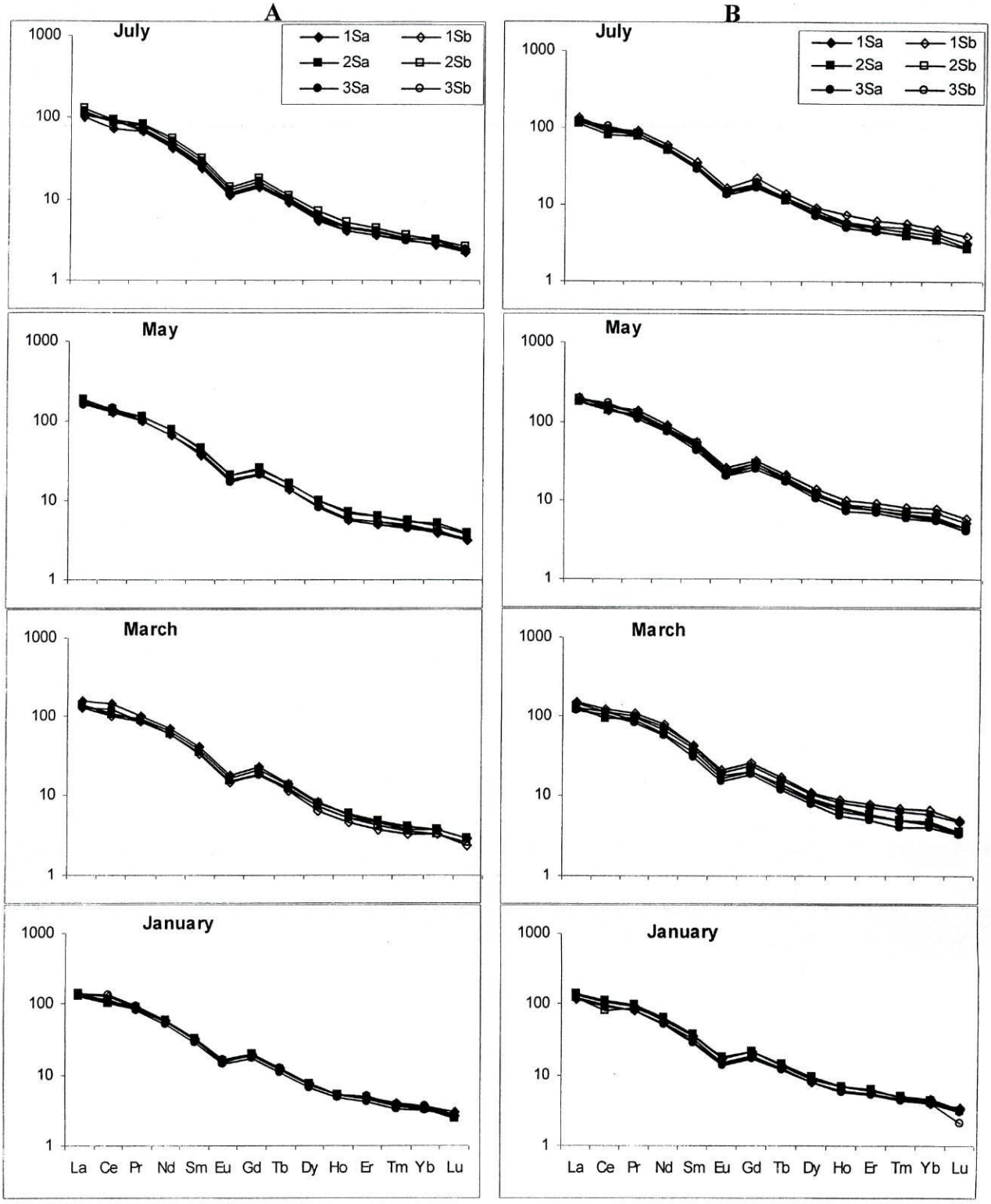

Fig. A.2.2. Plot of chondrite-normalised concentrations of rare earth elements against atomic number (y-axis in logarithm) obtained for the vineyard soil samples collected at surface (S_a) and at 20 cm depth (S_b), in the three selected sites (1S, 2S, 3S), in the old (A) and young (B) vineyards, in four different months. Sample $3S_b$ of the old vineyard of the month of March was lost.

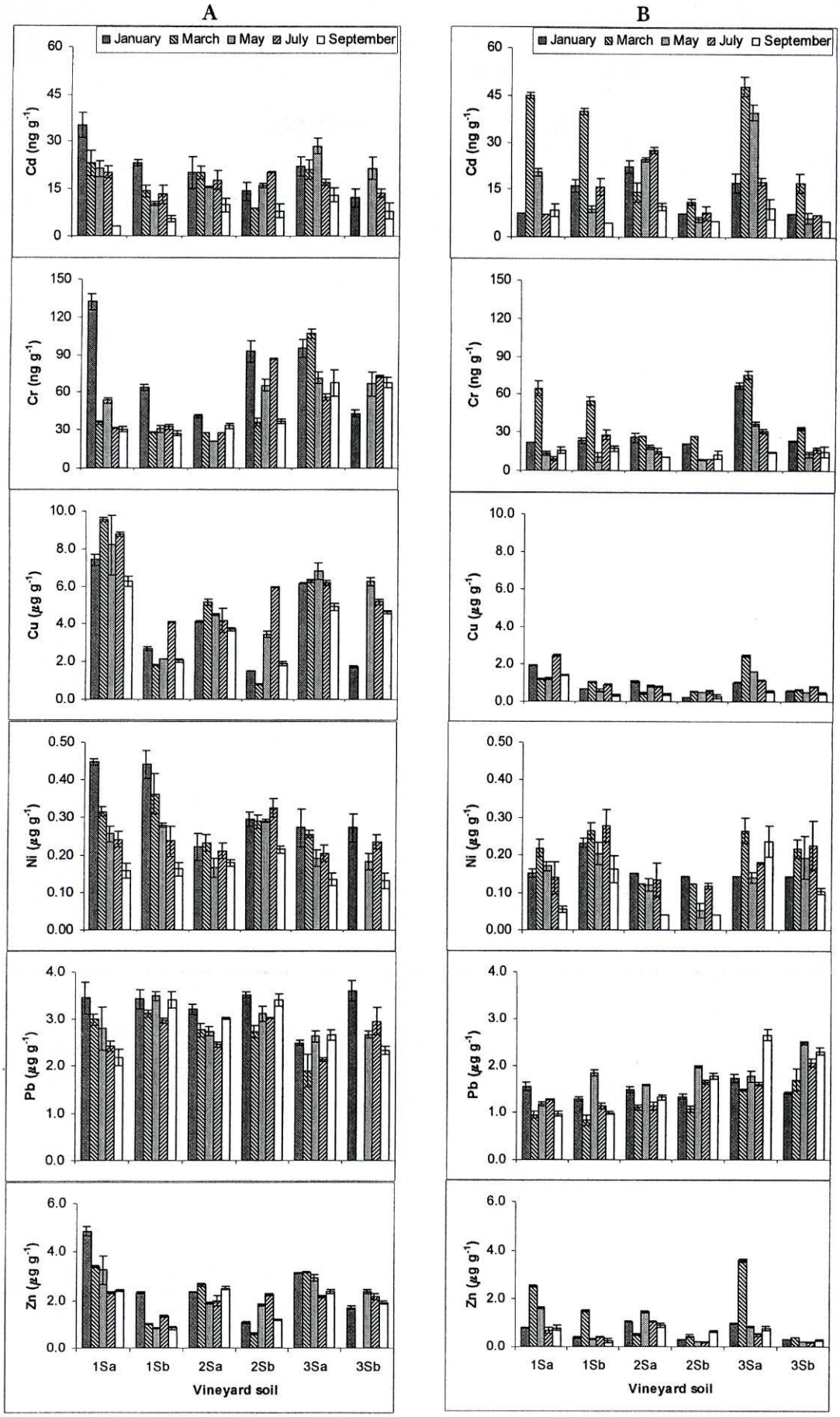

Fig. A.2.3. EDTA soil extract concentrations of Cd, Cr, Cu, Ni, Pb and Zn (per g of dry soil) obtained in five different months for the soil samples collected at surface (S_a) and at 20 cm depth (S_b), in the three selected sites (1S, 2S, 3S), in the old (A) and young vineyards (B),. Sample $3S_b$ of the old vineyard of the month of March was lost.

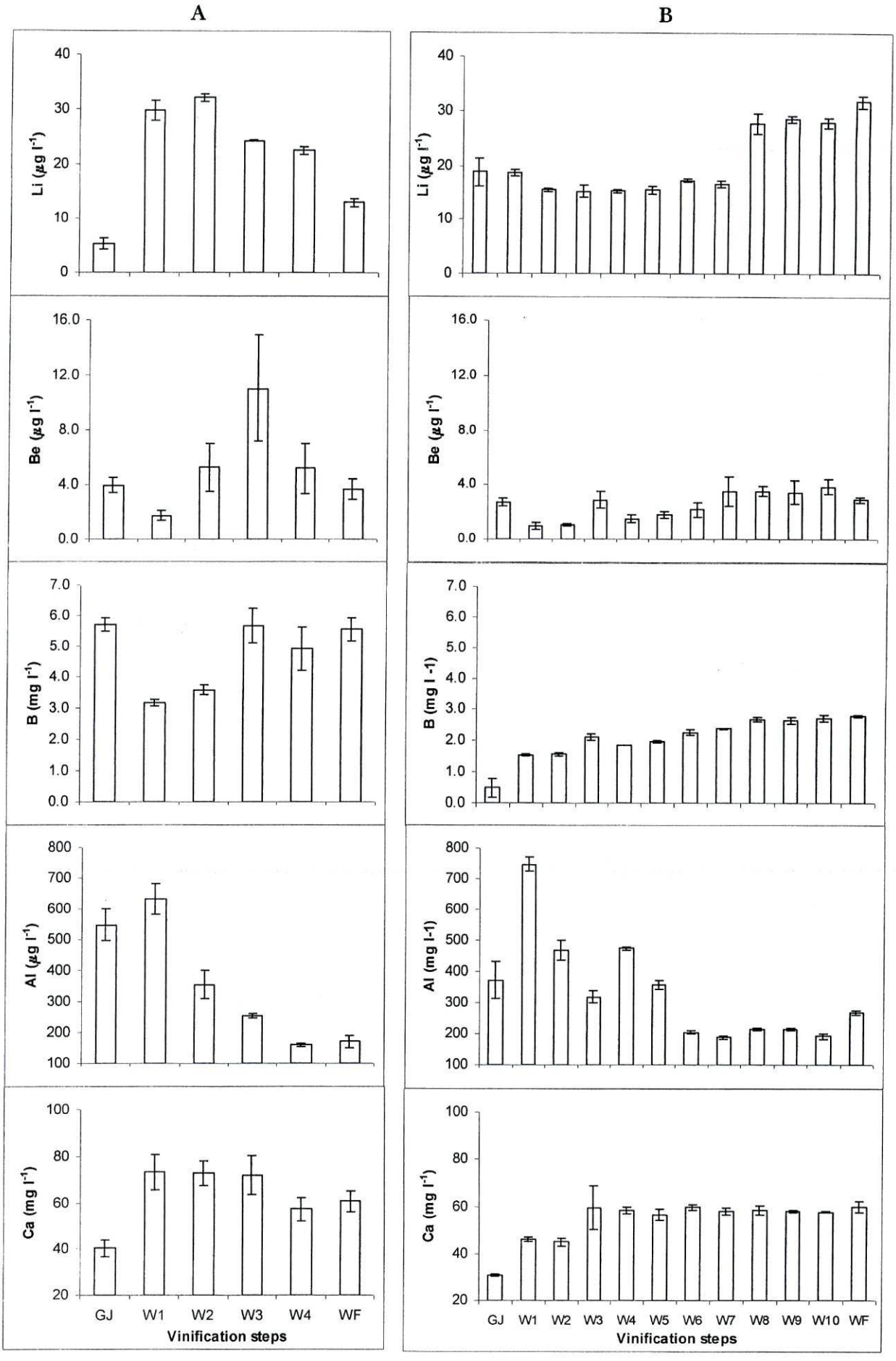
A
B
Li (µg l-1)
Be (µg l-1)
B (mg l-1)
B (mg l -1)
Al (µg l-1)
Al (mg l-1)
Ca (mg l-1)
GJ W1 W2 W3 W4 WF
GJ W1 W2 W3 W4 W5 W6 W7 W8 W9 W10 WF
Vinification steps
Vinification steps

Fig. A.2.4.

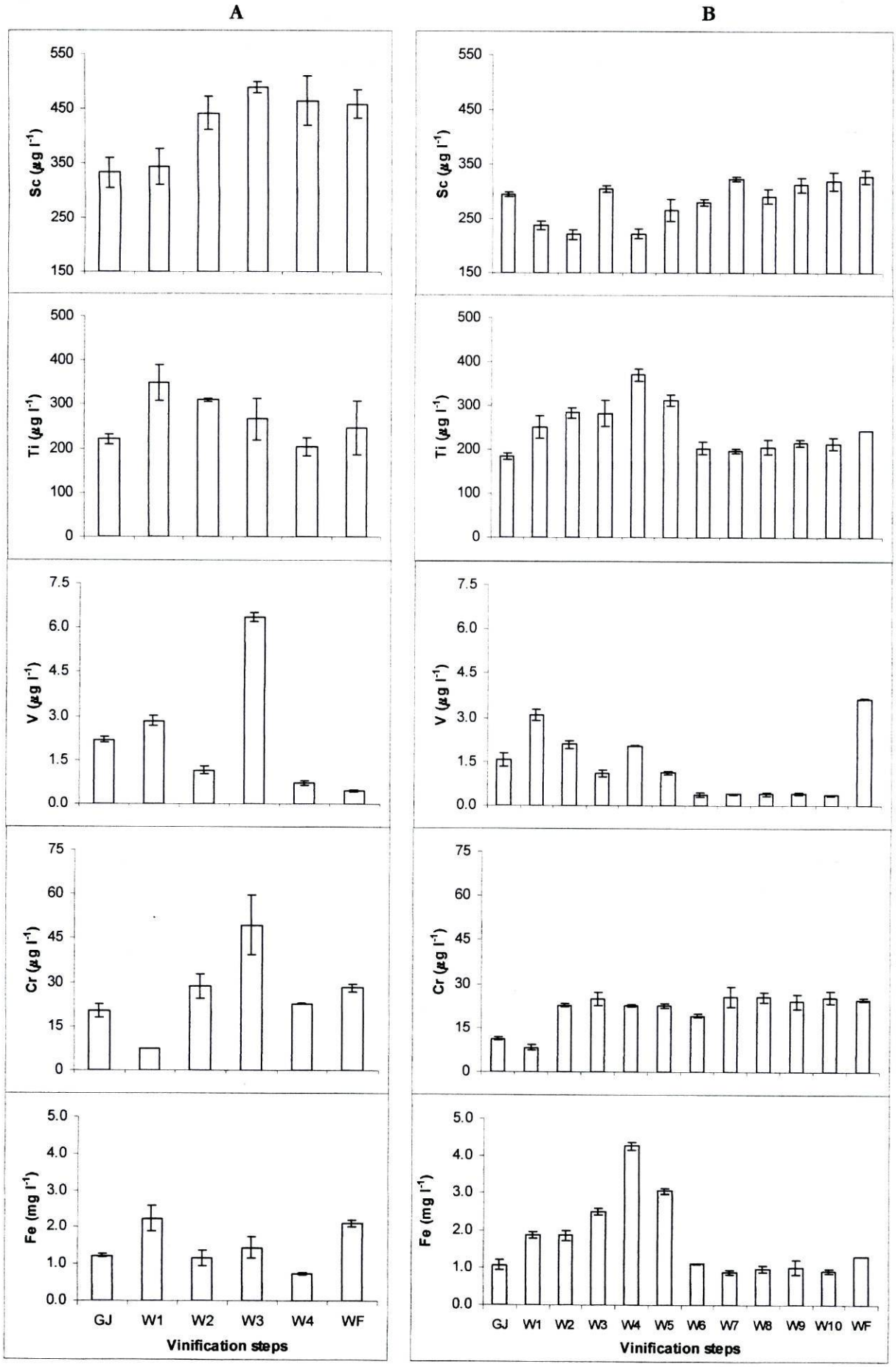

Fig. A.2.4. *continuation*

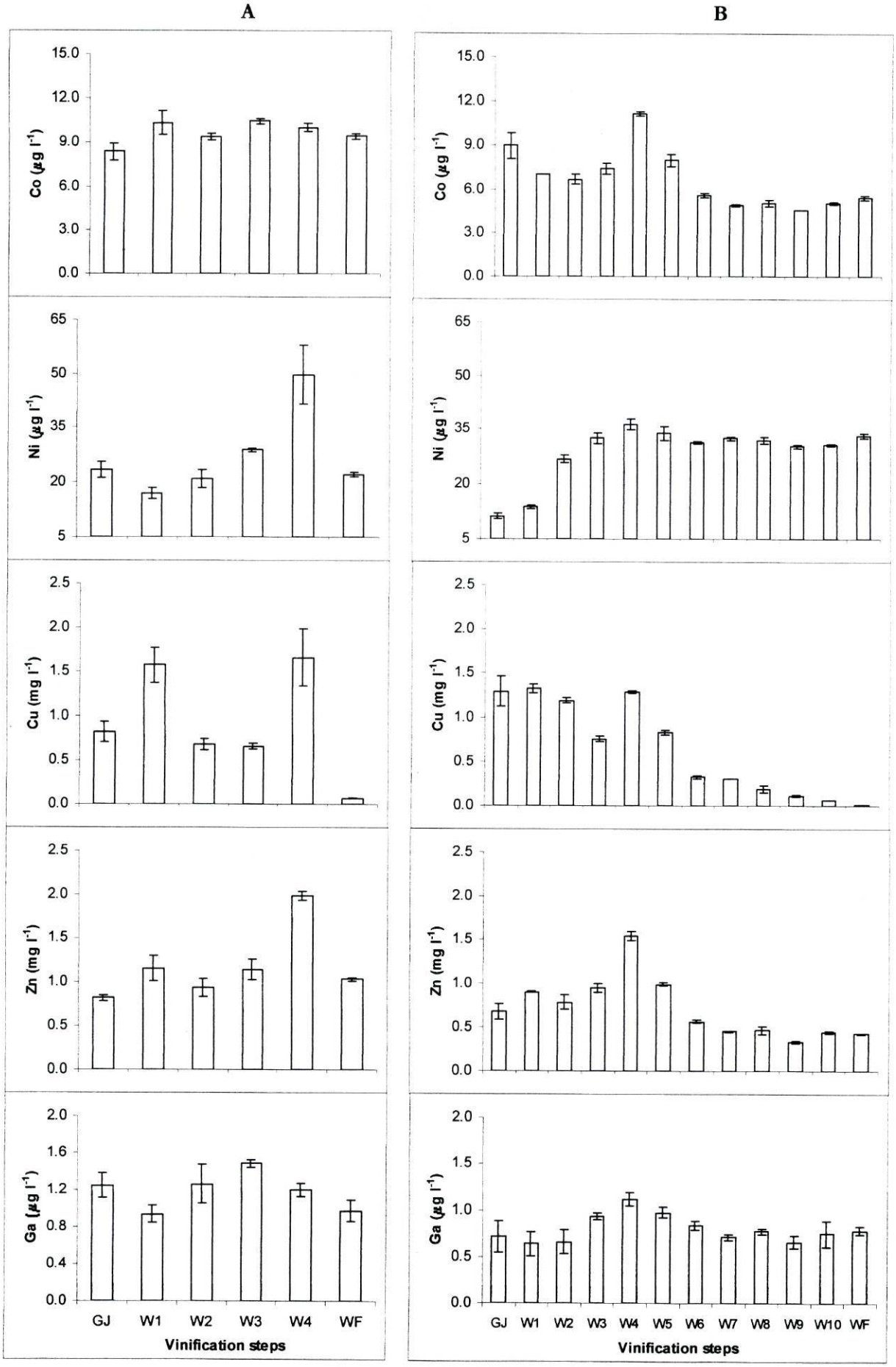

Fig. A.2.4. *continuation*

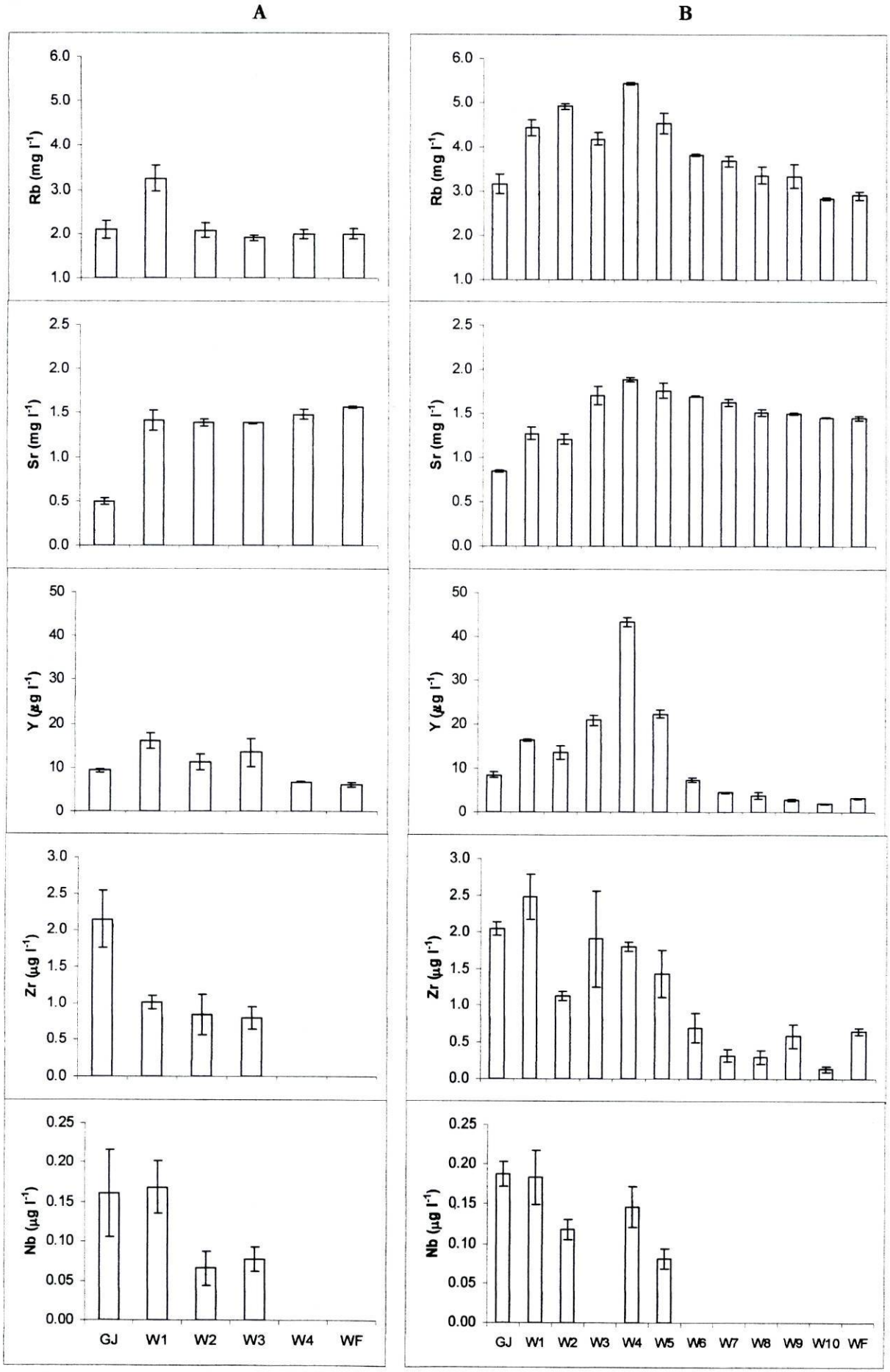

Fig. A.2.4. *continuation*

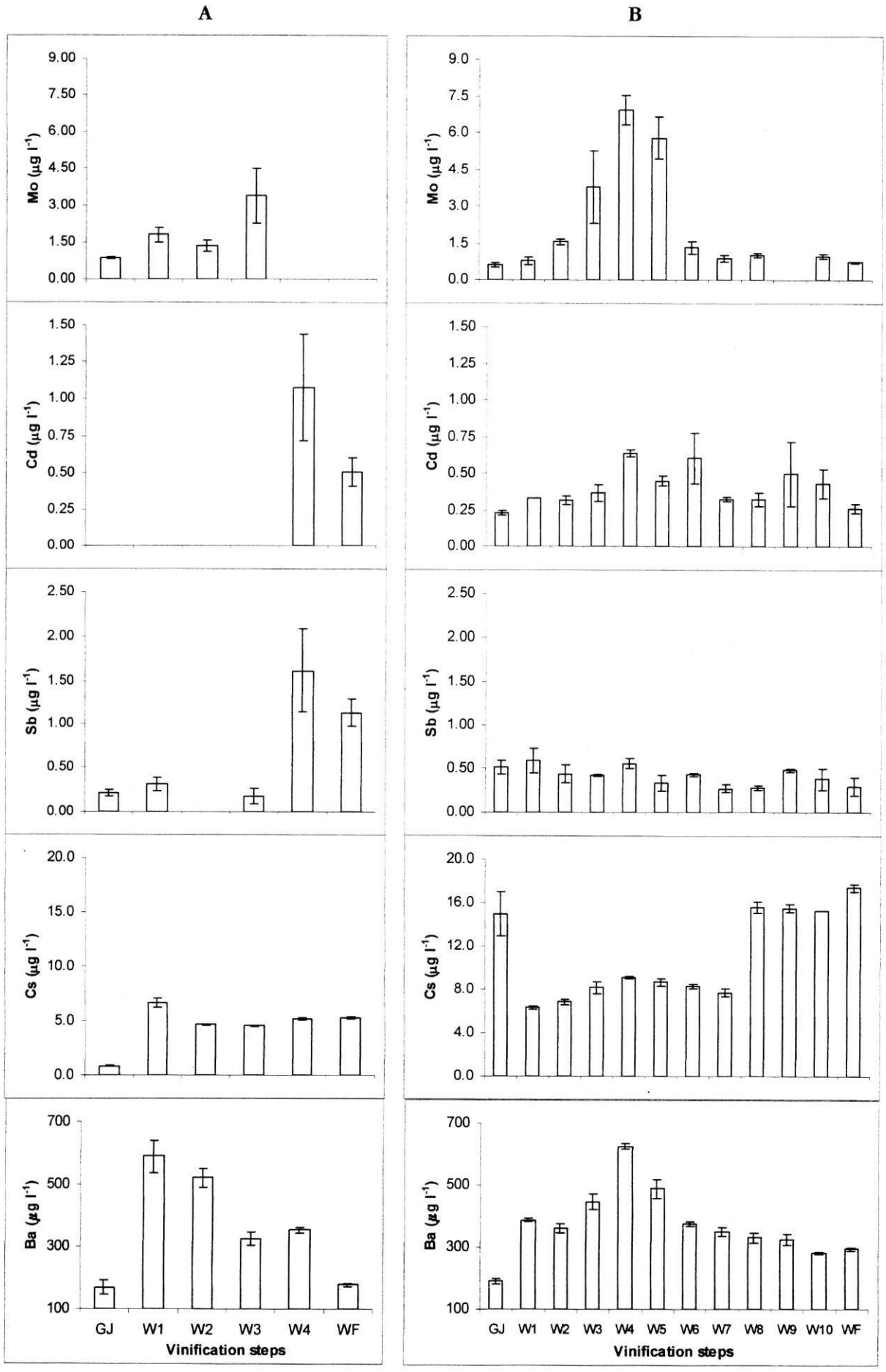

Fig. A.2.4. *continuation*

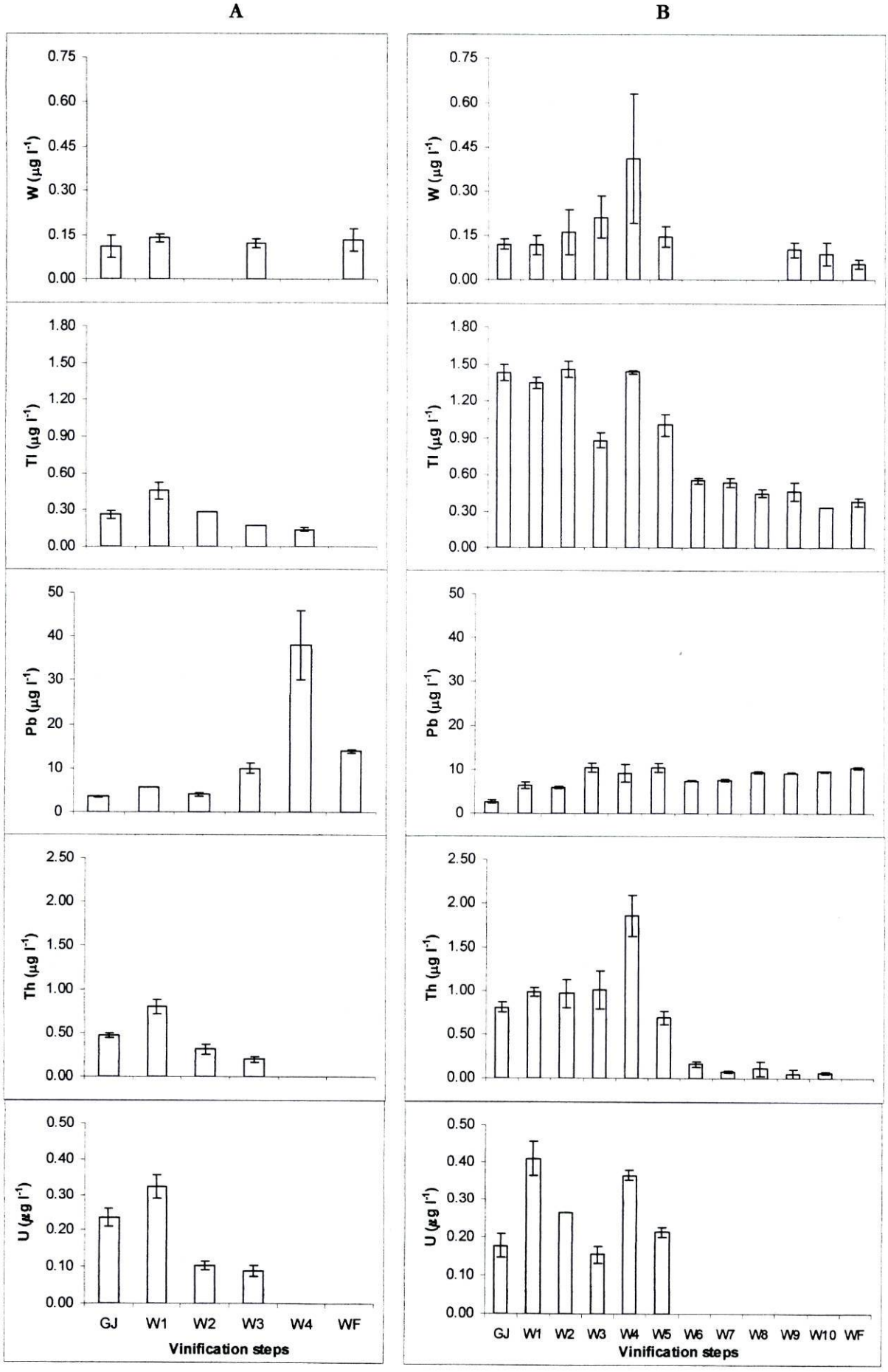

Fig. A.2.4. *continuation*

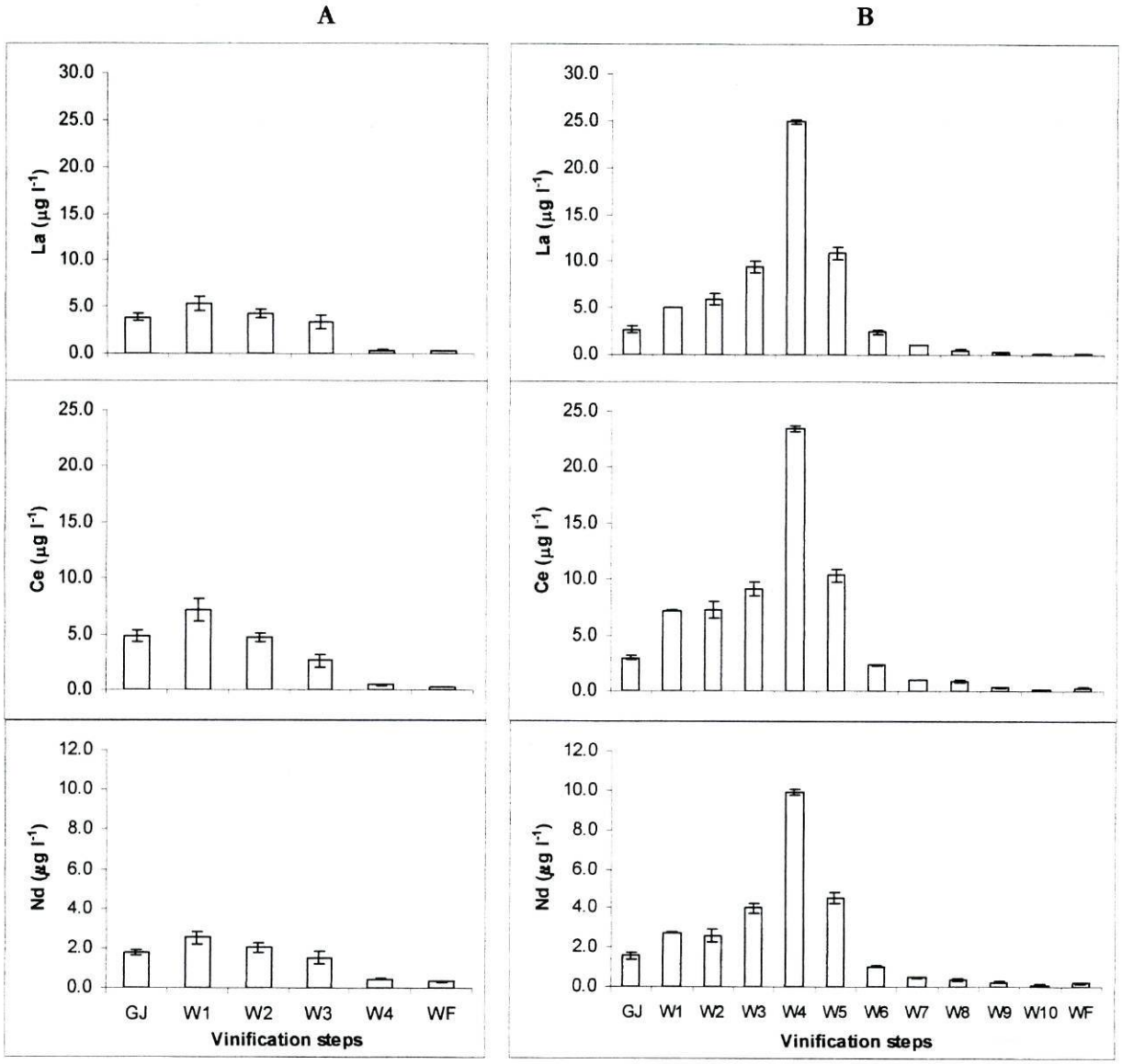

Fig. A.2.4. *continuation.* Total concentration of Li, Be, B, Al, Ca, Sc, Ti, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Ga, Rb, Sr, Y, Zr Nb, Mo, Cd, Sb, Cs, Ba, W, Tl, Pb, Th, U and La, Ce and Nd representing the REEs (in µg l^{-1}) obtained for the grape juice (GJ), in all the samples collected in several points during the entire vinification procedure (samples W1 to W10 in table and W1 to W4 in fortified wine) and in its final stage (WF). (A) red fortified wine produced with grapes from the old vineyard; (B) red table wine produced with grapes from the young vineyard. Values not presented were below the limit of quantification.

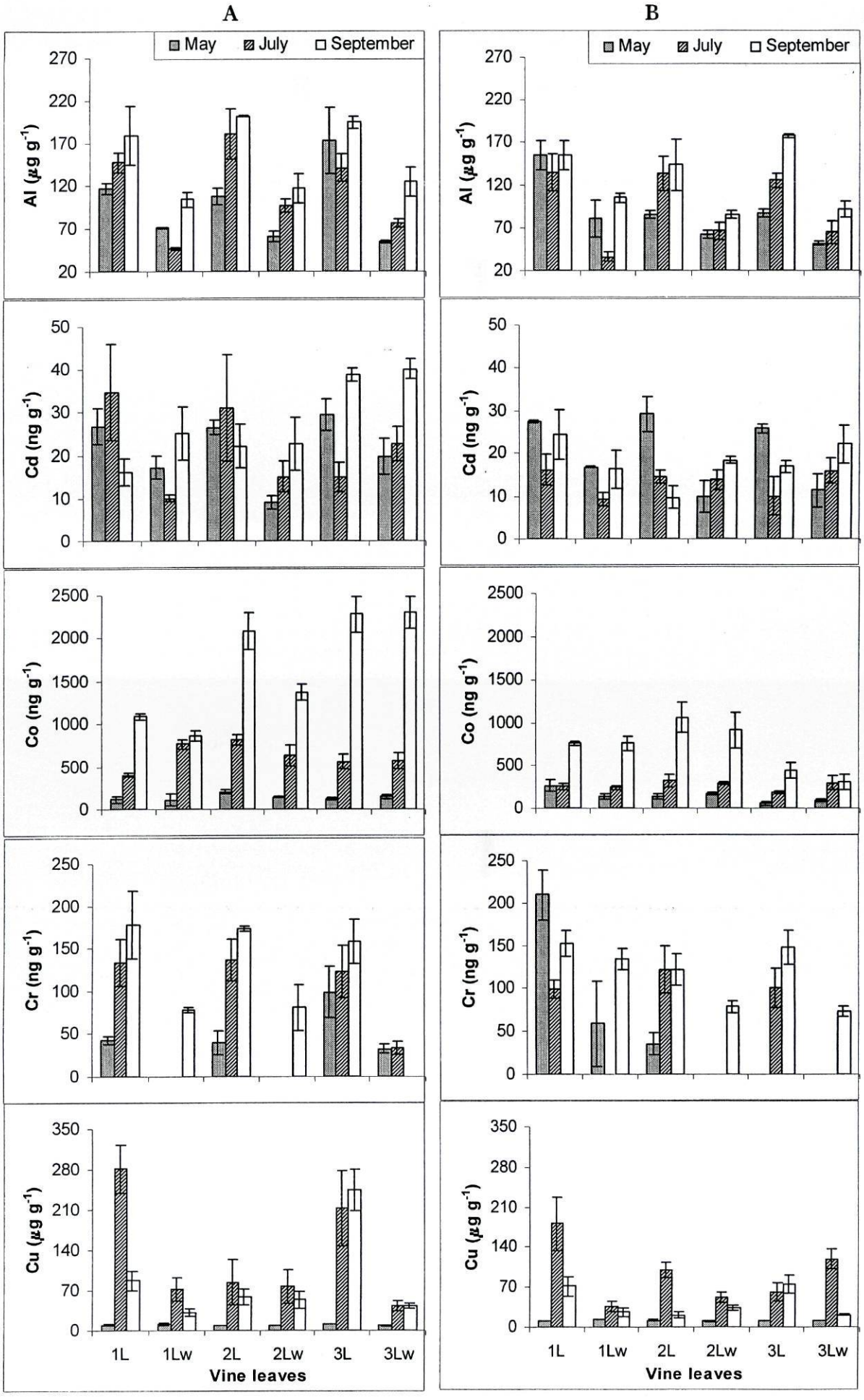
A
B
May
July
September
Al (µg g-1)
Cd (ng g-1)
Co (ng g-1)
Cr (ng g-1)
Cu (µg g-1)
1L
1Lw
2L
2Lw
3L
3Lw
Vine leaves

Fig. A.2.5.

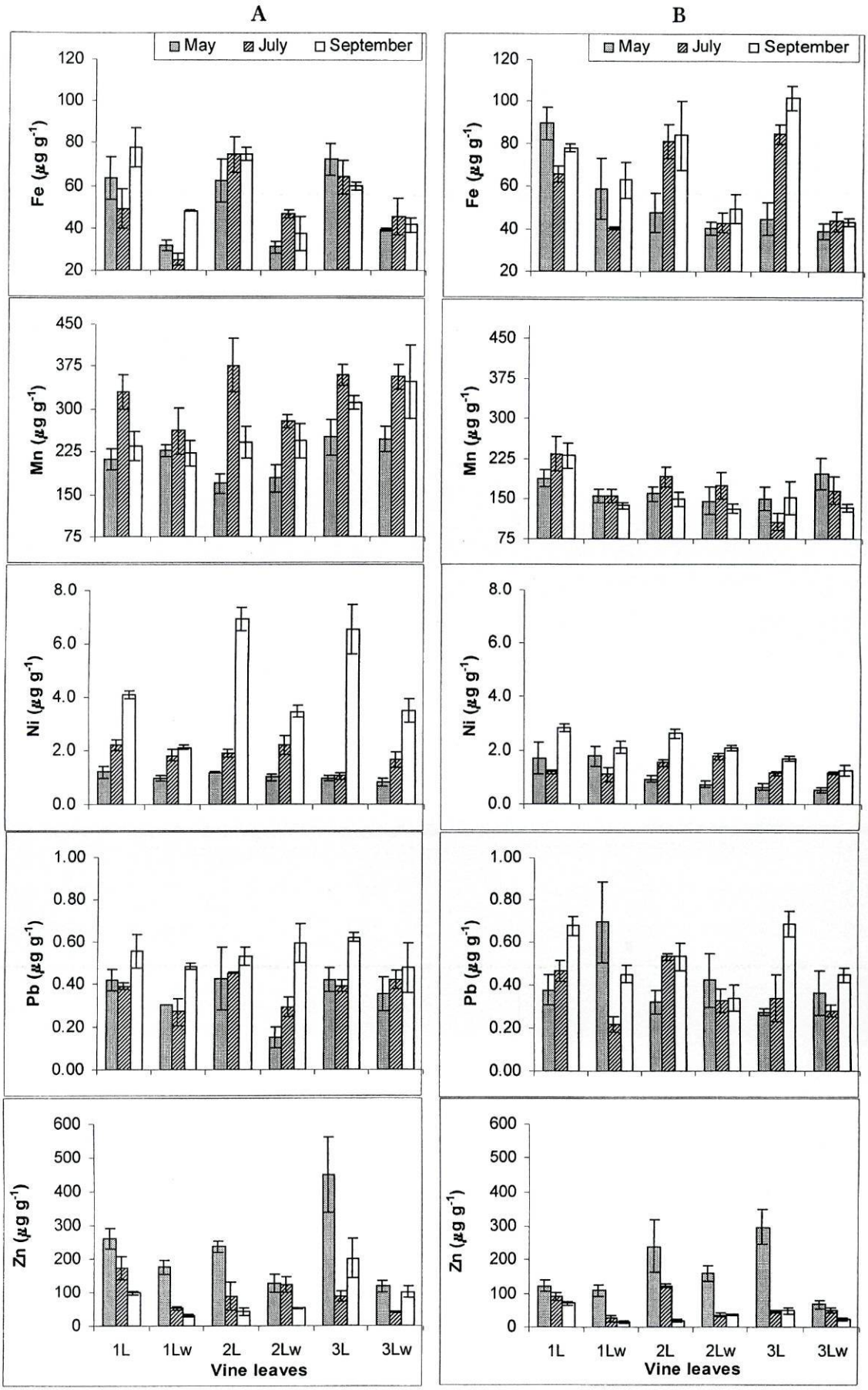

Fig. A.2.5. *continuation.* Concentration of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn (per g of dry vine leave) obtained in the vine leave samples washed (L_w) and non-washed (L) collected in the three selected sites (1L, 2L, 3L), in the old (A) and young (B) vineyards, in three different months. Cr was below the limit of detection (LOD = 6.4 ng g^{-1}) in the old vineyard samples 1Lw and 2Lw of May and July; and in the young vineyard samples 1Lw of July, 2Lw and 3Lw of May and July; and 3L of May.

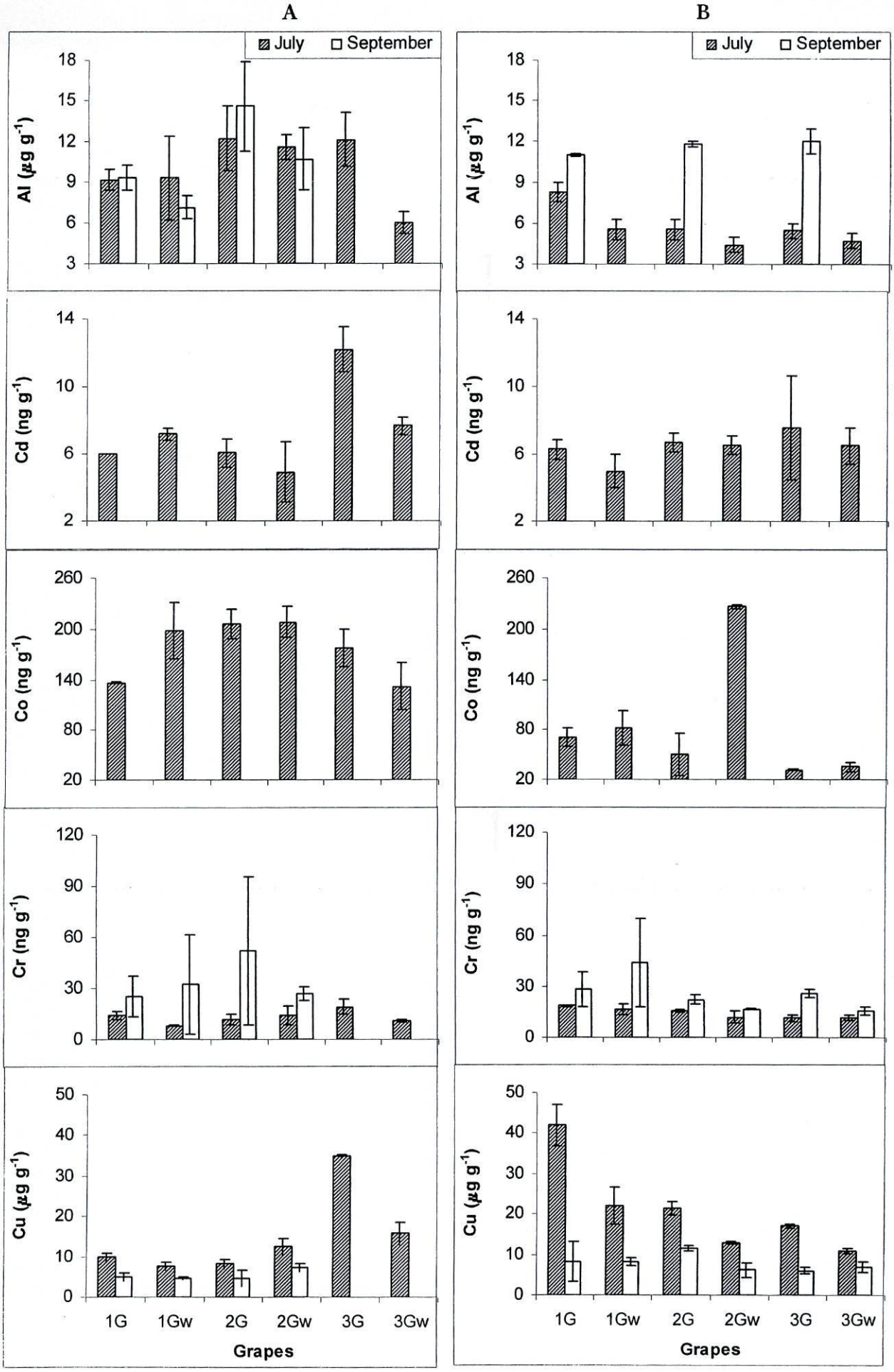
A
B
July
September
Al (µg g-1)
Cd (ng g-1)
Co (ng g-1)
Cr (ng g-1)
Cu (µg g-1)
1G
1Gw
2G
2Gw
3G
3Gw
Grapes

Fig. A.2.6.

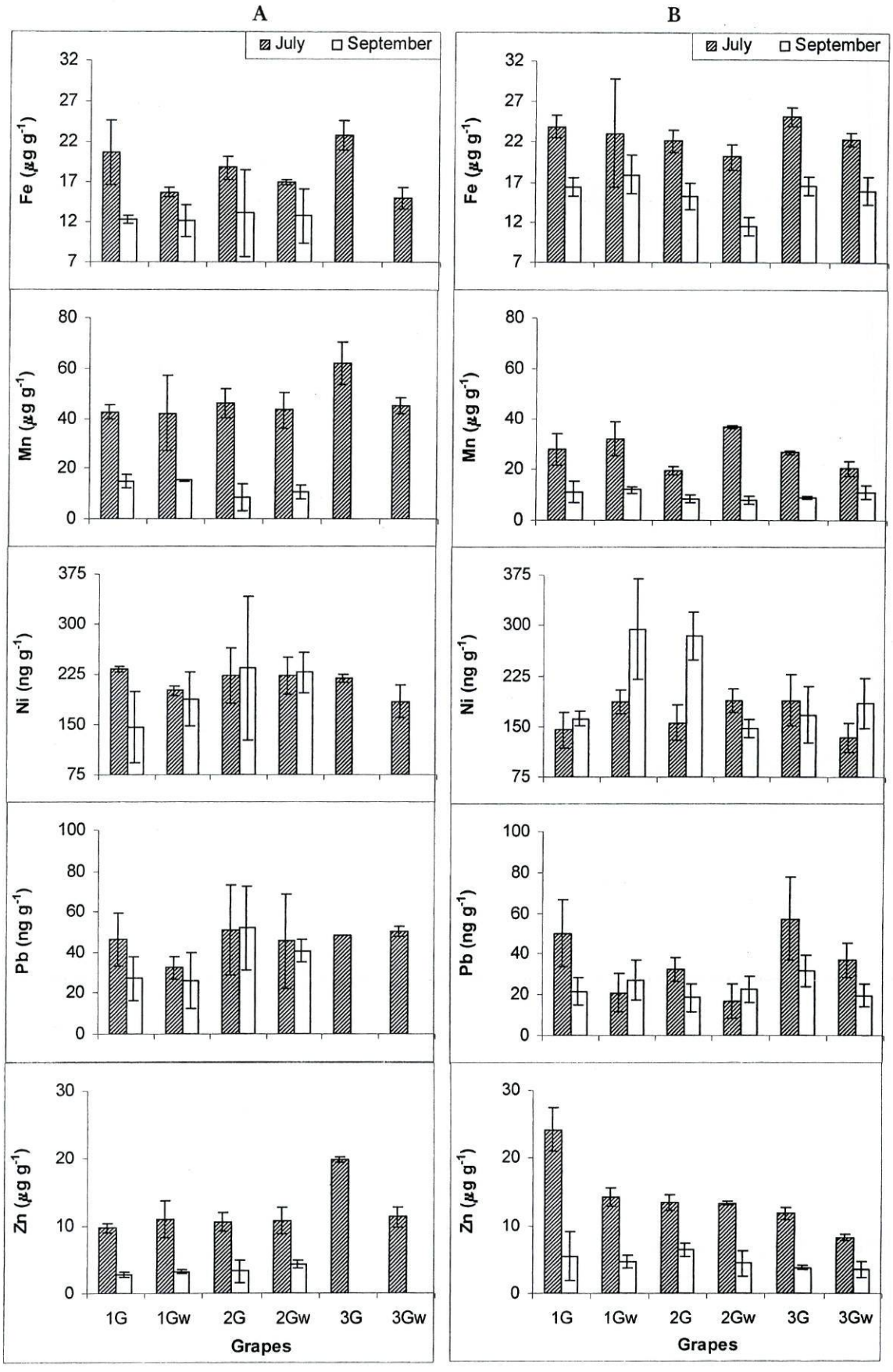

Fig. A.2.6. *continuation.* Concentration of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn (per g of dry grape) obtained in the grape samples washed (G_w) and non-washed (G) collected in the three selected sites (1G, 2G, 3G), in the old (A) and young (B) vineyards, in two different months. Samples 3G of the old vineyard of the month of September were lost. Al was below the limit of detection (LOD = 5.0 µg g^{-1}) in the young vineyard samples 1Gw, 2Gw and 3Gw of September. Cr and Co were below the LOD (LOD = 4.5 and 30.1 ng g^{-1}, respectively) in all samples.