Rare earth elements in grapes and soil. Study of different soil extraction methods

Éléments chimiques rares dans le raisin et le sol. Étude de différentes méthodes d'extraction de sol.

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Abstract

Lanthanides, together with scandium and yttrium, make up the group of Rare Earth Elements (REEs). An official method for analysis of the bioavailable REEs accumulated by plants, depending mainly on soil characteristics, chemical speciation in soil and the specific ability of the plant, is still lacking.

In this study we analysed the content of 13 REEs (yttrium, Y; lanthanum, La; cerium, Ce; praseodymium, Pr; neodymium, Nd; samarium, Sm; europium Eu; gadolinium, Gd; dysprosium, Dy; holmium, Ho; erbium, Er; thulium, Tm; ytterbium, Yb) in Chardonnay grapes in relation to the content in the soil, extracted using different methods in order to assess which of the extractants used could best reflect the amount of elements taken up by the plant.

The vineyard, located in north-eastern Italy, is characterised by a silt loam, calcareous, alkaline soil.

Four different extraction methods were tested: (1) aqua regia microwave digestion; (2) with DTPA, $CaCl_2$ and TEA; (3) with ammonium acetate and (4) with ammonium nitrate.

The amount of REEs extracted followed the order: aqua regia > DTPA > CH_3COONH_4 > NH_4NO_3. Compared to the "so-called total" content in soil, the sum of the REEs extracted with DTPA, ammonium acetate and ammonium nitrate was roughly 0.80%, 0.065% and 0.002% and each individual element was extracted in amounts of <2.9%, <0.5% and <0.2% respectively. Only 7 elements (Y, La, Sm, Eu, Dy, Er, Tm) were found in quantifiable amounts after extraction with ammonium nitrate.

The concentration of individual REEs in berries would seem to correspond best to the concentrations extracted using aqua regia.

Keywords: Rare Earth Elements, berries, soil, soil extraction, ICP-MS

Introduction

Lanthanides, together with scandium and yttrium, make up the group of rare earth elements (REEs). Within the REEs two subgroups are recognised: light elements with a lower atomic mass (LREEs: lanthanium, La; cerium, Ce; praseodymium, Pr; neodymium, Nd; samarium, Sm; and europium Eu) and heavy elements (HREEs: gadolinium, Gd; terbium, Tb; dysprosium, Dy; holmium, Ho; erbium, Er; thulium, Tm; ytterbium, Yb; lutetium, Lu and yttrium, Y) with a higher atomic mass and a smaller ionic radius. In some cases, Sm, Eu, Gd, Tb and Dy are defined as middle elements, MREEs (Tyler, 2004). REEs distribution shows a particular pattern: their content decreases with increasing atomic weight and, according to the Oddon-Harkins rule, each element with an even atomic number is more frequent than the next odd one (Kabata-Pendias, 2001). During weathering processes and soil-plant transfer REEs could be fractionated into LREEs and HREEs (Kabata-Pendias, 2001; Ding et al., 2006). Nowadays considerable attention is focused on the study of REEs, due to their use in the electronics industry and in agriculture to increase crop yield (Tyler et al.. 2004). REEs have been also investigated in traceability studies to assess the possibility of their being useful for effectively determining the geographical origin of food, wine in particular (Oddone et al., 2006).

Research on REEs is mainly carried out in China, the country with the largest reserves of these elements, where their intense use in industry and agriculture has led to accumulation in the environment. For ecological, physiological and traceability studies it is necessary to know the bioavailable fraction that is actually accumulated by a plant and which depends mainly on soil characteristics, chemical speciation in soil and the specific ability of the plant (Kabata-Pendias, 2001). The total concentration in soil is often considered not to be a good indicator of this (Chen et al., 1996). An official method for analysis of bioavailable REEs is lacking but different methods have been tested and compared by many authors. In particular, the reagents frequently used are: diluted organic or inorganic acids, chelating agents, buffered or unbuffered salt solutions (Ure et al., 1993).

With a sequential extraction fractionation procedure on soil it has been shown that the water soluble fraction contains less than 0.1% of the total REEs; the exchangeable, bound to carbonate, to organic matter or to Fe-Mn oxides fraction contains almost 10-40% of total REEs while the residue (non bioavailable fraction) contains more than 60% and up to 90% of total REEs. In particular MREEs seem to be potentially more bioavailable, being present in the residual fraction in a lower proportion (Cao et al., 2000). The extractable REEs are often bound to organic matter or to Fe-Mn oxides (Cao et al., 2000). REEs in water, soluble, exchangeable, bound to carbonate and organic matter obtained after soil extraction with water, $Mg(NO_3)_2$, diluted HCl and $H_2O_2 + MgCl_2$ respectively, are significantly correlated to the REEs content in alfalfa (Cao et al., 2000). CH_3COOH extracted REEs are correlated to the REEs content in corn and rice leaves (Li et al., 1998) whereas $CaCl_2$ and CH_3COOH extracted REEs have been shown to be in agreement with the shoot content of wheat (Lu et al., 2003). Recently, Fang et al. (2007), compared different soil extraction methods for the evaluation of LREEs bioavailability for wheat: DTPA and EDTA but not $CaCl_2$ and CH_3COOH extractable REEs were significantly correlated to wheat content.

The results for different extractants and for the correlation between soil and plant contents are often contradictory and this variability is due to differences in both soil and plant species. Different species collected in the same site show different REEs content and patterns (Wyttenbach et al., 1998). Hence it is important to study each particular soil-plant system.

In this work we studied the REEs content and pattern in Chardonnay berries in comparison with that in soil extracted using different methods. The aim was to assess the ability of the different extractants used to estimate the bioavailability of REEs for *Vitis vinifera*. This knowledge could be useful in traceability studies for determining the geographic origin of grapes and wine.

Material and methods

Information about vineyard position and characteristics, procedures for vegetal material sampling and preparation and the method for REEs analysis carried out with an ICP-MS are given in Bertoldi et al., 2008. The soil was collected in spring from 2 layers (Top-soil: 0-30 cm and Sub-soil: 30-60 cm) in 2 areas for each of 4 parcels. Each sample was separately air-dried, sieved < 2 mm after sorting out the plant debris and visible fauna, and stored in closed plastic pots until the time of analysis. For the analysis of the "so-called total" REEs content, an aliquot of the sample was ground in order to pass it through a 0.2 mm sieve. All the results are given per unit of dried soil. Standard particle-size determination was performed using a hydrometer and sieve analysis (Gee and Or, 2002). Soil pH was measured in a 1:2.5 (wt/wt) soil suspension. Active $CaCO_3$ was determined after extraction with ammonium oxalate 0.2N and hot titration with $KMnO_4$ 0.2N (Drouineau, 1942), and total $CaCO_3$ using HCl attack (ISO 10693:1995). Organic matter (OM) and total nitrogen content were determined using a elementary analyser (Elementar VARIO MACRO CN, Germany). The cation exchange capacity (CEC) was determined using $BaCl_2$ and triethanolamine (TEA) at pH 8.2 method (ISO 11260:1994).

Four different extraction methods were used:

(1) <u>aqua regia extraction</u> (ISO 11466:1995 modified as afterwards indicated): 0.5 g of soil sample were added to 1.5 ml of H_2O_2, 4.5 ml of HCl (37%) and 1.5 ml of HNO_3 (96%) and then digested in a microwave system (MARS EXPRESS, CEM, USA) equipped with 100 ml PTFE vessels. The mineralization conditions are specified in the table below:

Power (W)	Pressure (psi)	Temperature(°C)	Ramp (min)	Hold (min)
800	150	105	10	3
800	250	135	10	3
800	350	175	10	12
800	200	105	0	5

The digested samples were diluted 100 times before carrying out ICP-MS analysis to quantify the "so-called total" REEs content.

(2) <u>DTPA extraction</u> (Lindsay and Norwell, 1978): 25 g of soil were extracted with 50 ml of diethylene triaminepentaacetic acid (DTPA) 0.005 M, $CaCl_2$ 0.01M and triethanolamine (TEA) 0.1 M pH 7.3 for 2 hours.

(3) <u>ammonium acetate extraction</u> (SSIR 42 method 5A8): 5 g of soil were extracted with 100 ml of ammonium acetate 1M pH 7 for 1 hour

(4) <u>ammonium nitrate extraction</u> (ISO/DIS 19730): 20 g of soil were extracted with 50 ml of ammonium nitrate 1M for 2 hours.

The extracts (2), (3) and (4) were then centrifuged at 3500 rpm for 5 minutes, filtered with a PVDF filter and diluted 10 times before analysis. In the case of ammonium acetate extraction alone the samples were diluted 3 times before ICP-MS analysis.

All the materials were washed with diluted nitric acid and rinsed with ultrapure water.

Results and discussion

The calcareous soil developed on a post Würm alluvial fan but had been subjected to extensive digging work in 1987, before the vineyard was planted, so the pedological horizons are not currently distinguishable up to a depth of at least 60 cm.

The results of physical and chemical analysis of the soil are given in Table 1. The soil studied was a silt loam, calcareous, alkaline soil with medium cationic exchange capacity, "active" $CaCO_3$ on average, well provided with organic matter but poor in nitrogen. Top and sub soil had very similar characteristics except for pH, percentage of clay and silt and in particular organic matter and N content, which were significantly higher in the top soil (Mann-Whitney Test, $p<0.05$), as expected.

	Top-soil (0-30 cm)	Sub-soil (30-60 cm)
pH in water	8.1	8.2
sand %	41	41
silt %	49	47
clay %	10	11
total $CaCO_3$ (g/kg)	482	511
"active" $CaCO_3$ (g/kg)	26	25
OM (g/kg)	32	25
total N (g/kg)	0.9	0.7
CEC (cmoli(+)/kg)	13	13

Table 1 Soil characteristics of the 2 layers collected. The results shown are the median of 8 sub-samples.

These differences, although statistically significant, are agronomically negligible and do not lead to statistical differences in top- and sub-soil REEs content for aqua regia and DTPA extracts. Only a few statistical differences (Mann-Whitney Test, $p<0.05$) existed for the other 2 extraction methods used. Moreover, the root system is probably present in both layers and even deeper, therefore we will present the data here without distinguishing between the top and sub-soil.

The REEs content in soil extracts is shown in Table 2, whereas berry content is given in Bertoldi et al., 2008.

extractant	Y	La	Ce	Pr	Nd	Sm	Eu
aqua regia	7.24	21.2	44.7	4.90	19.9	3.56	0.710
DTPA	0.185	0.149	0.176	0.037	0.170	0.038	0.009
CH_3COONH_4	0.006	0.013	0.013	0.003	0.018	0.004	0.003
NH_4NO_3	0.0002	0.0002	<0.00006	<0.000004	<0.0001	0.0001	0.001

extractant	Gd	Dy	Ho	Er	Tm	Yb
aqua regia	2.85	1.72	0.299	0.762	0.097	0.578
DTPA	0.043	0.032	0.006	0.016	0.002	0.012
CH_3COONH_4	0.005	0.002	0.0004	0.001	0.0001	0.0005
NH_4NO_3	<0.0001	0.00004	<0.000004	0.00001	0.00001	<0.00002

Table 2 REEs content (mg/kg soil dried weight, median values without distinction between the top and sub soil, n=16) obtained in the 4 different soil extracts.

The content in the aqua regia soil extract was very similar to that reported by Diatloff et al., (1996) for different Australian soils. Ce is the most abundant element and, with La and Nd, made up about 80% of the sum of REEs analysed, as already shown for berries (Bertoldi et al., 2008). Wutscher and Perkins (1993) reported lower content for Florida soils but noted the predominance of Ce, La and Nd.

With respect to the total content reported in the literature (after extraction with a different mix of acids and HF) in soil or in the upper continental crust (Kabata-Pendias, 2001, Wyttenbach et al., 1996), the "so-called total" content measured in this work was obviously lower, since the predominant content of REEs is present in the residual fraction (Cao et al., 2000), which is not attacked by aqua regia. The HREEs presented a more marked decrease than the LREEs. The amount of REEs extracted using the 4 methods compared followed the order: aqua regia > DTPA > CH_3COONH_4 > NH_4NO_3 as expected according to their different extraction properties. Compared to the "so-called total" content in soil, the sum of the REEs extracted with DTPA, ammonium acetate and ammonium nitrate was roughly 0.80%, 0.065% and 0.002% and each individual element was extracted in amounts of <2.9%, <0.5% and <0.2% respectively.

The concentration of individual REEs in berries largely reflected those in the soil (Fig. 1). The aqua regia extractant seems to better mimic the ability of the *Vitis* to take up these elements (see r_s values in Fig. 1): only Eu showed a marked shift from the regression line. Ammonium nitrate extracted only 7 elements (Y, La, Sm, Eu, Dy, Er, Tm) in quantifiable amounts and the Eu deviation from regression line is definitely marked (Fig. 1). Because of the low fraction extracted, REEs quantification with this method is difficult and not very effective. At all events, with the exception of Eu, the other 7 quantified elements were well correlated with the berry content.
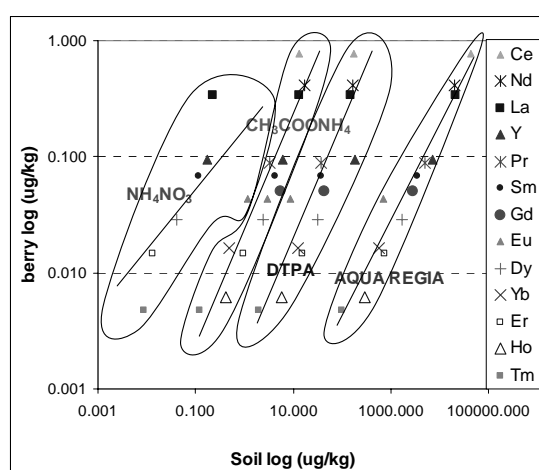

Figure 1 The content of each REEs content in the berry (μg/kg of fresh weight) is shown against the soil extracts (μg/kg of dried weight). For clarity, in the graph the median values reported on a logarithmic scale are represented. (Aqua regia extract r_s= 0.963; CH_3COONH_4 extract r_s=0.931; DTPA extract r_s=0.872; NH_4NO_3 extract r_s=0.747, Spearman's correlation, p<0.01).

Figure 2 shows the REEs normalised distribution pattern in berries and in the different soil extracts. These values are calculated by dividing the content of each REEs in the samples by the content of the same REEs in the upper continental crust (UCC value) and are shown agains the REEs series. Normalisation with the REEs content of a reference sample (e.g. UCC, post archean australian shale (PAAS) or chondrites) allows the elimination of the Oddon-Harkins effect and better and detailed visualisation of the pattern, fractionation or anomalies (Wyttenbach et al., 1996; Zhengguin et al., 2001). The UCC-normalised graphs (Fig. 2) also show the similarities between the pattern of REEs in berries and in the soil extracted with aqua regia, except for Eu and Tm. The Tm content in berries is very low, close to the detection limit for this element, so any considerations regarding it are not very reliable. The berries showed a decrease in HREEs (except for Tm), as for aqua regia extraction. The behaviour of Y, more similar to that of HREEs despite its low atomic weight, could be explained by its small ionic radius. The good correlation between the aqua regia REEs extraction level and vegetal tissue content has also been reported by Wutscher and Perkins (1993). The ammonium acetate and DTPA extraction patterns do not represent the Ce content in berries well. Cerium is mainly present in soil as Ce^{+4}, considered to be a less soluble form, and for this reason could not be extracted well using the CH$_3$COONH$_4$ and DTPA usually used to estimate the bioavailable content of elements. Besides Ce, extraction with DTPA also did not reproduce the Y and Eu content in berries. Only the ammonium acetate and ammonium nitrate extractions presented a Eu pattern similar to that noted in berries.
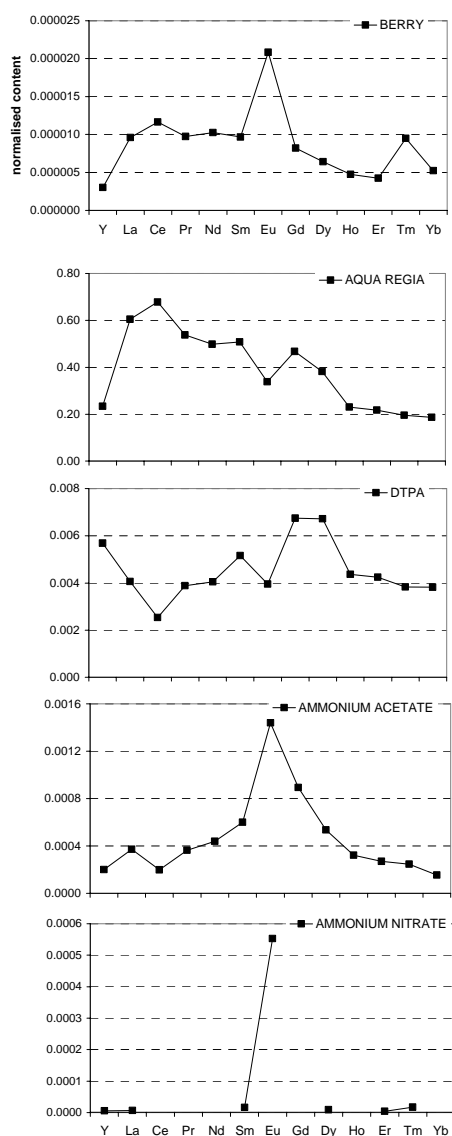
Figure 2 UCC-normalised REEs pattern in berries and in the 4 different soil extracts.

Conclusion

This research confirms that is very difficult to mimic the very complex physiological and biochemical mechanisms developed by the plant to take up different mineral elements from the soil using simple chemical extraction. For REEs, the aqua regia extractant seemed to best mimic the behaviour of the *Vitis* but this method is time-consuming and uses potentially hazardous reagents.

If only data about Y, La, Sm, Dy, Er and Tm content are required, ammonium nitrate extraction, although the least efficient, can provide satisfactory estimation of REEs content in berries.

These observations need to be confirmed by analysing the REEs content in soil-plant system sampling over several years and in different soils.

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