



Relationship among geochemical elements in soil and grapes as terroir fingerprintings in *Vitis vinifera* L. cv. “Glera”



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ABSTRACT

Prosecco, one of the most popular sparkling wines in the world, is produced in Italy. For this reason, it is important to develop a scientific method for determining geographic origin in order to prevent fraudulent labelling. To establish the relationship between geochemistry of vineyard soil and chemical composition of grape, a geochemical characterization of “Glera”, a *Vitis vinifera* cultivar from Italian Region, Veneto was undertaken. We evaluated the relationship between major and trace elements in soil and their concentrations in “Glera” grape berries in vineyards belonging to five localities in the Veneto alluvial plain, all included in the Controlled Designation of Origin (DOC) area of Prosecco. A statistically significant correspondence between the soil and grape was observed for Sr. Multivariate analysis (LDA) allowed discrimination of samples of soil and grape berries from each single winery according to the geographic origin. The elements that could establish a reliable correspondence between the geolithological features of the vineyard soil and the chemical composition of grape berries are: Sr, Ba, Ca, Mg, Al, K, Zn, B, Ni, Co.

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1. Introduction

The increasing international demand for sparkling wine has recently required more intense controls to avoid falsification and fraudulent use of denomination labels (Lenglet, 2014). The European label “Protected Designation of Origin” (PDO) identifies a product strictly associated to a region or location whose characteristics are bound to a specific geographical environment (Cadot et al., 2012) within the established concept of “terroir”. The International Organization of Vine and Wine (OIV) defined the “terroir” as “a concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivicultural practices develops, providing distinctive characteristics for the products originating from this area” (Tomasi et al., 2010). The term describes a particular vine linked to a wine region characterized by a specific climate area, a geological setting, a specific wine district and typical organolep-

tic characteristics of the wine. From a geological point of view, the terroir has been defined as the geochemistry of soil, surface and ground water, or as the characteristics of agricultural food products (quality, brand, taste) which interact with climate, soil, vine variety and geology (Wilson, 1998; Haynes, 1999; Van Leeuwen and Seguin, 2006; Costantini and Bucelli, 2008).

It is known that the Pinot noir, Chardonnay, and Pinot Meunier grapes employed in the production of renowned wines as “Grand Cru” champagne, “Premier Cru” red, and “Commune” belong to specific geological conditions, respectively the formations “Permand marl” (a ferruginous oolite layer of Middle-Upper Jurassic), “Dalle nacrée” (pearly flagstone, Middle Jurassic) and “Digonella” (marly limestone, Middle Jurassic) (Wilson, 1998). The best white wine of the Chablis region, France, is obtained by Chardonnay vineyards planted on Kimmeridgian limestone (Malm-Upper Jurassic) (Huggett, 2006).

The studies on terroir are based on the concept that the chemical elements of the soil may be transferred to the plant and afterwards to the finished product (Greenough et al., 2005; Petrini et al., 2014; Protano and Rossi, 2014). Previous research showed that the distribution of rare earth elements (REE) in rocks was maintained in vineyard soil and in vine tissues (Censi et al., 2014; Pepi et al., 2016a)

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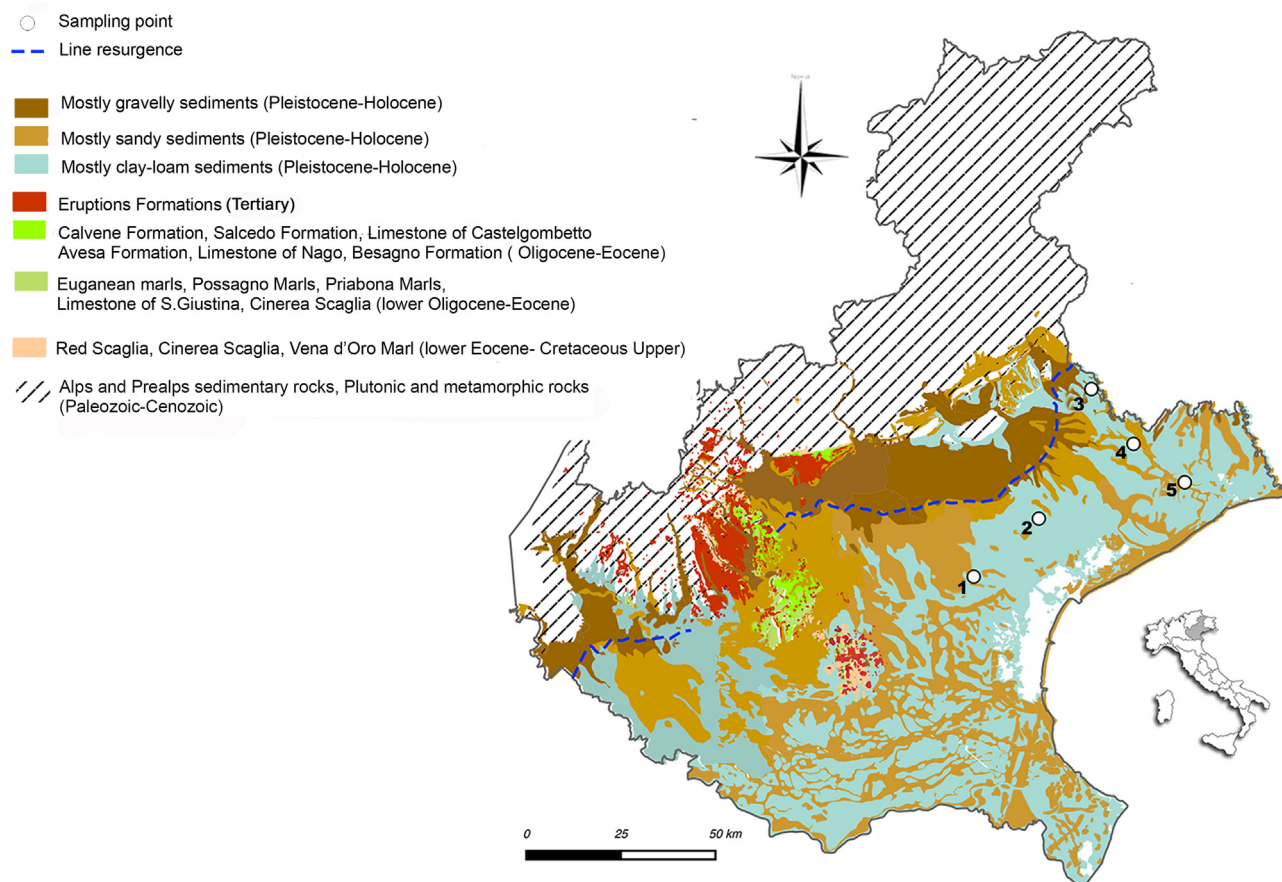


Fig. 1. Geological map of the Veneto Region (Italy) showing the location of the five “Glera” wineries studied: “Pattarello” (1), “Bottazzo” (2), “Gaiarine” (3), “Aleandri” (4), “Nardin” (5).

Table 1
Median concentrations of major (%) and trace elements (ppm) in soil samples of the five “Glera” vineyards, analyzed by XRF. A non-parametric multiple test (Test di Kruskal-Wallis) was applied. n = number of samples; P-values: ns = not significant; * < 0.05; ** < 0.01; *** < 0.001. Experimental values correspond to the median \pm standard deviation (SD).

wt%	Aleandri n = 6	Bottazzo n = 6	Gaiarine n = 6	Nardin n = 6	Pattarello n = 6	p-value
SiO ₂	46.2 \pm 1.3	47.1 \pm 2.8	65.0 \pm 0.7	60.9 \pm 0.3	59.9 \pm 0.5	***
TiO ₂	0.58 \pm 0.02	0.61 \pm 0.1	0.90 \pm 0.01	0.84 \pm 0.01	0.77 \pm 0.01	***
Al ₂ O ₃	11.3 \pm 0.6	12.5 \pm 1.0	16.3 \pm 0.5	15.0 \pm 0.2	19.5 \pm 0.7	***
Fe ₂ O ₃	4.12 \pm 0.21	4.44 \pm 0.5	8.14 \pm 0.7	7.06 \pm 0.1	7.04 \pm 0.4	***
MnO	0.08 \pm 0.01	0.11 \pm 0.01	0.11 \pm 0.01	0.14 \pm 0.01	0.12 \pm 0.01	***
MgO	8.81 \pm 0.36	8.69 \pm 0.7	2.32 \pm 0.12	3.94 \pm 0.1	3.09 \pm 0.2	***
CaO	25.6 \pm 2.1	23.4 \pm 3.8	4.26 \pm 1.7	8.82 \pm 0.3	4.14 \pm 0.4	***
Na ₂ O	0.33 \pm 0.05	0.68 \pm 0.03	0.56 \pm 0.01	0.50 \pm 0.01	0.98 \pm 0.02	***
K ₂ O	2.53 \pm 0.07	2.28 \pm 0.2	2.29 \pm 0.16	2.45 \pm 0.05	4.16 \pm 0.1	***
P ₂ O ₅	0.08 \pm 0.01	0.12 \pm 0.02	0.20 \pm 0.05	0.12 \pm 0.03	0.25 \pm 0.05	***
ppm						
Ba	179 \pm 10	199 \pm 22	354 \pm 9.1	311 \pm 8.7	609 \pm 24.3	***
Co	16.3 \pm 1.0	15.8 \pm 0.6	21.7 \pm 1.9	24.5 \pm 1.3	18.2 \pm 1.3	***
Cr	61.1 \pm 6.1	47.2 \pm 7.9	132 \pm 5.9	113 \pm 3.5	81.7 \pm 6.0	***
Ga	11.6 \pm 1.3	12.0 \pm 2.5	15.5 \pm 0.5	15.7 \pm 1.3	21.3 \pm 0.5	***
La	58.4 \pm 1.3	56.3 \pm 5.8	79.5 \pm 2.3	78.1 \pm 2.7	74.3 \pm 5.1	***
Nb	8.35 \pm 0.9	8.80 \pm 1.3	16.6 \pm 1.6	11.6 \pm 4.0	17.1 \pm 1.0	***
Nd	12.8 \pm 3.4	12.5 \pm 2.5	34.3 \pm 5.4	24.1 \pm 5.8	38.3 \pm 3.4	***
Ni	43.8 \pm 3.0	26.2 \pm 4.1	63.9 \pm 2.8	60.5 \pm 1.1	33.4 \pm 9.7	***
Pb	21.2 \pm 8.6	23.8 \pm 13	33.8 \pm 6.2	28.7 \pm 4.3	48.3 \pm 5.9	***
Rb	54.6 \pm 4.9	46.5 \pm 11	108 \pm 9.5	101 \pm 12	165 \pm 9.2	***
Sc	15.7 \pm 2.2	16.9 \pm 2.8	17.5 \pm 2.0	17.5 \pm 2.3	15.3 \pm 0.9	n.s
Sr	166 \pm 11	160 \pm 23	100 \pm 4.9	106 \pm 16	101 \pm 7.9	***
V	87.6 \pm 5.1	82.2 \pm 7.9	108 \pm 1.4	110 \pm 2.5	92.8 \pm 4.4	***
Y	10.9 \pm 2.0	14.2 \pm 2.8	26.9 \pm 2.1	17.8 \pm 4.67	26.9 \pm 3.0	***
Zr	85.8 \pm 12	98.2 \pm 11	252 \pm 21	134 \pm 33.8	217 \pm 7.4	***
Cu	37.7 \pm 15	46.2 \pm 19	142 \pm 26	49.7 \pm 20.3	91.2 \pm 8.3	***
Th	2.50 \pm 1.3	4.55 \pm 1.6	7.70 \pm 4.3	7.25 \pm 4.4	12.1 \pm 5.3	**
Zn	43.2 \pm 4.3	42.8 \pm 6.8	108 \pm 11.7	77.7 \pm 2.7	112 \pm 7.2	***

and that the concentrations of REE changed according to species and soil type (Wytenbach et al., 1998; Oddone et al., 2009). Major and trace element distribution in grape berry samples of cultivars Moscato d'Asti and Sauvignon Blanc (Aceto et al., 2013; Censi et al., 2014), and Lambrusco (Durante et al., 2016) has been shown to be related to geographic origin.

Studies aimed to trace the soil-plant links through the concentration of trace elements have been focused on grapes (Angelova et al., 1999; Jakubowski et al., 1999; Protano and Rossi, 2014; Pepi et al., 2016b) and on wine (Almeida and Vasconcelos, 2003; Jos et al., 2004; Coetzee et al., 2005; Martin et al., 2012). Other studies concerned the distribution of trace and ultra-trace elements in grape skin, seeds and flesh (Cabanne and Donèche, 2003; Rogiers et al., 2006; Bertoldi et al., 2009, 2011; Young et al., 2010; Amorós et al., 2013; Pepi et al., 2016a).

The use of isotope ratios has also been applied alone or in association with trace element concentrations as geochemical marker of grapes and wines from Spanish and Italian regions (González et al., 2011; Marengo and Aceto, 2003; Aceto et al., 2013; Petrini et al., 2014; Baffi and Trincherini, 2016; Durante et al., 2016; Pepi et al., 2016b). The analyses of mineral profiles in grapes are usually conducted by atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) or ICP-mass spectrometry (ICP-MS) (González and Pena-Méndez, 2000; De La Guardia and Gonzalez, 2013). A recent review emphasized that the combination of instrumental analyses with multivariate statistics may successfully classify grapes and wines according to geographical origin and winemaking processes (Versari et al., 2014).

The aim of this investigation is to establish the territorial fingerprints of “Glera”, a *Vitis vinifera* cultivar from Italian Region, Veneto, through the relationships among local geo-lithological features, geochemistry of vineyard soils and chemical composition of grapes within the Region Veneto. This cultivar is the major one employed in the production of the renowned Controlled Designation of Origin (DOC) wine “Prosecco”: a detailed geochemical characterization of Glera berries could be relevant not only for territorial identification but also for prevention of fraudulent labelling.

2. Materials and methods

Sampling areas

The studied vineyards belonged to five distinct wineries located in the Region Veneto, within the Veneto-Friuli alluvial plain (Fig. 1). From a geological point of view, the substrate is characterized by recent fine sediments, aged from Pleistocene to Holocene. The sampling areas belonged to the basins of rivers Brenta (winery Pattarello), Livenza-Tagliamento (wineries Aleandri and Nardin), Piave (wineries Bottazzo and Gaiarine). The first three wineries were located on clay-loam alluvial sediments, while the other two on sandy alluvial sediments. The sampling areas are characterized by a continental climate with annual temperature range between 11.5 °C and 13.5 °C and temperature average slightly above 20 °C. The rainfalls are more or less equally distributed along the year, with averages about 800 mm/y and 1100 mm/y respectively in the low and high alluvial plain, and about 2000 mm/y on Prealps (Barbi et al., 2012).

Grapevines of *Vitis vinifera* L., cultivar “Glera”, used for the Controlled Designation of Origin (DOC) wine “Prosecco”, were grafted on three rootstocks, 420A and Kober 5bb (*Vitis berlandieri* & *Vitis riparia*), and Richter 110 (*Vitis berlandieri* & *Vitis rupestris*). The vines were trained with vertically oriented canopies, according to “Sylvoz” and “Double Guyot” pruning methods. Rows were oriented N-S and vine spacing was 2.3 m × 1.1 m in each study site.

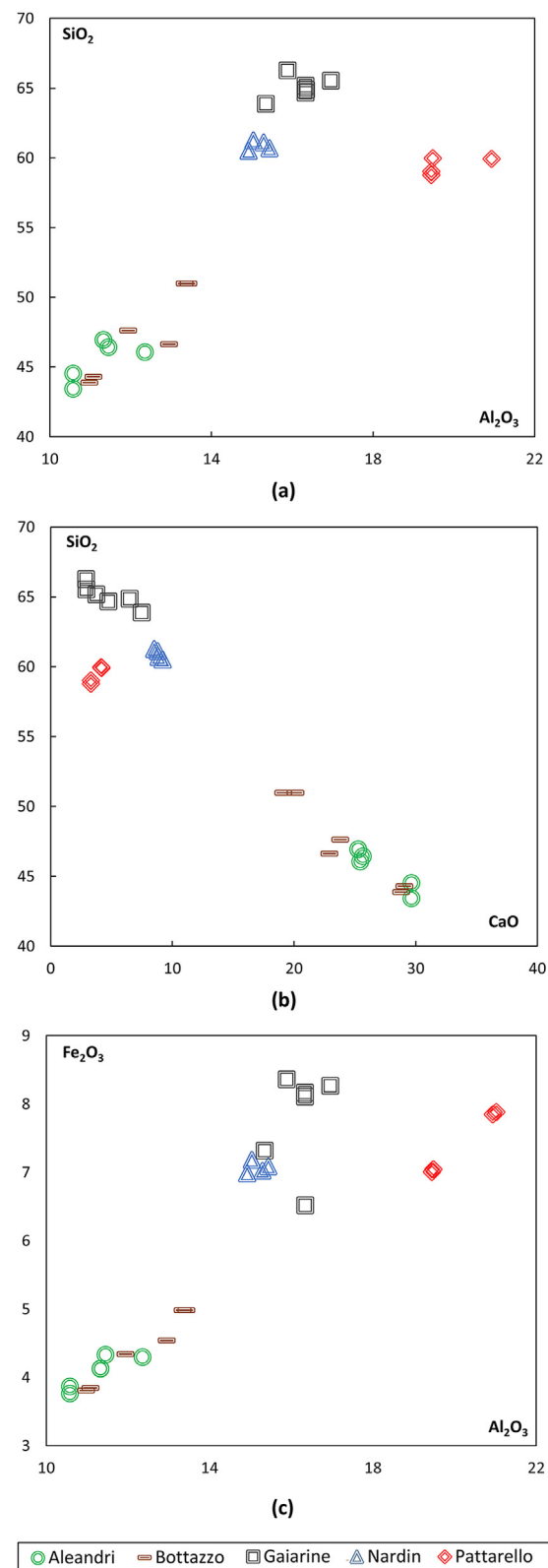


Fig. 2. Bivariate plots of abundances of SiO₂ vs Al₂O₃ (a) and CaO (b), and of Fe₂O₃ vs Al₂O₃ (c) in soil samples from the five “Glera” vineyards indicated in Fig. 1. Values of oxides are expressed in %.

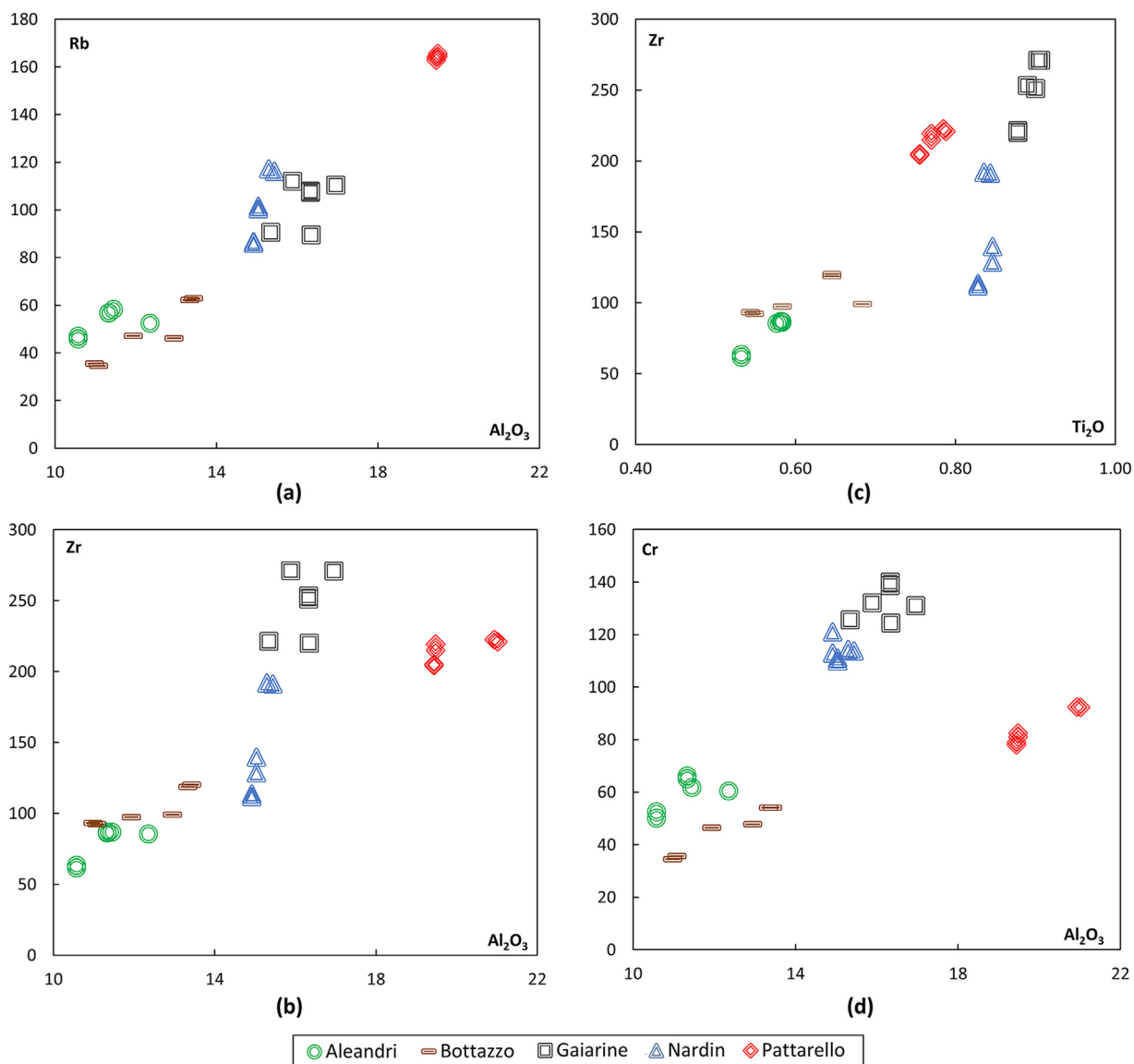


Fig. 3. Bivariate plots of abundances of Rb vs Al₂O₃ (a), Zr vs Al₂O₃ (b), Zr vs TiO₂ (c) and Cr vs Al₂O₃ in soil samples from the five “Glera” vineyards indicated in Fig. 1. Values of trace elements are expressed in ppm and values of oxides in %.

Soil sampling was carried out in the five experimental fields by means of an Edelman auger (Eijkelkamp Soil & Water, Giesbeek, The Netherlands). For each of the five sampling areas, six soil samples were collected at regular intervals at the depth of 60 cm and at 50 cm of distance from the vine: each sample was collected in triplicate. At harvest time, for each of the five sampling sites nine vineyard samples, each containing 10 grape clusters, were freshly picked and put in polyethylene bags at 4 °C. All clusters were completely destemmed in laboratory and about 300 berries for each sampling site for each area were immediately frozen at –20 °C for analysis.

2.2. Sample treatments

2.2.1. Soil

The soil samples were dried at 105 °C for 24 h to eliminate the hygroscopic water and then ground in an agate mortar. Afterwards, about 4 g of grounded soil were layered in a cylinder and covered by 15 g of powdered boric acid: the layers were then pressed by an hydraulic press to obtain the pellets suitable for X-Ray fluorescence

(XRF). The pellets were analysed by XRF in a wavelength dispersive spectrometer ARL ADVANT^{XP} (Thermo Fisher Scientific, Waltham, Massachusetts). Simultaneously, about 0.6 g of powder from each sample was heated for about 12 h at 1000 °C, cooled and weighed again to determine the weight loss on ignition (LOI).

2.2.2. Grape berries

The samples (about 300 berries each) were washed with milliQ[®] water (resistivity 18.2 MΩ cm⁻¹), paying attention not to damage them in order to avoid juice loss. The samples were centrifuged at 12,600 rpm in a Centrika Metal centrifuge (Ariete, Florence, Italy) separating the juice residue (JR) from solid residue (SR), according to a previously established protocol (Pepi et al., 2016a,b).

A quantity of 4 g of JR was accurately weighed in a Teflon digestion vessel, capacity, 43 × 60 mm size (VWR International, Milan, Italy), adding 3 mL of HNO₃ (65% in distilled water, Suprapur[®], Merck KGaA, Darmstadt, Germany) and 3 mL of H₂O₂ (37% in distilled water, Suprapur[®], Merck). Samples were pre-treated in an ultrasound water bath for about 30 min at room temperature to homogenize the mixture. Each sample was then heated at

Table 2

Median concentrations of elements in juice residue of grapes from “Glera” vineyards, analyzed by ICP-MS. The values are expressed in ppm ($\mu\text{g/g}$) from B to Zn, and in ppb ($\mu\text{g/kg}$) from Ag to Zr. All abbreviations as in Table 1.

ppm	Aleandri N=9	Bottazzo N=9	Gaiarine N=9	Nardin N=9	Pattarello N=9	p-value
B	2.26 ± 0.7	2.73 ± 0.2	1.36 ± 0.2	2.73 ± 0.03	2.66 ± 0.54	***
Ca	62.2 ± 13.9	69.9 ± 15.9	92.1 ± 19	45.3 ± 6.8	57.4 ± 9.3	***
Cu	1.10 ± 0.32	0.87 ± 0.5	1.44 ± 1.1	0.64 ± 0.18	2.16 ± 0.7	*
Fe	0.72 ± 0.4	0.77 ± 0.02	0.55 ± 0.3	1.12 ± 0.4	1.32 ± 0.4	n.s
K	1892 ± 753	1325 ± 329	1689 ± 336	1834 ± 251	1704 ± 78.9	n.s
Mg	59.4 ± 17.3	52.7 ± 12.4	46.4 ± 12	58.9 ± 8.4	59.7 ± 8.72	n.s
Mn	0.29 ± 0.1	0.45 ± 0.21	0.31 ± 0.05	0.28 ± 0.07	0.38 ± 0.09	n.s
Na	4.75 ± 1.8	2.89 ± 1.46	3.41 ± 1.0	3.55 ± 0.5	4.24 ± 1.3	n.s
Rb	1.89 ± 1.1	1.49 ± 0.05	0.73 ± 0.2	0.56 ± 0.4	1.25 ± 0.3	*
Zn	0.37 ± 0.03	0.30 ± 0.05	0.33 ± 0.01	0.27 ± 0.1	0.24 ± 0.1	n.s
Ag	0.38 ± 0.11	0.48 ± 0.11	0.18 ± 0.09	0.20 ± 0.1	0.55 ± 0.13	**
Al	248 ± 50.4	267 ± 90.3	245 ± 66	83.1 ± 27	311 ± 71	***
As	16.0 ± 8.1	19.8 ± 9.6	22.0 ± 8.0	10.9 ± 6.9	15.5 ± 12	n.s
Ba	27.4 ± 12.3	21.3 ± 7.9	34.3 ± 7.2	15.3 ± 2.1	29.2 ± 3.4	***
Co	1.45 ± 0.8	1.78 ± 0.21	2.43 ± 0.5	2.06 ± 0.3	2.89 ± 0.9	*
Cr	2.90 ± 0.9	0.75 ± 0.4	1.37 ± 0.4	0.73 ± 0.4	3.48 ± 0.9	*
Ga	3.13 ± 1.3	1.26 ± 0.8	1.80 ± 1.0	1.14 ± 0.4	1.26 ± 0.9	n.s
Mo	5.48 ± 1.0	3.99 ± 1.1	11.6 ± 3.8	12.2 ± 5.2	3.62 ± 1.2	n.s
Ni	6.18 ± 4.3	11.1 ± 4.3	13.5 ± 8.8	9.48 ± 5.8	20.4 ± 9.3	n.s
Pb	2.00 ± 0.84	3.25 ± 1.5	0.91 ± 0.6	1.23 ± 0.2	1.22 ± 0.53	*
Sr	113 ± 30	107 ± 25	95.6 ± 21	80.3 ± 3.4	81.2 ± 6.6	***
Y	0.17 ± 0.10	0.15 ± 0.05	0.15 ± 0.03	0.17 ± 0.09	0.26 ± 0.08	*
Zr	1.05 ± 0.40	0.75 ± 0.06	0.64 ± 0.1	0.64 ± 0.4	0.78 ± 0.08	***

Table 3

Median concentrations of elements in solid residue of grapes from “Glera” vineyards, analyzed by ICP-MS. The values are expressed in ppm ($\mu\text{g/g}$) from B to Zn, and in ppb ($\mu\text{g/kg}$) from Ag to Zr. All abbreviations as in Table 1.

ppm	Aleandri n=9	Bottazzo n=9	Gaiarine n=9	Nardin n=9	Pattarello n=9	p-value
B	4.41 ± 0.2	4.55 ± 0.8	2.73 ± 0.4	3.86 ± 0.8	4.48 ± 1.4	**
Ca	450 ± 65	407 ± 39	331 ± 112	212 ± 107	177 ± 72	***
Cu	4.92 ± 0.3	4.39 ± 0.2	5.17 ± 0.4	1.76 ± 0.7	8.54 ± 2.9	n.s
Fe	4.81 ± 0.4	3.40 ± 0.9	5.75 ± 0.9	3.72 ± 1.1	7.13 ± 1.1	n.s
K	2071 ± 88	2466 ± 387	3174 ± 869	3368 ± 1103	3326 ± 488	n.s
Mg	141 ± 9.4	112 ± 29	67.4 ± 15	91.3 ± 28	60.5 ± 17	***
Mn	2.36 ± 0.4	1.28 ± 0.6	0.77 ± 0.3	0.75 ± 0.2	1.14 ± 0.5	***
Na	2.79 ± 1.1	4.39 ± 1.2	2.42 ± 1.1	3.06 ± 0.3	3.66 ± 0.6	n.s
Rb	3.80 ± 1.8	3.12 ± 0.5	1.35 ± 0.6	1.26 ± 0.3	3.08 ± 1.0	*
Zn	1.68 ± 0.1	0.92 ± 0.2	1.03 ± 0.34	0.58 ± 0.2	0.68 ± 0.2	***
Ag	1.50 ± 0.3	2.00 ± 0.3	1.56 ± 0.87	0.71 ± 0.2	2.36 ± 1.0	**
Al	364 ± 64	533 ± 57	126 ± 19	219 ± 81	616 ± 140	***
As	16.8 ± 3.1	15.3 ± 7.3	25.0 ± 4.6	14.5 ± 1.9	13.9 ± 6.3	n.s
Ba	334 ± 49	172.1 ± 15	304 ± 105	134 ± 31	355 ± 65	***
Co	6.92 ± 2.3	4.83 ± 1.3	6.00 ± 1.2	5.00 ± 1.7	4.64 ± 1.6	n.s
Cr	8.12 ± 7.1	2.69 ± 1.9	7.81 ± 4.5	5.15 ± 1.5	4.65 ± 1.4	n.s
Ga	10.1 ± 8.9	5.16 ± 3.4	9.00 ± 4.1	4.23 ± 1.8	9.87 ± 3.3	n.s
Mo	20.8 ± 2.4	9.68 ± 3.6	17.2 ± 9.4	1.69 ± 0.4	12.8 ± 4.5	***
Ni	52.4 ± 2.9	56.9 ± 9.7	15.2 ± 3.7	33.5 ± 4.9	20.5 ± 2.4	***
Pb	5.31 ± 1.3	2.95 ± 0.7	0.90 ± 0.6	2.28 ± 0.6	4.92 ± 1.1	n.s
Sr	994 ± 130	676 ± 64	918 ± 103	670 ± 138	927 ± 125	***
Y	0.30 ± 2.0	1.14 ± 0.5	0.17 ± 0.07	0.16 ± 0.1	0.36 ± 0.1	**
Zr	2.99 ± 0.6	2.94 ± 0.9	2.32 ± 1.0	0.74 ± 0.2	2.21 ± 1.0	***

110–140 °C for 3 h until complete drying and resuspended in 2 mL HNO_3 .

An amount of 2.5 g of SR was accurately weighed in the Teflon vessel, adding 4 mL HNO_3 and 3 mL H_2O_2 . Samples were pre-treated in an ultrasound water bath for 1 h at room temperature, heated at about 180 °C for 4 h until complete drying and resuspended in 3 mL HNO_3 . All JR and SR samples were transferred in plastic (Perfluoroalkoxy-copolymer, PFA) flasks and made up to 100 mL with highly purified Milli-Q® water. As a control and for correction of instrumental drift, an internal Rh-Re standard was added to each sample to a final concentration of 10 ppb and all samples were analysed by inductively coupled plasma-mass spectrometry (ICP-

MS) using a Thermo Electron Corporation X series spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts).

2.3. Analytical determinations

The determination of major and trace elements in soils was performed by X-ray fluorescence, using a wavelength dispersive spectrometer ARL ADVANT'XP (Thermo Fisher Scientific, Waltham, Massachusetts). The chemical composition of soils was expressed as a weight percentage of the following oxides (SiO_2 , TiO_2 , Al_2O_3 , Fe_2O_3 , MnO , MgO , CaO , Na_2O , K_2O , P_2O_5) and as ppm for the

following trace elements: Ba, Co, Cr, La, Nb, Ni, Pb, Rb, Sr, V, Y, Zn, Zr, Cu, Ga, Nd and Sc.

Accuracy was generally lower than 2% for major oxides and less than 5% for trace elements determinations: the detection limits for major oxides were 0.03–0.05% and for trace elements 1–2 ppm (Lachance and Trail, 1966; Saccani et al., 2014).

The chemical elements in JR and SR were determined by inductively coupled plasma-mass spectrometry (ICP-MS) using a Thermo Electron Corporation X series spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts). The elements determined were Al, Ag, B, Ba, Ca, Co, Cr, Cu, Fe, Ga, K, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sr, Zn, Y and Zr. Accuracy was generally lower than 15% for all elements. The detection limits were 1–10 ppb for Al, Ca and Fe, 0.1–1 for Mg, K and Na, and less than 0.1 ppb for all other elements.

The accuracy of the analysis of soil samples was checked by NIST 2709 and USGS GXR-2 certified reference materials. The stan-

dard reference materials for ICP-MS were SRM 1547–Peach Leaves and SRM 1567a – Wheat Flour (National Institute of Standards and Technology, Gaithersburg, Maryland).

2.4. Statistical analysis

The Kruskal-Wallis non-parametric test (with post-hoc Dunn's test) was employed to establish the differences among groups in all data from soil, and from JR and SR samples. Linear discriminant analysis (LDA) was found to be the most appropriate multivariate statistical technique for the interpretation of all data. In this technique the user must assign a classification group to all sample data: the differences among these predetermined groups describe combinations of variables (Rencher, 2002). The variables were evaluated by means of stepwise LDA, using a Wilks Lambda test (p -value < 0.01) and an F-statistic factor (Rencher, 2002). All

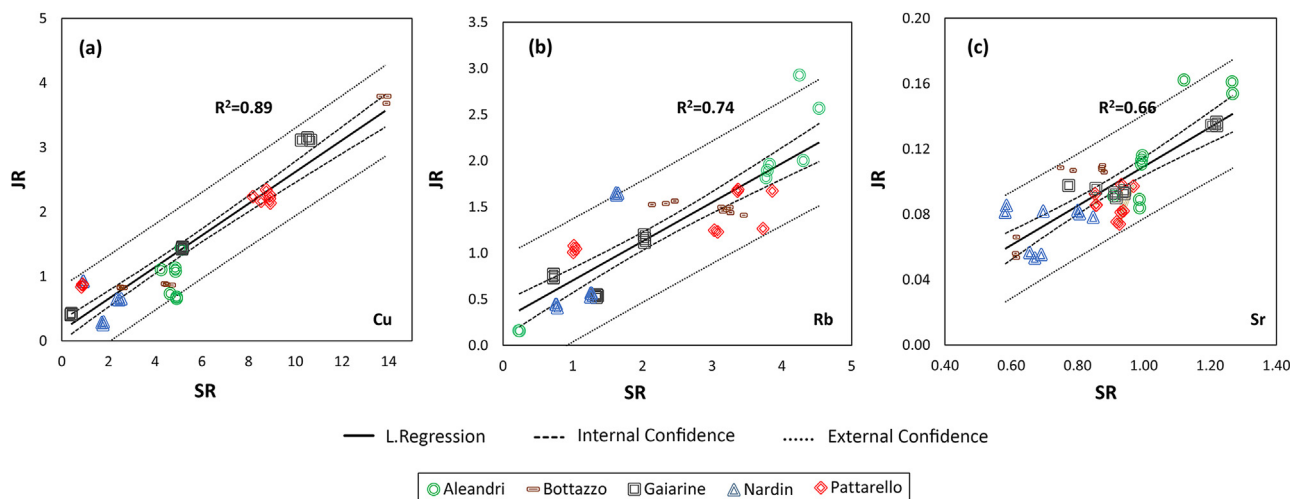


Fig. 4. Linear regression values of concentrations of Cu (a), Rb (b) and Sr (c) in juice residue (JR) vs solid residue (SR), showing a significant correlation ($R^2 > 0.60$) with an internal and external 95% confidence interval. Values of trace elements are expressed in $\mu\text{g/g}$.

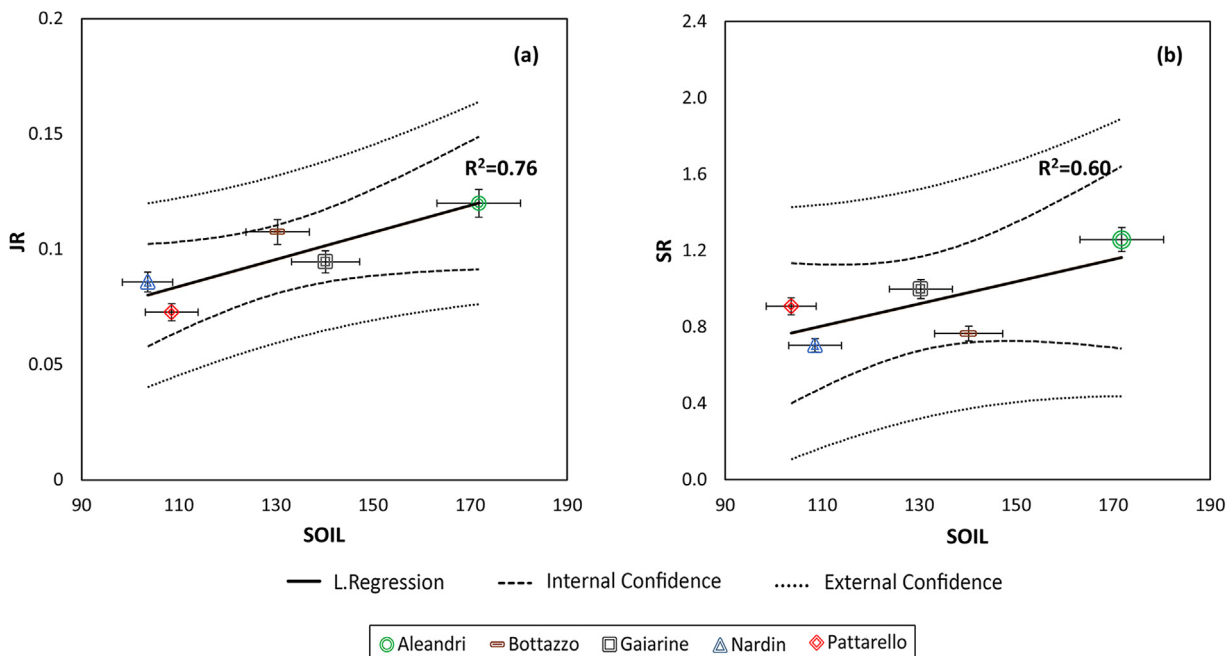


Fig. 5. Average values and standard deviation of concentrations of Sr in soil vs JR (a) and SR (b) in the five vineyards studied. Linear regression and confidence intervals as in Fig. 4. Values of Sr are expressed in $\mu\text{g/g}$.

data analyses were carried out by the software XLSTAT (Version 2015.5.02, Addinsoft, Paris, France).

3. Results and discussion

3.1. Soil characterization by XRF analyses

Table 1 reports the chemical composition of collected soil samples from each vineyard. Statistically significant differences ($p < 0.05$) were obtained for most values of major and trace elements. The highest concentration value for major elements and for all vineyards was SiO_2 , followed by Al_2O_3 , Fe_2O_3 , MgO and CaO . Examining the differences in oxide concentrations among vineyards, CaO resulted much higher in Aleandri and Bottazzo vineyards in comparison to Pattarello, Gaiarine and Nardin. Concerning trace elements, the highest concentration was Ba, followed by Sr, Rb and Zr. The highest concentrations of Cr were detected in vineyards Nardin and Gaiarine, and those of Zr in Pattarello and Gaiarine. Generally, the concentrations values of major and trace elements apparently changed according to geographical origin of soils.

The bivariate plots of concentrations of major element oxides in Table 1 are shown in Fig. 2. The comparison between Al_2O_3 and SiO_2 (Fig. 2a) suggests a higher concentration of quartz minerals in vineyards Nardin, Gaiarine and Pattarello, and a lower one in Aleandri and Bottazzo, confirming previous results obtained by X-ray powder diffraction (XRD) by Petrini et al. (2014). Comparing CaO and SiO_2 (Fig. 2b), a higher concentration of calcite and dolomite is detected in vineyards Aleandri and Bottazzo, according to previous data obtained by XRD (Petrini et al., 2014). The higher values of Al_2O_3 vs Fe_2O_3 detected in vineyards Nardin, Gaiarine and Pattarello are probably due to clay minerals and Al/Fe hydrous oxides present in alluvial deposits (Fig. 2c).

The bivariate plots of concentrations of trace elements are shown in Fig. 3. The values of Al_2O_3 vs Rb, Zr, and Cr are respectively shown in Fig. 3a–c, and those of TiO_2 vs Zr in Fig. 3d. In all vineyards the values of Al_2O_3 vs Rb supports the presence of clays minerals, related to high content of aluminosilicates in soil fine sediments (Fig. 3a). The correlation Al_2O_3 vs Zr (Fig. 3b) higher in Nardin and Gaiarine vineyards suggests the presence of silic minerals and their residues, related to Zr-rich sediments. The presence

of metal-rich phyllosilicates in all vineyards, with higher values in Nardin, Gaiarine and Pattarello, revealed by values of Al_2O_3 vs Cr (Fig. 3c), suggests the presence of chlorite in soil sediments, as previously reported for Po River Delta (Di Giuseppe et al., 2014). The values of TiO_2 vs Zr (Fig. 3d) follow the same pattern of Al_2O_3 vs Cr and suggest the presence of Ti-rich complex silicates. The Ti to Zr ratio could be used to identify the soil origin (Hutton, 1977; Ishiga et al., 2013; Schaetzel and Anderson, 2005).

Generally, all vineyard soils showed a heterogenous siliclastic and carbonate composition whose distribution changed according to geographic origin. The high values of Ti, Zr and Cr support a high degree of weathering of all soils examined (Schaetzel and Anderson, 2005). In addition, the data in Fig. 3c and d indicate that Cr and Zr can be used as discriminatory elements for geographical origin of the examined soils.

3.2. Major and trace elements in grape berries and soil

Chemical compositions of juice residue (JR) and solid residue (SR) of grape berries collected in the five vineyards are respectively reported in Tables 2 and 3. According to the results shown in the two tables, the concentration values of all elements are higher in SR than in JR, supporting previous data obtained with slightly different protocols (Teissedre et al., 1994; Rogiers et al., 2006; Bertoldi et al., 2009, 2011; Young et al., 2010).

In JR there was a slight prevalence of statistically significant differences ($p < 0.05$) in element concentrations (Table 2). The highest values of major elements in JR (Table 2) were respectively K, Ca, Mg, and Na, but the only statistically difference among vineyards was that of Ca. Concerning trace elements, the highest values were respectively Rb, Al, Sr and Ba, and all these values had statistically significant differences among vineyards.

In SR (Table 3) about two-thirds of values were statistically significant ($p < 0.05$). The highest concentration values of major elements in SR were K, Ca, Mg, B and Na: all were statistically significant except K and Na. For trace elements, the highest values were respectively Sr, Rb, Al and Ba, all statistically significant.

The concentration values of all elements in JR were plotted against those in SR and the linear regression values were calculated. Only three elements (Cu, Rb and Sr) showed a significant correlation

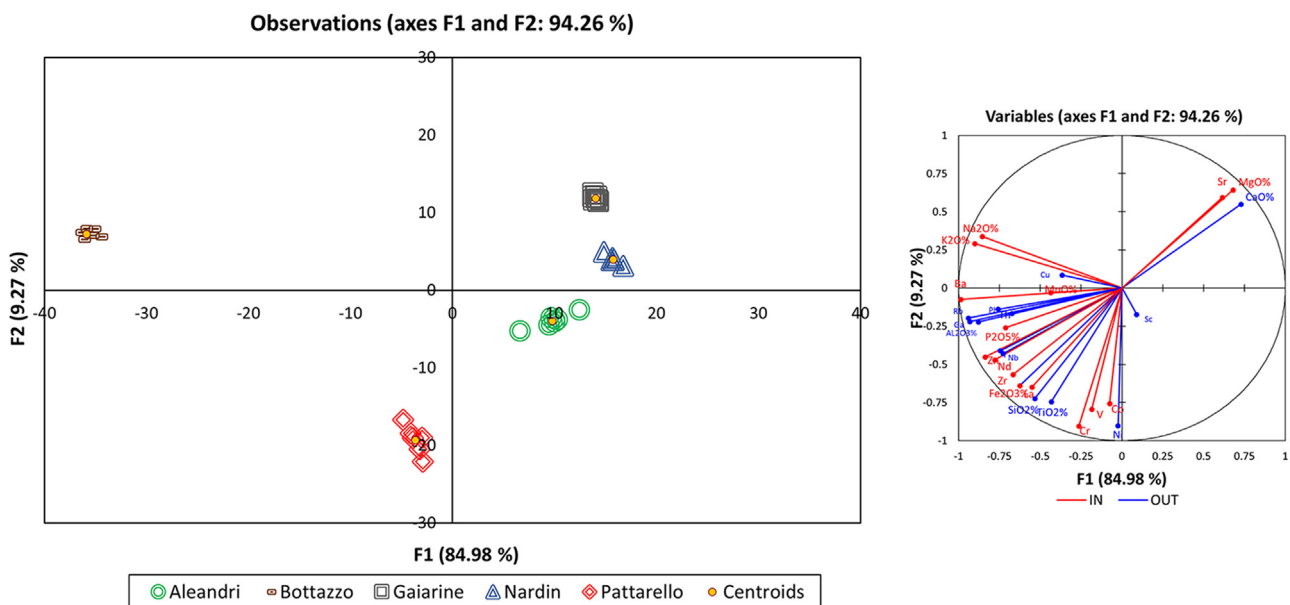


Fig. 6. Linear discriminant analysis (LDA) plot of the values of element concentrations in soils of the five vineyards shown in Table 1. The factor loading plot is shown on the right side: F1, first discriminant function; F2, second discriminant function.

($R^2 > 0.60$) with an internal and external 95% confidence interval (Fig. 4). The highest correlation was that of Cu, probably related to agronomic practices (Vitanović et al., 2008; Volpe et al., 2009; Fregoni and Viticoltura di Qualità, 2013; Vystavna et al., 2014). For trace elements, the Rb values could be related to the interchangeability of this element with K, previously documented in plants (Kabata-Pendias, 2011). The effect of Rb on plant growth and the development may depend on its concentration and on relative abundance of K and Na (El-Sheikh and Ulrich, 1970). Moreover, a possible deficiency of available K in soils could be at least compensated by uptake of Rb (Tyler, 1997; Drobner and Tyler, 1998; Kabata-Pendias, 2011).

Based on the above data, all JR and SR values of major and trace elements were correlated to the respective element concentration in the soils of the five vineyards. The only element that showed a significant correlation with soil concentration (respectively $R^2 > 0.76$ in JR and $R^2 > 0.60$ in SR) was Sr (Fig. 5). It is known that Sr is interchangeable with Ca according to the plant metabolic requirements: its uptake may be increased by Ca and probably by Mg, and decreased by K and Na (Kabata-Pendias, 2004). Experimental data confirm that an increased level of Ca in the growth medium stimulates Sr uptake by plants (Kabata-Pendias, 2011). Previously obtained $^{87}\text{Sr}/^{86}\text{Sr}$ data for the same vineyards (Petrini et al., 2014) support the hypothesis that the positive linear correlation detected for Sr in the five vineyards is due to the Sr concentration in soil.

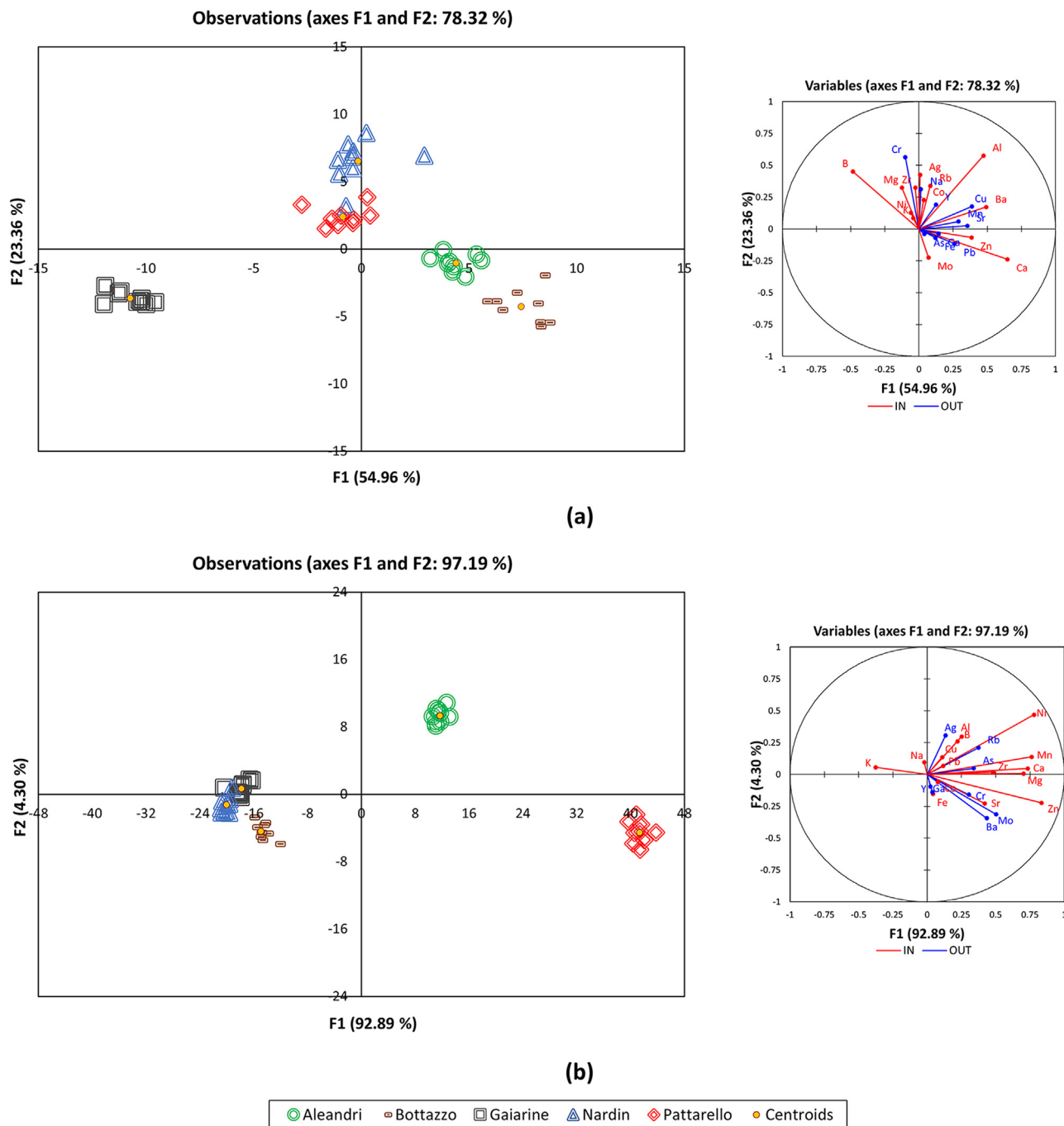


Fig. 7. Linear discriminant analysis (LDA) plot of the values of element concentrations in JR (a) and SR (b) of the five vineyards, respectively shown in Tables 2 and 3. The factor loading plots are shown on the right side: F1, first discriminant function; F2, second discriminant function.

3.3. Multivariate analysis by LDA of major and trace elements

3.3.1. Soil

Stepwise linear discriminant analysis (LDA) using a Wilks Lambda test (p -value < 0.01) and an F-statistic factor (Rencher, 2002) were applied to all data of major and trace elements in soils from the five vineyards examined, by the software XLSTAT. Among all functions calculated by the program, two were chosen as best discriminating functions among all data sets (Rencher, 2002). Both discriminating functions were associated to the values of major elements and trace elements shown in Table 1, and the plot of the first discriminant function (F1) against that of the second one (F2) is shown in Fig. 6. The LDA analysis is clearly able to discriminate the soil samples according to their geographical origin, with a validation of 73.05%, confirming that it is possible to identify each one of the five wineries (Fig. 6). The highest discrimination among soils is linked to F1, in which the elements selected by the forward stepwise process for the discriminant plot are MgO, MnO, Na₂O, K₂O, P₂O₅, Ba, Co, Cr, La, Nb, Sr, V, Zn, Zr.

3.3.2. Juice and solid residue

The LDA analysis was applied in the same conditions to the data of juice residue (JR) (Fig. 7a) and solid residue SR (Fig. 7b), respectively shown in Tables 2 and 3. The two functions F1 and F2 were selected as previously described (Rencher, 2002). The elements selected by the forward stepwise process for the discriminant plot were Ag, Al, B, Ba, Ca, Co, Rb, Sr, Zn, Zr in JR, and Al, B, Ba, Ca, Mg, Mn, Ni, Sr, Zn, Zr in SR.

The LDA model yielded 93.33% of sample validation for JR (Fig. 7a) and 97.78% for SR (Fig. 7b). According to F1 and F2, the two different grape residues yielded separate groups for each winery, thus the LDA analysis was clearly able to discriminate all JR and SR samples according to their geographical origin. The results in JR and SR showed that the plants accumulate elements in different parts depending on geochemical properties of the soils. These data confirmed that each winery could be identified by its geographic origin.

The LDA multivariate analysis enabled to successfully discriminate the geochemical composition (thus the geographical origin) of soil and Glera grape berries from the five different wineries. Based on this analysis, the elements useful as possible geochemical markers for provenance of Glera grape berries, in order of relevance, are Sr, Ba, Ca, Mg, Al, K, Zn, B, Ni, Co.

4. Conclusions

Major and trace elements in soil and grape berries of “Glera”, a *Vitis vinifera* cultivar from two Northern Italian Regions, were evaluated as possible geographical markers for territoriality of products made in Italy. The analysis of soils yielded a geological characterization for each of the five wineries examined. The study reveal the different concentrations of major and trace elements in juice residue and solid residues of grape berries. This study provides a new simplified protocol, based on geochemical markers, allowing rapid identification of the geographical origin of grapes. Comparing results on soil and on grape berries, the only element that provide a clear geochemical correlation between soil and grape berries is Sr. However, the LDA multivariate analysis allowed to detect geochemical correlations of other elements besides Sr, namely, Ba, Ca, Mg, Al, K, Zn, B, Ni, and Co. All these elements could establish a reliable correspondence between the geolithological features of the vineyard soil and the chemical composition of juice and solid residues. Overall, besides combined element characteristics assessed by LDA multivariate analysis, Sr turn out as key marker of soil provenance for the geographical origin of the Glera grape.

The relationship between soil and grape berries confirm that major and trace elements could act as geochemical markers of Glera grapes according the geological area. These elements could be also useful to establish geochemical fingerprintings for testing the origin of Prosecco wine, in order to protect the Made in Italy trademarks against falsifications and fraudulent use of denomination labels.

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