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(Article begins on next page)

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Tracing the *"terroirs"* via the elemental composition of leaves, grapes and derived wines in cv Nebbiolo (*Vitis vinifera* L.)

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Abstract

Thirty-nine samples of leaves and berries from eight sites and sixteen related samples of wines coming from seven sites were analyzed by means of inductively coupled plasma - atomic emission spectroscopy (ICP-AES). Twenty elements (Al, Ba, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Ni, Sb, Si, Sr, Ti, V, Zn, Zr) were analyzed. The aims of this study were to determine whether some elements could be used to trace the origin of grapes and wines and whether rootstock and variety may contribute to the selective uptake of these elements. In addition, to improve the classification by geographical origin, this study evaluated whether the presence of some elements is more dependent on the environment or on the rootstock/variety. Principal component analyses (PCA) were applied to leaf, berry and wine elemental composition to distinguish among the sites. A non-parametric test was used to compare the means of the five plots of leaves and berries for the rootstock and variety mineral composition.

A good separation was obtained among the leaf, grape and wine samples with respect to their geographic origin. Ba, Mn, Si, Sr and Ti had the most discriminant power to separate the sites of the Alpine area from the sites characterized by calcareous clay soils of the Langhe area. Rootstock and variety had effects on the selective absorption of several elements in grapes and leaves but the grape mineral content seemed more efficient in discriminating samples than leaf mineral content. Although Ba, Si and Ti absorption showed the influence of rootstock and variety, they played a major role in discriminating the geographical origin of the samples.

1. Introduction

The production of wines with denomination of origin is a pillar of the European wine industry. Among those that stand out for their quality and economic importance are wines from Italy with Denomination of Origin Controlled (DOC) and Guaranteed (DOCG), where the conformity of the product is controlled by a number of public bodies per EU law (Reg. 479/2008) and Italian national laws (Leg. Decree 61/2010). Compliance with the wine DOC requires that both the varietal composition and the grape and wine geographical origin have to be guaranteed. Recent studies have shown that elemental analysis can be a valuable tool for identifying the geographical origin of wine (Marengo and Aceto, 2003; Greenough et al., 1997; Gonzalvez et al., 2009, Coetze et al., 2005) and how different wines can be classified according to the basic profile and based on the area of origin of the grapes. Other studies show some relationships between the ground source, the geological origin and the behavior of the vine (Greenough et al. 2005, Conradie et al. 2002; Larcher et al. 2003).

The element composition seems to be a powerful instrument to discriminate wines and grapes coming from different geographical origins and for use as a sort of fingerprinting to trace the origin of the vegetal products (Bertoldi et al., 2011; Aryiama et al., 2007). Based on the literature, using the mineral distribution for classification purposes requires consideration of global factors. In particular, these factors include i) the different origins of the grape metal content (from geological processes or by human addition and pollution) (Marengo and Aceto, 2003); ii) the property of the soil on which the vineyards are located; iii) the capacity of the vine (variety, rootstock, eco-physiology) to absorb the element from the soil; and iv) the contribution of fertilization during the grape-growing cycle.

It is well known that the variety and rootstock can affect the mineral uptake (Fekete et al., 2012; Lehoczky and Kocsis, 1998, Scienza et al., 1986), but previous studies do not consider their effect in the mineral accumulation in the berry.

Therefore, the aims of this study were to determine whether some elements could be used to trace the origin of grapes and wines as well as whether the rootstock and variety may contribute to the element uptake. In addition, we were interested in defining whether the presence of some elements is dependent on the local environment or is rootstock/variety specific, to perform a better geographical origin classification. Grapes, leaves and wines were analyzed using atomic spectrometry. This method is fast and easy to use once the procedure proposed in literature is optimized. This procedure, coupled with multivariate statistical analysis, is particularly suitable for the purposes of wine classification.

2. Materials and methods

2.1. Sites

The sampling sites were located in northwestern Italy: Treiso (44º41.253' N 8º05.030' E), Sinio (44º36.093' N 8º00.570' E) and Monforte (44º35.667' N 7º57.617' E) in South Piedmont, Mezzomerico (45º36.549' N 8º35.835' E), Briona (45º32.739' N 8º29.952' E) and Agliè (45º22.494' N 7º47.524' E) in North Piedmont, Pont Saint Martin (45º36.202' N 7º47.267' E) in Aosta Valley and Sondrio (46º10.280' N 9º44.785' E) in Lombardy (Fig. 1). In these areas, the Nebbiolo grape variety (*Vitis vinifera* L.), one of the most important Italian cultivars used for the production of world-renowned red DOC and DOCG wines, is widely grown.

2.2. Dataset

The dataset comprised three main groups of samples: leaves, grapes and related wines, all picked during the 2010 season (Table 1). The datasets created to compare geographic origin were composed of 39 samples of leaves and grapes picked at eight sites (Table 1a) and of 16 samples of wine coming from seven sites (Table 1b). The comparisons between two

rootstocks, 420A and Kober 5BB (K5BB), and two varieties (Nebbiolo and Barbera) were assessed in two sites, Mezzomerico and Treiso, Mezzomerico and Monforte, respectively. For the rootstock comparison, 15 samples of cv Nebbiolo were used (Table 1c); the comparison between varieties was based on 12 samples (Table 1d). The concentration of twenty elements were determined (Table 2): 17 in the leaf samples and 18 in the grape and wine samples (Table 3).

2.3. Leaf and grape group samples

At each site, a different number of sampling plots was identified (Table 1). Each sampling plot contained five contiguous vines. At ripening, 10 berries per each bunch were picked and pressed. Soluble solid content (22-24 °Brix), total acidity (7.25-10 g/L as tartaric acid) and pH (3.10-3.30) in the must were then assessed. In addition, the leaf opposite the basal cluster of each shoot, was sampled for each vine. The set of samples obtained from the five plants of the plot represented the samples used for the elemental analysis.

2.4. Winemaking procedure

At commercial harvest, 60 kg of grapes, divided into two replicates, were collected from the same plants used to make elemental determinations for leaves and grapes; to achieve the minimum quantity for the winemaking, grapes were also collected from adjacent plants. Grape berries were destemmed and crushed in enological little steel tanks (100 L). The must was fermented using the *Saccharomyces cerevisiae* yeast strain BRL97 (Lallemand, Montreal, Canada), and maceration was carried out for 12 days. The fermentation temperature was maintained at 27±1 ºC. Two pumping-over without aeration for each day were performed. After this maceration/fermentation stage, the grape pomace was pressed by a small pneumatic press (volume 400 L, Velo SpA, Italy) with a maximum pressure of 1.20 bar. After adjusting

the free sulfur dioxide content to 60 mg/L to avoid malolactic fermentation, the wines were filtered and bottled in 0.75-L glass bottles.

2.5. Chemical analysis

2.5.1. Sample preparation

Procedures for sample preparation, optimized for each type of sample based on wet digestion methods (Matusiewicz, 2003), are described in brief. Leaves were washed with distilled water after collection, dried at room temperature and ground with an electric grinder. Grape samples were dried at 150 °C in an oven and then ground with a mortar . One gram of the powders was then transferred in Teflon vessel and treated three times at 100 °C, alternating between 2 mL of *aqua regia* (concentrated hydrochloric acid and nitric acid, 3:1 v:v) and 1 mL of hydrogen peroxide solution (30 %), waiting 30 min between each addition of reagents. The solution was maintained at 100 °C for 1 h, then cooled, diluted to known volume and analyzed.

Wine samples (2 mL) were transferred to a Teflon vessel and mineralized for 2 h at 100 °C with 3 mL of concentrated nitric acid. The solution obtained was neutralized, diluted to known volume with ultrapure water and analyzed. Each analysis was performed in triplicate.

All chemicals (nitric acid, hydrochloric acid, hydrogen peroxide and ultra-pure water) were purchased for Atomic Spectrometry at analytical grade from Sigma-Aldrich (Milan, Italy).

2.5.2. Instrumental conditions

Analyses were performed by means of Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) ICAP 6300 (Thermo Scientific, Waltham, MA, US). Emission wavelengths of the elements of interest were chosen by optimizing the instrumental

parameters to obtain good sensitivity and accuracy and limit interference between different element emissions; values of selected wavelengths are reported in Table 2.

To ensure proper repeatability of the results obtained from the analysis of mineral elements, the instrument operating conditions must remain constant. For these determinations, the following values were used: power of torch 1550 W, pump speed 50 RPM, auxiliary gas flow 0.50 L/min, nebulization gas flow 0.70 L/min.

2.6. Multivariate statistical methods

All of the data were normalized and submitted to the principal component analysis (PCA) (Kwan and Kowalski, 1978). The new variables, the principal components (PCs), were orthogonal to each other, and they were calculated to maintain most of the information of the original dataset in the least possible number of new variables. Plotting PC scores makes it possible to perform a graphical inspection of the data to reveal biological meanings hidden in the dataset. Numerous PCAs were performed for each dataset to identify a pool of variables justifying an opportune level of variance and useful for discriminating sites, rootstock or varieties on the basis of the leaf, berry and wine element concentration. To evaluate sampling adequacy and to test the correlations between variables, the Kaiser-Meyer-Olkin (KMO) index and the Bartlett's test of sphericity were applied, respectively. To estimate the degree of classification obtained with the analytical data coming from the rootstock and variety comparisons, the Mann-Whitney non-parametric test was used to test the differences between group means. The efficiency of this test is greater than the t-test on non-normal distributions, and it is nearly as efficient as the t-test on normal distributions.

All statistical analyses were performed with SPSS for Windows, ver. 17 (IBM, New York, USA).

3. Results and discussion

3.1. Geographic comparison

The concentration of all of the elements analyzed in the leaf blades, whole berries and wines were within the ranges known for leaves (Fekete et al., 2012; Blaich and Hedwig, 1997), grapes (Bertoldi et al., 2011) and wines (Taylor et al., 2003; Aceto and Marengo, 2003) (Table 3). After carrying out the Z-Score normalization procedure, the PCA was applied to the different dataset to identify the most powerful elements (Table 4) in discriminating the geographical origin of the samples consistent with the leaf, berry and wine element composition.

3.1.1. Leaves

When PCA was applied to the Ba, Mg, Mn, Si, Sr and V concentration in the leaves, the Kaiser-Meyer-Olkin (KMO) test of sampling adequacy was 0.74 and the Bartlett's test of sphericity was highly significant for the correlations between these variables. The first two PCs explained 82.8 % of the total variance (57.4 % and 25.4 %, respectively), and only those PCs showed an Eigenvalue greater than one (3.4 for PC_1 and 1.5 for PC_2), well representing the information from the original data matrix. V, Ba, Si and Mn were mainly correlated with the PC_1, whereas Sr was correlated with PC_2 (Fig. 2a, Table 4).

The model including the elements listed above was powerful for the discrimination of sites (Fig. 2d). PC_1 was able to discriminate the geographic areas, clustering them into three major groups: Northern Piedmont (NEA, NEB, NEM), Lombardy, Aosta Valley and Monforte (NESO, NEP, NEMO) and Sinio and Treiso (NES, NET), thanks to the concentration of Ba, Si and V, which decreased from right to left in the chart. PC_2 discrimination pattern showed the power of the Strontium (Sr) in distinguishing the individual sites included in a similar area due to its decreasing concentration from top to bottom in the chart. The Sr concentration was

highest in Sinio and lowest in Lombardy (NESO). The geological matrix of the South Piedmont, is mainly limestone whereas for the sites of geological Alpine origin (NEA NEB, NEM, NESO, NEP), it is mainly granitic siliceous (AAVV, 2000; Failla et al., 2000; Mannini et al, 2010; Tropeano, 1984).

3.1.2. Grapes

When PCA was applied to the Ba, Mg, Mn, Si, Sr, V and Ti concentration in the grapes, the KMO test was 0.70, and the Bartlett's test of sphericity was highly significant. The first three PCs explained 83.0 % of the total variance (47.0 %, 21.0 % and 15.0 %, respectively), and they also had Eigenvalues greater than one (3.3 for PC_1, 1.50 for PC_2 and 1.0 for PC_3). Mg, Ba, Si, Mn were mainly correlated with PC_1; V and Ti with PC_2; and Sr with PC_3 (Fig. 2b, Table 4).

The model allowed to discriminate and group the sites from Southern Piedmont (NES, NET, NEMO), where the geological matrix is mainly limestone, with respect to the sites of geological Alpine origin (NEA, NEB, NEM, NESO, NEP). The grapes from South Piedmont sites were characterized by lower concentration of Mg, Ba, Si and Mn in the grapes (Fig. 2e).

3.1.3. Wines

When PCA was applied to wine element composition, KMO and the Bartlett's test showed the best discriminant power of the model with Ba, Mn, Si, Sr and Ti. The first two PCs explained 78.4 % of the total variance (55.8 % and 22.6 %, respectively) showing Eigenvalues of 2.8 for PC_1 and 1.1 for PC_2. Mn, Sr, Ba and Si were correlated with PC_1 and Ti was correlated with PC_2 (Fig. 2c, Table 4). This model allowed to group the wines produced in the Alpine Region, whereas wines from Sinio (NES) and Monforte (NEMO) were well separated because of their levels of Sr and Ti, respectively (Fig. 2f).

Using the statistical analyses performed on the samples of leaves, grapes and wines coming from the different sites, it has been possible to discriminate the geographical origin of the samples. The separation of the sites on the base of leaf-blade element composition indicated that vines probably accumulate Ba, Mg, Mn, Si, Sr and V based on the soil composition and structure (Kabata-Pendias, 2004). It is known that the Langhe vineyards, where the samples NEMO, NES and NET were collected, are characterized by calcareous limestone soils (pH > 8), whereas the soils of Mezzomerico, Briona, Agliè, Sondrio and Pont Saint Martin tend to have an acidic or sub-acidic pH, with significantly lower percentages of clay (Failla et al., 2000; Mannini et al, 2010; Tropeano, 1984). It is well known that at high pH and in clay-rich soils Ba (Lamb et al., 2013), Sr (Kowalenko and Ihnat, 2010) and Mg (Fregoni, 2005) are more available for plants; Si and Ti availability seems to be related to soil total concentration more than to soil pH (Sangster and Hogson, 1986; Wójcik et al., 2010), whereas Mn and V, are more available in soil with low pH (Fregoni, 2005; Larsson et al., 2013). Nevertheless, the foliar and berry content is also regulated by the synergism and antagonism between elements, as well as by the specific genetic resistance mechanisms which are active when certain elements, in particular those having toxic effects for plants, such as Ba, Mn and Sr, are too concentrated in the soil nutrient solution (Fregoni, 2005; Lamb et al., 2013). For these reasons, a high bioavailability of a specific element in soil does not necessarily mean a high concentration in plant organs.

The mineral elements of wines useful in the identification of their geographical origin were largely the same as those used for leaves and grapes, although with quite different discriminating power. Additionally, we obtained a clear separation between the South Piedmont and Alpine regions (North Piedmont, Aosta Valley and North Lombardy). Wines and leaves from Sinio, contained the greatest concentration of Sr and wines from Monforte contained the highest concentration of Ti. The concentration of Sr also allowed to highlight

the differences and similarities among sites in the Alpine region: in fact, we observed similarities not only between Briona and Mezzomerico, which are geographically close (Fig. 1), but also between Agliè and Sondrio, which are relatively far apart. In contrast, Sr was not a primary indicator for the description of the sites by means of elemental composition of leaves and grapes, which may indicate a likely different behavior during winemaking. However, it was evident that the relationship among the elements in the wine were different from those in leaves and grapes; in the leaves, Sr was more concentrated in Sinio samples and less in Sondrio; in the wines, Sr was more concentrated in Sinio but less in Briona and Mezzomerico.

It seems possible to trace the geographic origin of the wine samples following the Ba, Mn, Sr, Si and Ti concentration, also in a small geographic area, confirming the results of several studies comparing commercial wines originating from different parts of the world (Marengo and Aceto, 2003; Sen and Tokatli 2014; Taylor et al., 2003).

3.2 Rootstock effect

As described in section 2.2, the rootstock assimilation of several soil elements was assessed by analyzing the mineral profile of leaves and grapes (Table 3) of Nebbiolo vines grown in two sites (Table 1c) and testing the differences by means of a single Mann-Whitney non-parametric test. Elements that showed different accumulation ($p \leq 0.05$ Mann-Whitney U test) in leaves and grapes as a function of the rootstock (Nebbiolo grafted onto 420A or K5BB in Mezzomerico and Treiso) are reported in Table 5.

K and Ti in Mezzomerico, and Ca, Cr, Mo, Ni and Ti in Treiso were similarly influenced by the rootstock in leaves and grapes. In addition Ca, Cr and Ti in the leaves and Co, K, Mg, Mo, Ni, Sb, Si, Ti in the grapes, permit to separate the rootstocks in both sites; only Ti was similarly influenced at both sites and in leaves and grapes (Table 5a), confirming the already

established discriminant power of this last element. The differences were not always rootstock-specific for all elements because their uptake may change among the sites. This is probably related to the dynamics of uptake and translocation of the individual elements, which are influenced by synergies and competition among elements, factors that are objectively difficult to evidence clearly.

Using PCA, it was possible evaluate the impact of the rootstock on mineral assimilation in leaves and grapes. When PCA was applied to the leaf samples, Ca, Cr, K, Mo, Ni, Ti, and Sb levels showed a KMO test of sampling adequacy of 0.74, and the Bartlett's test of sphericity was highly significant for the correlations between these variables. The first two PCs explained 95.1 % of the total variance (70.5 % and 24.6 %, respectively), showing an Eigenvalue greater than one for PC_1 (4.9) and PC_2 (1.7). Mo, Sb, K and Cr were mainly correlated with PC_1, whereas Ca and Ti were mainly correlated with PC_2 (Fig. 3a, Table 6). Using PC_1 (Fig. 3c), it was clearly possible to discriminate the geographic origin of the samples, and the rootstock selectivity became evident on the basis of PC_2, but mainly for the Treiso samples. Considering the leaf content, Ca and Ti seemed to be more "rootstock-specific" elements, and 420A seems more able to absorb these elements than the K5BB.

In the grape model, PCA for Co, Cr, K, Mg, Mn, Mo, Ni, Si and Ti showed a KMO test of sampling adequacy of 0.73, and the Bartlett's test of sphericity highly significant for the correlations between these variables. The first two PCs explained 83.0 % of the total variance (45.7 % and 37.3 %, respectively), showing an Eigenvalue greater than one (4.1 for PC_1 and 3.4 for PC_2). Mg, Mn, K, Cr and Si levels (associated with PC1) seem to be more site specific; the uptake of Ti, Mo, Co, Ni, associated with PC2, seems to be more rootstock-specific (Fig. 3b). Mo, Ni and Ti mainly accumulated in grapes of vines grafted onto 420A, while grapes from vines grafted onto K5BB accumulated more Co. The berries coming from Mezzomerico were richest in Cr, Mg, Mn, K and Si, in contrast to the berries coming from

Treiso (Table 6a, Fig. 3d). The berry model was more useful than the leaf model for separating both sites and rootstocks, showing that the analysis of berries could be more than enough to discriminate sites or rootstocks.

3.3. Varietal selectivity

Using the same statistical approach as performed in section 3.2, the variety effect on the uptake selectivity was compared analyzing leaf and berry mineral content from two sites (Mezzomerico and Monforte) where both Nebbiolo and Barbera varieties were grown (Table 1d).

Like that for the rootstocks, different behaviors of leaf and berry accumulation emerged in relation to the specific sites (Table 5b). Significant differences in mineral accumulation emerged between Nebbiolo and Barbera regarding the accumulation of Ba, Ca, Cu, K, Mg, Mn, Sb, Sr, Ti and V in the leaves and of Ba, Co, K, Mn, Mo, Ni, Sb, Si, Sr and V in the grapes. Only five elements in leaves (Ba, Cu, K, Sb, V) and four in grapes (Ba, Ni, Sb, Si) showed differences between varieties in both sites. In Mezzomerico, the differences between varieties were more accentuated. The only common elements between leaves and berries that differentiate the two varieties in both sites were Ba and Sb.

PCA was applied to the leaf Ba, Cr, K, Mg, Mn, Si and Sr content: KMO test of sampling adequacy was 0.64 and the Bartlett's test of sphericity was highly significant for the correlations between the variables. The first two PCs explained 79.7 % of the total variance (54.2 % and 25.5 %, respectively), and only those PCs showed an Eigenvalue greater than one (3.8 for PC_1 and 1.8 for PC_2), synthesizing the great part of the information of original data matrix. K, Ba, Cr, Mg, were mainly correlated with the PC_1, whereas Mn was mainly correlated with PC_2 (Table 6b, Fig. 4a). In the leaf model (Fig. 4c), PC_1 discriminated the geographic origin of the samples, while PC_2 mainly discriminated the varieties in particular in

Mezzomerico. In Mezzomerico leaves concentration of Ba, Mn, Mg and Si was higher than in Monforte where, instead, Cr and K were more concentrated (Fig. 4c). Mn and, to a lesser extent, Mg and Si (Table 6b), had a relevant power in discriminating both sites and varieties. In Mezzomerico, where the differences were clearer, Barbera showed higher Mg and Si amounts and lower Mn accumulation than Nebbiolo whereas in Monforte this was less evident.

PCA applied to the berry Ba, Ca, Cr, Mg, Mn, Ni, Ti, V and Zr levels showed a KMO test of sampling adequacy of 0.75, and the Bartlett's test of sphericity was highly significant for the correlations between these variables. The first three PCs explained 88.9 % of the total variance (58.6 %, 18.0 % and 12.2 %, respectively), showing an Eigenvalue greater than one (5.3 for PC_1, 1.6 for PC_2 and 1.1 for PC_3), and synthesizing most of the information of the original matrix. Zr, Ca, Ti, Ba, Cr, Ni and Mn were mainly correlated with the PC_1, whereas V was mainly correlated with PC_2, and Mg with PC_3 (Table 6b, Fig. 4b). As observed for the leaf, also in berry models PC_1 was clearly able to distinguish the sample geographic origin, while PC_2 was mainly related to the variety mineral uptake/translocation selectivity (Fig. 4d). Barbera berries tended to be richer in V than Nebbiolo: for both varieties, the composition depended on the site. As already observed in the comparison of rootstocks, the model based on the grape elemental content seemed more efficient in discriminating among the sites and varieties.

4. Conclusions

This study investigated the possibility of characterizing and tracing the *terroir* of origin of grapes, leaves and wines made with two different cultivars (Barbera and Nebbiolo) and considering the effect of two rootstocks (420A and Kober 5BB). The ICP-AES analysis of the mineral composition coupled with multivariate statistical analysis appears to be a useful

method for characterizing and classifying samples from different geographic areas, even when the geological background was similar. The mineral elements of leaves, berries and wines showing the most geographic discriminating power were Ba, Mn, Sr and, to a lesser extent, Si and Ti. Levels of Ca, Cr and Ti in leaves and of Co, K, Mg, Mo, Ni, Sb, Si and Ti in grapes mainly depended on the rootstock selectivity in both studied sites; Ba, K, Sb and V in leaves and Ba, Sb, in berries were mainly linked to the variety selectivity.

The grape mineral content appeared to be associated with the variety and rootstock, and it seemed more efficient in discriminating samples than leaf mineral content. In addition, although there was a clear selectivity for Ba, Si and Ti due to the variety or rootstock, these elements still allowed the identification of the origin of the samples, confirming their validity as tracers of the geographical origin.

From the second part of our still ongoing study, seems emerge that the models used for tracing geographic proveniences and varieties may work independently of the vintage. The vintages, in their turn, appear to be discriminated on the base of models that take into consideration other mineral elements such as those the uptake of which depends on the seasonal meteorological conditions.

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Table 1. The group of leaf, grape (a) and wine (b) samples considered for the geographic determination and the group of leaf/grape samples considered for the rootstock (c) and variety (d) mineral uptake selectivity comparison.

		Symbol	Variety	Rootstock	Site	No. of sampling plots
a)	**leaf/grape** samples considered for the **geographic** comparison	NEA	Nebbiolo	420 A	Agliè	3
		NEB	Nebbiolo	420 A	Briona	3
		NEM	Nebbiolo	420 A/ K5BB	Mezzomerico	6
		NEP	Nebbiolo	K5BB	Pont S. Martin	9
		NEMO	Nebbiolo	K5BB	Monforte	3
		NES	Nebbiolo	420 A	Sinio	3
		NESO	Nebbiolo	420 A	Sondrio	3
		NET	Nebbiolo	420 A/K5BB	Treiso	9
b)	**wine** samples considered for **geographic** comparison	NEA	Nebbiolo	420 A	Agliè	2
		NEB	Nebbiolo	420 A	Briona	2
		NEM	Nebbiolo	420 A/ K5BB	Mezzomerico	4
		NEP	Nebbiolo	K5BB	Pont S. Martin	2
		NEMO	Nebbiolo	K5BB	Monforte	2
		NES	Nebbiolo	420 A	Sinio	2
		NESO	Nebbiolo	420 A	Sondrio	2
c)	**leaf/grape** samples considered for **rootstock** comparison	MEA	Nebbiolo	420 A	Mezzomerico	3
		MEB	Nebbiolo	K5BB	Mezzomerico	3
		NETA	Nebbiolo	420 A	Treiso	3
		NETB	Nebbiolo	K5BB	Treiso	6
d)	**leaf/grape** samples considered for **variety** comparison	BAM	Barbera	K5BB	Mezzomerico	3
		BAMO	Barbera	K5BB	Monforte	3
		NEM	Nebbiolo	K5BB	Mezzomerico	3
		NEMO	Nebbiolo	K5BB	Monforte	3

Table 2. List of the elements analysed in leaves, grapes and wines and emissions wavelength utilized for the ICP-AES analysis.

	Wavelengt considered (*nm*)		
Al	167.079	**Mn**	257.079
Ba	233.527	**Mo**	202.030
Ca	273.690	**Ni**	221.647
Co	228.616	**Sb**	206.833
Cr	267.761	**Si**	251.611
Cu	324.754	**Sr**	421.552
Fe	238.204	**Ti**	334.941
K	769.896	**V**	309.311
Li	670.784	**Zn**	213.800
Mg	279.079	**Zr**	343.823

Table 3. Element concentration range in the leaf, berry and wine samples. For leaves and berries, analyses were done on dry matter.

		concentration range		
Elements	**Units**	**leaves**	**berries**	**wines**[a]
Al	mg/kg	104-176	4-28	0.5-1.8
Ba	mg/kg	0.25-54	0.65-7	0.04-0.17
Ca	g/kg	15-42	1-2	0.6-1.1
Co	mg/kg	n.d.	0.11-1.5	0.06-0.12
Cr	mg/kg	0[b] - 91	0.01 - 1	n.d.
Cu	mg/kg	89-163.7	10-113	0.08-0.22
Fe	mg/kg	n.d.	10-36	n.d.
K	g/kg	27-356	7-24	1.4-2
Li	mg/L	n.d.	n.d.	0.03-0.07
Mg	g/kg	1.4-4	0.35-0.92	73-110
Mn	mg/kg	34-705	1.7-22	0.27-1.5
Mo	mg/kg	0[b] – 3	0.02 – 0.5	0.03-0.14
Ni	mg/kg	2.7-40	0.2-0.9	0.04-0.21
Sb	mg/kg	0.3-42	n.d.	0.01-0,05
Si	mg/kg	155-904	4-86	6.5-11.5
Sr	mg/kg	27-167	2.7-8.4	0.18-0.68
Ti	mg/kg	0.23-5	0.01-1.1	0.11-0.19
V	mg/kg	0[b] - 4	0.9 – 2.8	0.13-0.2
Zn	mg/kg	0.5-94	3.4-20	0[b]-0.66
Zr	mg/kg	0.2-13	0.03-1.37	0.02-0.07

[a] *all wine values are expressed in mg/L.*
[b] *0=values under the sensitivity threshold of the instrument.*
n.d. *not determined*

Table 4. Eigenvectors of the Principals Components (PCs) with Eigenvalues > 1, derived from the PCA performed for the geographic comparison on leaves, grapes and wines. In bold the variables more correlated to the principal extracted components.

	Leaves		Berries			Wines	
PCs	1	2	1	2	3	1	2
Ba	**0.92**	-0.07	**0.83**	0.03	-0.08	**0.81**	0.25
Mg	0.57	**-0.62**	**0.85**	0.40	-0.15	----	----
Mn	**0.76**	0.59	**0.82**	-0.27	0.09	**0.90**	-0.09
Si	**0.87**	-0.01	**0.83**	-0.13	0.10	**0.71**	-0.38
Sr	-0.29	**-0.85**	-0.32	0.53	**-0.74**	**-0.90**	-0.12
V	**0.93**	-0.25	0.48	**0.80**	0.17	----	----
Ti	----	----	-0.44	0.56	**0.64**	0.03	**0.95**

Table 5. Elements in Nebbiolo leaves and berries that showed a significant different accumulation (p≤0.05 Mann-Whitney U test) when grafted on 420 A and K5BB rootstocks (a). Elements in Nebbiolo and Barbera leaves and berries that showed a significant different accumulation (p≤0.05 Mann-Whitney U test) (b).

	(a) Rootstock				(b) Variety			
	Mezzomerico		Treiso		Mezzomerico		Monforte	
	leaves	grapes	leaves	grapes	leaves	grapes	leaves	grapes
Al		0.05						
Ba				0.02	0.05	0.05	0.05	0.05
Ca	0.05		0.02	0.01	0.05			
Co		0.04		0.01		0.04		
Cr	0.05		0.02	0.01				
Cu					0.05		0.05	
K	0.05	0.02		0.02	0.05	0.05	0.05	
Mg		0.03		0.01	0.05			
Mn				0.01	0.05	0.05		
Mo		0.02	0.02	0.02		0.05		
Ni		0.02	0.02	0.01		0.05		0.05
Sb		0.02		0.01	0.05	0.05	0.05	0.04
Si		0.05		0.02		0.05		0.05
Sr					0.05	0.05		
Ti	0.05	0.05	0.02	0.01	0.05			
V				0.01	0.05	0.05	0.03	
Zn			0.02					

Table 6. Eigenvectors of the Principals Components (PCs) with Eigenvalues > 1, derived from the PCA used for studying rootstock and variety effects. In bold the variables more correlated to the principal extracted components.

| | Rootstock (a) | | | | | Variety (b) | | | | |
| | Leaves | | Grapes | | | Leaves | | Grapes | | |
PCs	1	2	1	2	PCs	1	2	1	2	3
Ca	-0.01	**0.96**	----	----	Ba	**0.81**	0.31	**-0.87**	0.41	0.20
Co	----	----	0.08	**-0.90**	Ca	----	----	**0.91**	-0.01	0.01
Cr	**0.93**	-0.35	**0.90**	0.14	Cr	**-0.80**	-0.25	**-0.85**	0.39	-0.09
K	**0.96**	-0.26	**0.91**	0.06	K	**-0.87**	-0.34	----	----	----
Mg	----	----	**0.94**	0.06	Mg	**0.74**	0,61	-0.24	-0.62	**0.70**
Mo	**0.98**	0.11	-0.12	**0.93**	Mn	0.60	**-0.71**	**-0.76**	0.56	0.25
Mn	----	----	**0.94**	-0.13	Ni	----	----	**0.67**	-0.31	0.35
Ni	-0.67	0.62	-0.44	**0.84**	Si	**0.68**	0.61	----	----	----
Sb	**0.97**	-0.21	----	----	Sr	-0.58	0.50	----	----	----
Si	----	----	**0.81**	0.16	Ti	----	----	**0.88**	-0.01	0.21
Ti	-0.25	**0.94**	0.03	**0.95**	V	----	----	0.50	**0.67**	0.51
					Zr	----	----	**0.92**	0.21	0.24
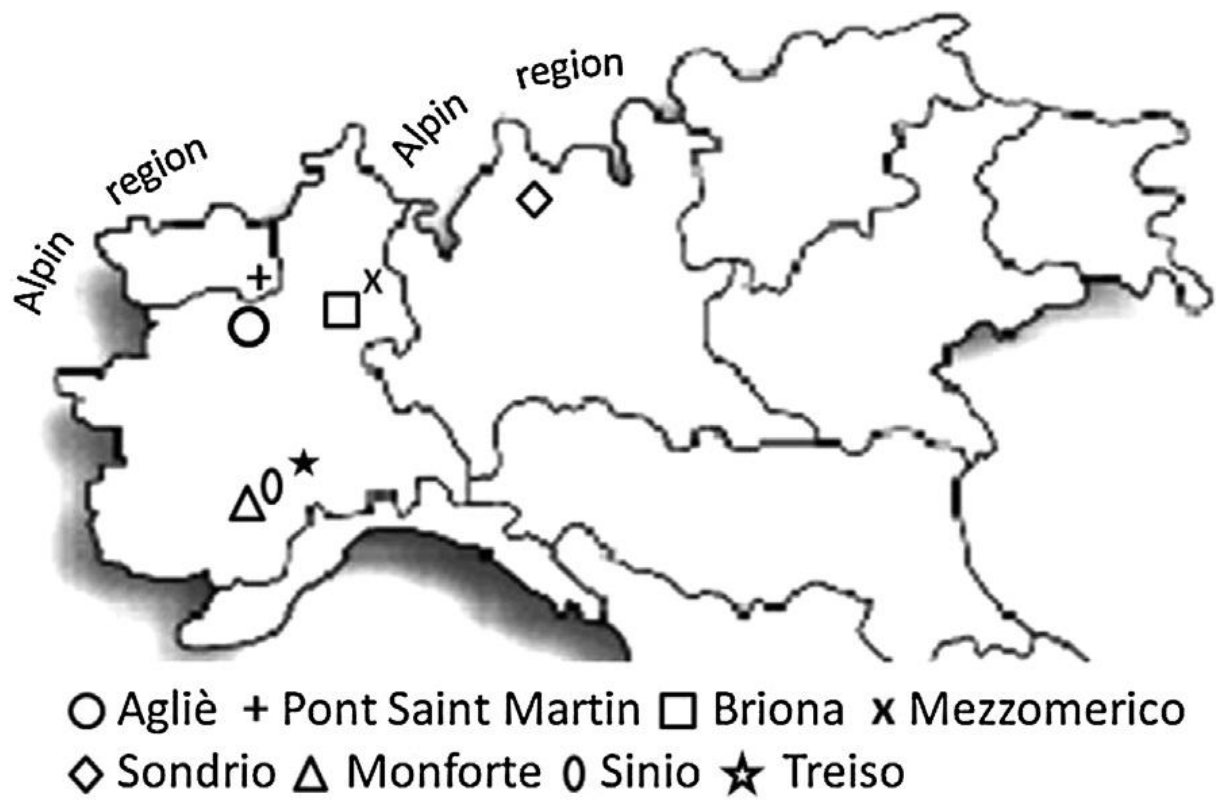
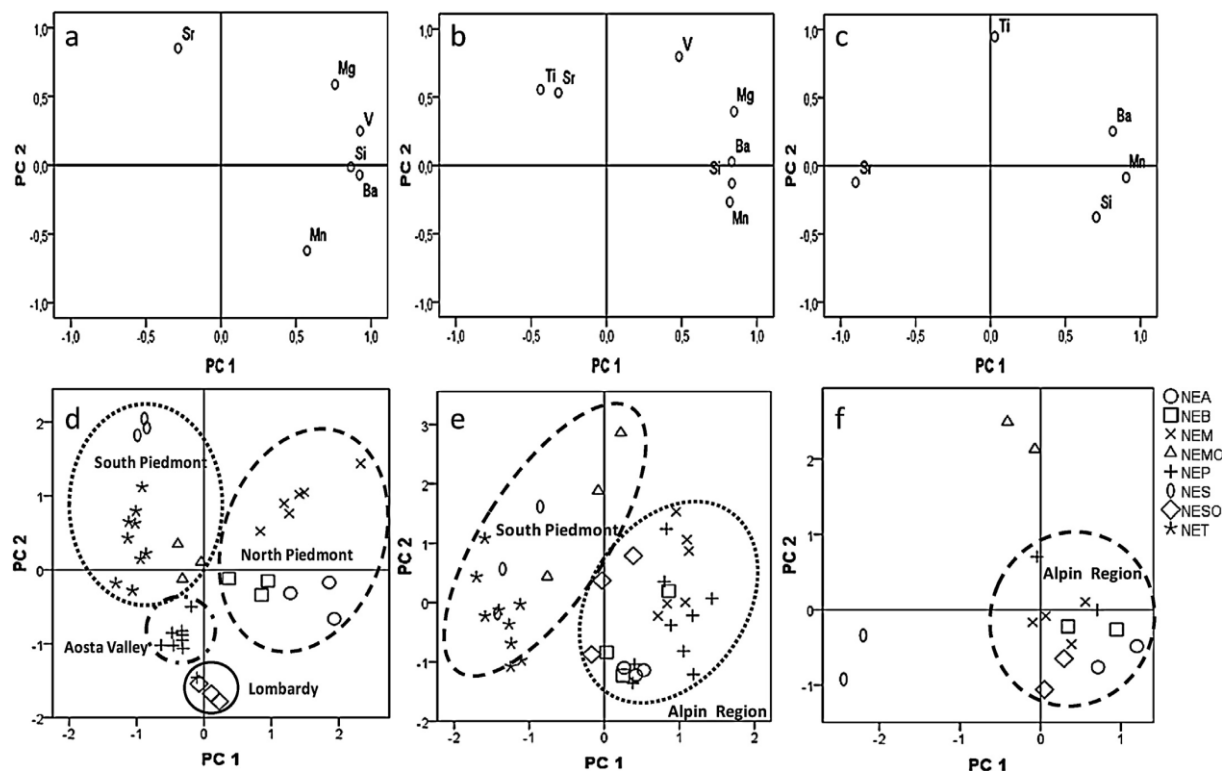Fig. 1. Geographic locations of experimental sites.

Fig. 2. Distribution of the variables (above) and samples (below) based on the PCA models considering the most discriminant variables for leaves (a and d), berries (b and e)and wines (c and f) and aiming to discriminate the geographic origin of the Nebbiolo samples. The symbols represent the replicates from each site; for legend, see Fig. 1 andTable 1a).
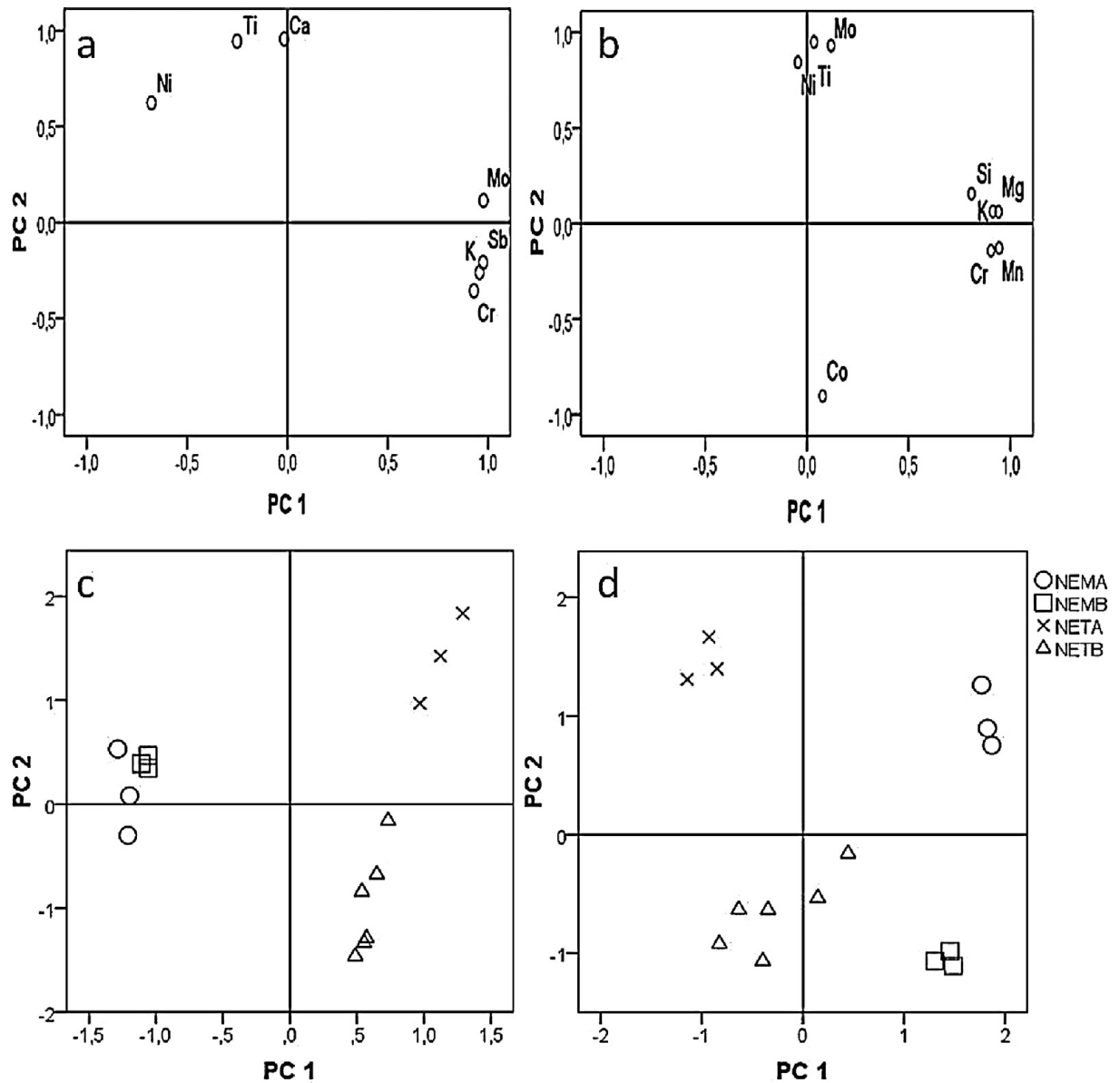
Fig. 3. Distribution of the most discriminant variables (above) and of the samples (below) based on the PCA models applied to leaves (a and c) and berries (b and d) ofNebbiolo grafted on two rootstocks. PCA aimed to discriminate the rootstock uptake selectivity. The symbols represent the replicates from each site. NEMA = Mezzomerico420A, NEMB = Mezzomerico K5BB, NETA = Treiso 420A, NETB = Treiso K5BB.
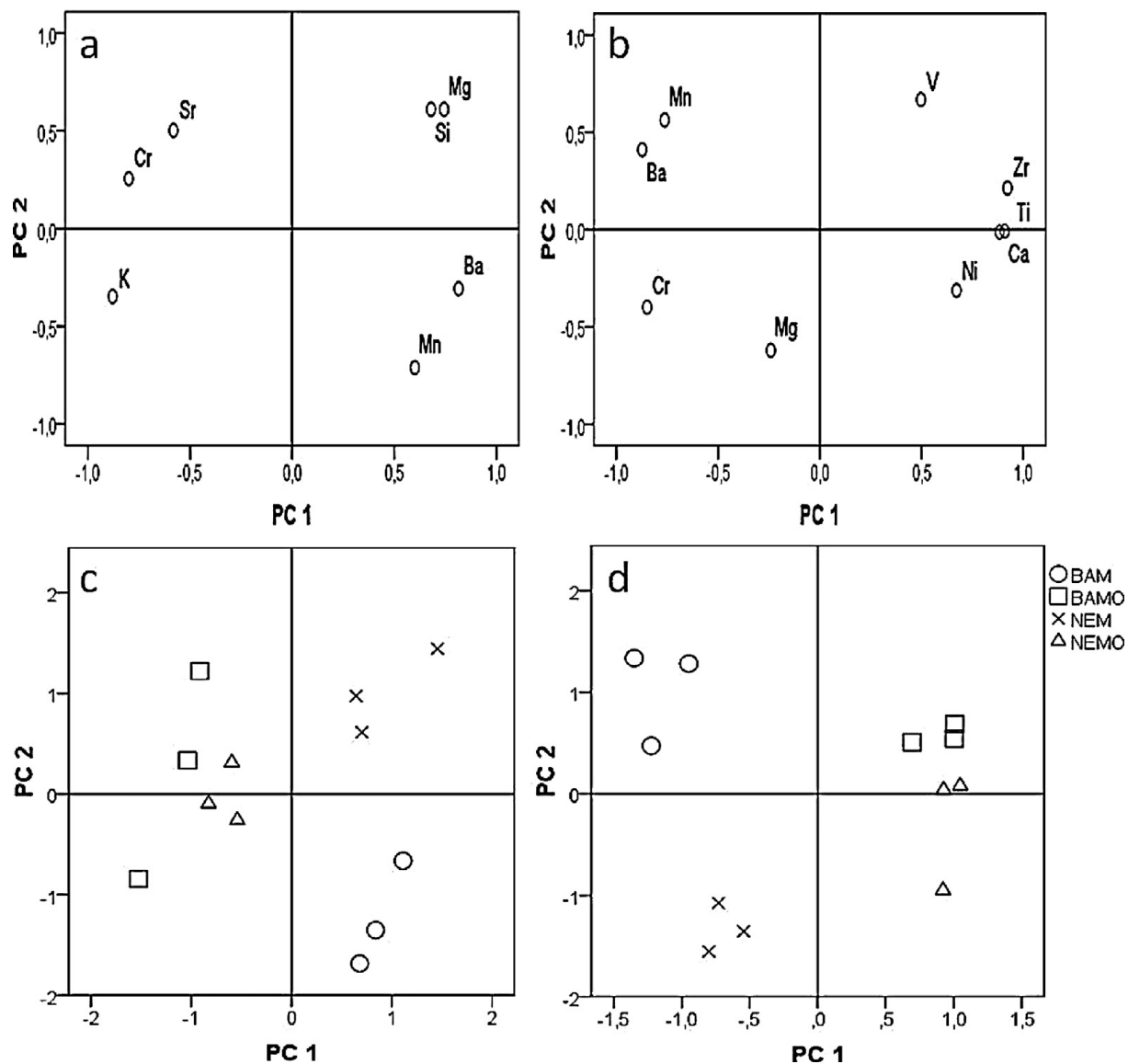
Fig. 4. Distribution of the most discriminant variables (above) and of the samples (below) based on the PCA models applied to leaves (a and c) and berries (b and d) ofNebbiolo and Barbera varieties grafted onto the same rootstock. PCA aimed to discriminate the variety uptake selectivity. The symbols represent the replicates from eachsite. NEM = Nebbiolo Mezzomerico, NEMO = Nebbiolo Monforte, BAM = Barbera Mezzomerico, BAMO = Barbera Monforte.