

1 Article

2 Authentication and traceability study on *Barbera* 3 *d'Asti* and *Nizza* DOCG wines: the role of trace- and 4 ultratrace elements

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13 **Abstract:** *Barbera d'Asti* - including *Barbera d'Asti superiore* - and *Nizza* are two DOCG
14 (Denominazione di Origine Controllata e Garantita) wines produced in Piemonte (Italy) from
15 *Barbera* grape variety. Differences among them arise in the production specifications in terms of
16 purity, ageing and zone of production, in particular with concern to *Nizza*, which has more
17 stringent rules and can therefore be considered as the one with the highest market value, with even
18 three-fold more average prices. To guarantee producers and consumers, authentication methods
19 must be developed in order to distinguish among the different wines. As the production zones
20 totally overlap, it is important to verify whether the distinction is possible or not according to
21 metals content, or whether chemical markers more linked to winemaking are needed. In this work,
22 Inductively Coupled Plasma (ICP) elemental analysis and multivariate data analysis are used to
23 study the authentication and traceability of samples from the three designations of 2015 vintage.
24 The results show that, as far as elemental distribution in wine is concerned, work in the cellar,
25 rather than geographic provenance, is crucial for the possibility of distinction.

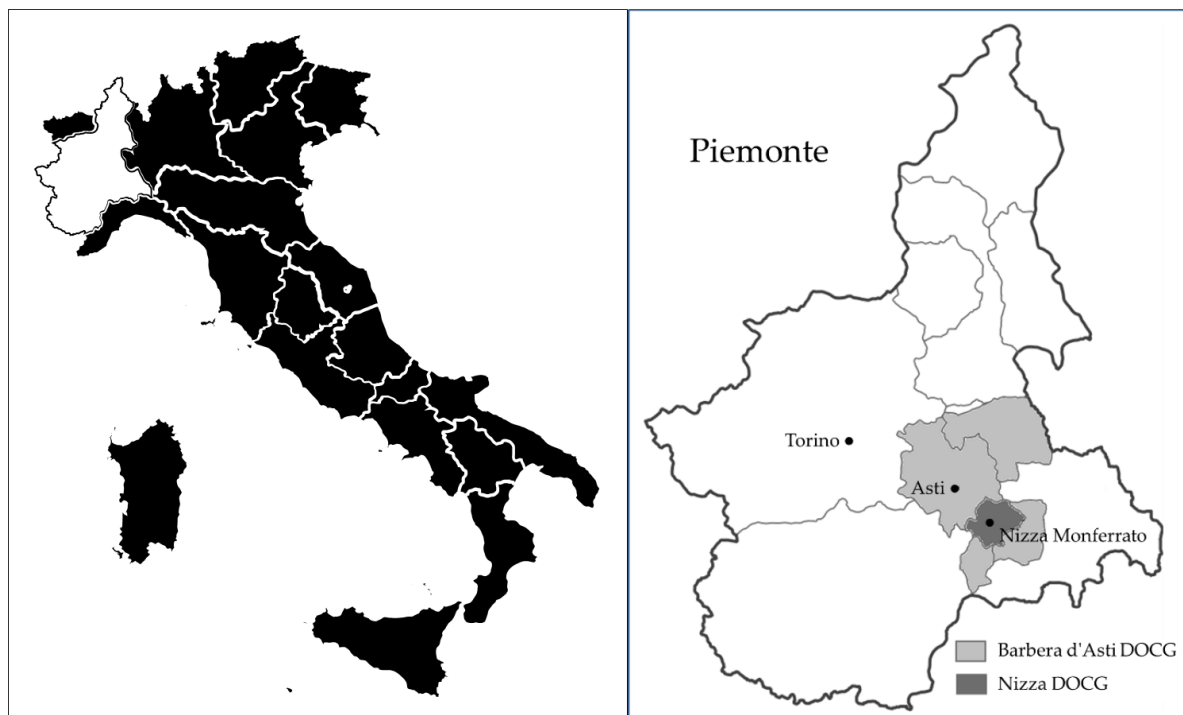
26 **Keywords:** ICP-MS; trace elements; wine; *Nizza*; *Barbera*; authentication

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28 1. Introduction

29 *Barbera d'Asti* DOCG and *Nizza* DOCG are two high-quality wines produced in Piemonte (Italy)
30 from *Barbera* grape variety (*Vitis vinifera*), an autochthonous vine cultivated in that region since 16th
31 century. The designation *Barbera d'Asti* was firstly labelled as DOC (Denominazione di Origine
32 Controllata) in 1970, approved with DPR 09/01/1970 [1] and later on as DOCG (Denominazione di
33 Origine Controllata e Garantita) in 2008, approved with DM 08.07.2008 [2]; the designation involved
34 116 communes in the Asti province and 51 communes in the Alessandria province for a total surface
35 of 53 Km² (5,300 Ha), of whom nearly 40 Km² (4,000 Ha) claimed in 2018. The DOCG designation
36 provided also the possibility of using an additional, finer specification as *Barbera d'Asti superiore* for
37 wines produced with minimum ageing of 14 months, 6 of whom in barrique; moreover, there was
38 the possibility of adopting the three specific labelling *Barbera d'Asti superiore sottozona Colli Astiani*,
39 *Barbera d'Asti superiore sottozona Nizza* and *Barbera d'Asti superiore sottozona Tinella* in the case of wines
40 produced, within the whole *Barbera d'Asti* area, in the three corresponding geographic sub-zones,
41 considered as the more suitable in terms of quality. Recently the *Barbera d'Asti superiore sottozona*
42 *Nizza* has been elevated to the rank of a new DOCG [3] called simply *Nizza*, according to more severe
43 rules that included production in only 18 communes inside the Asti province, located around Nizza

44 Monferrato (Figure 1), for a total area under vines of 7.2 Km² (720 Ha), of whom nearly 2.0 Km² (200
45 Ha) claimed in 2018.



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47 **Figure 1.** Production zones of *Barbera d'Asti/Barbera d'Asti superiore* and *Nizza*.

48 The main differences between *Barbera d'Asti*, *Barbera d'Asti superiore* and *Nizza* designations are
49 shown in Table 1.

50 **Table 1.** Differences between *Barbera d'Asti*, *Barbera d'Asti Superiore* and *Nizza* designations

Parameter	<i>Barbera d'Asti</i>	<i>Barbera d'Asti Superiore</i>	<i>Nizza</i>
Production zones	116 communes in the Asti province and 51 communes in the Alessandria province	116 communes in the Asti province and 51 communes in the Alessandria province	18 Communes in the Asti province
Altitude	not above 650 m a.s.l.	not above 650 m a.s.l.	between 150 and 350 m a.s.l.
Exposure	suitable for ensuring ripening of the grapes. North exposure is excluded for new plants	suitable for ensuring ripening of the grapes. North exposure is excluded for new plants	exclusively hilly with exposure from south to south west - south east
Alcohol content	12.00% vol. minimum	12.50% vol. minimum	13.00% vol. minimum
Ageing	4 months minimum	14 months minimum, 6 of	18 months minimum, 6 of

			whom in wood	whom in wood
Minimum total acidity	4.5 g/l.		4.5 g/l.	5.0 g/l
Minimum non-reducing extract	24.0 g/l		25.0 g/l	26.0 g/l
Ampelographic composition	Barbera (85% minimum), Freisa, Grignolino and Dolcetto, alone or jointly (15% maximum).		Barbera (85% minimum), Freisa, Grignolino and Dolcetto, alone or jointly (15% maximum).	Barbera 100%

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As it can be seen, specifications in *Nizza* designation are more severe in terms of purity, ageing and zone of production; they were chosen in order to produce wines with recognised higher quality. It is therefore to be expected that *Nizza* is generally considered the finest among the wines obtained from *Barbera* vine; on the Italian market, indeed, *Nizza* is sold at even three-fold average prices with respect to *Barbera d'Asti*.

To guarantee producers and consumers, authentication methods must be developed in order to distinguish between *Barbera d'Asti*, *Barbera d'Asti superiore* and *Nizza* wines. Among the different chemical markers available, one possibility is using trace- and ultra-trace elements as discrimination variables [4–7]. A particular focus must be given on the discrimination power of lanthanides. It is well known their role in providing a link between a specific territory and foodstuffs that originate from it, as a consequence of their homogeneous chemical behaviour which is not fractionated in the passage between soil, plant and food final product [8–10]. As far as wine is concerned, our previous work [11] and other works suggested that its production chain can cause fractionation of the original soil fingerprint. The role of other trace- and ultra-trace elements is however less understood.

Considering that the production zone of *Nizza* is totally contained within that of *Barbera d'Asti* (Figure 1), in this work we wanted to verify whether the distinction between *Nizza*, *Barbera d'Asti superiore* and *Barbera d'Asti*, listed according to their market value from the more expensive to the less one, is possible on the basis of the distribution of trace- and ultra-trace elements. It must be remembered that these wines come from very small areas: 40 Km² (4,000 Ha) for *Barbera d'Asti/Barbera d'Asti superiore* DOCG and nearly 2 Km² (200 Ha) for *Nizza* DOCG. ICP elemental analysis and multivariate data analysis were used at the purpose. Samples of wines were mostly from 2015 vintage. Moreover, in order to evaluate the correlation between soil and wine, we analysed samples of soils taken at the various locations of the producers of *Nizza*. The samples of *Barbera d'Asti* and *Barbera d'Asti superiore* were provided by the same producers of *Nizza*, so we can consider that the reference soils are the same.

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2. Materials and Methods

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2.1. Materials

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High-purity water from a Milli-Q apparatus (Milford, MA, USA) was used in the study. TraceSelect hydrogen peroxide 30%, nitric acid 69% and hydrochloric acid 37% were purchased from Fluka (Milan, Italy). Polypropylene and polystyrene vials, used respectively for sample storage and analysis with an auto-sampler system, were kept in 1% nitric acid and then rinsed with high-purity water when needed. Elements stock solutions (Inorganic Ventures, Lakewood, NJ, USA) were used for external calibration and internal standardization.

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2.2. Sample collection

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Soil samples were taken at the producers' locations. In each place, 1 Kg of soil was collected at a depth of 30 cm in order not to include surface contamination.

88 Wines were obtained directly from each producer. Bottles were kept in a cellar and opened only
89 at the moment of analysis.

90 2.3. Sample treatment

91 Soil samples were treated according to a standardised procedure: soil was dried at 105°C for 24
92 h, after which 1 g was sieved (ϕ 0.2 mm) and extracted with 20 mL of hydrogen peroxide for 20 min
93 and then with 12 mL of aqua regia on a heating plate for 2 h under reflux. The resulting solution was
94 diluted to volume in a 100 mL volumetric flask with high-purity water.

95 Wine samples were diluted 1:10 with a nitric acid 1% solution containing In 10 ppb as internal
96 standard for the ICP-OES and ICP-MS determination of almost all elements; K, P, S Mg, Ca and Na
97 were determined on wine samples diluted 1:100 with the same solution. After opening a bottle, the
98 first 10 mL were discarded in order to avoid contamination from the cork. Care was taken in every
99 manipulation passage, in particular when wine was collected with a micropipette to prepare the
100 diluted solution: this was carried out discarding the first volume collected, so as to avoid
101 contamination from the pipette tip.

102 2.4. ICP-OES Analysis

103 For major and minor elements, analyses were performed with a Spectro (SPECTRO Analytical
104 Instruments GmbH, Kleve, Germany) Genesis ICP-OES simultaneous spectrometer with axial
105 plasma observation. Instrumental parameters were as follows: RF generator, 40 MHz; RF, 1300 W;
106 plasma power, 1400 W; plasma gas outlet, 12 L/min; auxiliary gas flow rate, 0.80 L/min; nebuliser
107 flow rate, 0.85 L/min; pump speed, 2.0 mL/min. The following elements were determined: Na
108 (589.592 nm), K (766.491 nm), Mg (279.553 nm), Ca (317.933 nm), B (249.773 nm), P (213.618 nm), Si
109 (251.612 nm), Al (396.152 nm) and S (180.731 nm). A multi-element standard solution was prepared
110 starting from Inorganic Ventures (Christiansburg, Virginia - USA) CCS-4 and CCS-5 multi-element
111 standards containing 100 mg/L for each element; the solution was diluted in order to obtain 10, 5, 1,
112 0.5 and 0.1 mg/L solutions in 1% nitric acid solution. The limits of detection (LOD) and the limits of
113 quantification (LOQ), calculated respectively as 3 and 10 times the standard deviation of blank
114 measurements [12], are shown in Table 2.

115 2.5. ICP-MS Analysis

116 For most trace- and ultra-trace elements, analyses were performed with a Thermo Fisher
117 Scientific (Waltham, MA, USA) XSeries 2 model Inductively Coupled Plasma Mass Spectrometer.
118 The instrument is equipped with a quartz torch with silver PlasmaScreen device, a quadrupole mass
119 analyser, a lens ion optics based upon a hexapole design with a chicane ion deflector and a
120 simultaneous detector with real-time multichannel analyser electronics, operating either in analogue
121 signal mode or in pulse counting mode. The inlet system included an ESI PC³ Peltier Chiller
122 (Elemental Scientific, Omaha, NE) set at +2°C, incorporating a PFA micro-flow concentric nebulizer
123 and a cyclonic spray chamber. Instrument and accessories are PC controlled by PlasmaLab v.
124 2.6.2.337 software provided by Thermo Fisher Scientific. Instrument parameters can be found in
125 Aceto et al., 2019 [11].

126 Measurements were carried out mostly in standard mode. For some analytes the CCT-KED
127 (Cell Collision Technology-Kinetic Energy Discriminator) mode was used to eliminate polyatomic
128 interferences: to do this, an H₂/He 8/92% gas mixture was introduced before the quadrupole mass
129 analyser at a flow of 5.00 mL/min. Parameters were as follows: dual mode detection with peak
130 jumping; dwell time 10 ms (standard mode) or 25 ms (CCT-KED mode); 25 sweeps; 3 replicates for a
131 total acquisition time of 60 s.; isotopes used: ⁷Li, ⁴⁵Sc, ⁴⁹Ti, ⁵¹V, ⁵²Cr, ⁵⁵Mn, ⁵⁶Fe, ⁵⁹Co, ⁶⁰Ni, ⁶³Cu, ⁶⁴Zn,
132 ⁷⁵As, ⁷⁷Se, ⁷⁹Br, ⁸⁵Rb, ⁸⁸Sr, ⁸⁹Y, ⁹⁰Zr, ⁹³Nb, ⁹⁷Mo, ¹⁰⁸Pd, ¹¹¹Cd, ¹²⁰Sn, ¹²¹Sb, ¹²⁷I, ¹³³Cs, ¹³⁷Ba, ¹³⁹La, ¹⁴⁰Ce, ¹⁴¹Pr,
133 ¹⁴⁴Nd, ¹⁴⁷Sm, ¹⁵³Eu, ¹⁵⁸Gd, ¹⁵⁹Tb, ¹⁶³Dy, ¹⁶⁵Ho, ¹⁶⁷Er, ¹⁶⁹Tm, ¹⁷⁴Yb, ¹⁷⁵Lu, ¹⁹⁷Au, ¹⁹⁹Hg, ²⁰⁵Tl, ²⁰⁸Pb, ²⁰⁹Bi, ²³²Th
134 and ²³⁸U. Among these, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, and Bi isotopes were determined in
135 CCT-KED mode. Interferences were evaluated as follows: CeO⁺/Ce⁺ < 2% and Ba²⁺/Ba⁺ < 3%. A

136 stability and performance test was performed before each analysis session by monitoring ${}^7\text{Li}$, ${}^{59}\text{Co}$,
 137 ${}^{115}\text{In}$ and ${}^{238}\text{U}$ masses and the ${}^{59}\text{Co}/{}^{51}\text{Cr}$ ratio > 15 for CCT-KED mode. Background signals were
 138 monitored at 5 and 220 m/z to perform a sensitivity test on the above reported analyte masses. The
 139 limits of detection (LOD) and the limits of quantification (LOQ), calculated respectively as 3 and 10
 140 times the standard deviation of blank measurements [12], are shown in Table 2.

141 A multi-element standard solution was prepared starting from Inorganic Ventures
 142 (Christiansburg, Virginia - USA) CCS-1, CCS-2, CCS-4, CCS-5 and CCS-6 multi-element standards
 143 containing 100 mg/L for each element; this solution was diluted in order to obtain 10, 5, 1, 0.5 and 0.1
 144 $\mu\text{g/L}$ solutions in 1% nitric acid solution. Isotopes responses were corrected by dedicated internal
 145 standards using ${}^{115}\text{In}$ at 10 $\mu\text{g/L}$.

146 **Table 2.** LOD and LOQ for the elements determined with ICP-OES and ICP-MS

Element	LOD	LOQ	Element	LOD	LOQ	Element	LOD	LOQ
K ¹	0.001 mg/L	0.005 mg/L	Pb ²	0.015 $\mu\text{g/L}$	0.048 $\mu\text{g/L}$	Y ²	0.3 ng/L	1.0 ng/L
P ¹	0.062 mg/L	0.206 mg/L	Ni ²	0.060 $\mu\text{g/L}$	0.199 $\mu\text{g/L}$	U ²	0.3 ng/L	1.1 ng/L
S ¹	0.133 mg/L	0.444 mg/L	Ti ²	0.071 $\mu\text{g/L}$	0.236 $\mu\text{g/L}$	Pd ²	1.4 ng/L	4.6 ng/L
Mg ¹	0.004 mg/L	0.015 mg/L	Cr ²	0.061 $\mu\text{g/L}$	0.203 $\mu\text{g/L}$	Cd ²	1.4 ng/L	4.5 ng/L
Ca ¹	0.002 mg/L	0.007 mg/L	Sc ²	6.9 ng/L	23.0 ng/L	Tl ²	0.2 ng/L	0.5 ng/L
Na ¹	0.007 mg/L	0.022 mg/L	Li ²	5.2 ng/L	17.2 ng/L	Hg ²	8.6 ng/L	28.5 ng/L
Fe ²	0.052 $\mu\text{g/L}$	0.173 $\mu\text{g/L}$	Mo ²	7.8 ng/L	26.0 ng/L	Gd ²	0.8 ng/L	2.6 ng/L
B ¹	0.043 mg/L	0.144 mg/L	Sn ²	10.2 ng/L	34.1 ng/L	Pr ²	0.1 ng/L	0.2 ng/L
Si ¹	0.245 mg/L	0.816 mg/L	As ²	23.5 ng/L	78.2 ng/L	Sm ²	1.2 ng/L	4.1 ng/L
Sr ²	0.004 $\mu\text{g/L}$	0.014 $\mu\text{g/L}$	Cs ²	0.8 ng/L	2.8 ng/L	Dy ²	0.5 ng/L	1.6 ng/L
Rb ²	0.022 $\mu\text{g/L}$	0.075 $\mu\text{g/L}$	Co ²	1.3 ng/L	4.4 ng/L	Th ²	0.1 ng/L	0.2 ng/L
Al ¹	0.006 mg/L	0.019 mg/L	Zr ²	3.3 ng/L	11.1 ng/L	Yb ²	0.3 ng/L	1.1 ng/L
Br ²	0.495 $\mu\text{g/L}$	1.649 $\mu\text{g/L}$	Nb ²	0.7 ng/L	2.4 ng/L	Er ²	0.4 ng/L	1.3 ng/L
Zn ²	0.189 $\mu\text{g/L}$	0.630 $\mu\text{g/L}$	Ce ²	3.4 ng/L	11.5 ng/L	Eu ²	0.9 ng/L	2.9 ng/L
Cu ²	0.045 $\mu\text{g/L}$	0.150 $\mu\text{g/L}$	Se ²	23.7 ng/L	79.0 ng/L	Bi ²	1.4 ng/L	4.8 ng/L
Mn ²	0.021 $\mu\text{g/L}$	0.070 $\mu\text{g/L}$	Au ²	3.1 ng/L	10.2 ng/L	Tb ²	0.4 ng/L	1.5 ng/L
I ²	0.346 $\mu\text{g/L}$	1.152 $\mu\text{g/L}$	Sb ²	4.2 ng/L	13.9 ng/L	Ho ²	0.1 ng/L	0.3 ng/L
Ba ²	0.072 $\mu\text{g/L}$	0.241 $\mu\text{g/L}$	La ²	0.7 ng/L	2.3 ng/L	Lu ²	0.2 ng/L	0.7 ng/L

V ²	0.005 µg/L	0.016 µg/L	Nd ²	1.1 ng/L	3.6 ng/L	Tm ²	0.1 ng/L	0.4 ng/L
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147 ¹ determined by ICP-OES.

148 ² determined by ICP-MS.

149 2.6. Analysis of Certified Samples

150 To check performance and recovery of the proposed sample treatment for soil, SRM 2586 (Trace
151 Elements in Soil Containing Lead from Paint) certified material from NIST was analysed according
152 to the described treatment. The results, detailed in Table 3, showed good agreement between the
153 certified and observed concentration values.

154 It was not possible, however, to have a certified sample for wine.

155 **Table 3.** Analysis of SRM 2586 certified soil material (Trace Elements in Soil Containing Lead from Paint)

Element	Certified values (mg/Kg)	Uncertainty	Found (mg/Kg)	s.d.
Li	25 ¹		74	0.60
Sc	24 ¹		11	0.04
Ti	6050	660	2310	
V	160 ¹		128	0.40
Cr	301	45	226	1.79
Mn	1000	18	937	
Fe	51610	890	48837	
Co	35 ¹		24	0.21
Ni	75 ¹		150	6.21
Cu	81 ¹		85	1.04
Zn	352	16	369	
As	8.7	1.5	3	
Se	0.6 ¹		3	
Sr	84.1	8.0	131.2	1.71
Y	21 ¹		19	0.16
Nb	6 ¹		3	
Ba	413	18	218	2.64
La	29.7	4.8	27.2	0.59
Ce	58	8	56.2	0.82
Pr	7.3 ¹		7.9	0.08
Nd	26.4	2.9	29.4	0.77
Sm	6.1 ¹		6.0	0.11
Eu	1.5 ¹		1.2	0.04
Gd	5.8 ¹		6.6	0.04
Tb	0.9 ¹		0.9	0.02
Dy	5.4 ¹		4.1	0.04
Ho	1.1 ¹		0.7	0.01
Er	3.30 ¹		2.11	0.05
Tm	0.5 ¹		0.3	0.01
Yb	2.64	0.51	1.68	0.03
Lu	2		0.3	0.001

Cd	2.71	0.54	3	
Hg	0.367	0.038	3	
Pb	432	17	3	
Th	7 ¹		14	0.10

156 ¹ indicative value.

157 ² not determined in SRM.

158 ³ not determined by us.

159 2.7. Data analysis

160 Multivariate data analysis was applied to the dataset composed of 57 variables (the elements
161 determined) and 51 samples of wine. Data analysis and graphical representations were performed
162 with XLSTAT v. 2012.2.02 (Addinsoft, Paris), a Microsoft Excel add-in software package.

163 3. Results and discussion

164 Thanks to the relatively low dilution ratio (1:10) and to the use of high purity reagents, it was
165 possible having good results from a large set of analytes. Indeed, concentrations were higher than
166 LOQ for all the analytes indicated in Table 2. All data (ranges in Table 4) resulted to be compatible
167 with the known ranges of elements in wine [13].

168 **Table 4.** Ranges of concentration in *Barbera d'Asti*, *Barbera d'Asti superiore* and *Nizza* wines

Element	min (mg/L)	max (mg/L)	Element	min (µg/L)	max (µg/L)	Element	min (ng/L)	max (ng/L)
K	418.2	911.1	Pb	2.26	143.5	Y	52	1995
P	154.4	741.0	Ni	17.1	115.9	U	10	1754
S	119.7	639.6	Ti	24.0	92.4	Pd	40	1237
Mg	87.1	346.7	Cr	8.31	45.8	Cd	94	901
Ca	56.1	121.7	Sc	30.6	45.6	Tl	141	620
Na	21.90	149.0	Li	5.40	37.2	Hg	1	568
Fe	0.04	14.99	Mo	1.15	16.8	Gd	6	541
B	2.27	5.91	Sn	0.032	16.5	Pr	2	538
Si	2.46	4.90	As	0.972	13.9	Sm	3	438
Sr	0.83	2.43	Cs	2.32	12.9	Dy	5	372
Rb	0.577	1.858	Co	1.20	8.04	Th	4	305
Al	0.78	1.79	Zr	0.784	7.90	Yb	8	202
Br	0.518	1.591	Nb	0.014	5.75	Er	5	201
Zn	0.109	1.416	Ce	0.035	4.69	Eu ¹	25	176
Cu	0.006	1.132	Se	1.044	3.54	Bi	1	92
Mn	0.036	0.885	Au	0.013	2.69	Tb	1	71
I	0.233	0.506	Sb	0.102	2.46	Ho	1	67
Ba	0.055	0.280	La	0.014	2.31	Lu	1	34
V	0.0003	0.264	Nd	0.005	2.12	Tm	1	29

169 ¹ values suffer from positive interference of Ba.

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171 In the following sections, we will discuss the possibility of using the elemental distribution, or
172 part of it, to distinguish between *Barbera d'Asti*, *Barbera d'Asti superiore* and *Nizza* wines. It must be
173 remembered that *Barbera d'Asti* and *Barbera d'Asti superiore* are indeed parts of the same designation,
174 i.e. *Barbera d'Asti* DOCG, therefore they are produced in the same geographic areas; in addition, the
175 territory of *Nizza* designation is totally contained inside that of *Barbera d'Asti*. Therefore, differences

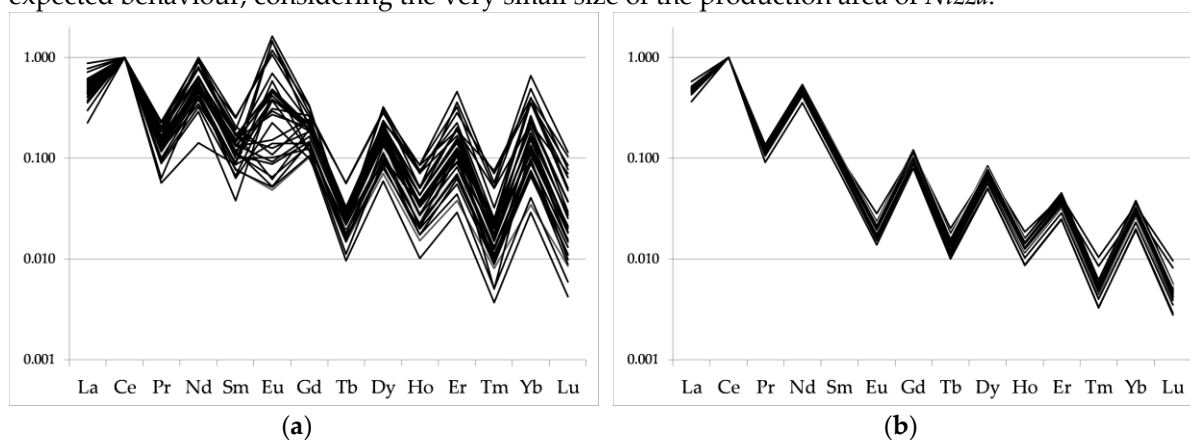
176 among these wines may be expected, rather than from soil, because of oenological practices and, in
 177 particular, of ageing (see Table 1).

178 3.1. Lanthanides

179 Our previous work on the use of lanthanides distribution as traceability markers [11] clearly
 180 indicated that the original fingerprinting given by soil is lost during the winemaking process. The
 181 same conclusion arose from other past works: Jakubowski et al. [14] in 1999 questioned the fact that
 182 rare earth elements (REE) distribution could be considered as reliable fingerprint for the geographic
 183 provenance of a wine. Nicolini et al. [15] and Castiñeira et al. [16] both advised that fining treatment
 184 with bentonite could lead to fractionation of the original trace element distribution in white wines.
 185 Rossano et al. [17] in their study on the influence of clarification, filtration, and storage on the
 186 concentration of REE in white wines, found that these processes provided a range of effects ranging
 187 from an overall increase to fractionation resulting in small increase of light REEs. As to red wines,
 188 Mihucz et al. [18] and Tatár et al. [19] found similar behaviours respectively in Romanian and
 189 Hungarian red wines.

190 The cited studies were mainly focused on the variation of *absolute* concentrations of lanthanides,
 191 or on the variation of their distribution along the wine chain without any reference to soil. In the
 192 present study we wanted to deepen the relationship between soil and wine, by comparing their
 193 distributions after normalisation to Ce according to the formula $[\text{Lanthanide}]_{\text{Ce-normalised}} =$
 194 $[\text{Lanthanide}]_{\text{sample}}/[\text{Ce}]_{\text{sample}}$. The lanthanides distributions of all our wine samples follow the
 195 Oddo-Harkins rule (Figure 2a, Ce-normalised data for *Nizza* wines, shown in logarithmic scale in
 196 order to highlight the differences on the heavy lanthanides that could not be properly appreciated
 197 under a linear scale). The behaviour of some lanthanides, however, is apparently unusual. In
 198 particular, the content of Nd, Dy, Er and Yb is higher than expected. This cannot be ascribed to
 199 isobaric interferences in the determination by ICP-MS: ^{144}Nd is isobaric with ^{144}Sm but its
 200 interference is automatically subtracted via software and the only known polyatomic interference is
 201 from $^{96}\text{Ru}^{16}\text{O}^+$ [20] which can be safely excluded being the level of Ru in our samples under LOD;
 202 ^{163}Dy has positive interference from $^{147}\text{Sm}^{16}\text{O}^+$ but ^{147}Sm accounts for only 15% of total Sm; ^{174}Yb has
 203 positive interference from $^{158}\text{Gd}^{16}\text{O}^+$ (^{158}Gd accounts for 25% of total Gd) but the Gd/Yb ratio is
 204 ranging from 0,304 to 3,618, so no correlation seems to exist. The behaviour of ^{167}Er could be
 205 explained in terms of positive interference from $^{151}\text{Eu}^{16}\text{O}^+$, as ^{151}Eu has in turn interference from
 206 $^{135}\text{Ba}^{16}\text{O}^+$, but no correlation exists indeed between ^{167}Er and $^{151}\text{Eu}^{16}\text{O}^+$, nor between ^{167}Er and ^{135}Ba .

207 By contrast, the lanthanides distributions determined in the corresponding samples of soil,
 208 collected at every location of *Nizza* producers (Figure 2b), are highly homogeneous and closely
 209 follow the Oddo-Harkins rule with a general lowering trend of heavy lanthanides. This is the
 210 expected behaviour, considering the very small size of the production area of *Nizza*.



211 **Figure 2.** Lanthanides distributions in samples of *Nizza* wines (a) and in the corresponding soils (b).

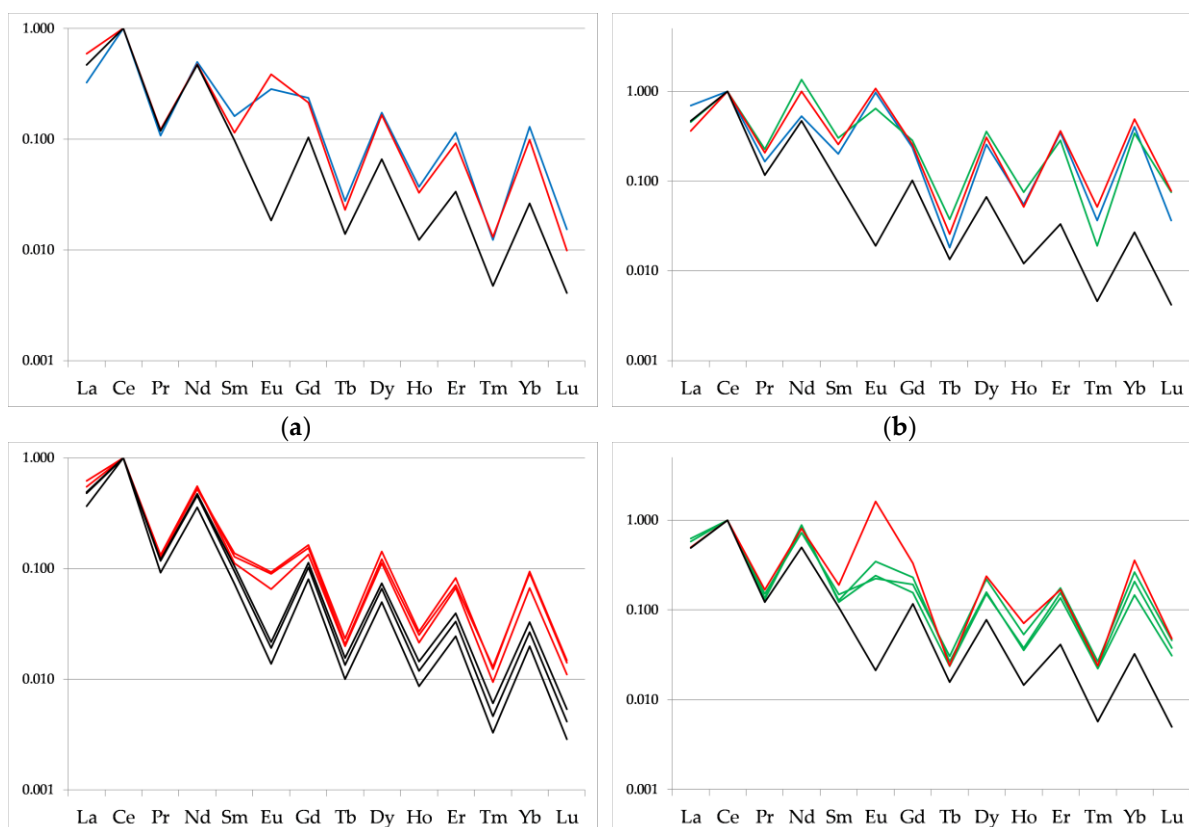
212 To evaluate numerically the different behaviour of lanthanides in wines and soils, as far as
 213 Ce-normalised data are concerned, the average RSD (calculated on all lanthanides except Ce) was
 214 55.2% in wines but only 10.0% in soil samples.

215 In the end, it must be accepted the fact that the winemaking processes had heavily influenced
 216 the lanthanides distribution, possibly as a consequence of the use of clarifying materials such as
 217 bentonite, as it was already cited in our previous work on Moscato d'Asti [21]; bentonites are indeed
 218 used by nearly all the producers of *Nizza* wine. According to these results, it is apparent that
 219 lanthanides cannot act as traceability markers as they are not representative of the original
 220 fingerprint, i.e. the distribution in soil. Not surprisingly, an attempt of distinguishing between
 221 *Barbera d'Asti*, *Barbera d'Asti superiore* and *Nizza* wines on the base of Ce-normalised data of
 222 lanthanides, using pattern recognition techniques, was unsuccessful (data not shown).

223 3.2. Comparison between wines and soils

224 It was possible to deepen the knowledge on the behaviour of lanthanides considering the cases
 225 where a winemaker produced two or three designations starting from grapes grown on the same or
 226 similar soil. Figure 3 shows some comparisons between wines and corresponding soils
 227 (Ce-normalised data, logarithmic scale):

- 228 (a) comparison between one *Barbera d'Asti* and one *Nizza* wine produced from the same vineyard:
 229 apparently, they show the same distribution, different from that of the corresponding soil;
 230 (b) comparison between one *Barbera d'Asti*, one *Barbera d'Asti superiore* and one *Nizza* wine
 231 produced from the same vineyard: again, the three wines have the same distribution, different
 232 from that of soil;
 233 (c) comparison between three *Nizza* wines obtained by a producer from grapes cultivated in three
 234 different but very close vineyards inside a small area: the three wines are more similar among
 235 themselves than to each respective soil;
 236 (d) comparison between three *Barbera d'Asti superiore* wines and one *Nizza* wine obtained by a
 237 producer from grapes cultivated in the same vineyards: the four wines are more similar among
 238 themselves than to soil.
 239



(c)

(d)

240 **Figure 3.** Comparison of lanthanides distributions in wines and in the corresponding soils in four cases (blue
241 line: *Barbera d'Asti* wine; green line: *Barbera d'Asti superiore* wine; red line: *Nizza* wine; black line: soil).

242 The results illustrated above highlight the fact that winemaking, irrespective of vintage and
243 ampelographic composition, is much more important in determining the final lanthanides
244 distribution in wine than the geochemical source.

245 3.3. Other trace- and ultra-trace elements

246 Despite the unsuccessful attempt of using lanthanides to distinguish between *Barbera d'Asti*,
247 *Barbera d'Asti superiore* and *Nizza* wines, we wanted to explore the behaviour of the other trace- and
248 ultra-trace elements. Indeed, many authentication studies on wines generically exploit the whole of
249 trace elements rather than only lanthanides [6,22–24]. Hopfer et al. [25], as an example, were able to
250 classify Californian wines according to their vineyard origin and their processing winery with
251 respect of soil elemental content and viticultural practices.

252 It is well known that winemaking treatments can affect the mineral content of wine.
253 Clarification with bentonites has strong effects in varying the original metal distribution [26], as
254 already pointed out with reference to lanthanides. Fermentation with different yeast strains
255 markedly affects the content of alkaline, alkaline-earth and transition metals [27]. In a recent study,
256 Catarino et al. [28] followed the trend of elements during winemaking, highlighting the role of the
257 different steps in modifying the original elemental composition in soil.

258 Pohl reviewed the possible sources of metals [13] in wine indicating the primary source as the
259 natural contribution from soil, regulated by the climatic condition during grapes growth; a
260 secondary source in the external impurities coming from environment, outside and inside the cellar
261 work; a third source in the oenological practices. Other sources of variation can be the following:

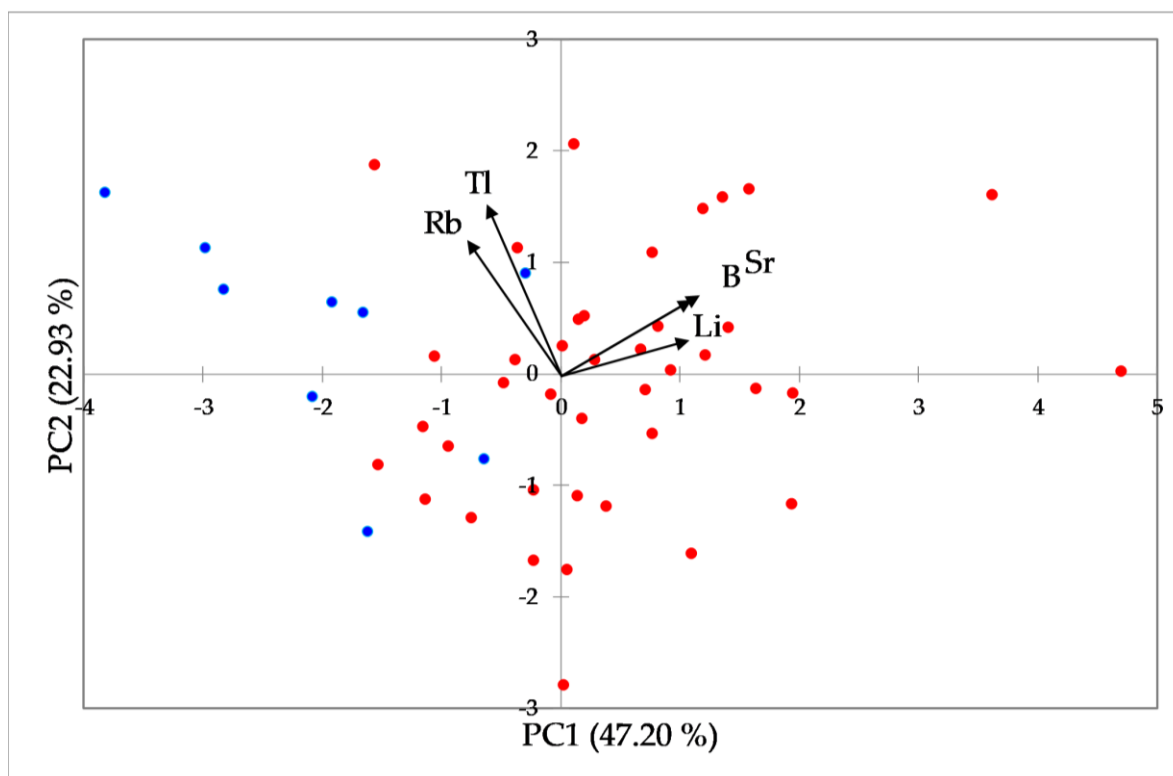
- 262 • pH of soil;
- 263 • type of rootstock;
- 264 • vine growing system;
- 265 • type of cultivar;
- 266 • time of harvest (it can change from one zone to another and from a farm to another, even at
267 short distances)
- 268 • type of collection (manual and/or mechanical)
- 269 • Transfer time (from vineyard to cellar) and temperature conditions
- 270 • Different types of processing that the product can undergo depending on the objectives of the
271 company grape pressing (time, duration, temperature)
- 272 • use of yeasts (usually different from a farm to another)
- 273 • duration of maceration and therefore of extraction from skins;
- 274 • further processing steps (ageing in steel, barrique - type of wood and provenance - or bottles);
- 275 • conservation conditions (temperature, relative humidity, etc.).

277 Another factor to be considered is of course the thermopluviometric trend, but in this work all
278 wine samples were from the same vintage.

279 After evaluating the role of lanthanides, in our study we used all the elements determined by
280 ICP-OES and ICP-MS to verify the possibility of discriminating between *Barbera d'Asti*, *Barbera d'Asti*
281 *superiore* and *Nizza* wines. The dataset was composed of 57 variables (the elements determined) and
282 51 samples (wines of the three designations). Principal Components Analysis (PCA) was used; data
283 were transformed into z-scores before analysis. However, no satisfactory results were obtained (data
284 not shown).

285 Better results were obtained after dividing the samples into two groups, the first containing
286 *Barbera d'Asti* wines and the second containing *Barbera d'Asti superiore* plus *Nizza* wines, i.e. the
287 younger wines against the more aged ones. A preliminary test by means of Analysis of Variance
288 (ANOVA) indicated that Li, Rb, Sr, B and Tl were the variables with the higher discriminating power

289 within this scheme. We then carried out PCA analysis using only these five variables: the results of
 290 PC1 vs PC2 score plot (Fig. 4), accounting for 70.13% of total variance, suggests that a good
 291 discrimination is achievable between the younger *Barbera d'Asti* (blue circles in figure) and the more
 292 aged *Barbera d'Asti superiore* and *Nizza* wines (red circles in figure).



293

294 **Figure 4.** PC1 vs. PC2 score plot obtained using Li, Rb, Sr, B and Tl. Blue circles: *Barbera d'Asti*
 295 samples; red circles: *Barbera d'Asti superiore* and *Nizza* samples. Black arrows indicate loadings.

296 The information arising from the loadings (black arrows in figure) indicates that *Barbera d'Asti*
 297 *superiore* and *Nizza* wines have a higher content of B, Li and Sr, while *Barbera d'Asti* wines have a
 298 higher content of Rb and Tl. Although alkaline and alkaline-earths elements are considered good
 299 indicators of geographical origin, in the present study their role must be considered in the light of
 300 oenological practises, being the origin of the samples nearly the same or at least too close to be
 301 discriminated (it must be remembered that the samples of *Barbera d'Asti* and *Barbera d'Asti superiore*
 302 analysed in this study come from producers of *Nizza*). Three factors must be considered:

- 303 1. The alcoholic content: Catarino et al. [28] showed that the concentration of Rb is inversely
 304 proportional to alcohol %, which is in agreement with our data if we consider that the average
 305 alcohol % is 14.2 for *Barbera d'Asti* wines and 14.7 for *Barbera d'Asti superiore/Nizza* wines.
- 306 2. The widespread use of bentonites by producers of these wines: Catarino et al. [26] showed that
 307 this treatment causes a strong fractionation of the original elemental distribution in musts; in
 308 particular Li, Sr and Tl were found to increase after bentonites treatment, while B and Rb
 309 decreased. However, bentonites are widely used in the production of all *Barbera* designations.
- 310 3. The main difference between *Barbera d'Asti* and *Barbera d'Asti superiore/Nizza* is ageing, which
 311 involves a more or less prolonged contact with barriques. Kaya et al. [29] studied the effect of
 312 wood aging on the mineral composition of wine; Sr was found to increase significantly in wines
 313 aged in wood, while for Li, Rb and Tl no significant effect was registered. These results partially
 314 confirm the differences found in our study with concern to Sr, which is higher in *Barbera d'Asti*
 315 *superiore/Nizza* than in *Barbera d'Asti*.

316 In the end, it is possible that the elemental differences arisen in this study be a combination of
 317 all the factors above described. The role of Tl is hard to be explained, considering that this metal

318 must be included in the group of contaminant elements of wine [30]. Even the role of B is still to be
319 accounted for.

320 4. Conclusions

321 The results obtained from the elemental analysis of *Barbera d'Asti*, *Barbera d'Asti superiore* and
322 *Nizza* wines show clearly that the distribution of metals in wine reflect the features of oenological
323 practises rather than the features of soil, in particular with concern to lanthanides. Nevertheless,
324 despite the fact that these three wines are produced in very close if not overlapping areas, it is
325 possible to discriminate the younger *Barbera d'Asti* from the more aged – and more valuable - *Barbera*
326 *d'Asti superiore* and *Nizza* according to the elemental content, using as chemical descriptors some
327 metals present at trace level concentration, that is Li, Rb, Sr, B and Tl.

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331 performed the experiments; M.A., E.R., M.P. and C.T. analysed the data; M.A. wrote the paper.

332 **Conflicts of Interest:** The authors declare no conflict of interest.

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