EVALUATION OF CONTAMINANT ELEMENTS IN PORTUGUESE WINES AND ORIGINAL MUSTS BY HIGH INTENSITY FOCUSED ULTRASOUND COMBINED WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

ÉVALUATION DES ÉLÉMENTS CONTAMINANTS DANS LES MOÛTS ET LES VINS PORTUGAIS PAR ULTRASONS DE HAUTE INTENSITÉ ET FOCALISATION COMBINÉ AVEC LA SPECTROMÉTRIE DE MASSE À PLASMA À COUPLAGE INDUCTIF

Sofia CATARINO[1,2*], José-Luís CAPELO[3],
António-Sérgio CURVELO-GARCIA[1] and Raúl BRUNO DE SOUSA[2]

1: INIAP, Estação Vitivinícola Nacional, 2565-191 Dois Portos, Portugal
2: Instituto Superior de Agronomia, Departamento de Química Agrícola e Ambiental
Tapada da Ajuda, 1349-017 Lisboa, Portugal
3: REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia,
Universidade Nova de Lisboa, 2829-516 Monte de Caparica, Portugal

Abstract: Portuguese musts and wines from several varieties and different vineyards were studied to evaluate their contents in contaminant elements, in two winemaking steps. HIFU (High Intensity Focused Ultrasound) combined with ICP-MS (Inductively Coupled Plasma Mass Spectrometry) was the methodology used. The variation from must to wines was a function of the element and its concentration. Be, Al, Mn, Co, Ni, Cu, Ga, Rb, Cd, In, Ba, Tl and U concentration in wines was lower than in musts. Cu depletion achieved 99% of the initial value. For Li, Fe, Zn, Sr, Cs, Pb and Bi, white and red samples presented different trends, suggesting the importance winemaking process. The levels of Cu, Zn, As, Cd and Pb were lower than the acceptable maximal limits established by the International Organisation of Vine and Wine and in general could be considered relatively low values.

Résumé : les teneurs de différents éléments (Li, Be, A, V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Cd, In, Cs, Ba, Hg, Tl, Pb, Bi et U) ont été évalués lors de la vinification dans les moûts et les vins issus de différents cépages portugais (*Vitis vinifera* L.),. Le traitement des échantillons a été réalisé par HIFU (Ultrasons de Haute Intensité et Focalisation) et les déterminations analytiques par ICP-MS (Spectrométrie de Masse à Plasma à Couplage Inductif). Les vins ont été obtenus par microvinification, sans addition d'additifs œnologiques autres que le dioxyde de soufre. Hormis les éléments As et Ag, on observe des taux de récupération de 98±5% (n=22) pour les moûts blancs et de 100±6% (n=21) pour les moûts rouges. Pour quelques métaux (Be, Al, Mn, Co, Ni, Cu, Ga, Rb, Cd, In, Ba, Tl et U), leur concentration dans les vins est plus faible que dans les moûts respectifs. La variation de la concentration des moûts pour les vins est fonction de l'élément et de leur concentration. Dans certains cas, la diminution de la teneur de Cu peut atteindre 99% de la valeur initiale. Les échantillons des vins blancs et rouges présentent des teneurs variables de Li, Fe, Zn, Sr, Cs, Pb et Bi, qui peut suggérer l'importance du procédé de vinification. Pour ces éléments, les teneurs minimum et maximum observées sont de 4,0 et 28,1 µg/L, 352 et 1,526 µg/L, 6 et 901 µg/L, 95 et 474 µg/L, 2,69 et 19,0 µg/L, 1,82 et 28 µg/L, et 0,004 et 0,07 µg/L, respectivement. Les teneurs de Cu, Zn, As, Cd et Pb sont inférieures aux limites établies par l'Organisation Internationale de la Vigne et du Vin (OIV) et, d'une façon générale, on peut les considérer comme des valeurs faibles.

Keywords: must, portuguese wines, contaminant elements, HIFU, ICP-MS
Mots clés : Moût, vins portugais, trace éléments, HIFU, ICP-MS

*Corresponding authors: sofia_catarino@clix.pt
evn.sofia.catarino@mail.net4b.pt

J. Int. Sci. Vigne Vin, 2006, **40**, n°2
©Vigne et Vin Publications Internationales (Bordeaux, France)

INTRODUCTION

The elemental composition of wines is influenced by environmental factors, such as geological and atmospheric phenomena, soil and climate, as well as by technological processing of the grapes, must and wine (ESCHNAUER, 1982; CURVELO-GARCIA, 1988; STOCKLEY and LEE, 1995; CURVELO-GARCIA and CATARINO, 1998; RIBÉREAU-GAYON *et al.*, 1998). Several sources contributing to the metal composition of a finished wine are known. An important part of the total concentration comes from the vineyard soil via the roots of the grapevine. The other part, considered as contamination, is introduced during the different processing stages, from the grape culture to the finished wine. Several research studies have been developed in the last decade in order to evaluate the potential sources of wine contamination (MCKINNON *et al.*, 1992; TEISSEDRE *et al.*, 1993; TEISSEDRE *et al.*, 1998a; LIMA *et al.*, 2004; NICOLINI *et al.*, 2004; GÓMEZ *et al.*, 2004). As an example, MÉDINA *et al.* (2000) have shown the influence of atmospheric pollution on the lead content of wines. Recently, SALVO *et al.* (2003) have studied the influence of different mineral and organic pesticide treatments on Cu, Zn, Cd and Pb contents in italian wines. The determination of some elements is of interest due to their toxicological or physiological properties, while others can lead to wine spoilage. Hence, the presence of metallic elements and their concentration may affect must and wine quality. Although the concentrations of only few elements in wines are currently under regulation, the increasing concern for health effects and environmental exposure will probably result in a longer list of potentially toxic elements to be monitored. To date, the International Organisation of Vine and Wine (OIV) define maximum concentration values for the elements Cu, Zn, As, Cd and Pb in wine (OIV, 2005) as follows: 1 mg/L, 5 mg/L, 0.2 mg/L, 0.01 mg/L and 0.2 mg/L respectively. Metal determination in must and wine can be performed only if matrix influences are eliminated or at least diminished. Must is a complex sample with a high organic content, mainly sugars and large agglomerations of organic matter. Therefore, a sample pre-treatment is required in order to destroy the organic matter which could interfere with the analytical determination by spectrometric techniques such as Inductively Coupled Plasma - Mass Spectrometry. Nowadays, microwave digestion (MWD) is widely used in the destruction of organic matter in samples with high sugar content (TEISSÉDRE *et al.*, 1993; CABRERA-VIQUE *et al.*, 1997; KRISTL *et al.*, 2002; GÓMEZ *et al.*, 2004). However, High Intensity Focused Ultrasound (HIFU) is also a useful technique for the extraction of metals associated with organic matter present in must. Recently, we have evaluated the suitability of this methodology in grape must (CATARINO *et al.*, 2005). It was demonstrated that lead concentration values were not statistically different when HIFU or MWD were used for sample treatment. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an excellent tool for metal analysis because it is a multielemental technique, with high selectivity and sensitivity and low analytical detection limits. The main purpose of this work was to evaluate the metal content in musts and corresponding wines from different grape varieties and regions from Portugal, by using HIFU sample treatment in conjunction with ICP-MS for metal quantification.

MATERIALS AND METHODS

I - APPARATUS

A Branson Sonifier 150 ultrasonic cell disruptor-homogeniser (100 W, 22.5 kHz, Branson Ultrasonics Corporation, USA) equipped with a 3-mm titanium micro-tip was used. Ultrasonic energy irradiation was fixed at 10% level with the 3-mm micro-tip. The Sonifier 150 has a digital LCD display which provides a continuous read-out of the watts delivered to the end of the probe (range 5-6 W in this work). Eppendorf cups (2 ml) were used throughout this work. A Wifug (London, UK) labor-50M centrifuge was used. The analytical measurements were carried out with a Perkin Elmer SCIEX Elan 9000 ICP-MS (Perkin-Elmer SCIEX, Norwalk, CT, USA) apparatus, equipped with a crossflow nebulizer, a Scott-type spray chamber made of Ryton and nickel cones. A peristaltic sample delivery pump with four channels, model Gilson, was used. Autosampler Perkin-Elmer AS-93 Plus was protected by a laminar-flow-chamber clean room class 100 (Reinraumtechnik Max Petek). Application software Elan - 6100 Windows NT (version 2.4), was used.

II - MATERIAL AND REAGENTS

Monoelement standard solutions of Be, Co, In (1,000 mg/L), from Merck (Darmstadt, Germany), and a solution of Mg, Cu, Rh, Cd, In, Ba, Ce, Pb and U (10 µg/L), from Perkin-Elmer (Connecticut, USA) were used for ICP-MS optimization procedures. Ultrapure concentrated HNO_3 (J.T.Baker, Phillipsburg, USA), C_2H_5OH (Lichrosolv, Merck) and Au (Merck, 1,000 mg/L) for washing, blank, and standard solutions were used. ICP-MS external calibration was established using a multielement standard solution with 30 elements (Perkin-Elmer, 10 mg/L). For ICP-MS internal standardisation standard solutions of Rh and Re (Merck, 1,000 mg/L), were used. H_2O_2 (Puriss. p.a.) from Fluka (Steinheim, Germany) was used for must treatment. Purified water (conductivity < 0.1 µS/cm) was produced using a Seralpur Pro 90CN apparatus (Seral, Ransbach-Baumbach, Germany). For sample and solution preparation only plastic material was used. All the material

©Vigne et Vin Publications Internationales (Bordeaux, France)

was soaked in 20% HNO_3 (v/v) for at least 24 h and rinsed several times with purified water before use.

III - ICP-MS DETERMINATIONS

The multielemental analysis of musts and wines was performed by ICP-MS quantitative approach (CATARINO *et al.*, *in press*). The operating conditions were optimized daily, by using an aqueous solution containing 10 µg/L of Mg, Ba, Ce, Cu, Cd, Rh, In and Pb, and monitoring the intensities of the isotopes ^{24}Mg, ^{103}Rh, ^{114}In, ^{208}Pb, ^{138}Ba and ^{140}Ce as well as the intensities at mass 69, 156 and 220 (corresponding to species $^{138}Ba^{2+}$, $^{140}Ce^{16}O^{+}$ and background, respectively). 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 d 3%) and of oxide ions (obtained by the intensities ratio CeO^{+}/Ce^{+}, always d 3%), precision better than 2 % and background < 30 cps. The Autolens system was optimised with a 10 ºg/L Be, Co and In solution, and Dual Detector calibration with a 200 µg/L solution with twenty-nine elements. Operating conditions were as follows: RF power of 1200W; sample uptake rate of 0.85 mL/min; nebulizer argon flow between 0.85 and 0.95 L/min. The wash solution contained 2 % HNO_3 (v/v), 1 % C_2H_5OH (v/v) and Au (200 µg/L). The Au was used to eliminate the Hg and U memory effect at nebulizer chamber. As internal standards Rh and Re, both at 10 µg/L concentrations, were used for blank solution, standards and samples preparation. Blank solution and standards contained 1 % HNO_3 (v/v), 1 % C_2H_5OH (v/v), 10 µg/L of Rh and Re, and 200 µg/L of Au. Since must do not contain C_2H_5OH, blank, standard and wash solutions used to analyse must samples were prepared without this compound. The must and wine samples, blank and standard solutions were prepared daily, in polyethylene tubes. In order to get signal stabilization, a sample read delay of 75 s was chosen. Between samples or standards, the sampling system was rinsed with a 2 % HNO_3 (v/v), 1 % C_2H_5OH (v/v), and 200 µg/L of Au solution for 75 s. External calibration was used and the appropriate interpolation was carried out for each element to determine its concentration in the corresponding calibration line. Isotopes monitored and concentration range (µg/L) for external calibration are the following: ^{201}Hg; ^{202}Hg (0.1; 0.2; 0.5); ^{9}Be; ^{59}Co; ^{71}Ga; ^{75}As; ^{82}Se; ^{107}Ag; ^{111}Cd; ^{115}In; ^{133}Cs; ^{205}Tl; ^{209}Bi; ^{238}U (0.05; 0.25; 0.5; 2.5); ^{7}Li; ^{51}V; ^{53}Cr; ^{60}Ni (0.25; 0.5; 2.5; 10); ^{65}Cu; ^{138}Ba; ^{206}Pb; ^{207}Pb; ^{208}Pb (0.5; 2.5; 10; 50); ^{27}Al; ^{55}Mn; ^{57}Fe; ^{66}Zn; ^{85}Rb; ^{88}Sr (2.5; 10; 50; 200). The selected isotopes were those free from isobaric or important matrix-induced interferences, when possible. Otherwise, suitable elemental equations were applied to correct isobaric and matrix-induced interferences. Since Pb isotope ratios may change from sample to sample, ^{206}Pb, ^{207}Pb and ^{208}Pb isotopes were measured. The ^{201}Hg and ^{202}Hg isotopes were added in order to increase the signal intensity. The experimental conditions used for the measurements were: dwell time = 50.0 ms; sweeps/reading = 30; reading/replicates = 1; replicates = 4; time per run = 235 s.

II - MUSTS AND WINES

Seven wines and original musts originated from 2003 and 2004 vintage years were used. Several grape varieties (*Vitis vinifera* L.) from different regions of Portugal, were used in this study. Samples A and B, both from white grapes, were treated with distinct pesticides. Samples C and D are different clones of Fernão Pires, a Portuguese white variety, growing in Óbidos and Caldas da Raínha vineyards. Samples E, F and G are from red varieties, the first two representing different clones of Aragonez from Alentejo, while G sample is from Syrah variety (Dois Portos vineyards).

III - MICROVINIFICATION PROCESSES

Microvinifications were performed at the winery of Estação Vitivinícola Nacional (Dois Portos, Portugal). The operating procedures were as follows: grapes from each white variety were destemmed/crushed, and immediately pressed for skin and must separation and sulfited with sulphur dioxide (100 mg/L). Musts were decanted at 4 ºC during 48 hours and fermentation continued for 11 to 12 days in 60 L stainless steel tank at 19 ºC. At the end of fermentation the wine was decanted from lees and stored in 20 L glass carboys at room temperature. After 9 to 10 months, the wines were racked and bottled in 750 mL glass bottles. Sulphur dioxide (40 mg/L) was added at bottling. Grapes from red varieties were processed, also separately, into wine according to the classical red wine technology. Grapes were destemmed/crushed and collected in 60 L stainless steel tanks, sulfited with sulphur dioxide (60 mg/L) prior skin fermentation at 25 ºC. The cap was punched down twice daily until it remained submerged. When alcoholic fermentation was nearly finished, after 5 days of maceration, the mash was pressed. The wine obtained was stored at 20 l carboys at room temperature until bottling. Sulphur dioxide (20 mg/L) was added at bottling. With exception of sulphur dioxide addition, in order to minimize potential contamination sources, namely those represented by enological additives, no other winemaking treatment was made.

VI - MUST SAMPLE COLLECTION

Exogenous contamination was avoided cleaning all the plastic bottles used for must sample collection with HNO_3 10 % v/v. The bottles were then rinsed gently with ultrapure water and dried. The must samples were obtained in duplicate immediately after the grapes were pres-

©Vigne et Vin Publications Internationales (Bordeaux, France)

sed (before sulphur dioxide addition), and 5 mL of HNO_3 (70 % v/v) were added to a sample volume of 0.1 L. The samples were stored at -10 ºC until they were analyzed.

V - MUST SAMPLE TREATMENT BY HIFU

Following the procedure previously described elsewhere (CATARINO *et al.*, 2005), to 0.5 mL must sample placed in eppendorf cups were added the following reagents: HNO_3 (70% v/v) + H_2O_2 (30 % v/v) (50 µl + 100 µL). Then, focused ultrasound was applied for 60 s at 10 % amplitude (5-6 watts delivered at the end of the 3 mm probe). Finally the sample was centrifuged for 5 min at 10,000 rpm and the supernatant was used for measurements. For each must sample, this treatment procedure was repeated several times in order to obtain the required volume to ICP-MS analysis.

RESULTS AND DISCUSSION

I - ULTRASONIC TREATMENT

Preliminary assays were performed to ensure the homogeneity and representativeness of the must samples investigated. Since must samples have large agglomerations of organic matter, eppendorf tips were modified in order to make weighting of the sample as precise as possible. In order to do so, the tip was cut 1 cm above its end, the hole of the tip being modified to a higher size. For each must, eight 0.5 mL samples were taken while stirring to ensure homogenization. The precision (RSD, n=8) was always lower than 2% (Must A: 0.590 ± 0.007 g); (Must B: 0.60 ± 0.01 g); (Must C: 0.58 ± 0.01 g); (Must D: 0.576 ± 0.006 g); (Must E: 0.57 ± 0.01 g); (Must F: 0.576 ± 0.005 g); (Must G: 0.59 ± 0.01 g). In order to control any contamination, as a quality control tool of the process, water was submitted to complete treatment procedure. Periodically, and between every 10 must samples, this procedure was repeated. Blank of HIFU sample treatment was subtracted to ICP-MS analytical results. Blanks of HIFU sample treatment had high levels of aluminium (nearly 300 µg/L) and vanadium (about 200 µg/L), probably due to ultrasonic probe. The low levels of vanadium in must make the measurement of this metal impossible. Aluminium content in musts and wine samples are high enough to allow its quantification.

II - MUST ICP-MS ANALYSIS

In an initial step of our work, the feasibility of ICP-MS to analyse must samples was assessed through recovery tests. White and a red must samples were spiked with 5 to 5,000 µg/L standard solution. Three different

Table I - Recoveries obtained for contaminant elements in must by ICP-MS analysis (ND: not determined).

Récupérations obtenues pour les éléments contaminants dans les moûts par l'analyse en ICP-MS (ND: non déterminé).

Element	Recovery		Element	Recovery	
	White must	*Red must*		*White must*	*Red must*
^{7}Li	95.1 ± 0.1	91 ± 2	^{85}Rb	96	97 ± 5
^{9}Be	95.6 ± 0.5	95 ± 2	^{88}Sr	100 ± 3	100 ± 2
^{27}Al	105 ± 4	112 ± 2	^{107}Ag	88	80
^{51}V	112 ± 2	111	^{111}Cd	90.1 ± 0.1	92 ± 2
^{55}Mn	100 ± 4	107 ± 2	^{115}In	94.01 ± 0.07	95 ± 2
^{57}Fe	99 ± 4	109 ± 4	^{133}Cs	91.6 ± 0.3	95 ± 2
^{59}Co	100.2 ± 0.1	102 ± 2	^{138}Ba	93 ± 1	97.1 ± 0.9
^{60}Ni	100 ± 1	99	^{201}Hg	105.5 ± 0.1	ND
^{65}Cu	103 ± 3	108.9 ± 0.5	^{205}Tl	90.9 ± 0.1	94 ± 3
^{66}Zn	101 ± 5	102	^{208}Pb	89.8 ± 0.7	92 ± 3
^{71}Ga	98.4 ± 0.4	100 ± 2	^{209}Bi	96 ± 2	97.1 ± 0.6
^{75}As	208.3 ± 0.3	218 ± 3	^{238}U	94.6 ± 0.3	97 ± 1

©Vigne et Vin Publications Internationales (Bordeaux, France)

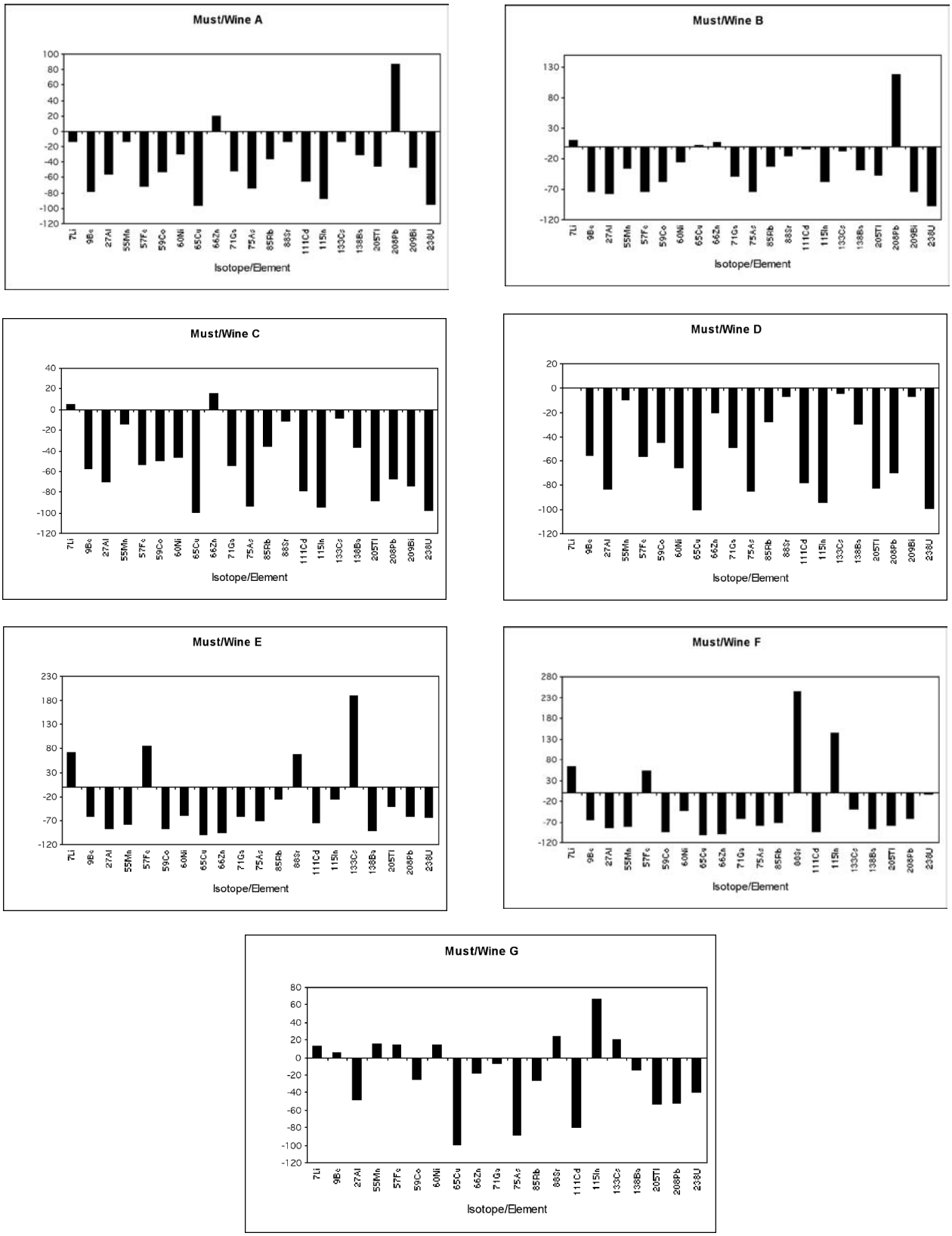


Figure 1 - Concentration change (%) of contaminant elements from must to wine

Variation de concentration (%) des éléments contaminants du moût pour le vin.

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table II - Levels of contaminant elements in musts and wines (µg/L).

Concentrations des éléments contaminants dans les moûts et les vins (µg/L).

Element /Isotope	A		B		C		D		E		F		G	
	Must	*Wine* pH = 3.12	*Must*	*Wine* pH =3.14	*Must*	*Wine* pH = 3.06	*Must*	*Wine* pH = 3.02	*Must*	*Wine* pH = 3.73	*Must*	*Wine* pH = 3.83	*Must*	*Wine* pH = 3.37
^{7}Li	4.6 (0.5)	4.0 (0.1)	5.021 (0.005)	5.7 (0.1)	24 (2)	25.2 (0.4)	13 (1)	13.0 (0.8)	6.8 (0.2)	11.7 (0.2)	5.5 (0.2)	9.0 (0.3)	24.8 (0.3)	28.1 (0.5)
^{9}Be	0.058 (0.003)	0.013 (0.003)	0.04 (0.009)	0.011 (0.003)	0.187 (0.006)	0.080 (0.007)	0.1330 (0.0007)	0.059 (0.002)	0.154 (0.002)	0.06 (0.01)	0.19 (0.02)	0.07 (0.03)	0.114 (0.003)	0.12 (0.05)
^{27}Al	301 (17)	134 (4)	526 (35)	119 (6)	671 (3)	201 (40)	685 (176)	116 (4)	1249 (20)	180 (39)	1501 (251)	236 (13)	527 (10)	273 (21)
^{51}V	ND	0.42 (0.04)	ND	0.38 (0.03)	ND	1.34 (0.03)	ND	0.90 (0.07)	ND	0.06 (0.02)	ND	0.16 (0.09)	ND	0.32 (0.06)
^{55}Mn	553 (4)	482 (12)	2076 (25)	1343 (32)	471 (26)	407 (10)	602 (30)	546 (25)	1738 (30)	382 (21)	1627 (51)	329 (6)	433 (2)	498 (12)
^{57}Fe	1212 (46)	352 (10)	1586 (98)	430 (12)	1144 (41)	540 (11)	1035 (22)	455 (24)	830 (20)	1526 (33)	892 (38)	1363 (27)	1192 (20)	1359 (41)
^{59}Co	1.7 (0.1)	0.81 (0.02)	2.1 (0.01)	0.90 (0.04)	2.4 (0.1)	1.22 (0.04)	2.47 (0.08)	1.36 (0.07)	2.39 (0.02)	0.32 (0.02)	2.2 (0.2)	0.15 (0.03)	3.35 (0.02)	2.5 (0.1)
^{60}Ni	13.8 (0.4)	9.7 (0.3)	13 (1)	9.8 (0.2)	14.1 (0.7)	7.5 (0.2)	16.5 (0.3)	7.6 (0.4)	17 (1)	7.1 (0.3)	13 (3)	7.7 (0.2)	14.1 (0.5)	16 (1)
^{65}Cu	372 (3)	14 (1)	361.96 (0.02)	368 (9)	9571 (211)	45.3 (0.8)	5577 (245)	23 (1)	1050 (18)	5.8 (0.2)	1067 (28)	6 (2)	7912 (75)	20 (5)
^{66}Zn	250.5 (0.7)	301 (34)	456 (18)	488 (40)	364 (10)	422 (13)	584 (63)	467 (27)	578 (57)	26 (5)	301 (34)	6 (2)	1092 (19)	901 (230)
^{71}Ga	3.6 (0.1)	1.73 (0.04)	3.60 (0.07)	1.84 (0.05)	3.76 (0.05)	1.73 (0.04)	3.3 (0.5)	1.7 (0.2)	3.0 (0.2)	1.17 (0.03)	3.51 (0.08)	1.31 (0.05)	4.4 (0.4)	4.1 (0.2)
^{75}As	ND	0.57 (0.03)	ND	0.59 (0.04)	ND	0.18 (0.01)	ND	0.30 (0.02)	ND	0.56 (0.02)	ND	0.43 (0.06)	7.141 (0.008)	0.81 (0.09)

^{82}Se	NQ	0.37 (0.06)	NQ	0.26 (0.06)	NQ	0.33 (0.09)	NQ	0.37 (0.04)	NQ	0.27 (0.06)	NQ	0.297	NQ	0.48 (0.07)
^{85}Rb	1283 (18)	823 (18)	1708 (34)	1158 (29)	2735 (60)	1533 (28)	2196 (98)	1560 (65)	2696 (47)	2022 (32)	2375 (67)	704 (13)	2503 (5)	1839 (35)
^{88}Sr	113 (2)	98 (2)	118 (2)	100 (3)	152.9 (0.6)	136 (3)	102 (6)	95 (4)	60.2 (0.8)	101 (1)	36.7 (0.9)	126 (3)	383 (3)	474 (9)
^{111}Cd	0.95 (0.01)	0.34 (5×10^{-9})	1.3 (0.1)	1.26 (0.09)	1.06 (0.01)	0.22 (0.01)	1.1 (0.2)	0.24 (0.02)	1.10 (0.04)	0.3 (0.5)	0.95 (0.04)	0.07 (0.02)	1.30 (0.01)	0.27 (0.01)
^{115}In	0.045 (0.008)	0.006 (0.005)	0.021 (0.004)	0.009 (0.002)	0.0175 (0.0007)	0.001 (0.002)	0.017 (0.004)	0.001 (0.002)	0.0145 (0.0007)	0.011 (0.008)	0.0165 (0.0007)	0.04 (0.03)	0.018 (0)	0.03 (0.03)
^{133}Cs	3.8 (0.1)	3.27 (0.09)	6.5 (0.1)	6.1 (0.1)	18.2 (0.4)	16.7 (0.3)	19.9 (0.9)	19.0 (0.7)	6.5 (0.1)	18.9 (0.4)	4.5 (0.1)	2.69 (0.05)	7.92 (0.06)	9.5 (0.2)
^{138}Ba	19.2 (0.5)	13.3 (0.2)	17.53 (0.05)	10.8 (0.3)	80 (2)	50.4 (0.8)	71 (2)	50 (2)	111.3 (0.8)	11.4 (0.3)	99 (3)	14.9 (0.3)	121.1 (0.2)	104 (2)
^{201}Hg	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
^{205}Tl	0.36 (0.02)	0.198 (0.004)	0.516 (0.009)	0.278 (0.004)	0.41 (0.01)	0.0487 (0.0006)	0.54 (0.03)	0.096 (0.005)	0.603 (0.009)	0.358 (0.002)	0.40 (0.01)	0.09 (0.03)	1.0025 (0.0007)	0.47 (0.04)
^{208}Pb	15.04 (0.09)	28 (0.4)	12.76 (0.09)	27.91 (0.09)	5.54 (0.02)	1.82 (0.02)	6.1 (0.3)	1.87 (0.08)	8.8 (0.2)	3.5 (0.4)	4.612 (0.008)	1.81 (0.05)	6.6 (0.1)	3.21 (0.04)
^{209}Bi	0.94 (0.05)	0.5 (0.3)	0.5 (0.1)	0.13	0.27 (0.03)	0.07 (0.01)	0.14 (0.01)	0.13 (0.02)	0.007 (0.008)	0.6 (0.2)	0.01 (0.02)	2 (1)	0.07 (0.04)	0.6 (0.3)
^{238}U	0.16 (0.04)	0.009 (0.005)	0.150 (0.008)	0.004 (0.002)	0.614 (0.004)	0.0143 (0.0006)	0.46 (0.06)	0.0057 (0.0006)	0.08 (0.01)	0.03 (0.01)	0.062 (0.003)	0.06 (0.05)	0.115 (0.001)	0.07 (0.06)

(Concentration values correspond to the mean of three independent replicates. Corresponding standard deviation are given in brackets. ND: not determined, NQ: not quantified)
(Les valeurs de concentration correspondent à la moyenne des trois répliques indépendantes. Les écarts-type correspondant sont présentés entre parenthèses. ND : non déterminé. NQ : non quantifié)

J. Int. Sci. Vigne Vin, 2006, **40**, n°2
©Vigne et Vin Publications Internationales (Bordeaux, France)

spikes of each element were performed. Table I shows the results which could be considered satisfactory except for As and Ag. Excluding afore mentioned elements the recovery averages were 98±5 % (n=22) for white must and 100±6 % (n=21) for red must. The high recovery percentage observed for As, approximately 200 %, probably means that severe polyatomic interferences (namely 40Ar35Cl species) were not correctly eliminated (MAY and WIEDMEYER, 1998). Taking this into account, the quantification of this element in must samples was not carried out. Ag recovery was about 80 %, in agreement with previous results observed in our laboratory.

III - CONTENTS OF ELEMENTS / CHANGES FROM MUST TO WINE

The levels of elements (mean of three independent replicates ± standard deviation) in musts and wines are shown in table II. The results in both musts and wine are expressed in µg/L in order to allow better must-wine comparison. Analytical results for V and As in musts are not shown because of the reasons explained previously. In addition, levels of Se in must and Hg in must and wine were below our quantification limits. To afford better observation and analysis of the results, figure 1 shows the changes of concentrations for each pair must-wine, expressed in percentage. Important losses (expressed as percentage) of Be, Al, Mn, Co, Ni, Cu, Zn, Ga, Rb, Cd, Ba, Tl and U occurred from musts to wines during alcoholic fermentation, probably due to precipitation as insoluble salts, namely sulphides. Furthermore, these phenomena are favoured both by sulphur dioxide and pH value, which, in part, could explain the differences between samples along the winemaking process (wines pH values are shown at table II). The tendencies for higher decreases of Mn and Co in red samples may be related to pH value. It should also be emphasized the almost complete depletion of Cu from must to wine, that could be explained by the low solubility of CuS. It is interesting to note that some elements showed different behaviour (throughout winemaking process) depending on sample type: thus, red wines showed considerable higher concentrations of Li, Fe, Sr, In and Bi than original musts; this trend was not observed for white wines. An explanation for this could be related to the maceration of solid parts of red grapes. Another hypothesis is the grape contamination with soil, a well know phenomenon (CURVELO-GARCIA and CATARINO, 1998). In white samples, Li concentrations remain quite constant from musts to wines, accordingly to results reported by GÓMEZ *et al.* (2004). In the present study, Pb was one of the few elements that increased concentration from must to wine (samples A and B), suggesting the existence of a source of contamination, although not identified. However, the final concentration of Pb is lower than the maximum accepted values allowed by the OIV (0.2 mg/L), thus the contamination introduced should be of no concern. For some samples (C, D, E, F and G) a main part of the Pb present in the must was removed, during alcoholic fermentation and the following vinification steps (figure 1). Such a decrease has been previously reported by various authors (TEISSEDRE *et al.*, 1993; HENICK-KLING and STOEWSAND, 1993). This elimination may be attributed to the reaction of Pb with hydrogen sulphide formed during yeast fermentation: Pb sulphide precipitate, is adsorbed to the yeast, and removed with the yeast lees. In addition, Pb may also be removed after insoluble complexes with proteins and polysaccharides are formed. It is also known that in wine a main part of the Pb is complexed with rhamnogalacturonan II (PELLERIN *et al.*, 1997). Regarding the contents of elements in both musts and wines, the ones could be considered at low concentration, moreover when confronted with published data. Be, In and U occurred in subtrace levels. The following elements were found in lower concentrations than those reported on literature: Be (THIEL *et al.*, 2004), Al (MCKINNON *et al.*, 1992; ESCHNAUER and SCOLLARY, 1995 and LOPEZ *et al.*, 1998), Ni (TEISSEDRE *et al.*, 1998a), Sr (GREENOUGH *et al.*, 1997) and V (TEISSÉDRE *et al.*, 1998b). Likewise other elements, levels of As and Cd were very low in all wines, moreover very distant from the OIV maximum acceptable values (2005), of 0.2 mg/L and 0.01 mg/L, respectively). HERCE-PAGLIAI *et al.* (2002) have reported levels of As in musts and wines in the range of 2.10-14.60 µg/L, while JAGANATHAN (2001) and THIEL *et al.* (2004) have found higher amounts (up to 22 µg/L). Recently, LIMA *et al.* (2004) observed 3-4 µg/kg and 0.58 µg/L of Cd in Azores musts and wines, respectively. Pb levels were also low, confirming a general tendency of decreasing in the levels of these elements observed in the last years. In 1994, SUDRAUD *et al.* related values between 14 and 450 µg/L. Recently, LIMA *et al.* (2004) reported an average concentration of 28.3 µg/L in Azores wines. The results obtained for the following elements are comparable to the amounts reported by different authors: Mn (SUDRAUD *et al.*, 1994; GREENOUGH *et al.*, 1997; TAYLOR *et al.*, 2003), Co and Ga (TAYLOR *et al.*, 2003; THIEL *et al.*, 2004), Ba (TAYLOR *et al.*, 2003), Tl (GREENOUGH *et al.*, 1997; TAYLOR *et al.*, 2003); Bi (GREENOUGH *et al.* 1997; TAYLOR *et al.*, 2003) and Zn (SUDRAUD *et al.*, 1994; GREENOUGH *et al.*, 1997; SALVO *et al.*, 2003). It should be referred that Zn levels are far from the OIV maximum acceptable value (5 mg/L). In the case of Rb, the levels are similar to those reported by SUDRAUD *et al.* (1994), in french wines, but higher than those founded by TAYLOR *et al.* (2003) or THIEL *et al.* (2004). Musts C, D and G showed high levels of Cu, probably due to grape treatment with copper sulphate. Sample B should be noted because it presents a peculiar behaviour:

©Vigne et Vin Publications Internationales (Bordeaux, France)

Cu concentration remains unchanged, perhaps due to the pesticide type used in vineyard treatment. The OIV maximum acceptable value for this element is of 1 mg/L. The results obtained for Cs are of particular interest as they could be considered high values (ESCHNAUER, 1982; GREENOUGH *et al.*, 1997; TAYLOR *et al.*, 2003; NICOLINI *et al.*, 2004). Finally, for some elements, namely Li, Mn, Zn, Sr, Cs and Ba, concentrations in must samples seem to be a reflex of provenance soil.

CONCLUSIONS

The change in the element concentration from must to wine depends on the element, its concentration and the sample type. Lower concentrations of the elements studied were found in wines. The major part of these decreases can probably be explained by precipitation of the elements, namely the heavy metals, as sulphured forms. Indeed, this emphasises the role played by alcoholic fermentation in the elimination of toxic elements contained in the grapes (must). In some cases, white and red samples presented different behaviour, suggesting the importance of winemaking process. Nevertheless, some elements remain constant from must to wine, independent of the winemaking process. Finally, the levels of all different elements were under the acceptable maximal limits established by the OIV and in general can be considered low both for musts and wines.

Acknowledgments: The authors are grateful to M. Cristina Clímaco, P. Clímaco and Goreti Botelho for providing the samples, to "PARLE - Project A" for financial support and to "FCT" for a PhD scholarship (POCI 2010, medida IV.3., BD/17237/2004).

REFERENCES

CABRERA-VIQUE C., TEISSEDRE P-L., CABANIS M-T. and CABANIS J-C., 1997. Determination and levels of chromium in French wine and grapes by graphite furnace atomic absorption spectrometry. *J. Agric. Food Chem.*, **45**, 1808-1811.

CATARINO S.C.G., 2000. Ocorrência de chumbo e cádmio em vinhos. *MSc Thesis*, Universidade do Porto, Portugal.

CATARINO S., Capelo J.L., Curvelo-Garcia A.S. and Vaião M., 2005. Focused ultrasound versus microwave digestion for the determination of lead in must by electrothermal-atomic absorption spectrometry. *J. AOAC Int.*, **88** (2), 585-591.

CATARINO S., CURVELO-GARCIA A.S. and BRUNO DE SOUSA R. (in press). *Measurements of contaminant elements of wines by inductively coupled plasma mass spectrometry: a comparison of two calibration approaches*. Talanta.

CURVELO-GARCIA A.S., 1988. *Controlo de Qualidade dos Vinhos. Química Enológica. Métodos Analíticos*. Instituto da Vinha e do Vinho, Lisboa, Portugal.

CURVELO-GARCIA A.S. and CATARINO S., 1998. Os metais contaminantes dos vinhos. Origens da sua presença, teores, influência dos factores tecnológicos e definição de limites. *Ciência Téc. Vitiv.*, **13** (1-2), 49-70.

ESCHNAUER H., 1982. Trace elements in must and wine: primary and secondary contents. *Am. J. Enol. Vitic.*, **33** (4), 226-230.

ESCHNAUER H.R. and SCOLLARY G.R., 1995. Aluminium in wein. *Wein-Wiss.*, **1**, 24-30.

FRANCESCO S., LA PERA L., DI BELLA G., NICOTINA M. and DUGO G., 2003. Influence of different mineral and organic pesticide treatments on Cd(II), Cu(II), Pb(II), and Zn(II) contents by derivate potentiometric stripping analysis in Italian white and red wines. *J. Agric. Food Chem.*, **51**, 1090-1094.

GREENOUGH J.D., Longerich H.P. and JACKSON S.E., 1997. Element fingerprint of Okanagan Valley wines using ICP-MS: relationships between wine composition, vineyard and wine colour. *Austral. J. Grape Wine Res.*, **3**, 75-83.

GÓMEZ M.D.L.C., BRANDT R., JAKUBOWSKI N. and ANDERSON J.T., 2004. Changes of the metal composition in German white wines through the winemaking process. A study of 63 elements by inductively coupled plasma-mass spectrometry. *J. Agric. Food Chem.*, **52**, 2953-2961.

HENICK-KLING T. and STOEWSAND G.S., 1993. Lead in wine. *Am. J. Enol. Vitic.* (Technical Brief), **44** (4), 459-463.

HERCE-PAGLIAI C., MORENO I., GONZALEZ G., REPETTO M. and CAMEAN A.M., 2002. Determination of total arsenic, inorganic and organic arsenic species in wine. *Food Addit. Contamin.*, **19** (6), 542-546.

JAGANATHAN J., 2001. A random testing of table wines for arsenic using electrothermal atomic absorption spectrometry. *Atom. Spectrosc.*, **22** (2), 280-283.

LIMA M.T.R., CABANIS M.T., MATOS L., CASSANAS G., KELLY M. and BLAISE A., 2004. Determination of lead and cadmium in vineyard soils, grapes and wines of the Azores. *J. Int. Sci. Vigne Vin*, **38**, 163-170.

LOPEZ F.F., CABRERA C., LORENZO M.L. and LOPEZ M.C., 1998. Aluminium levels in wine, beer and other alcoholic beverages consumed in Spain. *Sci. Total Environ.*, **220**, 1-9.

KRISTL J., VEBER M. and SLEKOVEC M., 2002. The application of ETAAS to the determination of Cr, Pb and Cd in samples taken during different stages of the winemaking process. *Anal. Bioanal. Chem.*, **373**, 200-204.

MAY T.W. and WIEDMEIYER R.H., 1998. A table of polyatomic interferences in ICP-MS. *Atom. Spectrosc.*, **19** (5), 150-155.

MCKINNON A.J., CATTRALL R.W. and SCOLLARY G.R., 1992. Aluminum in wine - its measurement and identification of major sources. *Am. J. Enol. Vitic.*, **43** (2), 166-170.

MÉDINA B., AUGAGNEUR S., BARBASTE M., GROUSSET F.E. and BUAT-MÉNARD P., 2000. Influence of atmospheric pollution on the lead content of wines. *Food Addit. Contamin.*, **17** (6), 435-445.

©Vigne et Vin Publications Internationales (Bordeaux, France)

NICOLINI G., LARCHER R., PANGRAZZI P. and BONTEMPO L., 2004. Changes in the contents of micro and trace elements in wine due to winemaking treatments, *Vitis*, **43** (1), 41-45.

OIV, 2005. *Recueil des méhodes internationales d'analyse des vins et des moûts*. Organisation Internationale de la Vigne et du Vin, Paris.

PELLERIN P., O'NEILL M.A., PIERRE C., CABANIS M-T., DARVILL A.G., ALBERSHEIM P. and MOUTOUNET M., 1997. Complexation du plomb dans les vins par les dimères de rhamnogalacturonane II, un polysaccharide pectique du raisin. *J. Int. Sci. Vigne Vin*, **31** (1), 33-41.

RIBéREAU-GAYON P., GLORIES Y., MAUJEAN A. and DUBOURDIEU D., 1998. *Traité d'œnologie. Chimie du vin, stabilisation et traitements.* Dunod, Paris.

SALVO F., LA PERA L., DI BELLA G., NICOTINA M. and DUGO G., 2003. Influence of different mineral and organic pesticide treatments on Cd(II), Cu(II), Pb(II), and Zn(II) contents determined by derivate potentiometric stripping analysis in Italian white and red wines. *J. Agric. Food Chem.*, **51**, 1090-1094.

STOCKLEY C.S. and LEE T.H., 1995. Much ado about lead in wine? An Australian review. *J. Wine Research*, **6** (1), 5-17.

SUDRAUD P., MÉDINA B. and GRENON J.P., 1994. Teneurs en éléments minéraux des vins. *J. Int. Sci. Vigne Vin*, **28** (1), 69-75.

TAYLOR V.F., LONGERICH H.P. and GREENOUGH J.D., 2003. Multielement analysis of Canadian wines by inductively coupled plasma mass spectrometry (ICP-MS) and multivariate statistics. *J. Agric. Food Chem.*, **51**, 856-860.

TEISSEDRE P.L., CABANIS M.T., DAUMAS F. and CABANIS J.C., 1993. Évolution de la teneur en plomb au cours de l'élaboration des vins des Côtes du Rhône et de la Vallée du Rhône. *R. F. OE. (cahier scientifique), 140 (Mars/Avril)*, 6-18.

TEISSEDRE P.L., CABRERA VIQUE C., CABANIS M.T. and CABANIS J.C., 1998a. Determination of nickel in French wines and grapes. *Am. J. Enol. Vitic.*, **49** (2), 205-210.

TEISSÈDRE P.L., KROSNIAK M., PORTET K., GASC F., WATERHOUSE A.L., SERRANO J.J., CABANIS J.C. and CROS G., 1998b. Vanadium levels in French and Californian wines: influence on vanadium dietary intake. *Food Addit. Contamin.*, **15** (5), 585-591.

THIEL G., GEISLER G., BLECHSCHMIDT I. and DANZER K., 2004. Determination of trace elements in wines and classification according to their provenance. *Anal. Bioanal. Chem.*, **378**, 1630-1636.

Manuscrit reçu le 16 janvier 2006 ; accepté pour publication, après modifications le 4 avril 2006

©Vigne et Vin Publications Internationales (Bordeaux, France)

 ©Vigne et Vin Publications Internationales (Bordeaux, France)