Multi-element Analysis of South African Wines and their Provenance Soils by ICP-MS and their Classification according to Geographical Origin using Multivariate Statistics

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Wines and their provenance soils from Robertson, Stellenbosch, Swartland and Walker Bay, four major wine-producing regions in the Western Cape Province of South Africa, were analysed by ICP-MS and the elemental composition was used in multivariate statistical analysis to classify the wines and soils according to geographical origin. In total, 67 wines, 29 white and 38 red, from 22 cellars and their corresponding soils were analysed for 26 elements (in order of mass number): Li, B, Al, Sc, V, Cr, Mn, Co, Ni, Cu, Zn, Rb, Sr, Zr, Nb, Mo, Cd, Sn, Sb, Ba, Ce, Nd, W, Tl, Pb and U in 1:2 diluted wines and microwave-digested soil samples. The elements Li, B, Sc, Mn, Co, Ni, Cu and Rb were identified, using a principle component analysis procedure, as indicators with the ability to discriminate between wines and soils from different geographical origins. A linear discriminant analysis procedure based on the log concentrations of the selected elements was applied to classify 96% of the wines and 100% of the vineyard soils correctly according to point of origin. It was verified that, for the wines and soils from four major wine-producing regions in the Western Cape, a correlation existed between the elemental composition of a wine and that of its provenance soil. This is an important prerequisite for the application of the fingerprinting methodology and a first result of this kind for South African wines.

INTRODUCTION

Baxter *et al*. (1997) first reported the possibility of determining the geographical origin of wine by multi-element ICP-MS analysis and using the concentration data in multivariate statistical analysis for classification. Since then, many studies in almost all wine-producing countries have explored this fingerprinting methodology based on multi-element data, with varying degrees of success. Many other wine-fingerprinting techniques using isotope ratios (Almeida *et al*., 2002; Barbaste *et al*,. 2002; Coetzee & Vanhaecke, 2005), organic compounds (Ogrinc *et al*., 2003; Garrido-Delgado *et al*., 2009), anthocyanins (Gómez-Ariza *et al*., 2006) and DNA analysis (Bowers *et al*., 1993) are also being developed. The discussion of these techniques is beyond the scope of this paper and only techniques based on multi-element analysis by ICP-MS will be discussed briefly.

Because of the complex nature of its matrix, the multi-element analysis of wine requires some care in order to obtain the accurate data necessary for fingerprinting. A number of studies have focused on the analysis of wine by ICP-MS, giving details about digestion procedures, matrix matching standards, and control of interferences (Stroh *et al*., 1994; Pérez-Jordán *et al*., 1998; Jakubowski *et al*., 1999; Tangen and Lund, 1999; Castiñeira Gómez *et al*., 2001; Almeida & Vasconcelos, 2002; Nikolakaki *et al*., 2002; Sauvage *et al*., 2002).

Studies in different wine-producing countries have shown that fingerprinting techniques using multivariate approaches have potential in obtaining information about the geographical origin of wines (Greenough *et al*., 1997; Pérez-Jordán *et al*., 1998; Jakubowski *et al*., 1999; Martin *et al*., 1999; Rebolo *et al*., 2000; Barbaste *et al*., 2002; Almeida & Vasconcelos, 2003; Marengo & Aceto, 2003; Taylor *et al*., 2003; Castiñeira Gómez *et al*., 2004; Gremaud *et al*., 2004; Thiel *et al*., 2004; Coetzee *et al*., 2005; Kment *et al*., 2005; Sperková & Sichánek, 2005; Angus *et al*., 2006; Iglesias *et al*., 2007). The technique is not simple, however, because many factors may influence the specific element composition of a wine that could result in the correlation with the provenance soil to fail. These factors include agricultural practices such as the use of fertilizers and pesticides (Almeida & Vasconcelos, 2003), pollution (Rosman *et al*., 1998), and the vinification process itself (Castiñeira Gómez *et al*., 2004, Nicolini *et al*., 2004). Some of these factors have been investigated, but the results reported in the literature are still inconclusive. The experimental verification of the correlation between the elemental composition of wine and that of its provenance soil has also not been investigated in sufficient detail, although some studies indicate an excellent correlation between the multi-element composition of the wine and the provenance soil (R = 0.994, n = 19, P < 0.01 (Almeida & Vasconcelos, 2003).

In a previous study (Coetzee *et al*., 2005) on the classification of South African wines from three wine-producing regions, the assumption was made that the chemical composition of a wine would reflect the chemical composition of the provenance

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soil for certain elements. This was, however, never proven. The main objective of this study was therefore to ascertain the correlation between the trace element composition of a wine and that of the provenance soil specifically for South African wine-producing regions. This would require obtaining reliable elemental composition data for the wines and their provenance soils. It is important to establish whether this link between soil and wine actually exists for South African wine regions in the process of validating the procedure and confirming its reliability and applicability. A second objective was to extend the number of regions to four in an attempt to assess the limitations of the classification procedure. Increasing the number of domains would also increase the degree of difficulty of obtaining a unique classification of the wines according to point of origin.

MATERIALS AND METHODS

Wine and soil samples

A total of 67 wine samples (29 white, 38 red) of the 2005 and 2006 vintages were collected from four wine-producing regions in the Western Cape: Robertson (16: 5 white, 11 red), Stellenbosch (17:11 white, 6 red), Swartland (22: 8 white, 14 red) and Walker Bay (12: 4 white, 8 red). All the wines were made from grapes produced in identified vineyards in the region. No wines that were blends of wines from other regions or wines made from grapes sourced from outside the region were included. Four wines sourced from the Groot Constantia cellar in the Constantia ward were included because the soil analyses showed similar soils in this ward to those that occur in the Stellenbosch region. Table 1 summarises the cellars and wine cultivars that were sampled for this project.

Soil samples were collected from the corresponding vineyards and therefore from the provenance soil for each of the wine samples. Three soil samples were taken from each vineyard (i.e. the block associated with a particular wine) at different positions identified as the "top", "middle" and "bottom". Soil samples were taken 30 cm below the surface with non-metallic grabs and sealed in Ziploc bags. From the Walker Bay region, only one soil sample was obtained from the centre of the block for each associated wine.

Sample preparation

Reagents

Nitric acid (Merck, p.a.) was purified by distillation in a sub-boiling quartz distillation apparatus from AF Analysentechnik (Model

TABLE 1

The 67 wines sampled from the four major wine-producing regions selected for this project.

Stellenbosch	Swartland	Robertson	Walker Bay
Delheim Sauv. Blanc	Alexanderfontein Chardonnay	Bonnievale Cab. Sauv.	Beaumont Chenin Blanc
Eikendal Sauv. Blanc	Kloovenberg Cab. Sauv.	Bonnievale Shiraz	Beaumont Malbec
Groot Constantia Chardonnay	Kloovenberg Shiraz	Clairvaux Chardonnay	Beaumont Merlot
Groot Constantia Merlot	Perdeberg Cab. Sauv. (SW4)	Clairvaux Merlot	Beaumont Pinotage
Groot Constantia Sauv. Blanc	Perdeberg Cab. Sauv. (SW9)	McGregor Shiraz (R5)	Hamilton Russell Chardonnay
Groot Constantia Shiraz	Perdeberg Chenin Blanc (SW1)	McGregor Shiraz (R1)	Hamilton Russell Pinot Noir
Jordan Chardonnay	Perdeberg Chenin Blanc (SW2)	McGregor Chardonnay	Hermanuspietersfontein Cab. Franc
Jordan Merlot	Perdeberg Chenin Blanc (SW3)	McGregor Cab. Sauv.	Hermanuspietersfontein Merlot
L'Avenir Chenin Blanc	Perdeberg Pinotage (SW6)	McGregor Sauv. Blanc	Hermanuspietersfontein Sauv. Blanc
Overgaauw Cab. Sauv.	Perdeberg Pinotage (SW7)	Roodezandt Cab. Franc	Newton Johnson Chardonnay
Overgaauw Chardonnay	Perdeberg Shiraz (SW5)	Roodezandt Cab. Sauv.	Newton Johnson Shiraz (W5)
Overgaauw Malbec	Perdeberg Shiraz (SW8)	Roodezandt Chardonnay (R10)	Newton Johnson Shiraz (W8)
Overgaauw Merlot	Porterville Cab. Sauv.	Roodezandt Chardonnay (R11)	
Simonsig Sauv. Blanc (ST9)	Porterville Chenin Blanc	Roodezandt Shiraz	
Simonsig Sauv. Blanc (ST10)	Porterville Pinotage	Rooiberg Merlot	
Simonsig Sauv. Blanc (ST11)	Porterville Ruby Cabernet	Rooiberg Shiraz	
Simonsig Semillon	Porterville Shiraz		
	Riebeek Cellars Chardonnay (SW10)		
	Riebeek Cellars Chardonnay (SW11)		
	Swartland Chardonnay		
	Swartland Merlot		
	Swartland Shiraz		

SAP_900IR). To ensure purity, survey scans were conducted regularly on the distilled acid and compared with commercially available Merck Suprapure HNO_3. The purity of the laboratory-prepared HNO_3 was comparable to the commercially available high-purity acid.

Ultrapure water (18.2 MΩ.cm) was obtained from a Milli-Q filtration system and used for preparing 1% nitric acid from distilled concentrated acid. Merck suprapure hydrogen peroxide (H_2O_2) was used in all microwave digestions of the wine and soil samples. Merck Guaranteed Reagent 98.8% ethanol, appropriately diluted, was used to prepare matrix-matched calibration standards for the diluted wine samples. Survey scans were obtained for the ethanol blanks to ensure that the unavoidable contamination from the ethanol was not interfering with the calibration of the instrument.

Wine sample preparation

The wine samples were diluted (1:2) with 1% nitric acid before analysis. The samples were made up to volume using distilled 1% HNO_3 after adding an appropriate volume of indium internal standard stock solution.

Soil sample preparation

Each of the soil samples was separated into three particle size fractions (> 500 µm, > 180 µm and < 180 µm) using standard laboratory Tyler screens. The < 180 µm fraction was used for digestion and analysis purposes. The method for microwave digestion using a Milestone Ethos system was the following: 1 g of soil, 2 ml of 65% HNO_3 and 4 ml of H_2O_2 micro-waved for 30 minutes at 180°C. Samples were then filtered after cooling and made up to 50 ml with Milli-Q water. The digested samples were further diluted (1:10) before analysis to reduce the level of total dissolved solids (TDS) so as to prevent the deposition of salts on the ICP-MS cones. The three soil samples per block were analysed individually and the element concentrations were then averaged for each block.

Blanks and standards

An appropriately diluted indium internal standard stock solution was prepared from 1 000 mg/L In ICP-MS standard solution (Alpha Aeser). For the analysis of all the wine and soil samples, blanks and standards, an indium internal standard was added to a final concentration of 10 µg/L.

A multi-element stock solution with a concentration of 1 000 µg/L was prepared from 1 000 mg/L single element standards (Alpha Aeser). Calibration standards for multi-element determinations in microwave-digested soil samples were prepared by appropriate dilution of the stock solution with 1% nitric acid. Matrix-matched calibration standards for 1:2 diluted wine samples were prepared by appropriate dilution of the multi-element stock solution with 1% nitric acid and ethanol at a similar concentration (7.5%) as that in the diluted wine samples.

The method blank for the 1:2 diluted wine samples was prepared to contain 7.5% ethanol in 1% nitric acid. The method blank for the digested soil samples was prepared by subjecting the solution used for digestion (2 mL 65% nitric acid plus 4 mL H_2O_2) to the microwave digestion programme and diluting to 50 mL with Milli-Q water.

Instrumentation

All analyses were carried out with a Thermo X-Series 2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) equipped with nickel cones, a peristaltic sample delivery pump and a Cetac auto sampler. Instrumental conditions for the ICP-MS were optimised, after completing the mass calibration and detector cross-calibration, by following a manual tuning procedure using Thermo Tuning Solution A containing the elements Li, Be, Co, Ni, In, Ba, Ce, Pb, Bi and U at 10µg/L, and As and Se at 100 µg/L. For data acquisition the ICP-MS was operated in peak jump mode, with a dwell time of 20 ms, 100 sweeps, and a forward RF power of 1400 W. Five replicate measurements were made. The isotopes measured were: ^{7}Li, ^{11}B, ^{27}Al ^{45}Sc, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{90}Zr, ^{93}Nb, ^{98}Mo, ^{111}Cd, ^{118}Sn, ^{137}Ba, ^{138}Ba, ^{140}Ce, ^{145}Nd, ^{182}W, ^{205}Tl, ^{207}Pb and ^{238}U. The elements selected are mostly metals and are considered to be useful as possible indicators of geographical origin, since they are not generally affected by vinification and are therefore in principle providing a link with the soil composition. Most of them have been tested in a number of previous studies (Almeida & Vasconcelos., 2003; Coetzee *et al*., 2005; Moreno *et al*., 2008).

To reduce molecular interferences, the data for ^{27}Al, ^{47}Ti, ^{48}Ti, ^{51}V, ^{55}Mn, ^{52}Cr, ^{60}Ni, ^{66}Zn, ^{65}Cu and ^{88}Sr were collected using Collision Cell Technology (CCT). Tuning in CCT mode using 7% H/He gas was carried out using the Autotune function and Thermo Tuning Solution A. To enhance the stability of the analyte signal, the sample uptake tubing of the peristaltic pump was regularly replaced.

RESULTS AND DISCUSSION

The wine samples were diluted as little as possible, in this case by a factor of two, to enhance the chances of determining elements such as Sc, V, Co, Ni, Cd, Ce, Nd, W, Tl and U, which have very low abundances in wine. This practice, however, increases the possibility of matrix interference. In this case, ethanol may affect the transport and nebulisation properties of the samples. Since the ethanol concentration in most of the wines selected for this study was between 13 and 15%, ethanol was added to all standards and blanks to a concentration of 7.5% so that it would resemble that in the analysed wine samples after dilution. The added ethanol, although of high purity, increased the levels of most elements in the blanks and therefore resulted in method detection limits (MDLs) up to 200% higher than the instrument detection limits (IDLs) in 1% nitric acid obtained for the Thermo Series 2 ICP-MS. MDLs were obtained by calculating the standard deviation (3s) per element from 10 readings of a method blank (7.5% ethanol and 1% nitric acid in ultrapure water), and then converting to concentration by using the sensitivity (from the equation for the calibration line) of each element. MDLs for the soil analyses were obtained in the same way using a digested blank instead of an ethanol blank.

Quality control procedures included the measurement of test wine, spiked wine, serial dilution, and interference check samples (ICS). The relative percentage difference (RPD = 100*(i-r)/i, where 'i' is the initial or known value and 'r' is the repeat value) between the repeat analyses of the test wine sample, measured after every 10 samples, was less than 10% for the selected elements. To assess possible matrix interference, serial dilution and spiked sample

TABLE 2

Average element concentrations (µg/L) in red and white wines from the Stellenbosch, Swartland, Robertson and Walker Bay regions.

Element	MDL	Stellenbosch			Swartland			Robertson			Walker Bay		
		White	Red	All	White	Red	All	White	Red	All	White	Red	All
[7]Li	0.23	3.2 ± 2.1	2.2 ± 1	2.9 ± 1.9	5.0 ± 2.4	5.5 ± 3.4	5.3 ± 3.1	43 ± 31	46 ± 51	45 ± 45	2.3 ± 2.1	2.6 ± 1.3	2.5 ± 1.6
[11]B	13	6 211 ± 1 695	9 831 ± 1 992	7 417 ± 2 472	3 958 ± 1 672	5 050 ± 1 916	4 653 ± 1 869	3 433 ± 941	6 015 ± 1 364	5 255 ± 1 723	1 767 ± 363	2 726 ± 681	2 327 ± 738
[27]Al	1.8	456 ± 515	338 ± 105	416 ± 422	215 ± 235	148 ± 39	172 ± 143	280 ± 113	124 ± 41	170 ± 99	232 ± 97	198 ± 180	212 ± 146
[45]Sc	0.41	4.4 ± 1.5	4.8 ± 1.9	4.6 ± 1.6	4.5 ± 2.7	6.5 ± 3.2	5.8 ± 3.1	13 ± 3.7	18 ± 5.4	17 ± 5.3	9.2 ± 2.2	11 ± 2.8	10 ± 2.6
[51]V	0.07	1.7 ± 1.4	0.25 ± 0.19	1.2 ± 1.3	17 ± 33	6.2 ± 3.4	10 ± 20	8.0 ± 8.9	3.5 ± 0.68	4.8 ± 5	40 ± 75	2.8 ± 0.74	18 ± 49
[52]Cr	0.08	11 ± 6.2	8.3 ± 1.4	9.8 ± 5.2	12 ± 9	12 ± 6.9	12 ± 7.5	9.5 ± 0.64	12 ± 2.6	11 ± 2.5	11 ± 11.8	5.4 ± 1.5	8.1 ± 7.9
[55]Mn	0.12	1 108 ± 809	1 449 ± 622	1 222 ± 751	699 ± 321	1 279 ± 477	1 068 ± 507	727 ± 74	1 090 ± 364	985 ± 349	800 ± 572	545 ± 99	651 ± 376
[59]Co	0.14	2.3 ± 1.2	0.94 ± 0.44	1.8 ± 1.2	2.3 ± 0.94	1.5 ± 0.46	1.8 ± 0.77	3.0 ± 0.97	2.6 ± 0.75	2.7 ± 0.81	1.8 ± 1.3	1.3 ± 0.19	1.5 ± 0.84
[60]Ni	0.47	8.3 ± 4	6.1 ± 1.9	7.6 ± 3.5	26 ± 35	15 ± 5	19 ± 21	16 ± 4.7	17 ± 3.8	16 ± 4	7.8 ± 4.5	9.0 ± 3.8	8.5 ± 3.9
[65]Cu	0.17	129 ± 144	53 ± 37	104 ± 124	142 ± 176	34 ± 18	73 ± 116	67 ± 56	115 ± 121	101 ± 107	63 ± 29	63 ± 52	63 ± 42
[66]Zn	0.14	576 ± 252	530 ± 172	560 ± 224	574 ± 202	408 ± 151	468 ± 185	462 ± 193	455 ± 192	457 ± 186	615 ± 265	418 ± 132	500 ± 213
[85]Rb	0.03	3927 ± 1 869	6 938 ± 4 196	4 931 ± 3 094	540 ± 223	728 ± 206	659 ± 227	359 ± 47	383 ± 82	376 ± 73	506 ± 244	514 ± 177	510 ± 197
[88]Sr	0.04	385 ± 119	685 ± 95	485 ± 182	402 ± 112	510 ± 157	471 ± 149	426 ± 94	710 ± 175	626 ± 203	368 ± 86	402 ± 92	388 ± 87
[90]Zr	0.02	2.0 ± 3	0.040 ± 0.04	1.3 ± 2.6	0.65 ± 1.4	0.037 ± 0.036	0.26 ± 0.88	1.2 ± 1.2	0.40 ± 0.71	0.63 ± 0.92	0.52 ± 1.1	0.023 ± 0.027	0.23 ± 0.69
[93]Nb	0.02	0.077 ± 0.095	0.0030 ± 0.002	0.052 ± 0.084	0.016 ± 0.03	0.0024 ± 0.00085	0.0072 ± 0.019	0.046 ± 0.063	0.015 ± 0.027	0.024 ± 0.041	0.032 ± 0.067	0.0011 ± 0.0011	0.014 ± 0.044
[98]Mo	3.2	< DL	< DL	< DL	4.8 ± 8.6	< DL	< DL	7.3 ± 7.6	< DL	3.6 ± 4.6	4.3 ± 3.5	< DL	< DL
[111]Cd	0.21	< DL	< DL	< DL	1.5 ± 3.6	< DL	0.62 ± 2.2	< DL	< DL	< DL	< DL	< DL	< DL
[118]Sn	2.2	< DL	< DL	< DL	< DL	< DL	< DL	5.84 ± 4.95	< DL	2.27 ± 3.45	2.37 ± 4.07	< DL	< DL
[121]Sb	0.01	0.95 ± 0.57	0.11 ± 0.074	0.67 ± 0.62	1.5 ± 1.2	0.26 ± 0.19	0.70 ± 0.94	0.66 ± 0.15	0.31 ± 0.25	0.41 ± 0.28	0.96 ± 0.3	0.57 ± 0.4	0.73 ± 0.41
[137]Ba	0.2	106 ± 50	84 ± 42	99 ± 47	156 ± 79	200 ± 71	184 ± 75	210 ± 60	301 ± 108	274 ± 104	80 ± 48	118 ± 39	102 ± 45
[140]Ce	0.29	0.66 ± 1.12	< DL	0.452 ± 0.951	0.36 ± 0.93	< DL	< DL	< DL	< DL	< DL	0.37 ± 0.63	< DL	< DL
[146]Nd	0.01	0.42 ± 0.71	0.046 ± 0.086	0.30 ± 0.6	0.27 ± 0.69	0.026 ± 0.027	0.12 ± 0.42	0.11 ± 0.22	0.017 ± 0.038	0.043 ± 0.12	0.33 ± 0.52	< DL	0.14 ± 0.36
[182]W	26	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
[205]Tl	0.02	0.85 ± 0.37	1.1 ± 1.3	0.93 ± 0.76	0.62 ± 0.21	0.68 ± 0.22	0.66 ± 0.22	0.15 ± 0.046	0.14 ± 0.052	0.14 ± 0.049	0.42 ± 0.2	0.33 ± 0.2	0.36 ± 0.2
[207]Pb	0.17	13 ± 5.5	13 ± 6.2	13 ± 5.5	24.90 ± 33.96	11.48 ± 11.76	16.36 ± 22.66	16.06 ± 8.41	10.55 ± 6.54	12.17 ± 7.33	4.55 ± 3.52	2.53 ± 1.43	3.37 ± 2.59
[238]U	0.03	0.08 ± 0.16	< DL	0.052 ± 0.13	0.19 ± 0.53	< DL	0.078 ± 0.32	< DL	< DL	< DL	0.094 ± 0.21	< DL	0.045 ± 0.13

tests were performed on four arbitrarily selected wine samples, two white and two red wines. The RPD for the serial dilutions was less than 10% except for Li, Al and Cu. The percentage recovery for the spiked elements was found to lie within the 80 to 120% interval that was set as an acceptable level of accuracy for the purpose of this study. An interference check sample (ICS), simulating typical concentrations of the major elements in the sample wine matrix, consisting of 50 mg Ca/L, 100 mg Na/L, 250 mg Mg/L, 500 mg K/L and an appropriate amount of ethanol, was

analysed to assess possible matrix and argon-related interferences. Elements prone to molecular interferences, namely ^{27}Al, ^{47}Ti, ^{48}Ti, ^{51}V, ^{55}Mn, ^{52}Cr, ^{60}Ni, ^{66}Zn, ^{65}Cu and ^{88}Sr, were measured in CCT mode to reduce these interferences. Because of the sensitivity loss, however, this mode was not used for measuring the very low levels of ^{45}Sc and ^{59}Co in the wine samples. These elements can be affected by carbon- and argon-based molecular interferences, such as $^{12}CO_2H$ and $^{13}CO_2$ in the case of ^{45}Sc, and Ca-OH and Ar-Na in the case of ^{59}Co. In these cases, the information obtained from
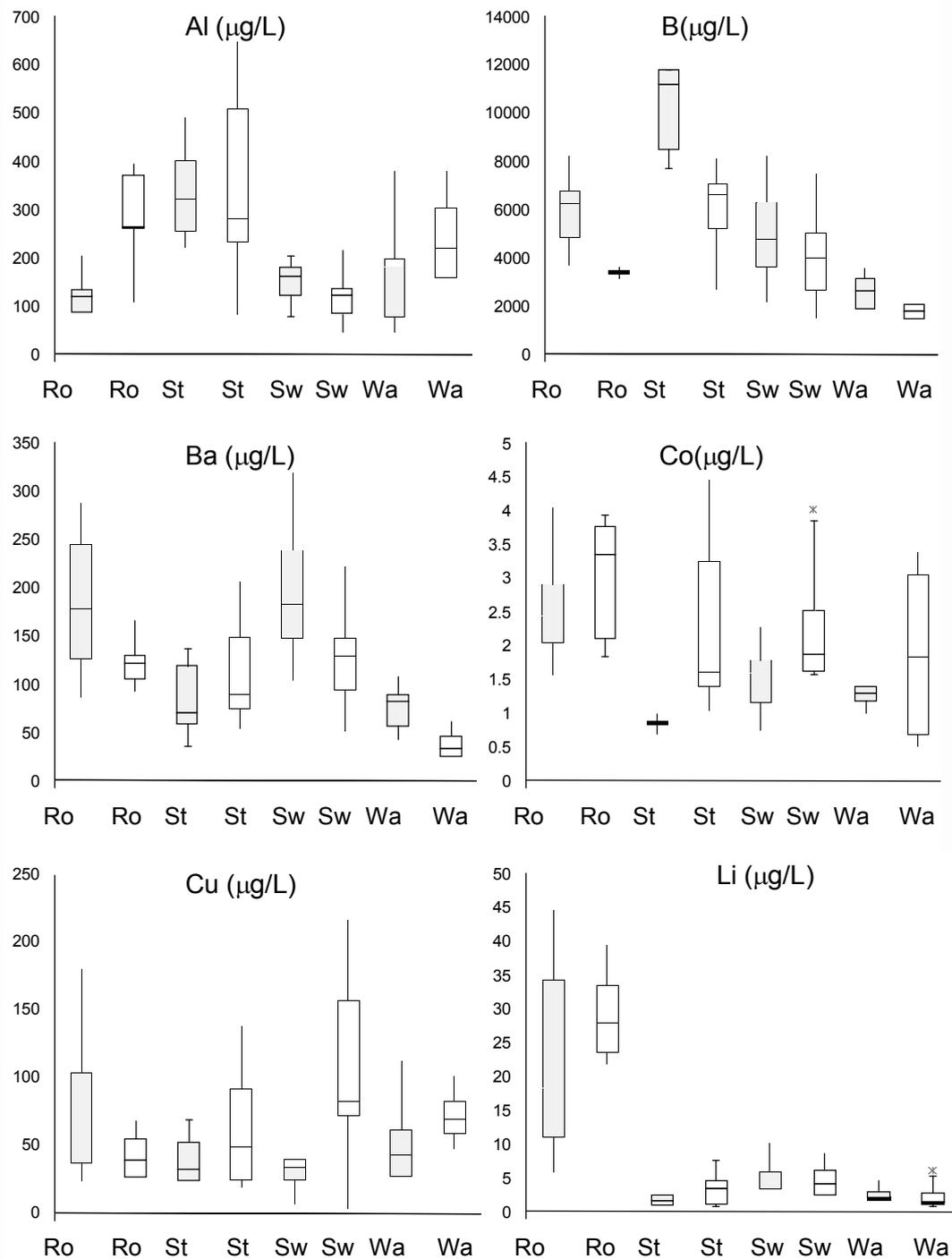

FIGURE 1(a)

Box plots of the concentrations of elements with indictor potential in white and red wines from four wine-producing regions: Robertson (Ro), Stellenbosch (St), Swartland (Sw) and Walker Bay (Wa). Boxes represent 50% of the data for each category and are shaded for red wines.

TABLE 3
Average element concentrations (µg/L) in provenance soils for red and white wines from Stellenbosch, Swartland, Robertson and Walker Bay regions.

Element	MDL	Stellenbosch			Swartland			Robertson			Walker Bay		
		White	Red	All	White	Red	All	White	Red	All	White	Red	All
[7]Li	0.93	365 ± 271	379 ± 175	370 ± 242	145 ± 68	94 ± 40	110 ± 55	381 ± 124	390 ± 164	394 ± 151	324 ± 281	244 ± 85	271 ± 166
[11]B	1.11	35 ± 19	42 ± 26	38 ± 21	127 ± 72	89 ± 81	101 ± 80	443 ± 195	292 ± 208	320 ± 212	109 ± 37	57 ± 27	74 ± 39
[45]Sc	6.23	76 ± 38	48 ± 29	67 ± 38	116 ± 41	90 ± 68	98 ± 61	71 ± 20	57 ± 24	60 ± 23	71 ± 21	52 ± 24	58 ± 24
[51]V	0.13	731 ± 298	527 ± 303	663 ± 312	1 016 ± 433	666 ± 396	776 ± 436	438 ± 101	356 ± 120	377 ± 117	1 810 ± 524	1 698 ± 705	1 735 ± 628
[52]Cr	0.03	424 ± 199	265 ± 149	371 ± 197	481 ± 175	362 ± 184	400 ± 188	711 ± 133	570 ± 245	604 ± 222	1 650 ± 1 095	1 140 ± 474	1 310 ± 730
[55]Mn	0.49	2 592 ± 2 259	976 ± 449	2 054 ± 2 007	2 741 ± 2 152	2 322 ± 1 992	2 454 ± 2 034	11 644 ± 10 916	10 397 ± 13 701	10 261 ± 12 625	1 148 ± 1 079	1 410 ± 1 602	1 322 ± 1 402
[59]Co	0.1	55 ± 54	17 ± 7.7	43 ± 48	3 489 ± 1 325	2 560 ± 1 519	2 853 ± 1 512	3 107 ± 584	3 271 ± 1 159	3 228 ± 1 014	246 ± 319	226 ± 207	233 ± 234
[60]Ni	0.46	147 ± 78	100 ± 49	131 ± 73	66 ± 54	42 ± 49	50 ± 51	326 ± 77	302 ± 118	308 ± 106	61 ± 97	65 ± 72	63 ± 76
[65]Cu	0.5	472 ± 206	404 ± 334	449 ± 255	358 ± 166	243 ± 151	279 ± 163	337 ± 94	276 ± 139	286 ± 129	174 ± 98	115 ± 93	135 ± 95
[66]Zn	0.33	314 ± 182	250 ± 181	292 ± 183	336 ± 282	216 ± 147	254 ± 205	580 ± 200	556 ± 203	559 ± 195	290 ± 338	254 ± 219	266 ± 249
[82]Se	1.16	23 ± 21	36 ± 15	27 ± 20	6.8 ± 4.1	6.2 ± 4.3	6.4 ± 4.2	< DL	< DL	< DL	< DL	< DL	< DL
[85]Rb	0.17	795 ± 506	555 ± 260	715 ± 451	394 ± 246	272 ± 101	310 ± 169	502 ± 130	416 ± 236	435 ± 208	332 ± 67	336 ± 159	334 ± 131
[88]Sr	0.47	294 ± 183	408 ± 383	332 ± 268	500 ± 818	249 ± 173	328 ± 487	1 152 ± 1 190	782 ± 862	849 ± 937	278 ± 249	333 ± 321	315 ± 289
[93]Nb	0.02	1.3 ± 0.85	1.2 ± 0.63	1.2 ± 0.78	0.49 ± 0.44	0.77 ± 0.65	0.68 ± 0.6	0.94 ± 0.67	0.97 ± 0.34	0.95 ± 0.43	0.33 ± 0.21	0.75 ± 0.32	0.61 ± 0.35
[98]Mo	44.22	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
[111]Cd	0.36	0.95 ± 1.4	3.0 ± 7.7	1.6 ± 4.6	0.69 ± 0.28	0.52 ± 0.28	0.57 ± 0.29	0.71 ± 0.55	0.61 ± 0.4	0.61 ± 0.43	0.95 ± 0.63	0.50 ± 0.54	0.65 ± 0.58
[118]Sn	37.88	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
[138]Ba	0.49	966 ± 601	592 ± 466	841 ± 583	5 110 ± 14 076	1 205 ± 1 270	2 438 ± 8 037	9 593 ± 10 975	3 266 ± 4 266	4 696 ± 7 006	621 ± 423	762 ± 544	715 ± 492
[140]Ce	0.29	951 ± 558	770 ± 326	891 ± 497	616 ± 329	550 ± 451	571 ± 415	808 ± 176	742 ± 281	758 ± 250	565 ± 151	516 ± 332	532 ± 278
[146]Nd	0.01	491 ± 290	392 ± 181	458 ± 262	362 ± 233	300 ± 224	319 ± 227	535 ± 128	468 ± 124	484 ± 124	325 ± 87	305 ± 214	312 ± 177
[182]W	134.21	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL
[205]Tl	0.24	6.5 ± 6.8	3.3 ± 1	5.5 ± 5.8	2.9 ± 1.1	2.2 ± 0.9	2.4 ± 1	3.2 ± 0.74	2.5 ± 1.5	2.7 ± 1.3	3.2 ± 1.7	3.0 ± 2.5	3.1 ± 2.2
[207]Pb	0.11	363 ± 210	399 ± 248	375 ± 222	292 ± 190	197 ± 113	227 ± 147	244 ± 48	254 ± 134	251 ± 114	408 ± 237	321 ± 192	350 ± 201
[238]U	0.07	33 ± 24	29 ± 8	32 ± 20	27 ± 12	17 ± 11	20 ± 12	7.6 ± 1.9	11 ± 14	10 ± 12	24 ± 8.6	21 ± 11	22 ± 10

the interference check sample (ICS) was used to assess overall interference. In the case of [59]Co, the overall interference was < 0.06 µg/L. This value included the possible Ca-OH and Ar-Na molecular ion interferences at mass 59. Wine samples were also microwave digested to remove the carbon matrix. The analytical results were compared with those obtained from the diluted wine samples. The element concentrations were essentially the same, suggesting relatively small matrix interference effects. This is in agreement with the results obtained in previous studies (Coetzee *et al*., 2005).

A summary of the average elemental composition (complete data set in Van der Linde, 2008) of the wine and soil samples is given in Tables 2 and 3 for the following 26 elements in order of mass number: [7]Li, [11]B, [27]Al [45]Sc, [51]V, [52]Cr, [55]Mn, [59]Co, [60]Ni, [65]Cu, [66]Zn, [85]Rb, [88]Sr, [90]Zr, [93]Nb, [98]Mo, [111]Cd, [118]Sn, [121]Sb, [137]Ba, [140]Ce, [145]Nd, [182]W, [205]Tl, [207]Pb and [238]U. Note that for all the wines, the average concentrations and the concentrations in individual wines were far below the maximum acceptable limits (MAL) for the five elements B (14 mg/L), Cd (0.01 mg/L), Cu (1 mg/L), Pb
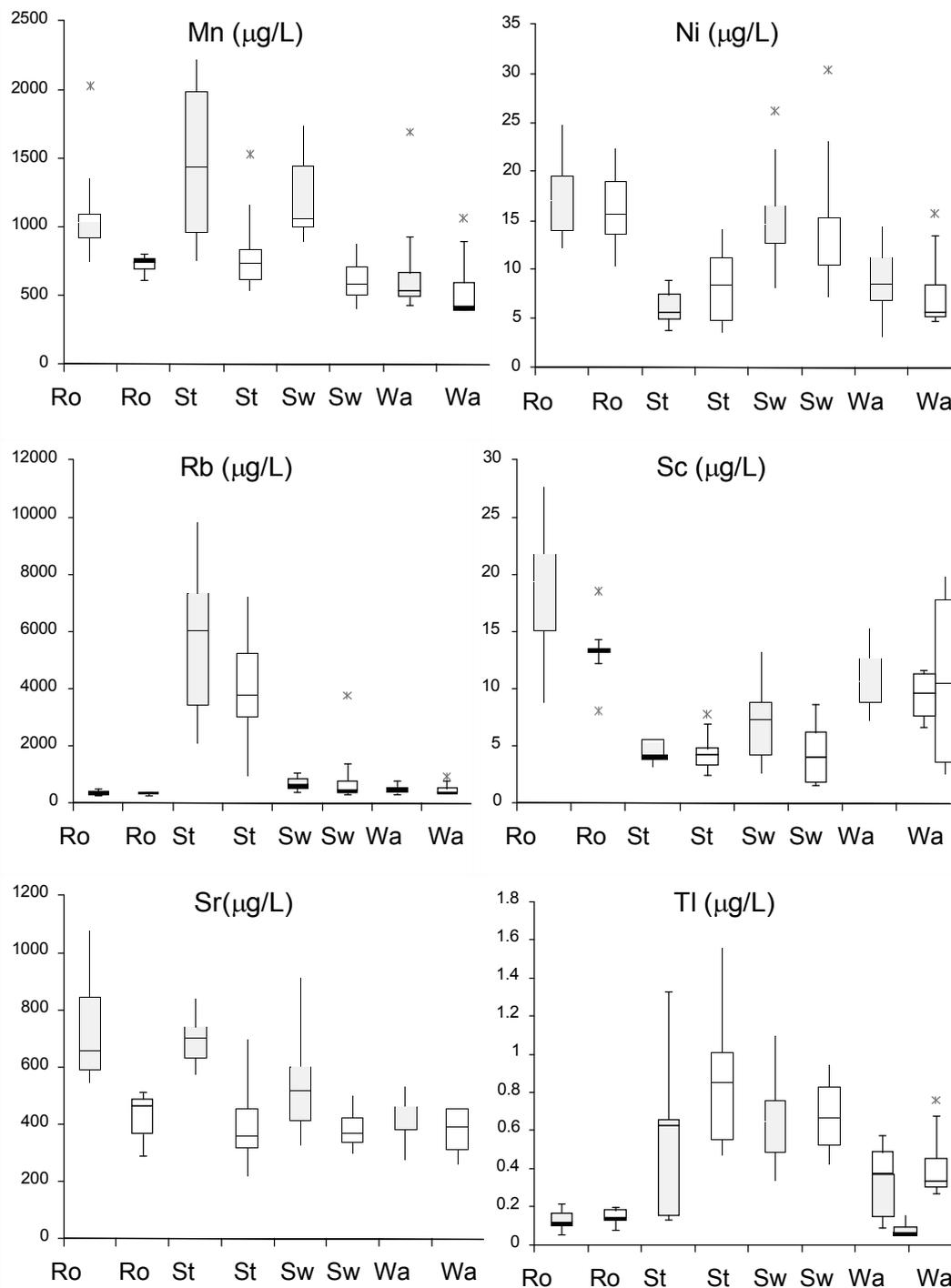

FIGURE 1(b)

Box plots of the concentrations of elements with indictor potential in white and red wines from four wine-producing regions: Robertson (Ro), Stellenbosch (St), Swartland (Sw) and Walker Bay (Wa). Boxes represent 50% of the data for each category and are shaded for red wines.
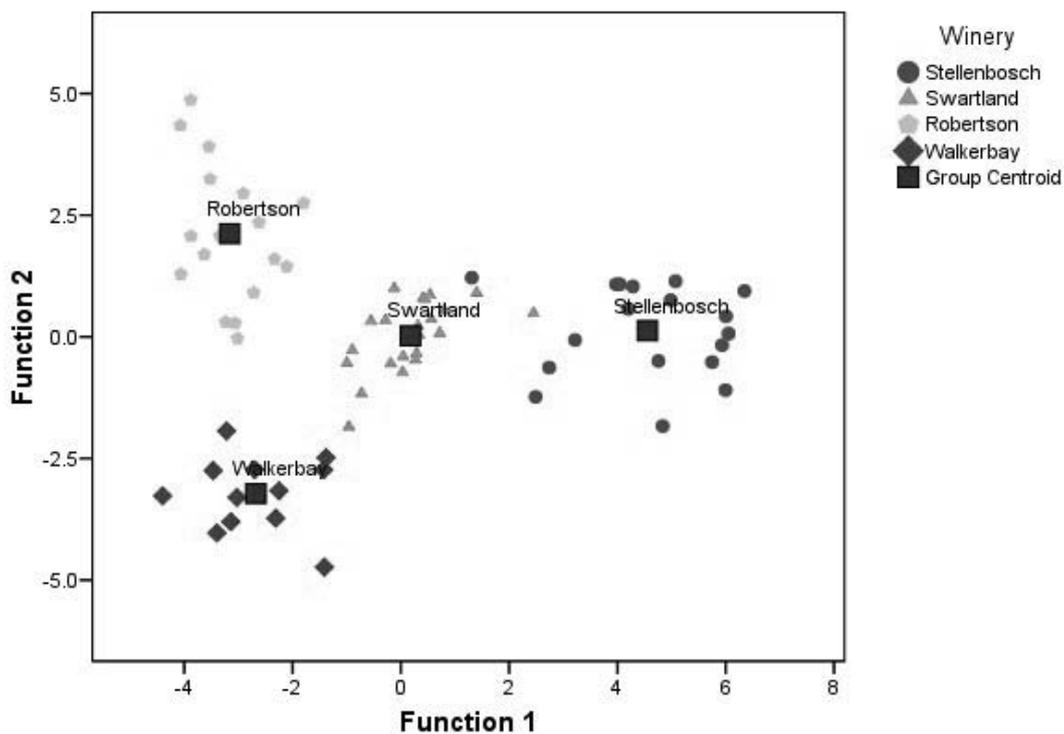
Canonical Discriminant Functions

FIGURE 2

Canonical discriminant functions plot of wines from the four regions based on the elements Li, B, Sc, Ni, Mn, Co, Cu and Rb.
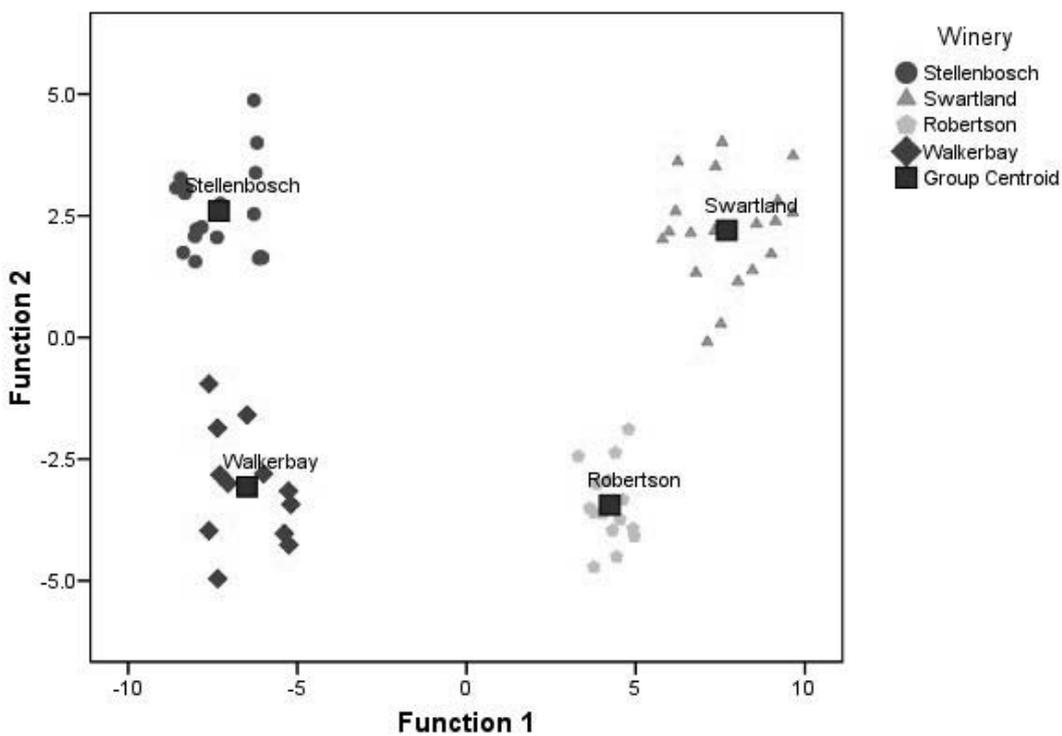
Canonical Discriminant Functions

FIGURE 3

Canonical discriminant functions plot of soils from the four regions based on the elements Li, B, Sc, Ni, Mn, Co, Cu and Rb.

(0.15 mg/L) and Zn (5 mg/L), as published by the International Organisation of Vine and Wine (OIV, 2008).

From an inspection of Table 2 it appears that higher levels of B generally occur in red wines than in white wines. No consistent pattern is observed for the other elements and it does not seem that red wines on average have higher trace element concentrations than white wines. Box plots provide a useful way of identifying trends in the data. Box plots of the range of concentrations (μg/L) of 12 elements that show potential as indicator elements in the classification of wines from the different regions through multivariate statistical analysis, namely Li, B, Al, Sc, Mn, Co, Ni, Cu, Rb, Sr, Ba and Tl, are shown in Fig. 1. The box length represents the interquartile range (IQR) and includes 50% of the data. The median is shown by the horizontal line in the middle of box. The upper edge of the box plot is the 3rd quartile (Q$_3$) and the lower edge is the 1st quartile (Q$_1$). The position of the median line relative to the quartile lines shows the skewness of the data. The whiskers represent the range of the data and are calculated from Q$_3$+1.5*(IQR) for the higher limit and Q$_1$-1.5*(IQR) for the lower limit. The asterisks in the plots indicate the maximum and minimum data values if they fall outside the upper or lower limits defined by the whiskers.

An inspection of Fig. 1a and b reveals that there is no general trend in die distribution of element concentrations in white and red wines. The notion that element concentrations should be higher in red than in white wine because skin contact is longer in the vinification process in the case of red wine, is not supported by the data. In the case of B, however, its concentration in the red wines from Stellenbosch and Robertson is indeed larger than in the white wines. The same trend, i.e. concentrations higher in red than white wines, occurs for Mn and Sr in Stellenbosch and Sr in Robertson. It is, however, not enough to support a generalisation on this count, although differences do exist. Data for red and white wines are therefore included in the data set for statistical analysis. If differences between trace element concentrations in red and white wine from within a region are smaller than typical differences between regions for a specific element, then a multivariate statistical analysis, such as discriminant analysis, should in principle be possible to explain the variability among the regions. In previous work (Coetzee *et al*., 2005) it was established that this was indeed the case.

It appears form the box plots that the concentration of Al is typically high in Stellenbosch wines, Co is high in Robertson and low in Stellenbosch, Li is high in Robertson, Rb is high in Stellenbosch, Sc is high in Robertson and low in Stellenbosch, and Tl is low in Robertson. These differences in element concentrations provide the basis for the fingerprinting procedure. The statistical significance of these differences and how they can be appropriated were investigated by means of principle component analysis (PCA) and linear discriminant analysis (DA).

Certain elements, namely [93]Nb, [98]Mo, [111]Cd, [118]Sn, [140]Ce, [145]Nd, [182]W, [207]Pb and [238]U, were excluded from the data selected for statistical analysis because the analytical uncertainty was too large in cases where the concentration levels were < QDL, where QDL is the quantitative detection limit equal to 10 x the MDL (see Table 2). The remaining elements were included in a factor analysis exercise to identify those elements that show the best potential for explaining the variability of the soils and wines among the different regions. The concentration data for the elements Li, B, Sc, Co, Mn, Ni, Cu and Rb, emerging from the PCA (principal component analysis) were then subjected to linear discriminant analysis (DA) using the SPSS statistical package. Discriminant functions, i.e. linear combinations of log$_e$-transformed concentrations of the selected elements, were derived that best differentiated among the four regions, namely Robertson, Stellenbosch, Swartland and Walker Bay. Log$_e$-transformed concentrations, bringing high and low abundances within the same range, allowed element concentrations used in the statistical procedures to vary by orders of magnitude.

The following discriminant functions based on linear combinations of the log$_e$ concentrations of the elements Li, B, Sc, Ni, Mn, Co, Cu and Rb were obtained to classify wines from the four regions included in this study.

f$_1$ = 0.362 ln(B) – 0.770 ln(Sc) + 0.231 ln(Ni) – 0.167 ln(Li) + 0.162 ln(Mn) + 0.903 Ln(Rb) + 0.195 ln(Co) – 0.021 ln(Cu)

f$_2$ = 0.689 ln(B) – 0.446 ln(Sc) + 0.010 ln(Ni) + 0.937 ln(Li) + 0.357 ln(Mn) – 0.053 Ln(Rb) + 0.133 ln(Co) + 0.272 ln(Cu)

These functions enabled 96% of the wines to be classified correctly. Only one wine, Simonsig Chenin Blanc from the Stellenbosch region, was incorrectly classified in the Swartland group. A graphical presentation of these functions, applied to the wine data set, is shown in Fig 2. It is clear from the plot that the wines are classified in four well-separated clusters and that the red and white wines are apparently randomly mixed within a cluster, making it unnecessary to run separate analyses for red and white wines. This result is in agreement with previous work (Coetzee *et al*., 2005) in which wines from only three regions were classified. As the number of groups (dependant variables) that need to be identified in the data set increases, the demands on the quality of the analytical data (variables) and the correct selection of indicator elements become greater. It is interesting to note that the group of eight indicator elements used in the discriminant functions include only two elements, namely Mn and Rb, that were also part of the group of nine indicator elements (Al, Mn, Rb, Ba, Sr, Se, Cs, W, Tl) applied previously in the three-region classification of South African wines (Coetzee *et al*., 2005).

The inclusion of B concentrations in the discriminant functions raises a question regarding the stated condition that a link must exist between the trace element composition of a wine and that of its provenance soil. In the case of B, the element is a very important micronutrient and is commonly applied as a borax foliar spray (Christensen *et al*., 2006) or soil spray in vineyards where the soils are boron deficient, such as in the Walker Bay region. This agricultural practice may therefore change the natural boron content in the provenance soil and in the vine and therefore eventually in the wine. The nature of this change may or may not have an effect on the usefulness of B as an indicator element for the classification of wines in this specific data set. B is, however, also included in the discriminant functions used in the successful classification of the corresponding soils and therefore supports the notion that agricultural practices may have changed the B content of the soil and vine in such a way that the element remains useful as an indicator element.

In the previous study (Coetzee *et al*., 2005), the assumption was made that the chemical composition of a wine would reflect the

chemical composition of the provenance soil for certain elements. This was, however, never proven for South African wines. However, the correlation between the chemical composition of a provenance soil and the trace element composition of wine was investigated elsewhere by Almeida and Vasconcelos (2003). Significant correlations were obtained between the multi-element composition of the wine and the provenance soil (R = 0.994, n = 19, P < 0.01) for the set of elements determined in common in the different types of samples.

In the current study, an indirect approach through discriminant analysis was used to verify the correlation between trace element composition of the wine and the provenance soil. The same set of elements that was used in the classification of the wines, namely Li, B, Sc, Ni, Mn, Co, Cu and Rb, was applied to the soil samples in a discriminant analysis procedure. A successful classification of the soil samples would confirm the required link between wine and provenance soil. The following discriminant functions were obtained for the soil sample database, with their graphical presentation in Fig. 3.

f_1 = 0.526 ln(B) − 0.199 ln(Sc) − 1.609 ln(Ni) − 0.098 ln(Li) + 0.015 ln(Mn) − 0.586 ln(Rb) + 2.070 ln (Co) − 0.007 ln(Cu)

f_2 = -0.967 ln(B) + 0.534 ln(Sc) − 1.753 ln(Ni) + 0.230 Ln(Li) + 0.248 ln(Mn) + 0.424 ln(Rb) − 0.015 ln(Co) + 1.488 ln(Cu)

These functions enabled 100% of the soils from the four regions to be classified correctly. The soil associated with the incorrectly classified Simonsig Chenin Blanc wine was correctly placed in the Stellenbosch area. The reason for the incorrect classification of the wine is not clear, but could be due to a sampling error.

The successful classification of the wines and soils, applying the same set of elements, supports the conclusion that, for certain elements, the trace element composition of the tested wines enables fingerprinting of the wines and their classification according to geographical origin. It is interesting to note that the four wines and soils from the Constantia ward classify correctly under the Stellenbosch region, thereby supporting the link between soil chemistry and wine composition in terms of trace elements.

CONCLUSION

The elements Li, B, Sc, Co, Mn, Ni, Cu and Rb were identified as indicators with the ability to discriminate between wines and soils from four major wine-producing regions using a linear discriminant analysis procedure based on the $\log_e$ concentrations of the selected elements. The element set differs from the set of indicator elements applied when only three regions were considered. This implies that the discriminant functions and the selected indicator elements will only be valid for a particular data set and will change according to the composition of the data set. If data for another region were to be added, for example, a different set of indicator elements and discriminant functions are likely to arise in the statistical analysis.

The most important conclusion drawn from this work is ,that for the wines and soils from four major wine-producing regions in the Western Cape, a correlation exists between the elemental composition of a wine and that of its provenance soil. This is an important prerequisite for the application of the fingerprinting methodology and a first result of this kind for South African wines. It implies that the fingerprinting methodology based on multi-element data and multivariate statistical analysis provides a promising modus operandi for the classification of South African wines according to point of origin. If properly developed, a chemically-based and independent system of classification would support the "wine of origin" classification system used in all wine-producing countries and help prevent fraudulent practices in the wine industry. The intraregional classification of wine using this methodology will be addressed in future work in order to assess its limitations and reliability.

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