Università degli Studi di Palermo

DIPARTIMENTO DI SCIENZE DELLA TERRA E DEL MARE
(Di.S.Te.M)

Ph.D. in Geochemistry

DOTTORATO DI RICERCA IN GEOCHIMICA - XXIV Ciclo (GEO/08)

THE BIOGEOCHEMICAL BEHAVIOUR OF RARE EARTH ELEMENTS AS A CONSEQUENCE OF PROCESSES OCCURING IN THE *VITIS VINIFERA L.* – SOIL SYSTEM

Ph.D. thesis by

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To those who believe in me…. Thank you!

Abstract

The geochemical behaviour of lanthanides and yttrium (Rare Earth Elements, REE) has been investigated mainly in geological systems as the REE exploitation in industrial and agricultural practices is progressively growing in the last years, to such an extent to become strategic materials. Extensive researches evidenced that REE capability to investigate processes occurring at the interface between different media such as the in depth investigation of REE behaviour is a matter of fact in many geochemical studies. These capabilities are a consequence of the chemical characters of REE that are exploited to investigate processes occurring during migrations of chemical elements in the soil-to-plant system. To carry out this research the *Vitis vinifera* was the chosen plant since it is one of the most important botanic species exploited for alimentary purposes.

During this research, changes of REE behaviour in the soil-*Vitis vinifera* system was assessed on specimens growing *off-soil* and *on-soil* conditions in order to evaluate if this behaviour is influenced by a plant effect in terms of elemental fractionations along the REE series. These studies have been obtained studying REE distributions in bioavailable and pseudo-total soil fractions and comparing them with REE contents in different plant portions during different growth stages. Changes in REE features induced by the different soil characters have been assessed producing the growth of *Vitis vinifera* specimens onto different soils and also grafted onto different rootstocks usually exploited in Sicily. All these experiments have been carried out under controlled lab conditions. All the indications provided by these experiments have been considered as preliminary basis to investigate the REE behaviour of *Vitis vinifera* specimens growing *in-field* onto different soils. The complicated Sicilian geology allowed us to recognise plants growing on very different soils, from metamorphic to eruptive and sedimentary parent rocks that gave us a comprehensive scenario of relationships occurring between REE in the bioavailable soil fractions and in the related vine berries.

The results indicate that REE fractionations in off-soil growing plants are negligible whereas REE distributions in plant organs of vine growth on-soil mainly depend on the composition of bioavailable soil fraction. Detailed recognitions of REE distributions in these plants confirmed that REE in Vitis vinifera behave according to two main mechanisms: REE scavenging in roots and REE complexation in aerial parts.

In particular, roots are the plant organs where REE are preferentially concentrated, in particular elements from Sm to Ho (middle REE, MREE) whereas REE complexation through the plant xylem is highlighted by Eu enrichments occurring in aerial plant parts. Eu-positive anomalies suggest that Eu^{3+} can form stable organic complexes in place of Ca^{2+} in several biological processes in xylem fluids. The possibility that Eu mobility in these fluids can be enhanced by its reductive speciation as Eu^{2+} cannot be ruled out. The assessment of the geochemical behaviour of REE according to Tetrad Effect Theory carried out confirms that REE coming from soil are scavenged onto root tissues or mineral surfaces whereas their behaviour in aerial parts of *Vitis vinifera* is driven by dissolved REE complexation. The results we achieved also suggest that the effective capability of REE to trace the geographical origin of berries is related to the awareness of a detailed soil database where substrata are strictly determined according to their major elements composition and mineralogy.

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Introduction

1.1 Background

Vitis vinifera L. is a plant cultivated since ancient times, and this is related to the importance this species has for people, both for alimentary and cultural reasons. Products derived from *Vitis vinifera* are so widely used that it is difficult to identify cultures that do not use them extensively. Therefore, recognition of the capacity of *Vitis vinifera* to extract minor and trace components from the growth substrate, as well as the destiny of these elements during processes occurring in the rhizosphere and their transport through the xylem towards the aerial parts of plants, represent an interesting research focus. Only a limited number of studies have been carried out on this topic (Bavaresco, 1997; Rodushkin et al., 1999; Chopin et al., 2008; Bertoldi et al., 2011; Ortiz-Villajos et al., 2012) and these studies were mainly focused on the accumulation of toxic species in *Vitis* berries (Bertoldi et al., 2013). Only a few case-studies have been carried out investigating lanthanides and Yttrium (namely Rare Earth Elements, REE) distributions in *Vitis vinifera* (Bertoldi et al., 2009; 2011), but the available data were not treated according to an approach highlighting the geochemical behaviour of these elements in the *Vitis vinifera*-soil system. On the contrary, REE concentrations were simply discussed according to a "classical" approach without any consideration of their particular chemical behaviour during multiple processes occurring in biological systems. The choice of a "classical" approach has reduced the possibility to obtain information about processes involving REE during the growth of *Vitis vinifera* that can be enhanced by the geochemical treatment of REE data through the use of normalised REE concentrations and the derivate characteristics as it is detailed below. REE's characteristics allow them to represent probably the best geochemical tracer of processes involving the occurrence of an interface between media with different chemical-physical characteristics. More information are provided by the employment of trace metal rather than major and/or minor components of involved solid phase. This happens because the amplification degree of process occurring at the interface is as much greater as the concentration gradient of an element between two phases at interface. This aspect makes REE the best choice to investigate processes occurring to

trace elements during their migration from soil to plant and its fruits (Tyler, 2004; Liang et al., 2008).

Although interface studies related to REE behaviour have focused on rock/water interactions (Michard et al., 1989; Bau & Dulski, 1996; Moller & Giese, 1997; Moller et al., 2003 and references therein), these have recently been extended towards biological systems, biofilms and bacterial colonies (Takahashi et al., 2002; 2005; 2007, Davranche et al., 2004; 2005; 2008). The binding between biological surfaces and dissolved REE occurs through O-donor groups that join into the REE coordination sphere forming surface complexes. Elements along the REE series are differently involved in this process depending on the involved binding groups on the biological surface and the physical-chemical conditions occurring in the system (Takahashi et al., 2010). Recently, Takahashi et al. (2010) showed that the REE complexation onto biological surfaces occurs by means of carboxylate and/or polyphosphate binding groups. The former ligands show larger affinity towards heavier REE, from Ho to Lu (HREE) whereas the latter O-donor groups preferentially fractionate middle REE, from Sm to Dy (MREE). However, the REE behaviour represents a suitable geochemical proxy of interface processes even if biological substrata are involved (Moriwaki et al., 2013). The REE behaviour in the *Vitis vinifera*-soil system can represent a very promising application of the analysis of distributions of these elements among different media, from the inorganic soil interface to the coexisting fluid phase and hence to the biological medium in the plant.

The present research was carried out studying the "geochemical behaviour" of REE during the uptake and the growth of *Vitis vinifera*. To investigate these phenomena is paramount to consider geochemical soil properties, soil-plant interaction and effect on plant of REE absorption and translocation. In order to achieve our goals first of all we will try to understand if REE mobilisation from root to aerial vine apparatus in an off-soil isolated system is the result of dilution effect that will occur with or without element fractionation. The next goal will be to evaluate the absorption process from substrate to plant; since soil is characterised by a complex mineralogy and moreover the mass soil effect may influence the absorption, to assess whether there are competing effects caused by different mass ratios of REE, the plants behaviour will be study in a off-soil totally available and equimolar REE system. This experiment would allow to highlight if the vine carries out preferential element absorption or if there is not discrimination among REE. After analysing these processes, the effected induced by

different soil characteristic with respect to vine will be investigated. Moreover, due to the sensitivity of *Vitis vinifera* towards phylloxera, cultivated *Vitis vinifera* are usually grafted onto different rootstock species, so that effects related to different rootstocks on REE behaviour in different parts of *Vitis* have to be evaluated, as well as the effect of different nature of vine variety; finally the correlation between different soil and berries will be investigated in field.

This approach is fundamental for a possible use of REE geochemical behaviour in the *Vitis vinifera*-soil system to provide geochemical indicators that can lead to discrimination between grapes grown on plants living on different soils.

1.2 The soil

1.2.1 Characteristics

The soil is not simply a mixture of unconsolidated material, resulting from the weathering processes of underlying rocks; but soil is a natural body, having both mineral and organic components in addition to physical, chemical, and biological properties. Soil properties, therefore, cannot be a simple reflection of the combined properties of all soil components (Kabata-Pendias, 2011).

The soil is composed by three phases: *solid phase*, consisting of inorganic components (rock detritus, detritic and authigenic minerals) and organic ones (composted waste, biomass, humic substances), *liquid phase* made of water and dissolved or dispersed substances and *gaseous phase*. Moreover, the chemical composition, mineral structure and dispersion of the solid components are important factors influencing soil properties.

The soil mainly consists of:

- *Primary* or *parent minerals*, including silicates, feldspar, olivine, pyroxenes, amphiboles, micas formed during the primary magmatic crystallization. Some primary minerals represent the less reactive soil component due to their chemical inertia during weathering and secondary processes.
- *Secondary* or *authigenic minerals* formed during processes occurring in soils. These phases consist of clay minerals, carbonates, sulphates, Fe, Mn, Al oxyhydroxides, usually characterised by strong reactive surfaces with large capability to adsorb chemical from the dissolved phase.

- *The organic soil component* (OM) often due to decomposed biological debris and from organic substance produced during processes involving the maturation of these biological materials. Both humic and fulvic compounds belong to this category. The organic matter and humic substances play a key role in several processes allowing to the improvement of the soil structure and its water retention capability. These components drive the adsorption of minor and trace elements during soil reactions.
- *The dissolved pool* representing the aqueous phase where inorganic and organic compounds are fractionated with respect to the other soil components.

The whole soil formation stages occur through two main processes starting from the alteration of primary minerals by their hydration in the physical-chemical reactions during the *weathering*. The *pedogenesis* represents the second stage resulting in the formation of a soil profiles from the unaltered parent rock to the mostly weathered products. Both these processes are hard to distinguish because they usually occur almost simultaneously, in the same sites and are closely related (Kabata-Pendias, 2011). The elemental mobility and availability during weathering is determined by the geochemical properties of the elements, the stability of the detritic minerals, the capability of these elements to be involved in the crystallization of secondary phases and the stability of their aqueous complexes under physical-chemical conditions occurring in the soil.

1.2.2 Cation exchangeable capacity (CEC) and colloids

The adsorption is defined as a temporary retention process of ions to the solid surface. Between soil constituents, clay minerals, oxides, hydroxides and humic substances present particular adsorbent properties and they constitute the so-called exchange complex of soil. Typically there is an electrostatic attraction between the exchanger solid and the ion in solution and the exchange reaction is fast, stoichiometric, reversible and selective.

The clay mineral surfaces are characterised by the presence of pH independent negative charges formed as a result of isomorphic substitution in which a cation with lower charge (i.e., Al^{3+}) replaces another cation with higher charge (i.e., Si^{4+}) generating a negative hole; while the presence of pH-dependent negative charges (at soil pH) on the humic substances is due to the deprotonation of the COOH groups. It determines the cations attraction (exchangeable type) on the exchanger surface and the weak electrostatic interactions formation (cation exchange).

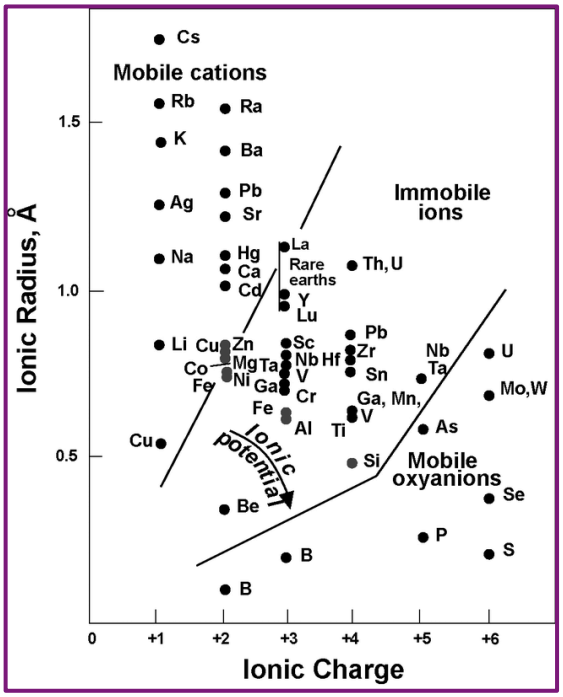
The ions have a different affinity adsorption: greater charge ions are fixed with greater force (Adriano, 2001; Violante, 2002). In addition, the adsorption also varies as a function of the binding capacity of the soil components that can only be due to electrostatic attractions or even to weak bonds (hydrogen bonding and/or Van der Waals' forces). The organic substances can bind mineral elements by weak (ion exchange) or strong interaction (chelation) to lead to the formation of soluble or insoluble compounds influencing the metals availability (Tyler, 2004). More than fulvic acid, between the main natural organic chelating acids we may also mention citric, oxalic, acetic and ascorbic.

Apart from the elemental binding in the colloidal pool that strongly changes the geochemical mobility of elements, the ion mobility is closely influenced by the ionic potential α, defined as:

$$\alpha = \frac{Z^{n\pm}}{r} \qquad \textbf{Eq. 1}$$

where $Z^{n\pm}$ is the ionic charge and *r* the ionic radius (Ottonello, 1997). According to their ionic potential values the ionic behaviour of elements can be summarised as follows (Figure 1).

Figure 1 Elements subdivision based on the ionic potential (Dongarrà & Varrica, 2004 modified)



Ions with $\alpha < 3$ (K^+, Na^+, Ca^{2+}, Fe^{2+}, Mg^{2+}) are the most mobile and they usually occur as aquoions and can be leached during the weathering if their parent minerals are soluble. Ions with $3 < \alpha < 9.5$ (i.e., Fe^{3+}, Al^{3+}) usually can form oxyhydroxides and are involved in the formation of clay minerals. Finally, ions with the highest α values usually are oxygen-reactive and form oxyanions (Dongarrà & Varrica, 2004; Kabata-Pendias, 2011). The occurrence of different oxidation states for several elements also influences their mobility in aqueous systems and soils.

1.2.3 The *Total* and *Pseudo-total* soil fractions

The *total* analysis of metals in soils provides very valuable information about the geological soil origin. Analysis for metals in solids can be carried out by two different approaches, namely direct analysis of the solid, for example, X-ray fluorescence (XRF) spectroscopy, or after decomposition of the matrix to liberate its metal content, for example by acid digestion.

Acid digestion involves the use of mineral or oxidizing acids and an external heat source to decompose the sample matrix. The choice of an individual acid or combination of acids is dependent upon the nature of the matrix to be decomposed and the research objective. If the research aim is a geochemical study, it may be necessary to determine the total metals content of soil, taking into account metals that are silicate-bound also, *i.e.* part of the silicate "backbone" (Dean, 2003). In this situation, the only appropriate acid to digest the silica is hydrofluoric acid (HF), because during HF digestion of silica-based materials, stable and soluble complex $[SiF_6]^{2-}$ are formed in solution. No other acid or combination of acids will liberate the metal of interest from the silica matrix.

As it is unlikely that the silicate-bound metals will leach during soil-plant interaction, digestion in strong acids such as HNO_3, HCl or mixture as aqua regia that do not dissolve the silicate matrix, can give an estimate of the maximum amounts of elements that are potentially available or mobilizable, so-called *pseudo-total* content, with changing environmental conditions. Therefore, *pseudo-total* content is a useful tool in the assessment of the long-term elements mobility. Such reagents do not mobilise trace elements from geological silicate parent materials but dissolve metal which largely enter the soil environment in non-silicate-bound forms (Rao et al., 2008 and references therein).

According to ISO 11466/95, the use of aqua regia to make a pseudo-total analysis

is perfectly acceptable in this situation, and has been used as a reference procedure in the preparation of soil and sediment reference material certified for extractable contents by the European Community of Bureau of Reference (BCR).

Unfortunately, today, most of the **Certified Reference Materials** (**CRMs**) are available only for heavy metals extractable content while for the REE; CRMs certify only the total content.

1.2.4 The *Bioavailable* soil fraction

The mobility of metals in soils depends upon the phases the metals occur in, and which chemical and physical processes these phases are subjected to (Rao et al., 2010).

The total elements content of the soil reflects the geological soil origin, but it is considered to be a poor indicator of the mobile elements fraction and therefore bioavailable. The bioavailable fraction is defined as the fraction of the soil elements that are available or may become available and can therefore be absorbed, metabolised or accumulated by organisms (Ehlken & Kirchner, 2002; Ehlers & Luthy, 2003; Feng et al., 2005; Rao et al., 2008). The bioavailable fraction is the result of several complex processes of mass transfer and absorption; therefore the soil properties, metal speciation, plant species and consequently the soil-plant interactions, determine the bioavailability of metals in soils (Ehlken & Kirchner, 2002; Kabata-Pendias, 2011).

The main difficulty in the practical application of bioavailability concept in the environment comes from the lack of a definite agreement on the methodologies to be used for its determination (Benson et al., 1994; Adriano, 2001). The study of the bioavailable elements has been addressed through various approaches including: method based on the free ions activity (free ion activity model; FIAM) (Campbell, 1995; Hough et al., 2005), isotope dilution method (Collins et al., 2003), diffusive gradients method (diffusive gradients in thin films; DGT) (Zhang & Davison, 1995), anodic stripping voltammetry method (ASV) (Sawamoto, 1999) and single or sequential extraction of soil (Rao et al., 2010). A good estimate of bioavailable concentration (which normally represents less than 10% of the total content) can be given by the sum of the soluble fractions, dissolved and weakly adsorbed to the solid phase in equilibrium with soil solution (Kabata-Pendias, 2011 and references therein).

The chemical extraction is the most used method to estimate the fraction of bioavailable elements in the short term; however it should be noted that the bioavailability of an element is linked to a dynamic process and it varies according to

abiotic and biotic parameters. In vivo, in addition to the soil characteristics, the plant absorption and extraction are also influenced by the physiology of the plant species, the particular chemical reactions that occur into the rhizosphere (desorption, adsorption, precipitation, dissolution) and the interactions between elements and the micro flora (Shan et al., 2003; Feng et al., 2005; Kabata-Pendias, 2011). So, different species grown on the same soil may have different element concentrations in their tissues.

The sequential extraction methods, while allowing to investigate the soil fractions in which the metals are bound (Tessier et al., 1979; Quevauviller et al., 1993), do not provide a real information about bioavailable metals fraction, because extracting agents only a bit similar to those naturally produced by plants are used. More accurate information can be obtained through extraction processes with solutions similar to those exudated by plants in the rhizosphere. Among the many extraction methods of the bioavailable soil fraction proposed in literature, the use of organic acids seems interesting because the plant extrude low-molecular-weight organic acids (**LMWOAs**) in rhizosphere soil (Shan et al., 2003 and references therein), and it uses these compounds to mobilise, complex and absorb various nutrient elements (Jones, 1998). The commonly used methods have been developed for the heavy metals or micronutrients extractions while the other trace elements, and in particular REE, are less studied. Recently, some authors (Fang et al., 2007; Rao et al., 2008 and references therein) have proposed chelating agents solutions such as DTPA (diethylene triamine pentaacetic acid) and EDTA (ethylene diamine tetraacetic acid) to determine the mobile trace element contents.

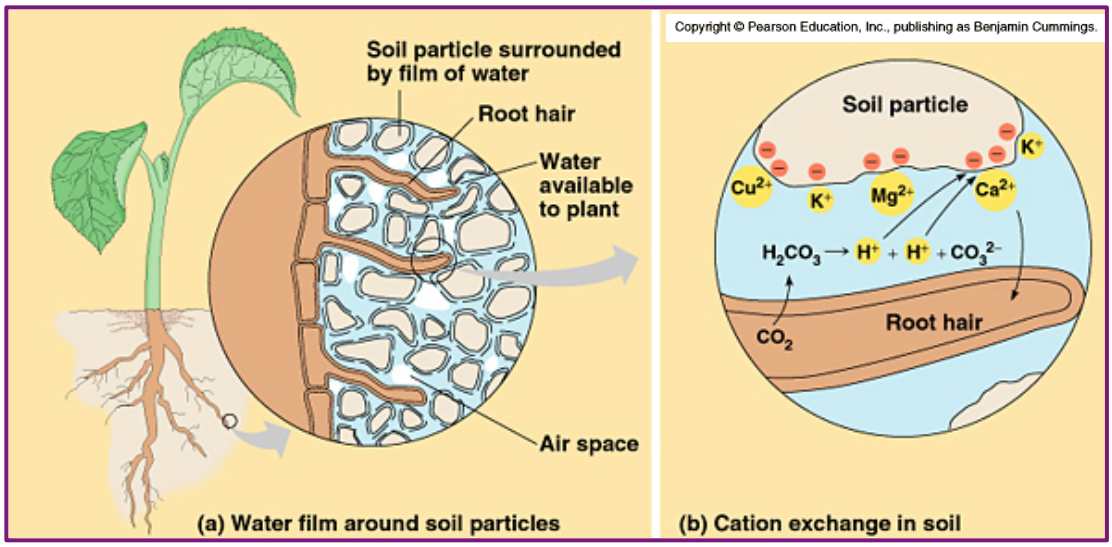
1.2.5 The rhizosphere

The rhizosphere is the soil volume whose chemical, microbiological and physical characteristics are influenced by the presence of plant roots. It is a few mm (1-20 mm) of soil immediately in contact with the roots, whose chemical-physical characteristics are significantly different from those of the surrounding soil (Gregory, 2006). In the rhizosphere, the plant roots may release large quantity and variety of organic materials including sugars, polysaccharides, organic acids, amino acids, mucilages, enzymes, cell walls. The released root exudates may affect the chemical elements availability (Gregory, 2006; Neumann & Romheld, 2002); indeed, the exudates contain complexing or chelating substances (phytosiderophores, organic acids) that can efficiently desorb and mobilise soil elements such as Fe and P from insoluble mineral soil phase by

formation of stable complexes re-absorbed by roots (Gregory, 2006); these mechanisms have also been observed in *Vitis vinifera L.* (Brancadoro et al., 1994; Jimenez et al., 2007).

The rhizosphere pH may differ from the "bulk soil" pH (Gerendas & Ratcliffe, 2002; Gregory, 2006); the root extrudes H^+ or OH^- and HCO_3^- (Figure 2) in stoichiometrically equal amount to the excess of cations or anions absorbed so as to maintain an electro neutrality condition in the root cell (Marschner, 1986). pH changes may also be due to organic anions release, the root respiration or microbial activity with consequences on solubilisation, desorption, adsorption of the soil elements (Gregory, 2006).

Figure 2 Soil leaching induced by root activity



The humic compounds increase the availability of elements for plant forming complexes with various micronutrients, but they also have a direct action on the plant metabolism carrying out an activity hormone-like (Jimenez et al., 2007).

A trace elements remobilisation in soil can occur as a result of several processes as:

- competition with other cations (present in high concentrations),
- release from Fe/Mn oxides and hydroxides due to redox condition changes,
- release following the carbonates dissolution by a pH decrease,
- formation of very stable soluble complexes by chelating agents,
- microbial activity that can change pH.

1.3 Vitis vinifera L.

1.3.1 Morphology and systematic

The grapevine belongs to the botanical species called *Vitis vinifera L.* Cultivated varieties (vines or grapevines) are classified into subspecies *sativa*, while wild forms, from which those households derived, are classified in subspecies *silvestris* (Table 1).

Table 1 Systematic classification of *Vitis vinifera* (Angelini, 2007)

Kingdom	***Plantae***
Subkingdom	*Tracheobionta* – vascular plant
Super division	*Spermatophyta* – plant with seeds
Division	*Magnoliophyta* – plant with flowers
Class	*Magnoliopsida* - dicots
Subclass	*Rosidae*
Order	*Rhamnales*
Family	*Vitaceae*
Genus	*Vitis L.*
Species	*Vitis vinifera L.* - grapevine
Subspecies	*Vitis vinifera L. ssp.* Sativa – domestic vine *Vitis vinifera L. ssp.* Sylvestris – wild vine

Other species of the genus *Vitis*, important for viticulture, are those from North America that have given rise to varieties used as rootstocks or for the production of grapes. Among species used for rootstocks production there are: *Vitis rupestris*, *Vitis riparia* and *Vitis berlandieri*; while *Vitis lambrusca*, *Vitis eastivalis* and *Vitis amurensis* are used to create fertile hybrids (Angelini, 2007).

The *V. riparia* has been used on soils with low limestone content and in temperate environment; the low vigor can anticipate the budding and maturation and induces a slightly lower productivity than other rootstocks. In Italy is no longer used for the low vigor, however, could be reintroduced in high-density installations.

The *V. rupestris*, whose the *Du Lot* selection is the most widespread, is characterised by an enough deep root system, good resistance to limestone, good grafting affinity and rooting of cuttings; it is not very resistant to drought but has great capacity of microelements absorption; remarkable sensitivity to infection by viruses.

The *V. berlandieri* is not used as such due to their very little rhizogenic capacity of cuttings, but the positive features are the great resistance to drought and limestone.

Generally in viticulture using rootstocks from crosses between these species, as often the intersection expressed positive features of both parents. Among the hybrid

rootstocks are three groups: the American hybrids, the American complex and the Euro-American hybrids.

In the first group, American hybrids are divided into:

1. *V. riparia x V. rupestris* (including 3306, 3309, 101-14, Schwarzmann, 16-108, 16-113 and 2A) have moderate vigor, suitable for good fertility levels soils, do not adapt to the drought southern regions, fear the humidity, the compactness of soil and the high rate of active limestone. They induce early maturation, which are suitable for the northern regions.
2. *V. berlandieri x V. riparia* (including 420A, Kober 5BB, SO4, Binova, 157-11, Teleki, 5C, 125AA, 161-49, 34EM, 225 Ru, 8B Ferrari, Cosmo 2 and 10 and RSB1) show greater affinity engagement than previous hybrids, better resistance to limestone, drought and increased vigor.
3. *V. berlandieri x V. rupestris*, (including **1103P**, **779P**, **140Ru**, 17-37, 99R, 110R, 775P, 1447P and R. Du Lot) are characterised by high vigor, good resistance to drought and active limestone (more than the previous groups).

The vine is a liana that can hold on itself thanks to special prehensile organs called *tendrils*. The wild vine behaves like a liana itself, is also able to invade and smother whole trees used as guardians. In rocky environment they take bushy and creeping aspect. Conversely men condition the growth habit in cultivated systems according to the type of wanted farming (Angelini, 2007).

The root system of the grapevine carries out anchoring function, hormone synthesis, water absorption and accumulation of reserve substances (carbohydrates) from epigeal area, which will subsequently be used by plant to the vegetative awakening. The root system is contained almost completely within one-meter depth of soil, but under certain optimal conditions of soil can reach 4-5 meters and expand laterally to some meter in dependence of the type of soil and the density of planting (Fregoni, 2005). It may constitute up to 60% of the vegetative biomass, although this percentage is highly variable depending on variety, rootstock, environmental conditions and seasonal variations.

The lignified part of plant (skeleton) consists of a main axis said *strain* or *stem*, from which secondary axes branching, up to one year *shoots* that are pruned every year. The leaves are placed individually on each node (alternating arrangement). At axilla of each leaf there is a lateral bud that can give rise to a lateral shoot. On second to fourth

node after the crown is present, in opposite position to leaf, the first cluster or the first tendril if the bud has not been able to differentiate (Angelini, 2007).

From ampelographic viewpoint, the leaf is an important organ of the plant, together with shoot, berries and seeds, because it can be used to discriminate the different varieties of *Vitis vinifera* and rootstocks. (Fregoni, 2005; Angelini, 2007).

The flowers of grapevine clustered in a so-called *inflorescence* or *cluster*; the *inflorescences* are inserted on the opposite node compared to the leaf. The cluster consists of a main axis (*rachis)* and it contains several ramifications. The vine can generate three basic types of flowers:

1. *male flowers* common in American vines (rootstocks) and rarely in clusters of European vines together with hermaphrodite flowers;
2. physiologically *female flowers* but morphologically hermaphrodites are on American vines or even on some European varieties;
3. *hermaphrodite flowers*.

Hence the American vines are mostly dioecious and European vines mostly hermaphrodites (Table 2) (Fregoni, 2005; Angelini, 2007). European grapevine have also bigger size in relation to all organs, which show a higher degree of polymorphism; phenological stages are less variable in American vines than those of European vines. (Angelini, 2007).

Table 2 Rootstocks classification based on flower sexuality

Rootstocks sex			
Male	**Female**	**Hermaphrodite**	**Imperfect (sterile)**
Rupestris Du Lot	101-14	1447P	99R
Ripari Glorie	16-16	1202C	196-17
779P	161-49		
110R	Kober 5BB		
1103P	41B		
140Ru	Salt Creek		
216-3	Fercal		
44-53			

The berry is formed by a membranous *epicarp* (skin). On the cuticle of some varieties can form a waxy layer called *pruina*. The *epicarp* contains: tartaric acid, phenolic compounds (anthocyanins and flavones), tannins, flavours, enzymes, etc. Under the skin there is the *mesocarp* with large cells filled with juice, and immersed in pulp there is the *endocarp* containing seeds. The mesocarp and the endocarp forming

the pulp. Finally, the shape of the berry is important because it is a distinctive character of varieties (Fregoni, 2005).

1.3.2 Physiology

Life cycle (Fregoni, 2005)

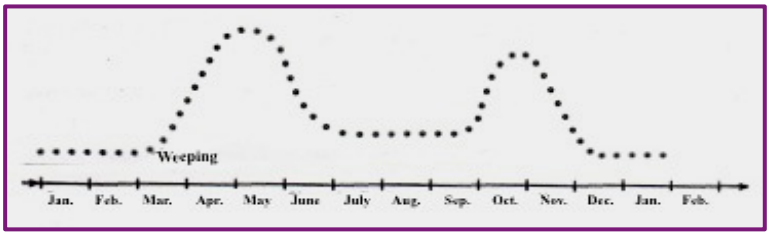
The life cycle of grapevine includes four main phases:

1. the *Juvenile phase* (from 2 to 3 years) is an unproductive period as C/N ratio is low. Root absorption greater than carbohydrates synthesis in leaves.
2. the *Increasing productivity phase* (from 3rd or 4th to 5th or 6th years) is characterised by an increase in C/N ratio in favour of carbohydrates. As well as an increase in flowers hormones synthesis and/or leaf synthesis promoters.
3. the *Constant productivity phase* (from 6th or 7th to 20th or 25th years). The production indeed is not constant, but follows a more or less high sine wave depending on varieties and cultivation technique. The C/N ratio shifts towards C and flowers hormones become prevalent on inhibitors.
4. the *Senescence phase or decreasing productivity* (from 25th years to plant death). The C/N ratio increases unto C for aging of all plant parts, in particular roots absorption capacity is reduced.

Annual cycle (Fregoni, 2005)

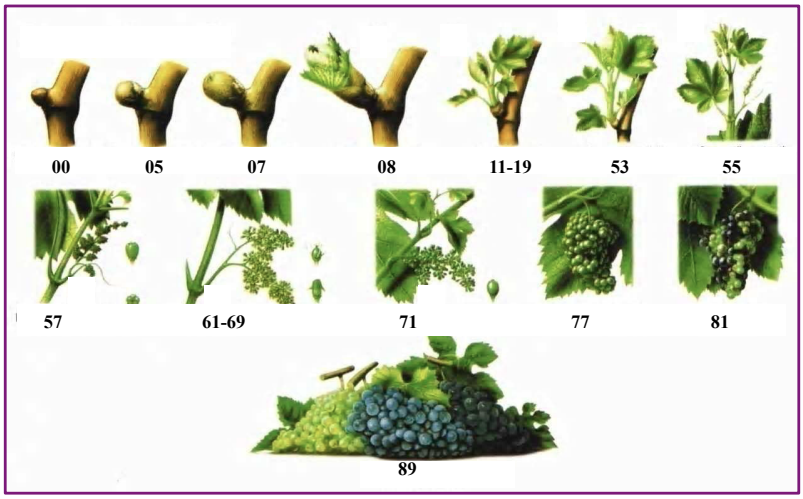
Finished unproductive period the grapevine carries out the annual cycle through different phenological stages, which can be divided into two sub cycles: *vegetative* and *reproductive*. The *weeping* phenomenon heralds the *vegetative* sub cycle that occurs with dripping lymph from pruning cuts about 15 days before budding; the weeping phenomenon is a consequence of rapid increase in absorption rate of the root system. From nutritional viewpoint root activity is characterised by two maxima, which do not correspond with critical period of more demanding phenological stages (Figure 3).

Figure 3 Fluctuation of root activity in the grapevine during the annual cycle (Fregoni, 2005)



In this period it occurs both maximum water and minerals absorption by plant and root growth and renewal, indeed more lymph loss as well as plant is very vigorous. In late Winter-Spring maximum vegetative development follows radical greater absorption period. Then budding starts through bud swelling, bud bracts opening and shoot emission; this phenomenon is controlled by both exogenous (such as temperature, altitude, latitude, exposure, photoperiod, pruning, soil fertility etc.) endogenous factors as hormonal stimuli, vigor (rootstock and/or variety), genotype, etc. Last stage is rest period, ranging from fall leaves until next vegetative recovery. In rest period occurring various biochemical and enzymatic processes on behalf of vine reserve substances; in our environment runs from late November-early December to late March-early April (Figure 4).

Figure 4 Biologische **B**undesanstalt, **B**undessortenamt und **CH**emische Industrie, **BBCH**, scale (modified) used to identify the phenological development stages of *Vitis vinifera*.



Legend: **00**: winter bud; **05**: "wool stage"; **07**: beginning of bud burst; **08**: bud burst or **budding**; **11-19**: leaves development; **53**: inflorescences clearly visible; **55**: inflorescences swelling, flowers closely pressed together; **57**: inflorescences fully developed, flowers separating; **61-69**: **flowering**; **71**: **fruit setting**; **77**: berries beginning to touch; **81**: **véraison**; **89**: **berries ripe for harvest**; [**97**: **end of leaf-fall**].

1.3.3 The rootstocks

A hundred years ago the hybrid rootstocks of the American species, as the previously mentioned V. rupestris, V. riparia and V. berlandieri, were developed to cope phylloxera emergence. However, the rootstock is today chosen primarily for its adaptability to environmental conditions and not for its phytosanitary tolerance (although phylloxera is still alive in our country). In Sicily the most diffused rootstocks

are: **140 Ru**, **1103 P**, **779 P**, 775 P and 110 R. Vitis riparia is always the "preferred" species by breeder due to good suitability in propagation by cuttings and/or graft. To acquire drought and limestone resistance it is hybridized with V. berlandieri, but rather surface roots of V. riparia (for example Kober 5BB and 420A) are favoured in fresh soil and with good retention capacity. Vitis rupestris conversely owns a deep root system and its hybrids with Vitis berlandieri are more suitable in droughty or complex soils with high CPI (*chlorotic power index*) values. Dense vineyards propagation (more than five thousand plants per hectare) and more frequent droughty years are helping diffusion of hybrids (as 1103 Paulsen), while 140 Ruggeri pay excessive vigor despite good drought resistance, saline soils and limestone adaptability. Reaction capacity to abiotic stresses and induction vigor are characteristics that also influence different rootstock ability to root system absorption, minerals accumulation and transfer (such as potassium) in plant (high in case of 1103 Paulsen) (Tosi, 2011).

V. Berlandieri x V. Rupestris: **1103 Paulsen**

Rootstock vigor slightly lower than Kober 5BB, especially in fresh and loose soils. It well adapts to loamy soils but not wet in the spring. It is very resistant to drought and to limestone (18-20%). It delays the vegetative cycle with a negative effect for red wines, but it is favourable for white wines whose musts have acid more balanced profile.

V. Berlandieri x V. Rupestris: **779 Paulsen**

Late Nineteenth Century Paulsen obtained it in Palermo; it is similar to 1103P but shows a more reduced vigor. Good drought and limestone resistance and soil compactness always characterise it, but in medium fertility soils it adapts too.

V. Berlandieri x V. Rupestris: **140 Ruggeri**

This very vigorous rootstock normally slows the vegetative cycle and maturation. It well resists in limy and clayey soils, wet in spring and drought in summer. With weak vines it should be used in very drought limy and needy soils.

1.3.4 Elemental behaviour in plants

The ions absorption by various plant species changes depending on surface radical, cationic exchange root capacity and exudates production; it is also influenced both by environmental conditions (temperature, photoperiod, humidity) that determine the root development and evapotranspiration rate and by soil characteristics (pH, Eh,

water condition, porosity, clay content, organic matter, oxides, CEC, mineral elements amount).

Nutrients can come into contact with the root system in various ways. The soil solution can transport elements near the roots that in turn may extend into soil in search of nutrients. The roots have their own cation exchange capacity that allows fixing ions, whose concentration increases in vicinity of the root itself.

The strategies used by plants to increase the nutrients absorption are multiple: increase of radical surface, mycorrhizal symbiosis, rhizosphere pH changes of 2-3 units, by release of peptides and exudates, acidifying and complexing agents (Gregory, 2006).

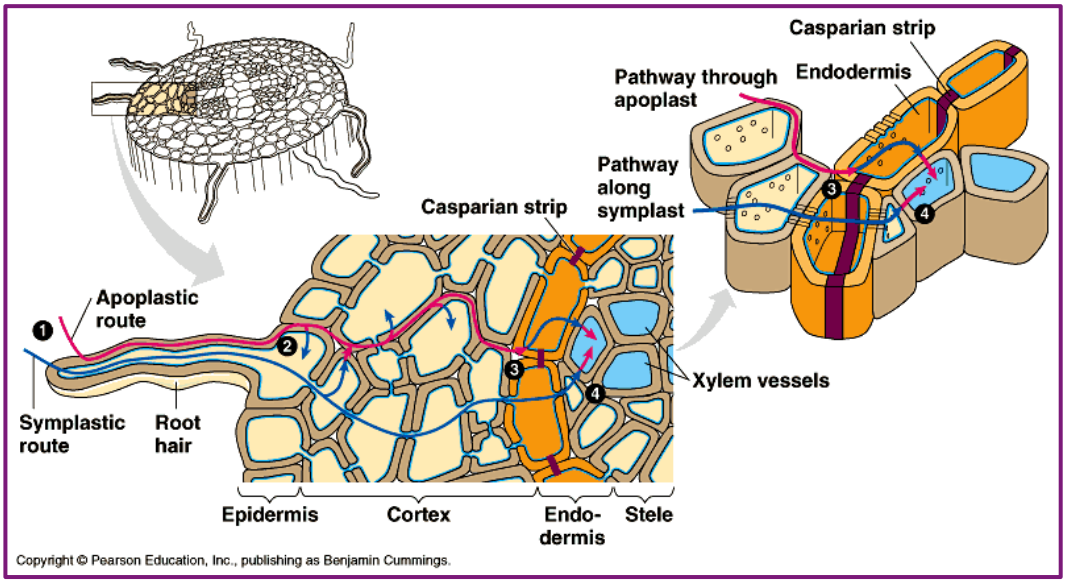
The soil elements can enter into plant through leaves or roots. The first way can be significant for some elements in contaminated particular environments (*i.e.*, proximity to foundries, mines, polluted areas) or in response to foliar treatments but generally the radical way is the absorption predominant mode.

As regards to the nutrient elements, due to large difference between the element concentration of the soil solution and that into the plant, it is evident the existence of an absorption and accumulation regulated by cell membrane which acts as a barrier to movement of soluble water ions. The absorption inside root cells is also influenced by the chemical-physical properties of elements: neutral species and ions with hydrated ionic small radius and small charge are absorbed more easily because there are fewer interactions with charged groups of cellular components. Indeed, plant uptake is uneven for cations and anions, and it involves a system perturbation. The excessive absorption of cations (with H^+ extrusion) determines an increase of the internal pH; the latter is balanced by the organic acids synthesis whose anions bind to absorbed cations and they are accumulated. Typically the trace elements are complexed by low molecular weight ligands: this allows them to remain in soluble form and to be transported; alternatively, they can be incorporated into metabolites. Due to aspecific absorption, no-nutrient elements having charge and/or hydrated ionic radius similar behave as the essential elements (Adriano, 2001).

Two different paths (Figure 5) can perform the transport from root cells towards the stem:

- *apoplastic* path, through the cell walls by diffusion or following the water flow,
- *symplastic* path directly through living cells.

Figure 5 Biological uptake of elements



Currently, the release mechanism of ions in the xylem vessels has not yet been clearly defined: some evidence seems to indicate that it is a process mediated by carriers (Adriano, 2001; Bais et al., 2006; Badri & Vivanco, 2009). In xylem vessels, the flow of water and solutes from root to apices is determined by radical pressure and especially by transpiration. During transport interactions can occur between soluble ions and charged negatively wall sites of the xylem vessels, or nutrients absorption by cells. These processes are influenced by the charge of ionic species and its concentration, by the complexing agents, by other ions, by the diameter and charge density of the xylem vessels. The various elements, especially in relation to its concentration and the plant species, can be accumulated in roots or transported to aerial organs.

The metabolism of macro- and micronutrients has been extensively studied but limited information exists on trace elements (Badri & Vivanco, 2009). The amount of these elements in plant is considered to be largely depending on the geochemistry of growth site (Kabata-Pendias, 2011). The behaviour of no-nutrient elements is often closely linked to nutrient elements with similar chemical-physical characteristics. The absorption and transport processes studied for main mineral elements have also been observed for some no-nutrient elements (Adriano, 2001).

Many not-essential elements are primarily stored in roots and only small proportions are transported in aerial organs. Generally, Ag, B, Li, Mo, Se and to a lesser extent Mn, Ni, Cd and Zn are easily transportable upwards; while Co, Cu, Cr, Pb, Hg and Fe are more closely linked to the root cells (Kabata-Pendias, 2011).

1.4 Rare Earth Elements

The REE are less well known but they are becoming gradually more interesting in environmental traceability studies. The rare Earth Elements are released into the environment as a result of their technological uses (construction of LED TV, construction of car accumulators, laser targeting systems, systems to produce green energy, …) (Bignami 2010; 2012) and, in particular areas, as fertilizers.

Even if they are being studied, their biological and geochemical behaviour in soil-plant system are not yet well known. Their similar chemical behaviour and/or the particular soil composition (a reflection of their geological origin) make REE a hopeful proxy for the geographical characterisation (Spalla et al., 2009): several studies seem to indicate that their distribution profile is similarly found in the Earth's crust, in soil and in the plants grown on it (Wang et al., 2003; Fang et al., 2007; Liang et al., 2008 and references therein); other authors have not observed a relationship between plant and soil contents or otherwise they have found an elements fractionation during absorption (Tyler, 2004 and references therein).

1.4.1 Geochemical characters

Lanthanides are a group of 14 elements from $_{58}$Ce to $_{71}$Lu produced by progressive filling of the *4f* orbital. Their chemical characters are a consequence of two main aspects:

- ✓ Since *4f* electrons occupy an inner position closer to the nucleus, elements from $_{58}$Ce to $_{71}$Lu have the same outer electronic configuration, corresponding to $[Xe]6s_2 5d_1$ (Shannon, 1976). *4f* electrons are not involved in chemical bonds. $_{57}$La is also associated with lanthanides having the same external electronic configuration.
- ✓ The progressive decrease of ionic dimensions caused by the poor shielding of the *4f* electrons (*lanthanide contraction*) produces slight changes of the Z^{3+}/r ratio that influence lanthanide reactivity, especially in aqueous systems. Yttrium is also usually associated with lanthanides having the same 3+ ionic charge and radius intermediate between Ho and Er (Shannon, 1976).

Lanthanides, La and Y are usually associated to form the Rare Earths group (REE). REE have a typical 3+ oxidative state; only Ce and Eu can occur as Ce^{4+} and Eu^{2+} under selected environmental oxidising or reducing conditions, respectively.

From a geochemical viewpoint, REE behave similarly but not identically during

CHarge and RAdius Controlled natural processes (CHARAC) such as those allowing magma crystallisations (Bau, 1996). The limited observed differences are related to the wide range of REE coordination numbers ranging from 6 to 12 leading to their different "geochemical compatibility" towards crystal lattices of minerals. Similar slight differences among REE also remain during aqueous reactions such as dissolved or surface complexation (adsorption). These differences are more evident between Y and Ho and allow their decoupling due to the different electronic configurations. The different REE behaviour between CHARAC and non-CHARAC (aqueous) processes and then between crystalline solids and aqueous fluids (Bau, 1996) makes REE probably among the best geochemical tracers for studying solid/liquid interfaces.

Since in both aqueous systems and during crystallization processes, the characteristics of REE change continuously with ionic radius along the series, the geochemical behaviour of REE can be evidenced through the shape of the sequence of REE normalised concentrations assessed by:

$$[\boldsymbol{REE_i}]_{\boldsymbol{n}} = \frac{[\boldsymbol{REE_i}]_{\boldsymbol{sample}}}{[\boldsymbol{REE_i}]_{\boldsymbol{reference}}} \qquad \textbf{Eq. 2}$$

(Taylor & McLennan, 1995) where the subscript "n" refers to the normalised concentration of a given sample with respect to a material taken as reference. By studying enrichments or depletions of single elements along the series, usually named "anomalies", the evaluation of "geochemical behaviour" of REE is carried out. These anomalies can be assessed according to the equation:

$$\frac{[\boldsymbol{REE}]_{\boldsymbol{i}}}{[\boldsymbol{REE}]_{\boldsymbol{i}}^{*}} = \frac{\boldsymbol{2}[\boldsymbol{REE}]_{\boldsymbol{i}}}{\{[\boldsymbol{REE}]_{\boldsymbol{i-1}} + [\boldsymbol{REE}]_{\boldsymbol{i+1}}\}} \qquad \textbf{Eq. 3}$$

where the subscript "i" indicates every element along the REE series whereas "(i-1)" and "(i+1)" are its immediate neighbour before and after within the series (Alibo & Nozaki, 1999). Features of normalised-REE patterns can also be evaluated considering enrichments or depletions of groups of REE subdivided into light REE, from La to Sm (LREE), middle REE, from Eu to Dy or Ho (MREE) and heavier REE, from Ho or Er and Lu (HREE) according to their atomic weight. Moreover, the sequence of the distribution coefficients values (K_d), calculated as reported in equation 4, between REE concentrations is measured in two interfaced substances, 1 and 2:

$$\boldsymbol{K_d} = \frac{[\boldsymbol{REE}]_{\boldsymbol{1}}}{[\boldsymbol{REE}]_{\boldsymbol{2}}} \qquad \textbf{Eq. 4}$$

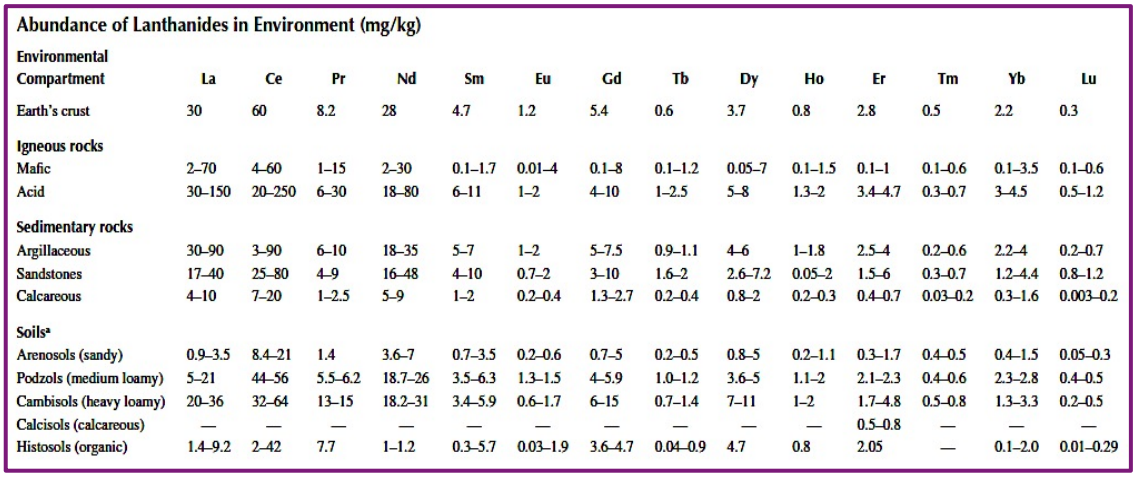
and can be split into four different intervals, La-Nd, Pm-Gd, Gd-Ho and Er-Lu, called tetrads. They are referred to as the first (t_1), second (t_2), third (t_3) and fourth tetrad (t_4), respectively. Peppard et al. (1969) suggested that this effect (thereafter called the "*Tetrad Effect*") could be related to the progressive filling of the *4f* orbital. The shape of tetrad effects has been defined as W-type or M-type if the splitting produces upward convex or downward convex features, respectively. Similar features have also been observed during several geochemical and biogeochemical processes (Masuda & Ikeuchi, 1979; Masuda et al., 1987; Kawabe, 1992; Irber, 1999; Bau, 1999; Monecke et al., 2002) and their occurrences were attributed to REE complexation with an inner-sphere mechanism either in a dissolved pool or onto surfaces. This suggestion makes the amplitude of tetrad effects a geochemical proxy to discriminate between strong, inner-sphere coordination bonds and simple adsorption or outer-sphere coordination processes. The amplitudes of tetrad effects can be evaluated according to equation 5 for the third and fourth tetrads:

$$\boldsymbol{t_3 = \sqrt{\frac{[Tb]_n * [Dy]_n}{[Gd]_n * [Ho]_n}}} \; ; \; \boldsymbol{t_4 = \sqrt{\frac{[Tm]_n * [Yb]_n}{[Er]_n * [Lu]_n}}} \qquad \textbf{Eq. 5}$$

and can represent tetrad curvatures due to the splitting of normalised-REE patterns (Irber, 1999). This curvature is significant if $t_i < 0.95$ and $t_i > 1.05$, leading to W- or M-type tetrad effects, respectively.

Due to their incompatible behaviour REE rarely form minerals and mainly are dispersed as isomorphous substituents in a wide spectrum of accessory minerals that mainly occur in highly evolved "crustal" rocks. Otherwise, due to their large affinity as regards of CO_3^{2-} groups, REE are enriched in carbonatites where they can crystallise REE-bearing carbonates (Laveuf and Cornu, 2009 and references therein). The strong chemical inertia of many accessory minerals, as some REE-bearing phosphates, allows to REE enrichments in residual soils where these minerals are concentrated explaining the largest REE deposits (Figure 6 and Table 3). Cao et al. (2000) have quantified the REE content in different soil fractions as a result of a sequential extraction: soluble fraction in water is less than 0.1% of the total content, the extractable fraction is < 2.5%, the fraction bound to the organic matter ranges between 2 and 19% and that linked to the Fe/Mn oxides varying between 5 and 42%. Finally, the residual fraction, obtained after HCl, HNO_3 and HF digestion contains most of the REE: from 51 to 92% of the total content depending on the soil type and the considered element.

Figure 6 Abundance of Lanthanides in rocks and soils (Kabata-Pendias, 2011)



Abundance of Lanthanides in Environment (mg/kg)

Environmental Compartment	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Earth's crust	30	60	8.2	28	4.7	1.2	5.4	0.6	3.7	0.8	2.8	0.5	2.2	0.3
Igneous rocks														
Mafic	2–70	4–60	1–15	2–30	0.1–1.7	0.01–4	0.1–8	0.1–1.2	0.05–7	0.1–1.5	0.1–1	0.1–0.6	0.1–3.5	0.1–0.6
Acid	30–150	20–250	6–30	18–80	6–11	1–2	4–10	1–2.5	5–8	1.3–2	3.4–4.7	0.3–0.7	3–4.5	0.5–1.2
Sedimentary rocks														
Argillaceous	30–90	3–90	6–10	18–35	5–7	1–2	5–7.5	0.9–1.1	4–6	1–1.8	2.5–4	0.2–0.6	2.2–4	0.2–0.7
Sandstones	17–40	25–80	4–9	16–48	4–10	0.7–2	3–10	1.6–2	2.6–7.2	0.05–2	1.5–6	0.3–0.7	1.2–4.4	0.8–1.2
Calcareous	4–10	7–20	1–2.5	5–9	1–2	0.2–0.4	1.3–2.7	0.2–0.4	0.8–2	0.2–0.3	0.4–0.7	0.03–0.2	0.3–1.6	0.003–0.2
Soils[a]														
Arenosols (sandy)	0.9–3.5	8.4–21	1.4	3.6–7	0.7–3.5	0.2–0.6	0.7–5	0.2–0.5	0.8–5	0.2–1.1	0.3–1.7	0.4–0.5	0.4–1.5	0.05–0.3
Podzols (medium loamy)	5–21	44–56	5.5–6.2	18.7–26	3.5–6.3	1.3–1.5	4–5.9	1.0–1.2	3.6–5	1.1–2	2.1–2.3	0.4–0.6	2.3–2.8	0.4–0.5
Cambisols (heavy loamy)	20–36	32–64	13–15	18.2–31	3.4–5.9	0.6–1.7	6–15	0.7–1.4	7–11	1–2	1.7–4.8	0.5–0.8	1.3–3.3	0.2–0.5
Calcisols (calcareous)	—	—	—	—	—	—	—	—	—	—	0.5–0.8	—	—	—
Histosols (organic)	1.4–9.2	2–42	7.7	1–1.2	0.3–5.7	0.03–1.9	3.6–4.7	0.04–0.9	4.7	0.8	2.05	—	0.1–2.0	0.01–0.29

Table 3 (a) Average concentration in the Earth's crust (Kabata-Pendias, 2011), (b) concentration range found in soils of different origins (Tyler, 2004)

Element	Earth's crust abundance (mg/Kg)[a]	Range contents in soil (mg/Kg)[b]
Y	20-33	7-60
La	30	5.5-33-2
Ce	60	11-68
Pr	8.2	1.3-7.5
Nd	28	0.3-53
Sm	4.7	0.9-4.6
Eu	1.2	0.22-0.83
Gd	5.4	1.0-4.8
Tb	0.6	0.15-0.65
Dy	3.7	0.9-3.74
Ho	0.8	0.20-0.74
Er	2.8	0.63-2.2
Tm	0.5	0.09-0.33
Yb	2.2	0.60-2.3
Lu	0.3	0.09-0.34

1.4.2 REE distributions in plants

Neither the REE contents of plant tissue nor their physiological functions have received much attention until the last decade. Robinson et al. (1958) reported, as the first one, high concentrations (up to 2300 mg kg^{-1}) of the total REE in hickory trees and pointed out the similarity between their contents in tree leaves and in exchangeable soil fraction. Laul et al. (1979) calculated the relative abundance of REE both in soil and plants and showed that concentrations of these elements in plants followed their occurrence in soil. Only recently, appropriate analytical techniques have facilitated both the investigation of their concentration levels in soils and plants and the study of their physiological functions (Hu et al., 2004; Tyler, 2004; Rao et al., 2008).

The soil characteristics and the chelating agents may influence the REE desorption from soil and modify the root absorption (Tyler, 2004). The absorption rate from soil is usually higher than the translocation rate towards the apices (Xu et al., 2002): in plant are observed larger quantities of REE in roots and then gradually smaller amounts in leaves, stems, flowers and finally in fruits (Cao et al., 2000; Hu et al., 2004). The REE accumulation in different wheat parts varies with growth stages, and at the maturity stage is in the order of roots > leaves > stems > grains (Ding et al., 2006). Moreover Fang et al. (2007) reported that soil pH and amorphous Fe oxides increased the REE content of wheat roots, whereas REE content of wheat shoots was independent of any soil properties.

The REE contents vary considerably (even by several orders of magnitude, from <1 to 15000 μg kg^{-1} d.w.) (Kabata-Pendias, 2011) in different species; higher levels are reported for bryophytes and lichens but especially for ferns and species of the genus *Carya* (*Juglandacee*) referred to as accumulating plants (Fu et al., 1998) (Table 4).

Table 4 REE in various terrestrial plant species (μg Kg^{-1} d.w.) (Kabata-Pendias, 2011 and references therein)

Element	Approximate Detection (%)	Various Land Plants[94]	Lichens and Bryophytes[94]	Cheatgrass[462]	Vegetables[94,462,547]	Rice Straw[1192]	Blueberry Tops[1085]	Pine Needles[1085]
La	100	3–15,000	400–3000	170	0.4–2000	88	130–340	260–300
Ce	100	250–16,000	600–5600	330	2–50	174	210–740	370
Pr	90	60–300	80–620	40	1–2	26	70–140	62–130
Nd	90	300	240–3000	150	10	84	73–130	150–160
Sm	90	100–800	60–800	35	0.2–100	20	24–49	30–32
Eu	80	30–130	20–170	8	0.04–70	10	0.82–5.6	4.9–5.3
Gd	80	2–500	60–560	37	<2	20	11–27	23–25
Tb	70	1–120	6–70	9	0.1–1	7	4.7–14	12–22
Dy	70	50–600	40–360	—	—	16	8.6–21	20–22
Ho	70	30–110	4–70	<20	0.06–0.1	5	2.5–4.2	3.9–5.1
Er	70	80–380	10–190	<500	0.5–2	10	1.5–7.7	6–6.8
Tm	50	4–70	1–26	50	0.2–4	4	1	1.1–1.7
Yb	50	20–600	10–900	20	0.08–20	10	2.5–10	8.2–8.5
Lu	40	30	1–20	3	0.01–60	5	1.2–2.3	1.9–2

In general, however, most of herbaceous and woody plants and edible vegetables, especially at the aerial portions, have fairly low levels.

Although there are some other reports on stimulating impact of REE on several processes in plants such as, seed germination, root growth, nodulation, chlorophyll production, these elements have not been proved yet to be essential to plants. For example the REE show effects on Ca function: the REE seem to have characteristics and effects similar to those of the Ca; they have comparable ionic radii and they are localised in the same cell sites (Hu et al., 2004). The La^{3+} can compete with the acceptors Ca channels in the cytoplasmic membrane, it can replace the Ca^{2+} onto extracellular binding sites or into many enzymes that may thus maintain same activity or be inhibited (Rangel, 1994). In pea seedlings was observed that the Eu could replace the Ca in calmodulin that still retains its binding ability (Amann, 1992).

1.5 Methods verification

1.5.1 Background

Reliable analytical methods are required for compliance with national and international regulations in all area of analysis. Therefore, every laboratory must operate in order to demonstrate, in an objective way, to be able to provide accurate and reliable data and this mainly through use of validated analytical methods. The term "*Validation*" means to confirm, by means of objective evidences, that the requirements for a particular use or intended application have been met. The validation process therefore has the aim to establish through the evaluation of all relevant parameters (analyst, equipment, methods, reagents etc.) whether the chosen method is valid for the determinations that are going to be performed. Validation applies to a defined protocol for the determination of a specified analyte and range of concentration in a particular type of test material used for a specified purpose. The validation procedure, as well as assessing the performance of an analytical method, provides the parameters to set the routine quality control in the application of the method. The aim on the routine quality control is to demonstrate that the method is under controlled conditions, or that the benefits are those expected. The key performance parameters that require attention during validation, vary from requirement to requirement and from method to method. Some of the most important parameters are:

- ✓ Linearity and working range;
- ✓ Detection limit;
- ✓ Quantification limit;
- ✓ Accuracy;
- ✓ Trueness and Precision.

1.5.2 Linearity and working range

In any quantitative method it is necessary to determine the range of analyte concentrations or property values within which the method may be applied. At the lower end of the concentrations range, the limiting factors are the values of the limits of detection and/or quantification. At the upper end of the concentrations range, limitations will be imposed by various effects depending on the instrument response system.

Within the "**working range***"* there may exist a linear response range. Within the linear range, signal response will have a linear relationship to analyte concentration or property value. The extent of this range may be established during the evaluation of the working range.

"*Linearity*" can be check informally by examination of a plot of residual produced by linear regression analysis of the response on the concentrations in an appropriate calibration set (Burke S., 2001).

The relationship of instrument response to concentration does not have to be perfectly linear for a method to be effective but the curve should be repeatable from day to day (ARPA guideline, 2003). Both the working range and the linear range may differ for different matrices, in agreement with the effects of interferences from the matrices themselves.

1.5.3 Detection limit

In general the "**detection limit** (**DL**)" may be described as the concentration of the analyte that gives an instrumental signal significantly different from the background signal. Although this parameter is widely used in many analytical documents, there is still no full agreement (between researchers or professional body) on the interpretation of the expression "*significantly different"* because it acquires different meaning referring to qualitative or quantitative analysis.

Recently, there has been an increasing trend to define the detection limit as the analyte concentration giving a signal equal to the blank signal, y_b, plus three standard deviations of the blank, s_b (In-House Method Validation, 2003).

$$\mathbf{Y_{DL} = y_b + 3s_b} \qquad \textbf{Eq. 6}$$

combined into:

$$\mathbf{DL = \frac{y_{DL} - q}{m}} \qquad \textbf{Eq. 7}$$

where *q* and *m* are respectively the intercept and slope of linear regression function.

In the method validation there should always be a distinction between "**instrument detection limit** (**IDL**)" and "**method detection limit** (**MDL**)". The former is based on measurement on reagent blank and the latter on measurement of a blank real sample that has been processed through all steps of the method. Clearly IDL is often far smaller than a MDL and it is inappropriate for method validation because does not take into account the matrix effects which may be relevant in determining detection limit (Thompson et al., 2002).

1.5.4 Quantification limit

Since detection limit provides a value with an extremely low degree of accuracy, it is necessary to establish another limit that defines the minimum amount of analyte in the sample that can be detected with a probability established *a priori*. As such, the "**quantification limit** (**QL**)" for chemical and physical measurement is introduced, which is defined as the lowest concentration of analyte which can be determined with an acceptable level of accuracy (repeatability). As a detection limit it can be obtained through several completely independent determination of analyte concentration in a typical matrix blank or a low level material, without reject negative results. QL is defined as the analyte concentration giving a signal equal to the blank signal, y_b, plus ten standard deviations of the blanks, s_b.

$$\mathbf{Y_{QL} = y_b + 10s_b} \qquad \textbf{Eq. 8}$$

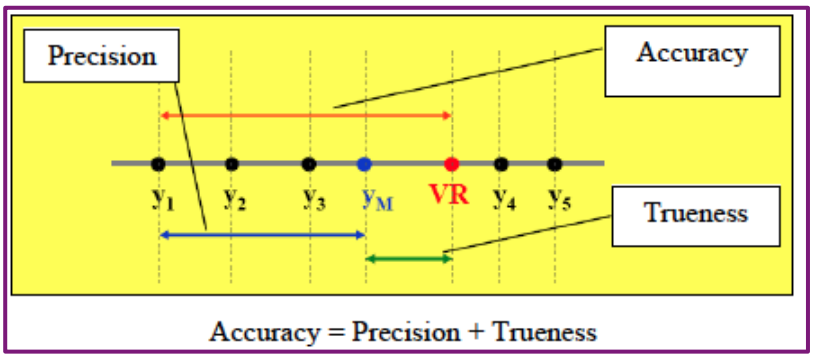
combined into:

$$\mathbf{QL = \frac{y_{QL} - q}{m}} \qquad \textbf{Eq. 9}$$

1.5.5 Accuracy

"**Accuracy**" expresses the closeness of agreement between a test result and the accepted reference value. Method validation seeks to quantify the likely accuracy of results by assessing both systematic and random effects on results. Therefore, accuracy is normally studied in terms of two components: **trueness** and **precision** (Figure 7).

Figure 7 Mathematical relationship between precision, trueness and accuracy



The "**trueness**" (of a method) represents how close the mean of a set of results (produced by the method) is to the true value; trueness is normally expressed in terms of "**bias**". "**Precision**" is a measure of how close the results are to one another, and is usually expressed by measures such as the standard deviation, which describe the spread of results.

1.5.6 Trueness

Trueness is expressed in terms of bias, with smaller bias indicating greater trueness. Typically it is determined by comparing the response of the method on a reference material to its known value (Thompson et al., 1999). *Certified Reference Materials* are traceable to international standard with a known uncertainty and therefore can be used to address all aspect of bias (method, laboratory, ect.) simultaneously, assuming that there is no matrix mismatch.

To check trueness using a reference material, the mean and standard deviation of a series of replicate tests are determined and compared to the characteristic values of the reference material by applying Student's t-test (Equation 10):

$$\mathbf{t_{calc}} = \frac{\mathbf{C_{CRM}} - x_m}{\sqrt{\frac{\mathbf{s_r^2}}{\mathbf{n}} + \mathbf{u_{CRM}^2}}} \leq \mathbf{t_{th(p,v)}} \qquad \textbf{Eq. 10}$$

where C_{CRM} is the certified value, x_m is the average obtained value, s_r is the standard deviation, n the number of performed replicates, u_{CRM} is the associated uncertainty with the reference material and ν are the degree of freedom calculated as follow:

$$\boldsymbol{\nu = \frac{\left(\frac{S_r^2}{N}+u_{CRM}^2\right)^2}{\left(\frac{S_r^2}{N}\right)^2} \cdot (N-1)} \qquad \textbf{Eq. 11}$$

If t_{calc} is less than t_{th} then it is possible to claim that the method provides accurate results to the significance level chosen and the recovery is equal to 1. The certified reference material should be as similar as the matrix under consideration during the validation; when interpreting the results, the uncertainty associated with the certified values should be taken into account.

1.5.7 Precision

According IUPAC, precision is "The closeness of agreement between independent test results obtained under stipulated conditions" (Thompson et al., 2002). The precision is usually stated in terms of standard deviation *(s)*, the relative standard deviation (RSD), or standard deviation of the mean *($S(x_m)$)* of an n number of replicates.

A measure of the dispersion of a set of n values is given by the equation

$$\boldsymbol{s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_m)^2}{(n-1)}}} \qquad \textbf{Eq. 12}$$

The standard deviation s, relative to the mean value x_m is equal to

$$\boldsymbol{RSD = \frac{s}{x_m}} \qquad \textbf{Eq. 13}$$

The $S(x_m)$ is a measure of the dispersion of a set of mean values obtained from n repeated measurements

$$\boldsymbol{S(x_m) = \frac{s}{\sqrt{n}}} \qquad \textbf{Eq. 14}$$

Precision depends only on the distribution of random errors and does not relate to the true or specified value. The size of precision depends on a variety of factors, including the number of parameters that are modified during the precision study and the level of variation in the operating conditions.

Materials and Methods

2.1 Materials and Reagents

2.1.1 Laboratory Materials

All lab ware was in polyethylene (PE), polypropylene (PP) or in Teflon and the calibration of all volumetric equipment was verified. A calibrated E42-B balance (Gibertini, Italy) was used to weigh all samples and standards.

2.1.2 Ultrapure water

High purity water, 18.2 MΩ cm, was obtained by Easy pure II purification system (Thermo, Italy).

2.1.3 Reagents

All chemicals used were of ultrapure grade. Ultra clean concentrated reagents used for sample analysis and cleaning plastic-ware were purchased from VWR International.

2.1.4 Metal Standards

Working standard solutions for each element (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) and internal standard solution (ISTD) containing ^{103}Rh were daily prepared by stepwise dilution of the multi-element stock standard solutions DBH, Merck or CPI International (1000 ± 5 μg mL^{-1}) in a HNO_3 1 % (w/w) medium.

2.1.5 Certified Reference Material

To test the analytical procedure applied to vegetable sample, the CRM *INCT-OBTL-5 Oriental Basma Tobacco Leaves* was used. The CRM, distributed by "'Institute of Nuclear Chemistry and Technology" in Warsaw, was made up of tobacco leaves with certified and known chemical composition.

To compare two mixtures to employ in acid digestion, the CRM *SRM 2711a Montana Soil II* was used. The CRM, distributed by "National Institute of Standards and Technology" (NIST) in USA, was made up of moderately contaminated soil with certified and known chemical composition.

2.2 Sample treatments

2.2.1 Soil samples

2.2.1.1 Sampling and storage

After elimination of grassy layer, the sampling was carried out using a pre-cleaned stainless steel spade and the sampled soil was stored in polyethylene bags. For each soil was collected the rhizosphere fraction (from 0 to 40 cm deep), so-called "*agronomic layer*", where there is the highest concentration of roots.

Soil samples were sieved to 5 mm to remove plant debris and any other foreign body, dried at 105 °C overnight, and then finely ground for a complete homogenization and stored in PE containers.

2.2.1.2 Pseudo-total soil fraction determination

An aliquot of 500 mg of soil sample was placed in a Teflon reactor, mixed with 2:1 v/v mixture of HNO_3 (65% w/w) : H_2O_2 (30% w/w) and subjected to digestion in a microwave oven.

The obtained solutions were diluted with ultra-pure water up to a final volume of 15 mL and storage (+4 °C) in PE centrifuge tubes. Prior to ICP-MS measurement, the solution was diluted 100 times with ultra-pure water.

2.2.1.3 Bioavailable soil fraction determination

Bioavailable soil fraction was carried out with a 5 mM DTPA solution prepared from the acid solid form (H_5DTPA) in ultrapure water. NaOH was gradually added to obtain a final pH solution equal to 5.0.

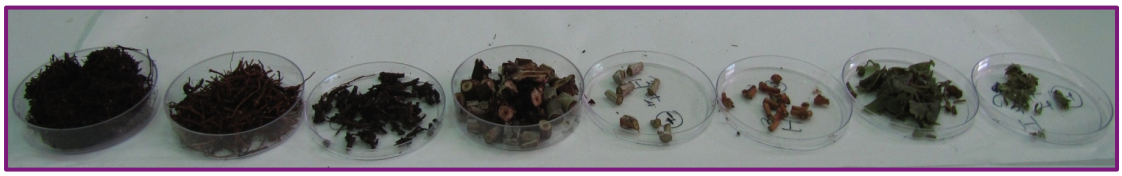
10 g of soil sample were added of 20 mL of a 5 mM DTPA solution in a PE centrifuge tube, shaken in an orbital shaker at 300 rpm for 24 hours at room temperature and successively centrifuged for 45 minutes at 5000 rpm. The surnatant filtered through a Whatman N° 41 filter paper and stored (+4 °C). Prior to ICP-MS measurement, the solution was diluted 50 times with 1% (w/w) HNO_3 solution.

2.2.2 Vitis vinifera samples

2.2.2.1 Sampling and storage

All plants were wholly sampled and the different organs separated. Each plant organ (Figure 8) was washed, cutted, dried at 105 °C, weighted and then finely ground for a complete homogenization and stored in PE containers.

Figure 8 Various organs of Vitis, from absorbing fine roots to apex of shoot



2.2.2.2 Sample preparation and analysis

An aliquot of 250 mg of vegetable sample was put in a Teflon reactor with 2:1 v/v mixture of HNO_3 : H_2O_2 and then digested in a microwave oven. The obtained solutions were diluted with ultra-pure water up to a final volume of 10 mL and storage (+4 °C) in PE centrifuge tubes until ICP-MS measurement.

2.2.3 Grapevine samples

2.2.3.1 Sampling and storage

The sampling was carried out using a ceramic scissors and stored in PE containers. Cluster samples were washed with ultra-pure water to remove any particulate matter present on the outer surface of the berry. Then berries were carefully separated from pedicel, weighed, dried at 105 °C, and then finely ground for a complete homogenization and stored in PE containers.

2.2.3.2 Sample preparation and analysis

An aliquot of 2.5g of berry sample was placed in a Teflon reactor, mixed with 2:1 v/v mixture of HNO_3 : H_2O_2 and subjected to digestion in a microwave oven. The obtained solutions were diluted with ultra-pure water up to a final volume of 15 mL and stored (+4 °C) in PE centrifuge tubes until ICP-MS measurement.

2.3 Instrumentations and operational parameters

A Microwave Accelerated Reaction System, MARS 5 Xpress^{TM}, was used for digestion of soil and vegetable samples. For each digestion cycle, reagent blanks were carried out also to keep under control memory effects. Operating conditions are shown in Table 5.

Table 5 Microwave digestion program

Stage	Power		Ramp (min)	Temp control (°C)	Hold (min)
	Max (W)	%			
1	600	100	10	200	60
2	800	100	-	200	60
3	1600	100	-	200	60
4	1600	100	-	200	60

An ICP-MS instrument (Agilent Technologies 7500ce Series Spectrometer) equipped with a collision cell, was used for the analyses of all the investigated trace elements. All parameters were daily optimized using a 1 ng mL^{-1} solution of ^{7}Li, ^{89}Y, ^{140}Ce, ^{205}Tl and to obtain maximum sensitivity, the instrument was tuned on ^{89}Y. Each solution was measured three times, and ICP-MS analyses were carried out with a classical external calibration approach using ^{103}Rh (1 mg L^{-1}) as internal standard to compensate for any signal instability or sensitivity changes during the analysis. Operating conditions are shown in Table 6.

Table 6 ICP-MS operating conditions and measurement parameters

RF power	1550 W
Sample uptake rate	0.400 mL min^{-1}
Coolant argon flow rate	15 L min^{-1}
Carrier argon flow rate	0.80 L min^{-1}
Make-up argon flow rate	0.25 L min^{-1}
Torch	Quartz
Nebuliser	MicroMist 200 µl
Sampler and Skimmer Cones	Nickel
Number of scans	3
Ion lens settings	Adjusted daily to obtain max. signal intensity
Washing time	1 min (HNO_3 5% v/v)
Oxide $^{156}CeO^{+}/^{140}Ce^{+}$ ratio	< 0.8%
Double charged $^{70}Ce^{++}/^{140}Ce^{+}$ ratio	< 0.5%
Measured isotope	^{89}Y, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{151}Eu, ^{158}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{173}Yb, ^{175}Lu
Internal standard	^{103}Rh 1 mg L^{-1}

2.4 Pseudo-total soil fraction method verification

According to Mackey et al. (2010) many of routine soil analyses are performed using EPA methods (for example, Method 3050B). These methods involve acid extraction of metals from the soils rather than total digestion; infact, these methods are convenient to use, involve use of less acid than total digestion and, in addition, some scientists use these methods to assess the maxim amount of metals during soil-plant interaction. Unfortunately, even in NIST Special Publication 260-172 to assist laboratories that use these EPA methods, information on the pseudo-total heavy metal content was provided but not for the REE series.

Our goal is clearly to avoid the use of aqua regia in order to limit the presence of Cl^- ions in solution that can create isobaric interferences, due to the formation of polyatomic species between Ba and Cl isotopes ($^{x}Ba^{y}Cl^{+}$).

Therefore, the CRM (*Montana Soil II*) was exclusively subjected to acid digestion to compare the efficiency of a mixture with oxidizing-complexing power, as the aqua regia, and a only oxidant mixture, as HNO_3 : H_2O_2. Independent aliquots of CRM were carefully weighed (500 mg), treated with both aqua regia and the HNO_3 : H_2O_2 (2:1 v/v) mixture respectively and subjected to microwave digestion. The CRM obtained values and its percentage recovery are shown in Table 7.

Table 7 Reported mass fraction and measured values (**mg Kg^{-1}**), standard deviation and recovery percentage of CRM 2711a *Montana Soil II* obtained both aqua regia and HNO_3:H_2O_2 mixture

Element	CRM 2711a *Montana Soil II*		Aqua regia			HNO_3:H_2O_2		
	C_{CRM}*	U_{CRM}*	C_m	s_m	Rec%	C_m	s_m	Rec%
La	*38*	*1*	22.1	0.2	58	23.1	0.2	61
Ce	70		44.0	0.4	63	45.7	0.4	65
Nd	*29*	*2*	19.4	0.1	67	20.0	0.2	69
Sm	**5.93**	**0.28**	3.65	0.06	61	3.79	0.04	64
Eu	*1.1*	*0.2*	0.71	0.01	65	0.74	0.02	67
Gd	5		3.58	0.06	72	3.69	0.06	74
Tb	0.8		0.483	0.008	60	0.506	0.008	63
Dy	5		2.63	0.03	53	2.77	0.07	55
Yb	3		1.50	0.03	50	1.52	0.04	51
Lu	0.5		0.2146	0.0003	43	0.236	0.004	47

*__Certified__, *reference*, and information mass fraction values

The CRM analysis allows comparing the two digestion mixtures. The obtained data with both mixtures are lower than the certificates values; these results were expected as our values were obtained after pseudo-total extraction while certified values were obtained by total digestion. For both examined mixtures, percentage relative standard deviation (RSD%) for all investigated elements was less than 2%. The pseudo-total amounts obtained by two extractant mixtures are comparable: 59% in aqua regia and 62% in HNO_3 : H_2O_2 mixture. The comparison highlights the similar extracting ability allowing to use them arbitrarily.

We decided to apply the comparison on soil with different physicochemical characteristic (unpublished data), to verify whether HNO_3 : H_2O_2 mixture could replace the aqua regia in the lanthanide series research. Infact, another way in which the results of this analytical method may be tested is by comparing them with those obtained by using a second (perhaps a reference) method (Miller & Miller, 2005).

In this case we had two sample means x_{m1} and x_{m2}. Taking the null hypothesis that the two methods give the same result, was necessary to test whether (x_{m1} - x_{m2}) differs significantly from zero. In order to test whether the difference between two sample variances is significant, the statistic F was calculated:

$$\boldsymbol{F = \frac{s_1^2}{s_2^2}} \qquad \textbf{Eq. 15}$$

where 1 and 2 was allocated in the equation so that F is always ≥1. In this case a pooled estimate, s, of the standard deviation can be calculated from the two individual standard deviations s_1 and s_2.

Whereby, to verify whether the difference among the two used mixture could be accounted for by random or systematic errors, a Student's t-test was employed. The statistic t parameter was calculated by the following equation for each element.

$$\boldsymbol{t_{calc} = \frac{|x_{m1} - x_{m2}|}{s \cdot \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}} \qquad \textbf{Eq. 16}$$

where x_{m1} and x_{m2} are the averages of the two set of measures, n_1 e n_2 are the samples size and s is the standard deviation assuming that the samples are drawn from populations with equal standard deviation, was calculated according to

$$\boldsymbol{s^2 = \frac{(n_1 - 1) \cdot s_1^2 + (n_2 - 1) \cdot s_2^2}{n_1 + n_2 - 2}} \qquad \textbf{Eq. 17}$$

Comparison of t_{calc} e t_{th} with a confidence level of 0.05 and n_1+n_2-2 degree of freedom (ν), Table 8 shows just one example, allowed to state that the differences between the two used mixtures are not significantly different ($t_{calc} < t_{th}$).

Since the concentrations of these elements, obtained after aqua regia digestion are comparable to those obtained by HNO_3:H_2O_2 mixture digestion for all analysed samples, it is likely that the two used mixtures extract same analyte amounts (unless of experimental error); therefore, for all samples of this thesis, was decided to perform digestions by HNO_3 : H_2O_2 mixture to avoid another source of error in analytical determinations.

Table 8 Mean value (**mg Kg^{-1}**, d.w.) and relative standard deviation % of a representative soil sample obtained both aqua regia and HNO_3:H_2O_2 mixture, and statistic F and t values

	HNO_3:H_2O_2		**Aqua regia**		**$F_{th(P=0.05)}$ = 19**		**$t_{th(P=0.05)}$ = 2.78**	
	C_m	***RSD %***	***C_m***	***RSD %***	**F_{calc}**	**outcome**	**t_{calc}**	**outcome**
Y	8.23	3.74	8.35	6.47	3.08	P	0.35	P
La	20.04	3.80	21.06	6.29	3.03	P	1.16	P
Ce	54.60	4.39	57.94	6.25	2.28	P	1.33	P
Pr	4.95	4.34	5.20	6.48	2.47	P	1.10	P
Nd	18.89	4.30	19.88	6.18	2.29	P	1.16	P
Sm	3.62	5.72	3.80	6.94	1.61	P	0.90	P
Eu	0.66	3.13	0.69	5.01	2.74	P	0.92	P
Gd	3.32	4.15	3.49	6.05	2.33	P	1.12	P
Tb	0.41	4.03	0.43	5.61	2.06	P	0.70	P
Dy	1.92	3.59	1.98	5.74	2.70	P	0.70	P
Ho	0.33	3.87	0.34	5.88	2.32	P	0.06	P
Er	0.88	3.74	0.84	5.93	2.92	P	0.60	P
Tm	0.10	2.49	0.10	6.95	7.37	P	0.65	P
Yb	0.60	3.91	0.60	7.06	3.18	P	0.24	P
Lu	0.08	3.71	0.08	8.91	5.56	P	0.33	P

2.5 Bioavailable soil fraction method verification

According to the literature, a relationships between the grapevine exudation (quantity and pH) and its physiological stage exists (Ohkawa, 1981), so it was decided to verify DTPA extracting capacity in order to simulate the existing conditions in nature and thus estimate the bioavailable REE content in soil that can be absorbed by the Vitis. Since the total concentration of organic acids in roots is typically around 10–20 mM (1–4% of total dry weight) (Jones, 1998), was evaluated the extracting capacity of DTPA solutions at four different concentrations 5, 10, 50 and 100 mM prepared as described in section 2.2.1.3. As show in Figure 9, the maximum amount of extruded exudates was reached at pH values included between 4 and 5.6 during diurnal changes, while range

between 5.2 and 5.6 in a seasonal cycle. Therefore, was chosen to fix the pH value of DTPA solutions to a halfway pH value equal to 5.0.

Figure 9 (a) Seasonal changes in level and pH of the exudate from vine (Ohkawa, 1981); (b) diurnal changes in amount and pH of the exudate from vine (Ohkawa, 1981)

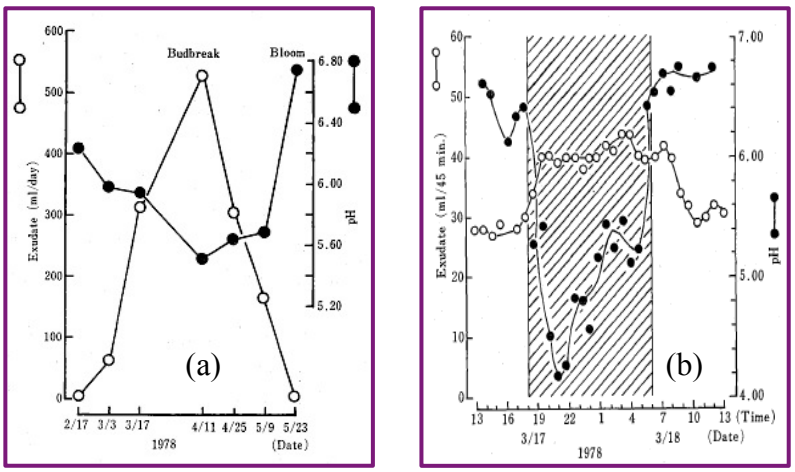


Table 9 shows the results obtained by treating a same soil with the different concentrations DTPA solutions.

Table 9 Mean value (C_m, **mg Kg^{-1}**) and percentage relative standard deviation (RSD%) in the bioavailable soil fractions extracted with different concentrations DTPA solutions from a **calcarenitic** soil

	DTPA **5 mM**		DTPA **10 mM**		DTPA **50 mM**		DTPA **100 mM**	
	C_m	***RSD %***	***C_m***	***RSD %***	***C_m***	***RSD %***	***C_m***	***RSD %***
La	0.91	2.0	1.04	1.4	2.47	1.4	2.46	1.3
Ce	9.75	3.4	6.11	1.2	12.11	0.5	10.57	1.0
Pr	0.27	3.2	0.28	0.8	0.69	1.8	0.70	1.3
Nd	1.14	2.8	1.18	1.3	2.89	1.3	2.92	1.2
Sm	0.25	3.0	0.27	2.4	0.65	0.7	0.66	0.8
Eu	0.06	4.7	0.06	3.0	0.14	1.7	0.14	0.5
Gd	0.35	3.3	0.35	1.6	0.82	1.1	0.81	1.0
Tb	0.04	5.7	0.05	1.3	0.11	1.8	0.11	1.4
Dy	0.24	2.8	0.25	1.9	0.59	1.6	0.58	0.6
Y	2.09	1.3	1.74	1.2	3.88	1.5	3.76	1.3
Ho	0.05	5.3	0.05	2.0	0.12	1.5	0.11	1.4
Er	0.14	3.1	0.14	2.0	0.32	2.1	0.31	1.2
Tm	0.02	2.9	0.02	2.3	0.04	0.9	0.04	1.2
Yb	0.13	3.0	0.13	0.8	0.28	1.1	0.26	1.9
Lu	0.02	2.9	0.02	0.4	0.04	1.4	0.04	1.3
Σ REE	**15.47**	**2.1**	**11.67**	**0.7**	**25.14**	**0.4**	**23.48**	**0.6**

The results show that increasing DTPA concentration a little increase in the extracting capacity for all investigated analytes was obtained. Using the lower

concentration solutions, 5 and 10 mM, the total REE amounts in the analysed solutions were equal to 15.47 and 11.67 mg Kg^{-1} respectively, while employing the more concentrated solutions, 50 and 100 mM, the extracted REE were 25.14 and 23.48 mg Kg^{-1} respectively.

In order to choose the best operating conditions, the extracting capacity of the different DTPA solutions was evaluated subjecting soil with different physico-chemical characteristic to the same single extraction method. In particular, in addition to the calcarenitic soil (Table 9) a carbonatic-marly-clay and a basaltic soils were also analysed. The results obtained treating a carbonatic-marly-clay soil, shown in Table 10, demonstrate, as for the calcarenitic soil, a greater extracting capacity by more concentrated solutions of DTPA.

Table 10 Mean value (C_m, **mg Kg^{-1}**) and percentage relative standard deviation (RSD%) in the bioavailable soil fractions extracted with different concentrations DTPA solutions from a **carbonatic-marly-clay** soil

	DTPA **5 mM**		DTPA **10 mM**		DTPA **50 mM**		DTPA **100 mM**	
	C_m	***RSD %***	***C_m***	***RSD %***	***C_m***	***RSD %***	***C_m***	***RSD %***
La	5.77	3.4	8.87	1.9	10.60	4.9	13.54	1.0
Ce	15.98	2.8	9.84	1.4	14.93	5.3	20.68	1.1
Pr	2.27	2.7	1.88	1.3	2.25	4.8	2.88	1.3
Nd	9.93	3.0	8.06	1.4	9.61	5.2	12.27	1.5
Sm	1.54	2.7	1.72	1.7	2.06	4.9	2.64	1.4
Eu	0.38	2.7	0.39	1.4	0.47	5.3	0.61	1.2
Gd	1.83	2.7	2.06	1.0	2.47	4.9	3.16	1.5
Tb	0.24	2.7	0.27	1.2	0.32	4.7	0.41	1.1
Dy	1.31	2.5	1.45	1.3	1.74	4.9	2.24	1.3
Y	11.69	2.7	11.14	1.1	13.09	5.6	16.53	1.1
Ho	0.26	2.8	0.28	0.9	0.34	4.7	0.44	1.5
Er	0.69	2.6	0.75	1.5	0.91	5.3	1.17	1.5
Tm	0.09	2.8	0.09	1.8	0.11	4.7	0.14	0.7
Yb	0.51	2.6	0.56	0.7	0.69	4.3	0.89	1.8
Lu	0.07	3.0	0.08	1.4	0.10	3.8	0.13	1.7
Σ REE	**52.54**	**1.3**	**47.44**	**0.6**	**59.70**	**2.2**	**77.74**	**0.5**

The solutions obtained after extraction with DTPA 5 and 10 mM show a content of REE equal to 52.54 and 47.44 mg kg^{-1} respectively, while those obtained after extraction with the more concentrated solutions the total amount of the investigated elements were 59.70 and 77.74 mg kg^{-1} respectively. The results obtained after treatment of the basaltic soil, listed in Table 11, clearly show that the DTPA extracting capacity is not a function of concentration. The results obtained by the more dilute solutions are 21.92 and 17.03 mg kg^{-1} respectively, while for the more concentrated solutions are 22.31 and 20.81 mg Kg^{-1} respectively.

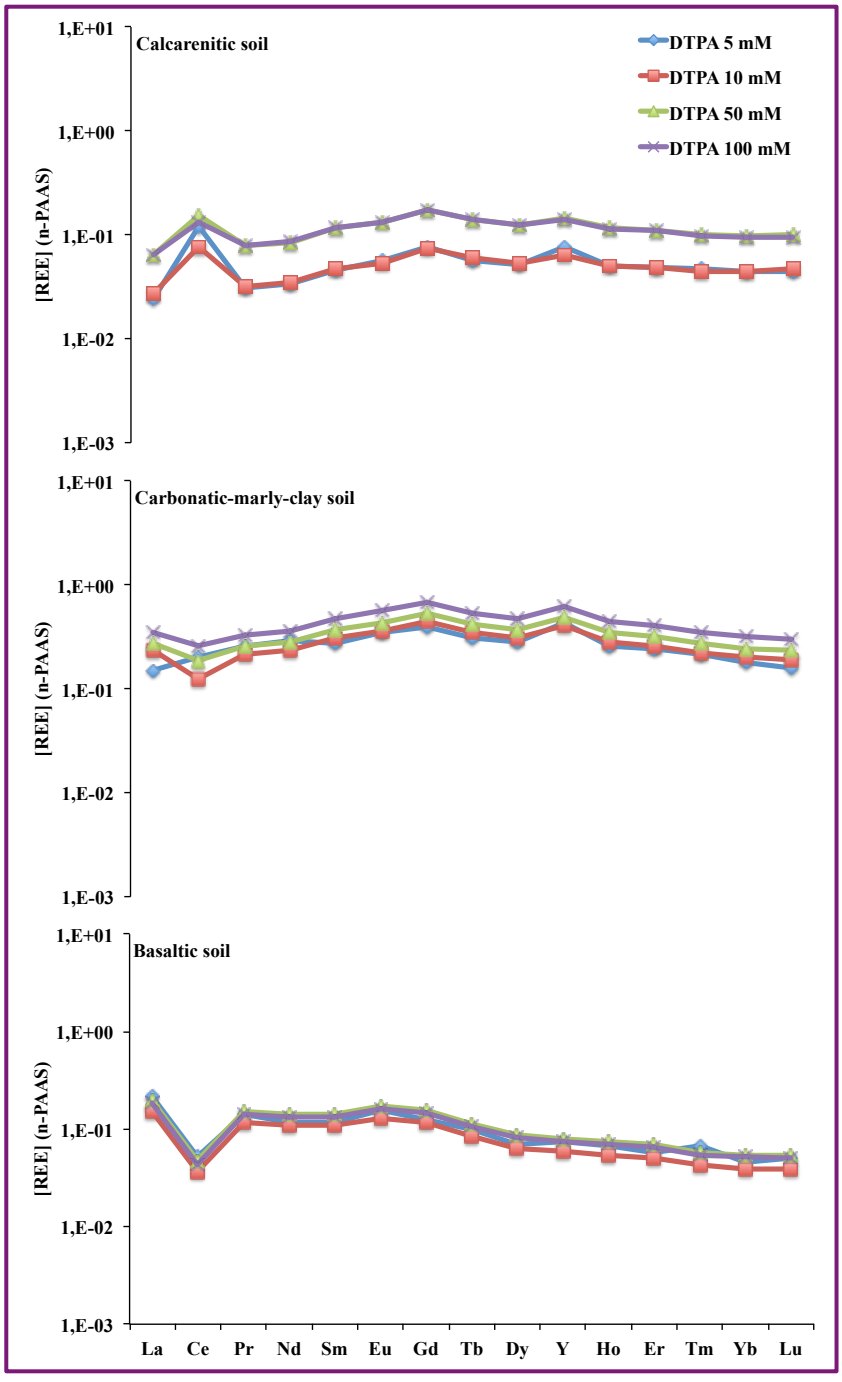
Table 11 Mean value (C_m, **mg Kg^{-1}**) and percentage relative standard deviation (RSD%) in the bioavailable soil fractions extracted with different concentrations DTPA solutions from a **basaltic** soil

	DTPA **5 mM**		DTPA **10 mM**		DTPA **50 mM**		DTPA **100 mM**	
	C_m	***RSD %***	***C_m***	***RSD %***	***C_m***	***RSD %***	***C_m***	***RSD %***
La	8.26	1.7	5.81	1.8	7.57	2.6	7.09	0.5
Ce	4.17	1.9	2.92	1.7	3.80	2.9	3.45	0.4
Pr	1.25	1.0	1.03	1.5	1.34	2.6	1.26	0.7
Nd	3.98	2.0	3.69	1.3	4.81	3.1	4.53	0.4
Sm	0.65	3.0	0.60	2.0	0.79	2.2	0.74	0.5
Eu	0.17	2.1	0.14	0.5	0.18	3.2	0.18	0.7
Gd	0.58	3.0	0.54	1.9	0.72	3.7	0.68	1.3
Tb	0.08	2.1	0.06	2.2	0.09	2.0	0.08	1.2
Dy	0.33	4.0	0.30	1.7	0.41	2.8	0.38	1.6
Y	2.04	2.0	1.59	1.7	2.13	2.8	1.98	0.6
Ho	0.07	1.9	0.05	1.3	0.07	3.8	0.07	1.2
Er	0.17	2.5	0.14	1.6	0.20	2.3	0.19	0.5
Tm	0.03	3.9	0.02	1.5	0.02	1.5	0.02	0.5
Yb	0.13	2.2	0.11	3.1	0.15	2.4	0.15	0.9
Lu	0.02	3.2	0.02	3.8	0.02	3.4	0.02	0.8
Σ REE	**21.92**	**0.9**	**17.03**	**0.8**	**22.31**	**1.2**	**20.81**	**0.2**

As shown in Figure 10, the REE pattern obtained treating different soil with DTPA at different concentrations are parallel, so we can conclude that the use of different DTPA concentration solutions did not cause a preferential REE extraction although in some cases the use of more concentrated solution returns a total REE amount slightly higher.

In the light of obtained results and according to Rao et al. (2010), subsequent determinations of bioavailable fraction in soil samples of this thesis, have been realised with a 5 mM DTPA solution. Differently by Rao et al. (2010), it was decided to maintain the pH solution to 5.0 with the aim to simulate as closely as possible the natural conditions generated by organic acids extruded by plant roots to mobilise soil nutrients.

Figure 10 PAAS-normalised patterns of the bioavailable soil fractions extracted with different concentrations DTPA solutions from three different soils



2.6 Performances method verification

2.6.1 Linearity and working range

To evaluate linearity of calibration curve for each element, residual distribution over the *x* values was evaluated. Moreover the data were subjected to Mandel's test to verify the validity of the linear model, and slope (m) and interception (q) were tested to statistically assess the possibility of the curve intersection with the zero point. The r squared value (R^2), indicating linearity between signal and concentration, was considered satisfactory when ≥ 0.998 for all the monitored isotopes during measurements in ICP-MS.

For all elements in the appropriate working intervals (*i.e.*, 0.001 – 500 μg L^{-1}), residual analysis indicated a normal distribution and the Mandel's test was positive; therefore, a statistically significant linear relationship existed. Table 12, for example, shows the R^2, m and q values for all considered elements obtained during berry samples determinations.

Table 12 Characteristic of calibration curves for each considered elements

Element	R^2	m	q
Y	0.999	2.39E-05	3.93E-05
La	0.999	2.36E-05	1.48E-04
Ce	0.999	2.18E-05	1.22E-04
Pr	0.999	2.69E-05	8.54E-05
Nd	0.998	4.62E-06	2.16E-05
Sm	1	3.94E-06	2.03E-05
Eu	1	1.26E-05	8.47E-05
Gd	1	6.64E-06	4.23E-05
Tb	1	2.69E-05	1.62E-04
Dy	1	6.54E-06	2.87E-05
Ho	1	2.61E-05	1.03E-04
Er	0.999	8.60E-06	6.52E-05
Tm	1	2.69E-05	1.06E-04
Yb	1	4.42E-06	1.69E-05
Lu	1	2.53E-05	1.00E-04

2.6.2 Detection and Quantification limit

To carry out instrumental detection and quantification limit (IDL e IQL) analyses, ten different aliquots of calibration blank (1% ultra-pure HNO_3) were measured. Behaviour of collected data was analysed according to the Shapiro-Wilks' test (P=0.05) to verify the normal distribution of obtained data, and according to David's (P=0.05),

Grubbs' (P=0.05) and Huber's tests (P=0.05) to verify the presence of outliers (Miller & Miller, 2005). The IDL and IQL in ng L^{-1}, according to equations 6-9, were calculated for each investigated analyte. Table 13 shows mean values and the standard deviations of replicates in and all values obtained, in ng L^{-1}, for IDL and IQL.

Table 13 Mean value and standard deviation (s) of Calibration Blank signal, IDL and IQL in ngL^{-1}

Element	Mean value	s	IDL	IQL
Y	0.12	0.18	0.66	1.92
La	3.50	0.59	5.26	9.36
Ce	3.21	0.10	3.51	4.22
Pr	0.10	0.25	0.83	2.55
Nd	0.81	0.40	2.00	4.78
Sm	1.61	0.28	2.46	4.44
Eu	0.25	0.25	1.00	2.74
Gd	0.18	0.25	0.92	2.66
Tb	0.16	0.26	0.94	2.76
Dy	0.37	0.24	1.07	2.72
Ho	0.20	0.24	0.92	2.60
Er	0.28	0.19	0.85	2.16
Tm	0.19	0.23	0.88	2.50
Yb	0.25	0.15	0.70	1.75
Lu	0.12	0.18	0.66	1.92

2.6.3 Accuracy

2.6.3.1 Trueness and Precision

Trueness and precision of the method were evaluated by subjecting the CRM *INCT-OBTL-5* (*Oriental Basma Tobacco Leaves*) to the entire analytical method.

The CRM was subjected to acid digestion with an exclusively oxidant mixture, HNO_3: H_2O_2, because in CRM monograph (Samczynski et al., 2011) the sample pre-treatment by acid digestion does not report further details. Eight independent aliquots of CRM were carefully weighed (250 mg), treated with HNO_3 : H_2O_2 (2:1 v/v) mixture and subjected to microwave digestion.

Data from procedural blank, obtained subjecting HNO_3 : H_2O_2 (2:1 v/v) mixture to entire method, were subtracted to CRM analysis. After controls carried out according the Shapiro-Wilks' (P=0.05), David's (P=0.05), Grubbs' (P=0.05) and Huber's tests (P=0.05) the mean value of the eight replicates and the standard deviations were calculated (Table 14).

Table 14 Mean value and standard deviation of the eight replicates obtained by HNO_3:H_2O_2 mixture, in μg Kg^{-1} (d.w.)

	C_m	s_m	RSD%
Y	1057	28	3
La	1637.17	68.59	4
Ce	2865.88	148.25	5
Pr	336	15	4
Nd	1286.06	49.21	4
Sm	256	7	3
Eu	62.0	0.9	1
Gd	270	6	2
Tb	33.8	0.4	1
Dy	188.1	2.2	1
Ho	35.9	0.5	1
Er	102	2	2
Tm	13.3	0.3	2
Yb	112	5	5
Lu	12.0	0.3	2

All values were higher than instrumental QL and into the linearity range of the calibration adopted. Relative standard deviation percentage (RSD%) for all investigated elements was between 1-5% in the performed test.

The trueness of method was evaluated comparing obtained results by acid digestion with certified values. The recovery percent and its standard deviation were also evaluated for the elements listed as information values in analysis certificate.

Recovery values of the certified elements (La, Ce, Nd, Sm, Eu, Tb, Er, Yb) obtained by HNO_3 : H_2O_2 mixture, showed in Table 15, are between 96 - 103%, while for the elements listed as informative values of the series the recovery range from 97 to 111%; only Lu shows a recovery of 72%.

In the case of repeated analyses of a CRM, the guideline ranges for the deviation from the certified values of the experimentally determined recoveries range from -20% to +10% for mass fraction range from 10 mg Kg^{-1} to 100 μg Kg^{-1}, while for concentration values near to tens μg Kg^{-1} recoveries range from -35% to +15% (2002/605/EC). Therefore data obtained after HNO_3 : H_2O_2 mixture digestion fall within the indicated ranges.

Table 15 Reported mass fraction of INCT-OBTL-5, recovery percent and its standard deviation

	CRM ($\mu g\ Kg^{-1}$)	U_{CRM}	Rec%	s_{rec}%
Y	963	20	110	3
La	**1690**	**45**	**97**	**4**
Ce	**2990**	**90**	**96**	**5**
Pr	321	10	105	5
Nd	**1330**	**55**	**97**	**4**
Sm	**264**	**7**	**97**	**3**
Eu	**60.2**	**2.1**	**103**	**1**
Gd	243	20	111	2
Tb	**34.7**	**1.2**	**97**	**1**
Dy	184.0	50.0	102	1
Ho	34.5	2.5	104	1
Er	**101**	**3**	**101**	**2**
Tm	13.6	0.3	98	2
Yb	**115**	**12**	**97**	**5**
Lu	16.7	6.6	72	2

To evaluate the efficiency of recovery on a statistical basis, discriminant function *t* (t_{calc}) is calculated (Eq. 10) and compared with the theoretical value (t_{th}) of *Student*'s *t* (Table 16).

Table 16 t_{calc} values, degree of freedom (ν) and t_{th} values for acid digestion of INCT-OBTL-5 obtained by HNO_3:H_2O_2 mixture

	t_{calc}	ν	t_{th}	outcome
Y	3.64	13	2.18	N
La	**0.93**	**22**	**2.07**	**P**
Ce	**1.06**	**18**	**2.10**	**P**
Pr	1.21	24	2.06	P
Nd	**0.73**	**108**	**1.98**	**P**
Sm	**1.02**	**53**	**2.01**	**P**
Eu	**0.85**	**1413**	**1.98**	**P**
Gd	1.33	6628	1.98	P
Tb	**0.77**	**2936**	**1.98**	**P**
Dy	0.08	13616394	1.98	P
Ho	0.54	31193	1.98	P
Er	**0.40**	**468**	**1.98**	**P**
Tm	0.89	91	1.99	P
Yb	**0.26**	**436**	**1.98**	**P**
Lu	0.70	16535690	1.98	P

The results obtained by statistical consideration, indicated that in acid digestion by HNO_3 : H_2O_2 mixture the recovery was unitary for all investigated elements ($t_{calc}<t_{th}$) except for Y than however falls within acceptability range of recovery (from -20% to +10%).

According to obtained results, the HNO_3 : H_2O_2 mixture has been regarded as the most appropriate and satisfactory for acid digestions carried out. Consequently, the determinations of examined analytes were performed by subjecting all samples (each organ of plants and/or different soils) to described preparative method.

Off-soil growth

3.1 Background

Only a limited biogeochemical literature about REE behaviour in plants occurs in order to investigate the influence of growth stages on the possible accumulation with respect to their soil content and their distribution in different plant parts (Ding et al., 2005; Babula et al., 2008). Moreover, these studies have never been addressed to *Vitis vinifera L*. They mainly have pointed out the wine composition and its changes induced by bentonite addition during manipulation wine procedures (Mihucz et al., 2006). Therefore, the first goal of this thesis was to determine REE fluxes in the substrate - grapevine system and to relate distribution of these elements in different parts of the vine. In order to investigate these phenomena it was paramount to consider several factors:

- ✓ geochemical soil properties,
- ✓ soil-plant interaction,
- ✓ effect on plant of REE absorption and translocation.

So, it was decided to treat "step by step" the system; first of all, it was studied whether, and in what way, the vine absorbs REE from substrate, and then we try to understand if the absorption takes place maintaining unchanged soil mass ratios. Since the mass soil effect may influence the absorption, to assess whether there are competing effects caused by different mass ratios of REE, it was decided to study the plants in an REE enriched and equimolar system. This experiment would allow to highlight if the vine carries out a preferential element absorption or if there is not discrimination among REE. The plant – substrate system, in which the latter is characterised by a complex mineralogy would not allow to carry out this investigation; therefore we chose to use a simple substrate obtained adding peat to inert silicate gravel, assuming that the latter has not chemical role in respect to roots (Saiano et al., 2004).

3.2 Off-soil experimental system

The off-soil experimental system consisted of one-year old Moscato d'Asti variety grafted on 1103 Paulsen. The whole system consisted of two sets of 15 plants

each separated into control plants (***Blank***) and Rare Earth Elements spiked plants (***Spiked***) (Figure 11). Both groups were placed in greenhouse and planted in pots containing 5 kg of a homogeneous substrate composed by peat : gravel = 2 : 3 w/w. Silicate gravel was used as drainage to prevent roots asphyxiation, while the peat was a simple mean of support to plant.

Figure 11 Off-soil experimental system: (a) at the beginning and (b) during the growth

The REE group was spiked in a single stage with an equimolar REE mixture (*2.5 µmol kg^{-1} of substrate*); this concentration, although higher than the normal level of the lithosphere, was chosen to study in a clear manner the individual competition of REE with respect to plant (Ding et al., 2006) eliminating any mass effect. Furthermore, the analytes were spiked in a single step to investigate the absorption as a function of the growth and physiological needs of the vines. During growth the plants were irrigated with a quantity of water such as to avoid material loss by leaching.

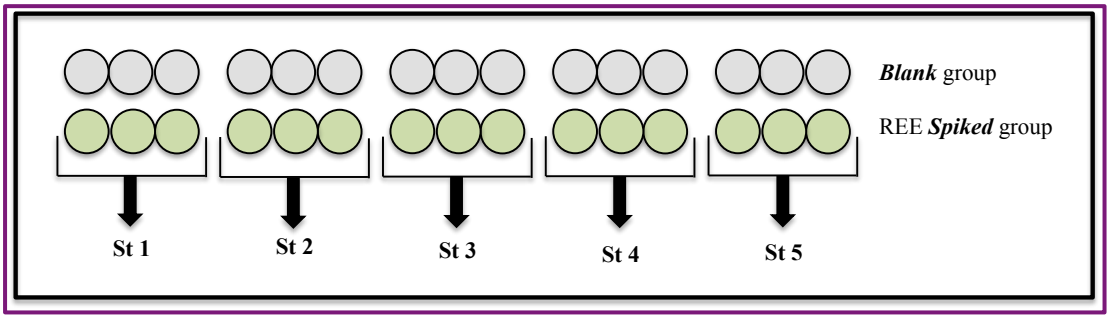
3.3 Sampling design

The plants were sampled in defined five phenological stages selected as a function of plant life and annual cycles (3 replicates for each group) (Table 17 and Figure 12).

Table 17 Times and phases of sampling

Stage	Phenological phases	Time from the pollution day (months)/ from budding (days)
St 1	Budding-flowering (**08/61-69**)	2 / +16
St 2	Fruit setting (**71**)	3 / +76
St 3	Véraison (**81**)	5 / +133
St 4	Harvesting (**89**)	7 / +197
St 5	Post-harvesting (**97**)	10 / +274

Figure 12 First experimental project



At each phenological stage, plants were wholly sampled, separating the different organs (Table 18), in order to assess the total metals content absorbed by plants and to study the elements translocation, highlighting the most involved organs. Each sample was treated as reported in sections 2.2.2.1 and 2.2.2.2 (Figure 13).

Table 18 List of various vine parts

Hypogeal	Epigeal
Fine roots (Ø ≤ 1mm)	Stem
Middle roots (1mm ≤ Ø ≤ 2mm)	Woody shoot (2/1 years old)
Woody roots (Ø ≥ 2mm)	Herbaceous shoot
	Apex of shoot
	Petioles
	Leaves
	Lateral shoot with its apex
	Petioles of lateral shoot
	Leaves of lateral shoot

Figure 13 Vine parts: from fine roots to apex of shoot

3.4 Results

3.4.1 Blank group

An accurate distribution assessment of the investigated analytes, during the phenological stages (from St 1 to St 5), was obtained by the REE concentration, expressed as μmol kg^{-1} dry weight (d.w.). The highest REE concentrations were shown in St 1 and St 2, followed by a decrease in the later stages (Table 19).

Table 19 Total REE (μmol Kg^{-1} d.w.) in the off-soil blank group during the 5 growth stage and average weight of three plants (g d.w.)

	Σ REE ± s	**Plants dry weight ± s**
St 1	107.1 ± 20.9	19.26 ± 3.03
St 2	105.4 ± 36.6	13.65 ± 3.16
St 3	68.1 ± 15.6	29.90 ± 5.76
St 4	22.53 ± 4.27	65.03 ± 8.69
St 5	20.29 ± 5.08	43.71 ± 5.96

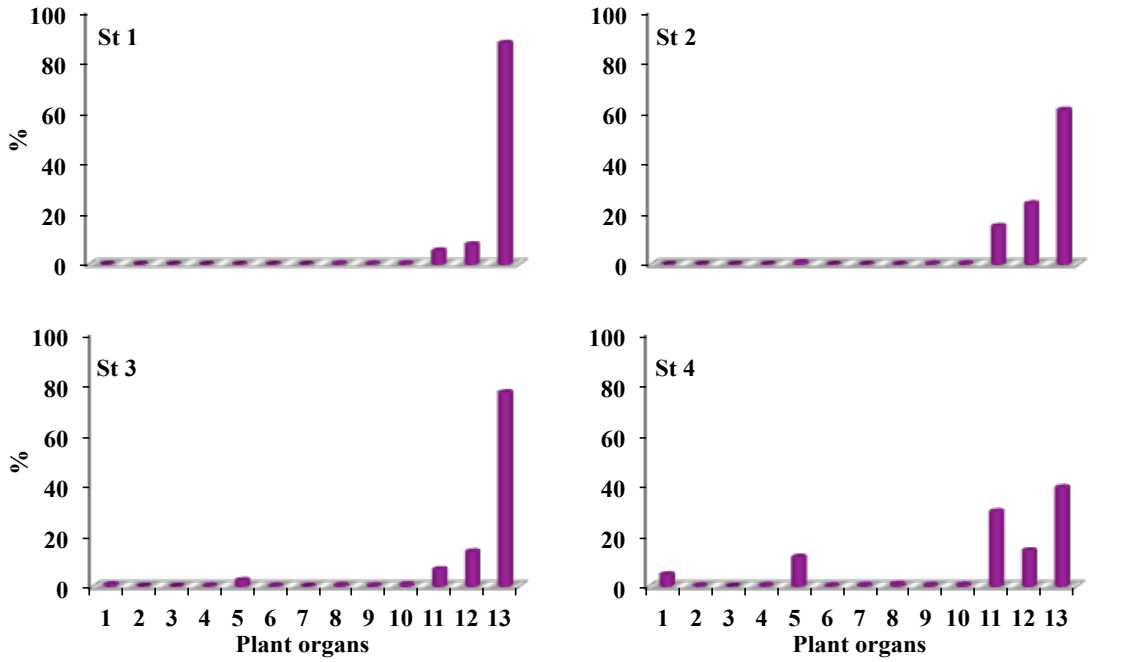
The maximum REE concentration in St 1 was 107.1 ± 20.9 μmol Kg^{-1} d.w., the concentration decrease from St 2 to St 4, from 105.4 ± 36.6 μmol Kg^{-1} to 22.53 ± 4.27 μmol Kg^{-1} respectively, is probably due to the significant increase in plants weight (from 13.65 ± 3.16 g d.w. to 65.03 ± 8.69 g d.w.) as a consequence of the aerial apparatus development that reaches the maximum in the harvesting time, causing a dilution effect of the studied analytes. The lowest concentration was observed in St 5 (20.29 ± 5.08 μmol Kg^{-1}). Contrarily to previous stages, this decreasing is not due to a dilution effect but it is a consequence of the rest period, characterised by the fall of leaves. Since the experimental substrate (peat and gravel) did not contain REE (Ferrat et al., 2012) the analytes amount in roots may derive from an absorption process by substrate in which the plants have been developed in the nursery during the first year of their life. The study of an isolated system, such as off-soil blank group, does not allow us to understand the absorption plant mechanism, but it can clearly highlight if analytes translocation processes occur during physiological plant development. To evaluate the REE distribution into plants, for each phenological stage, the total REE concentration (Table 20) and REE amounts regarding the individual plant organ were calculated.

Table 20 Total and REE amount per organ in the off-soil blank group during the 5 growth stage

	St 1	St 2	St 3	St 4	St 5
Plant Organs			**µmol/total plant (d.w.)**		
Leaves of lateral shoot	-	-	0.01144	0.06548	-
Petioles of lateral shoot	-	-	0.00057	0.00140	-
Apex of lateral shoot	-	0.00004	0.00064	0.00036	0.00869
Lateral shoot	-	-	0.00177	0.00372	0.00404
Leaves of herbaceous shoot	-	0.00877	0.04375	0.16598	-
Petioles	-	0.00020	0.00179	0.00220	-
Apex of shoot	0.00019	0.00025	0.00013	0.00422	-
Herbaceous shoot	0.00275	0.00022	0.00587	0.01123	0.03522
Woody shoot (1 year old)	0.00158	0.00152	0.00200	0.00441	0.00543
Woody shoot (2 years old)	0.00313	0.00207	0.01107	0.00622	0.01590
Woody roots	0.10576	0.21236	0.13251	0.42870	0.25733
Middle roots	0.15608	0.34106	0.27419	0.20328	0.24612
Fine roots	1.79300	0.87246	1.55139	0.56824	0.31418
Σ REE ± s	**2.06 ± 0.24**	**1.44 ± 0.37**	**2.04 ± 0.25**	**1.47 ± 0.20**	**0.89 ± 0.19**
RSD%	**11.5**	**25.9**	**12.3**	**13.4**	**21.0**

As previously described, during the plants growth the total REE concentration progressively decreases from 2.06 ± 0.24 to 0.89 ± 0.19 µmol/total plant (d.w.). The obtained values show that roots preferentially concentrate the largest REE contents, from 99.6% to 92.2% of the total amount from St1 to St 5 respectively (Figure 14).

Figure 14 Percentage distribution of total REE in off-soil blank group during the 5 growth stage



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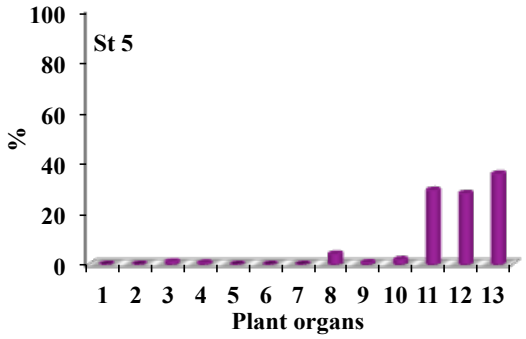


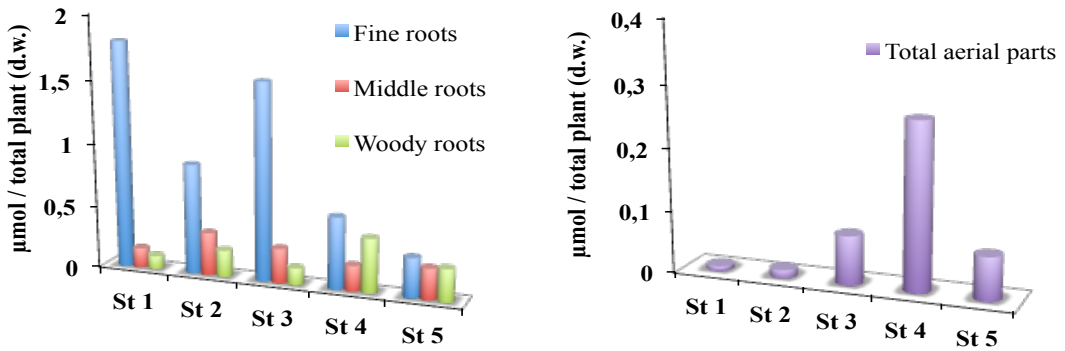
Legend: **1**: Leaves of lateral shoot; **2**: Petioles of lateral shoot; **3**: Apex of lateral shoot; **4**: Lateral shoot; **5**: Leaves of herbaceous shoot; **6**: Petioles of herbaceous shoot; **7**: Apex of herbaceous shoot; **8**: Herbaceous shoot; **9**: Woody shoot (1 year old); **10**: Woody shoot (2 years old); **11**: Woody roots; **12**: Middle roots; **13**: Fine roots.

It should be noted that each plant is a unique sample, consequently it is never possible to sample twice the same plant because the destructive methods of analysis do not allow it. The value of 1.44 ± 0.37 µmol determined in St 2 is probably due to a lower plant development and therefore a lower absorption of nutrients during growth in the nursery occurs. The dry matter content that in St 2 plants was 17.5% while in St 1 and St 3 plants was 20.9 % and 20.4% respectively suggests this hypothesis.

Data also indicate that greater REE contents occur in the finest roots, and this feature is mainly observed in the first three phenological stages, after which REE contents progressively decrease and a partial translocation of analytes to middle and woody roots takes place. In particular a decrease of the REE percentage content in the fine roots (from 86.9% in St 1 to 35.4% in St 5), corresponds to an increase in the middle and woody roots from 7.5% to 27.7% and from 5.1% to 29.0% respectively.

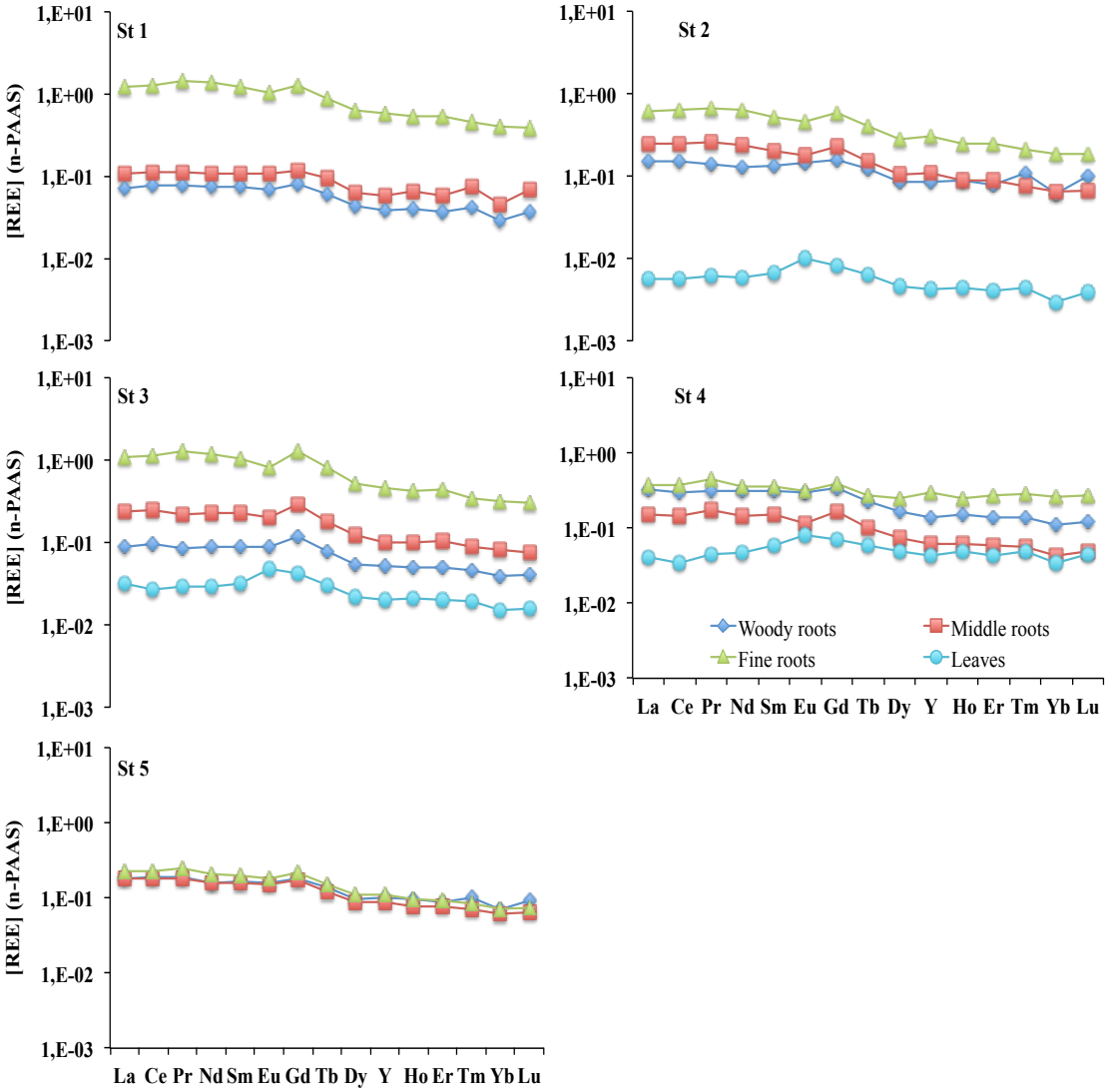
The results suggest a trace elements migration towards the aerial apparatus of plants: in fact the REE concentrations in leaves increased from 0.6% in St 2 to 11% in St 4. The translocation from root to aerial apparatus is better shown in Figure 15.

Figure 15 REE content in roots and aerial parts of off-soil blank group



Shale-normalised REE patterns of off-soil blank group, allow us to emphasize the behaviour of every analyte of REE series. Figure 16 shows the normalised patterns of each root type and leaves during plant growth; the observed behaviour of total REE content may also be deduced from the patterns. They show that each element of the REE series was equally transferred from fine roots towards middle and woody ones and then to aerial apparatus. Therefore, these data suggest that fractionation process do not occur during elemental translocation. REE patterns are characterised by slightly light REE (LREE) enrichment respect to heavy REE (HREE) without significant Ce and Eu anomalies. Since the REE content into plants comes from the previous absorption process, these features are probably due to the substrate composition in which plants grow during the first year of their life.

Figure 16 Shale-normalised REE patterns measured in roots and leaves of off-soil blank group during different growing stages (St 1-St 5)



3.4.2 Spiked group

Before considering REE distribution in each plant organ the absorbed percentage with respect to the total spiked amounts (2.5 μmol kg^{-1} of substrate per each analyte) expressed as average value of three plants during the 5 growth stages was compared (Table 21).

Table 21 Absorbed amount of total REE (μmol/total plant, d.w.) in the off-soil spiked group and the relative percentage during the 5 growth stage in respect to total spiked amounts

	μmol/total plant (d.w.) ± s	% REE ± s
St 1	10.66 ± 1.12	5.68 ± 0.60
St 2	10.06 ± 1.56	5.37 ± 0.83
St 3	10.06 ± 1.86	5.37 ± 0.99
St 4	4.34 ± 0.67	2.31 ± 0.36
St 5	2.52 ± 0.28	1.34 ± 0.15

Vine variability is well reported from many researchers and it cannot be avoided applying a destructive investigation method. In spite of this, we reported the data as percentage of REE amount with respect to the dry weight of plants. Data reported in Table 22 show a decrease of the total REE concentration from St 1 to St 4; a reverse trend instead characterises the transition between St 4 and St 5.

Table 22 Percentage of total REE amount with respect to dry plants weight in the off-soil spiked group and the plants dry weight (g d.w.) during the 5 growth stage

	% REE/total plant (d.w.) ± s	Plants dry weight ± s
St 1	86.5 ± 19.3	12.32 ± 2.43
St 2	58.9 ± 16.7	17.08 ± 4.05
St 3	23.06 ± 5.01	43.64 ± 4.97
St 4	5.36 ± 1.14	80.90 ± 11.91
St 5	13.29 ± 3.79	18.97 ± 4.99

The highest REE amount in St 1 with regard to St 2, contrary to those found in plants of blank group, reveals that spiked plants (before St 1 sampling) have absorbed the spiked elements from substrate. The decrease of measured concentration between St 2 and St 3, from 59% to 23%, accompanied by an increase of the plants dry weight, suggests a dilution of analytes within plant like to blank plants group. At the end of St 1, corresponding to the budding period, the plant does not continue to absorb elements

from the growth substrate but it uses the nutrients stored in the root apparatus. This status also continues during the St 3 and St 4 (Véraison and harvesting time, respectively), indeed a concentration decrease from 23% to 5% and a dry weight increase occurs. In contrast, an increasing concentration taking place between St 4 and St 5 (from 5% to 13%) coupled with plant weight depletion indicate a physiology mutation in system. The St 5, in fact, corresponds to the rest period in which plant is prepared to winter rest. During this period, the plant re-absorbs nutrients contained in falling leaves and petioles and a retake of root activity occurs. The latter hypothesis is confirmed by the increase in percentage of dry matter from 28% in St 4 to 40% in St 5.

To evaluate the REE distribution into plants for each phenological stage the total REE concentration into total plants and with respect to the individual plant organ were calculated (Table 23). The percentage distribution in each plant organ for all the examined stages, highlights the existence of translocation processes of investigated analytes but the highest REE content was again present in the roots assigned both to absorption (fine roots) and to nutrients reserve (middle and woody roots).

Table 23 Total and REE amount per organ in the off-soil spiked group during the 5 growth stage

	St 1	St 2	St 3	St 4	St 5
Plant Organs	**µmol/total plant (d.w.)**				
Leaves of lateral shoot	-	-	0.0094	0.1650	-
Petioles of lateral shoot	-	-	0.0008	0.0030	-
Apex of lateral shoot	-	-	0.0001	0.0006	-
Lateral shoot	-	-	0.0026	0.0097	0.0079
Leaves of herbaceous shoot	-	0.0198	0.0401	0.0950	-
Petioles	-	0.0004	0.0012	0.0009	-
Apex of shoot	0.0008	0.0001	0.00002	0.0032	-
Herbaceous shoot	0.0036	0.0040	0.0037	0.0121	0.0201
Woody shoot (1 year old)	0.0067	0.0003	0.0071	0.0036	0.0276
Woody shoot (2 years old)	0.0164	0.0061	0.0242	0.0031	0.0045
Woody roots	0.5013	0.3890	0.7982	0.7315	0.6040
Middle roots	0.9945	0.4371	0.6253	0.8310	1.0780
Fine roots	9.1340	9.2037	8.5509	2.4803	0.7796
Σ REE ± s	**10.66 ± 1.12**	**10.06 ± 1.56**	**10.06 ± 1.86**	**4.34 ± 0.67**	**2.52 ± 0.28**
RSD%	**10.5**	**15.5**	**18.5**	**15.5**	**11.3**

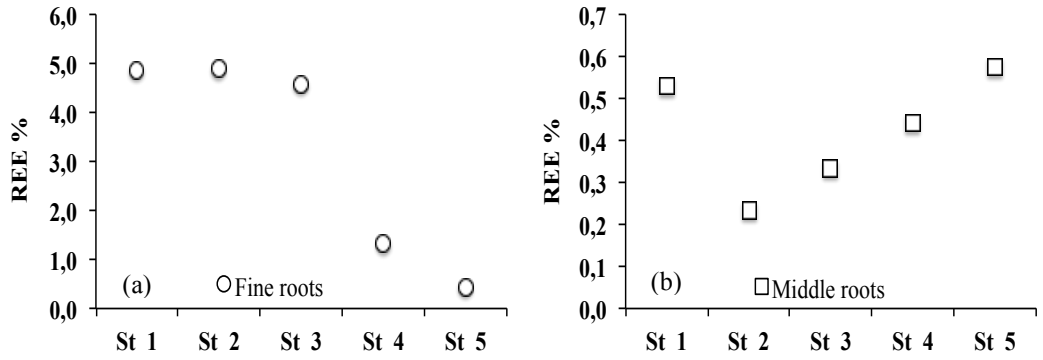
The percentage with regard to the total spiked amount (Table 24) was also calculated.

Table 24 Total and REE percentage per organ in the off-soil spiked group during the 5 growth stage

	St 1	St 2	St 3	St 4	St 5
Plant Organs			**% REE/total plant (d.w.)**		
Leaves of lateral shoot	-	-	0.00136	0.00515	0.00424
Petioles of lateral shoot	-	-	0.00501	0.08798	-
Apex of lateral shoot	-	-	0.00040	0.00158	-
Lateral shoot	-	-	0.00006	0.00031	-
Leaves of herbaceous shoot	0.00193	0.00213	0.00198	0.00644	0.01071
Petioles	-	0.01057	0.02139	0.05068	-
Apex of shoot	-	0.00022	0.00066	0.00048	-
Herbaceous shoot	0.00040	0.00006	0.00001	0.00172	-
Woody shoot (1 year old)	0.00355	0.00016	0.00377	0.00190	0.01473
Woody shoot (2 years old)	0.00875	0.00325	0.01293	0.00164	0.00242
Woody roots	0.2674	0.2075	0.4257	0.3901	0.3221
Middle roots	0.5304	0.2331	0.3335	0.4432	0.5749
Fine roots	4.87	4.91	4.56	1.32	0.42
Total % of REE ± s	**5.68 ± 0.60**	**5.37 ± 0.83**	**5.37 ± 0.99**	**2.31 ± 0.36**	**1.34 ± 0.15**

The analysis of absorbed REE amount in each plant organ shows that an early plant uptake occurs during St 1 (4.87 %) and St 2 (4.91 %), as indicated by the maximum percentage found in fine roots; after this stage it is observed a % REE decrease in fine roots and a linear increase in middle roots. The latter phenomenon is expected since during the next life cycle the middle roots will play a reserve role (Figure 17a-b). The woody roots, instead, show a percentage increase of REE content less significant.

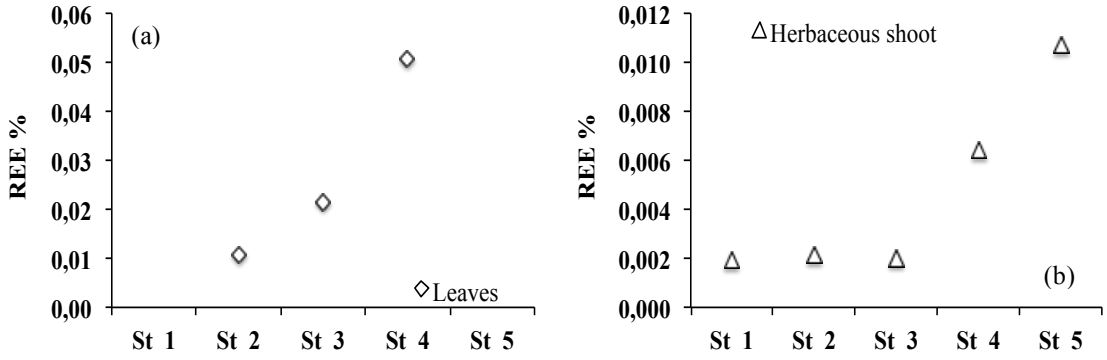
Figure 17 REE percentage in (a) fine and (b) middle roots during the 5 growth stage



The REE translocation continues towards aerial organs, the data show a significant REE increase in apex of shoot and in leaves from St 3 to St 4, when the

largest growth of aerial parts occurs. In these organs, the percentage REE content with respect to absorbed total amount ranges from 0.20% to 6.08% during the plant growth (Figure 18a). In contrast, the shoot that in the next life cycle will become woody shoot, shows a constant percentage content in the first three phases, while in the last two (St 4 and St 5) a percentage REE marked increase was observed (Figure 18b); during the plant growth percentage REE content with regard to absorbed total amount ranges from 0.03% to 1,11%.

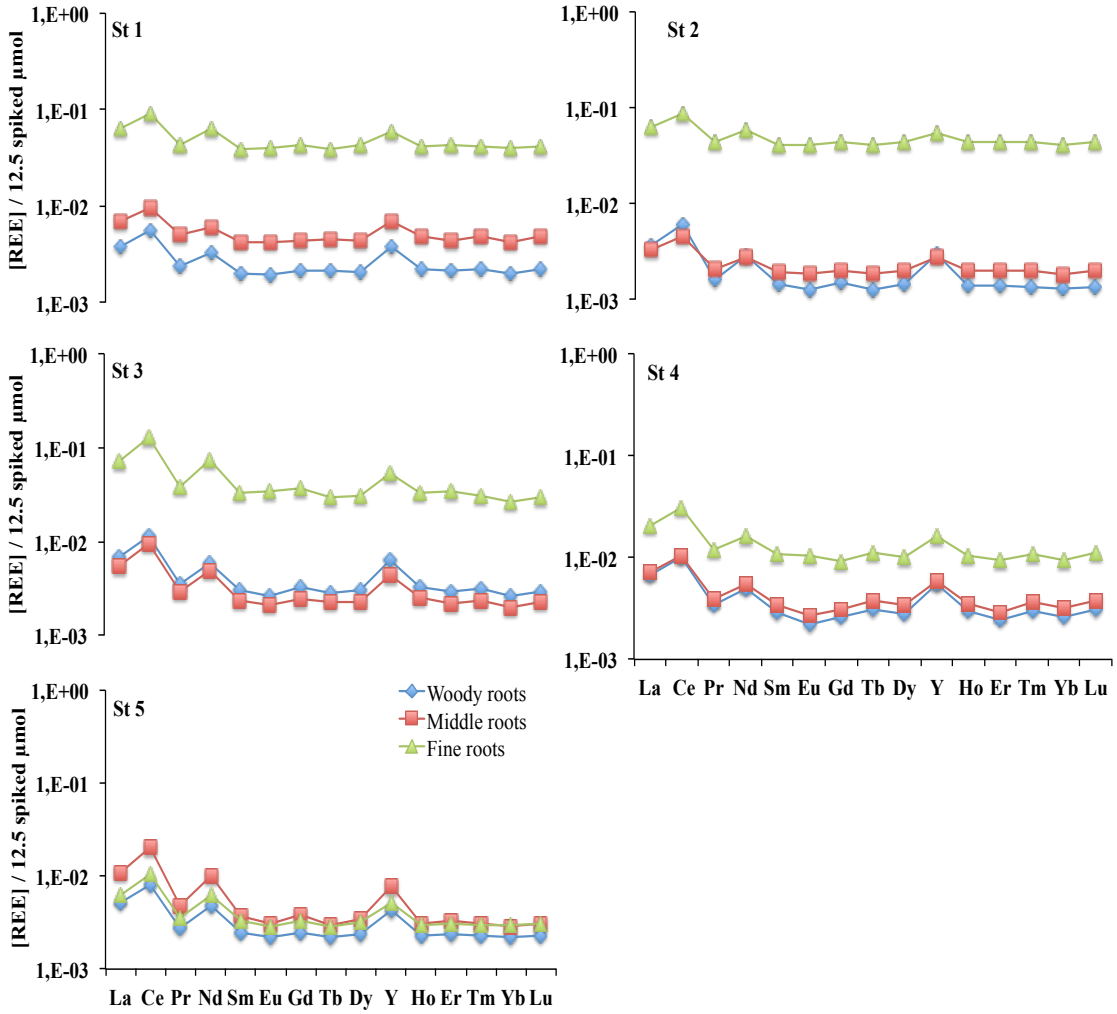
Figure 18 REE percentage in (a) leaves and (b) herbaceous shoot during the 5 growth stage



To evaluate the competition among REE during the uptake, normalised REE concentrations with respect to spiked amount (12.5 μmol) were assessed. The normalised patterns, shown in Figure 19 for each roots apparatus member during plant growth are characterised by a flat pattern from Sm to Lu.

This feature suggests that MREE and HREE during uptake are equally absorbed by roots. By contrast, LREE and Y deviate from the trend showing a higher apparent absorption with respect to the other elements of series. These features imply that Y behaves as a LREE, rather than a MREE. The "zig-zag" effect recognised in LREE normalised sequences suggests a preferential LREE scavenging onto the finest roots surfaces or the occurrence of substratum residua stuck in finest roots (Tyler, 2004; Hu et al., 2006a,b). Moreover, the possibility that analytical artefacts could interest these samples cannot be ruled out.

Figure 19 Normalised REE patterns to total spiked amount (*12.5 µmol for each elements*), measured in root of off-soil spiked group during different growing stages (St 1-St 5)

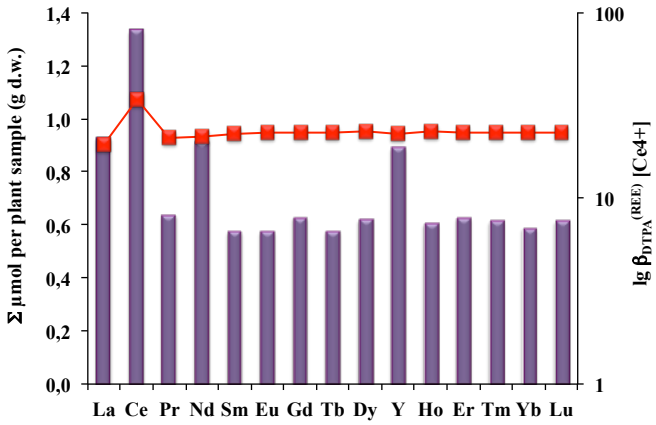


3.5 Discussion

The REE contents in roots are a consequence of two different interface processes between substrate and circulating fluids and between the latter solution and roots apparatus, respectively. The use of peat - gravel system has allowed us to focus our attention only on the second interface (circulating solution - roots). It is known from literature data (Moeller et al., 1965; Byrne & Li, 1995; Pourret et al., 2007), that the complexation constants REE – Humic acids (a constituent of peat) are lower than those REE - DTPA (root exudates simulator), so the first interface process is shifted towards the circulating fluids. The use of REE equimolar solution led to enhance possible competition phenomena with respect to the roots exudates. Although low, adsorbed REE contents do not show any preferential fractionation and the recognised REE

pattern mirrors the sequence of the stability constants for the REE-DPTA complex ($\beta_{DTPA}^{(REE)}$) (Fig. 20).

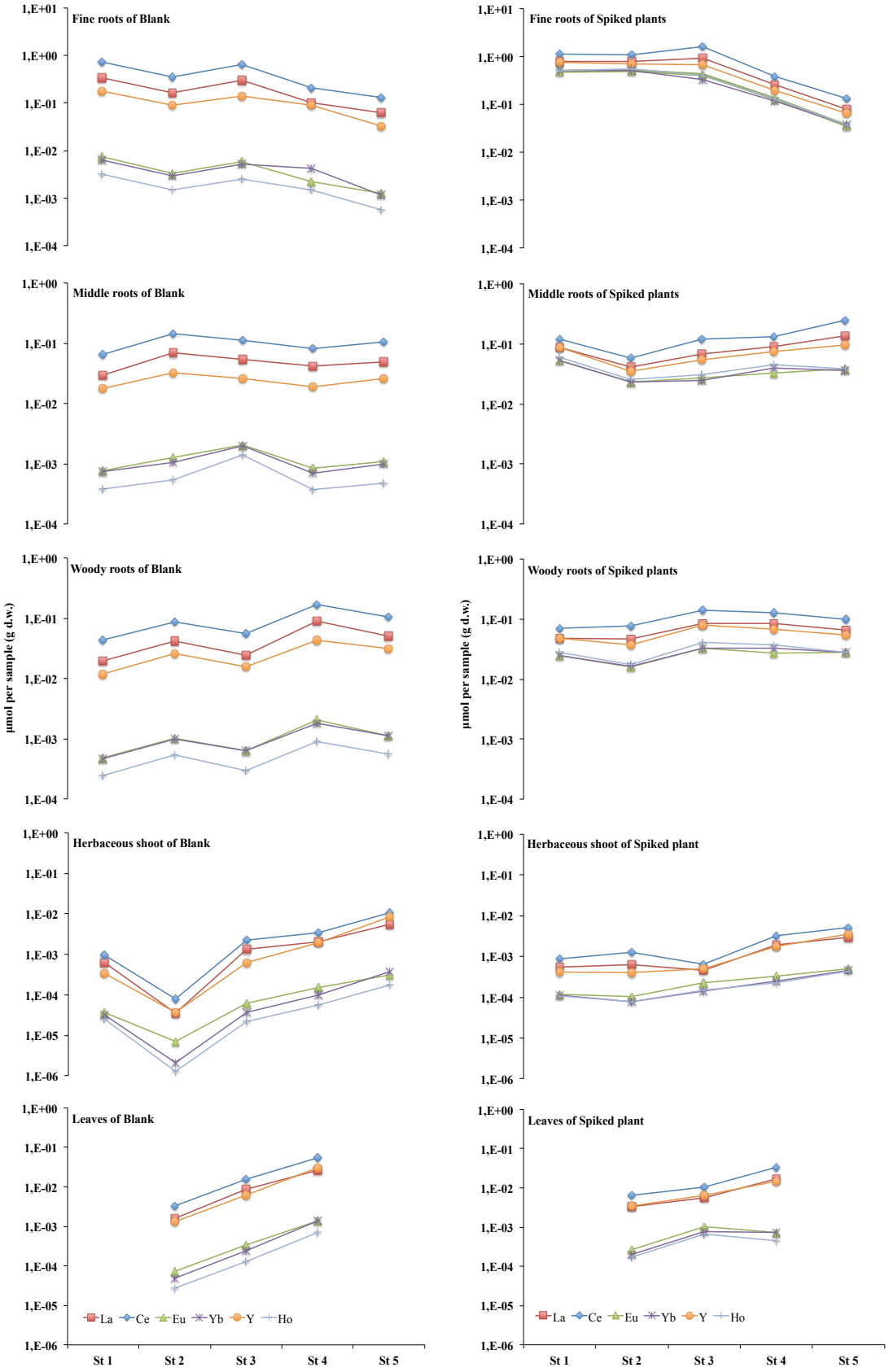
Figure 20 (left) Total absorbed µmols by spiked plants, (right) REE - DTPA stability constant $\beta_{DTPA}^{(REE)}$ (Byrne & Li, 1995)



The study of off-soil system suggested that REE absorptions and translocations within the plant occur in two different stages: an early REE absorption onto roots until attainment of equilibrium is followed by REE translocation towards aerial organs. This hypothesis is corroborated by evidences reported in Figure 21. Here, the preliminary and quick REE absorption onto the fine roots is followed by the partial REE translocation in middle and woody roots. Therefore, the REE concentrations increase in herbaceous shoots and in leaves.

The REE concentration of the aerial parts is about 1 or 2 order of magnitude lower than in roots. After absorption by the roots, REE may follow apoplastic pathway (through the cell wall) or the symplastic pathway (through the cytoplasm) to reach the aerial plant organs. Along the apoplastic pathway, REE moved into the cell wall by passive transport with respect to the diffusion gradient. Along the symplastic way, the transport of solutes occurred across the plasmalemma and ions entered into the cell within the cytoplasm (Brioschi et al., 2013 and references therein). Both apoplastic and symplastic way are interrupted by the *Casparian strip*, which is an ion-selective cell wall located in the endoderm of the roots. Our data are in agreement with what is known about the role of the Casparian strip, which blocks or at least modulates the transfer of ions to the aerial part of plants.

Figure 21 Both blank and spiked plants trends of representative REE elements during different growing stages. In order: fine, middle and woody roots, herbaceous shoots and leaves



On-soil growth

4.1 Background

After the analysis of the REE absorption and translocation process in vine in a model system, the next goal was to study how the REE absorption and diffusion were influenced by:

- soil natural variability, originated at different parent-rocks' expense;
- nature of several rootstocks varieties;
- nature of vine variety.

As described in section 1.3.3, many grapevine varieties are grafted on different rootstocks chosen according to soil, climatic and pedological characteristics of the production area. We have carried out these investigations by a model system consisting of three different varieties of rootstocks: *V. berlandieri x V. rupestris* (***1103 Paulsen***, ***779 Paulsen*** and ***140 Ruggeri***). On the contrary, the effect induced by changes of the vine variety was evaluated using the ***Moscato d'Asti*** and ***Sauvignon blanc*** varieties grafted on 1103P. Both rootstocks and vine variety were planted on different soils (calcarenitic parent rock, carbonatic-marly-clay precursor and basaltic parent materials). The chosen rootstocks were selected since these are the most exploited in Sicily due to their characteristics. The selected soils are among the most representative in Sicily and show very distinctive mineralogical characters. In any case, soil characteristics were not limiting factors for all rootstocks and varieties.

4.2 Soil collection

The soils were collected from distinct geographic areas of Sicily (Figure 22), in detail were collected in:

1. "Fossa della Garofala" located inside the Orleans Park in Palermo (38°6'22.66"N - 13°20'57.32" E, 53 m Sea level) with calcareous matrix (**PA-2**);
2. San Giuseppe Jato (Pa) (37°59'2.33" N-13°11'44.23" E, 360 m Sea level) with limy-clayey matrix (**PA-1**);
3. Viagrande (Ct) (37°37'19.36" N-15°5'41.87" E, 460 m Sea level) with basaltic matrix (**ET-1**).

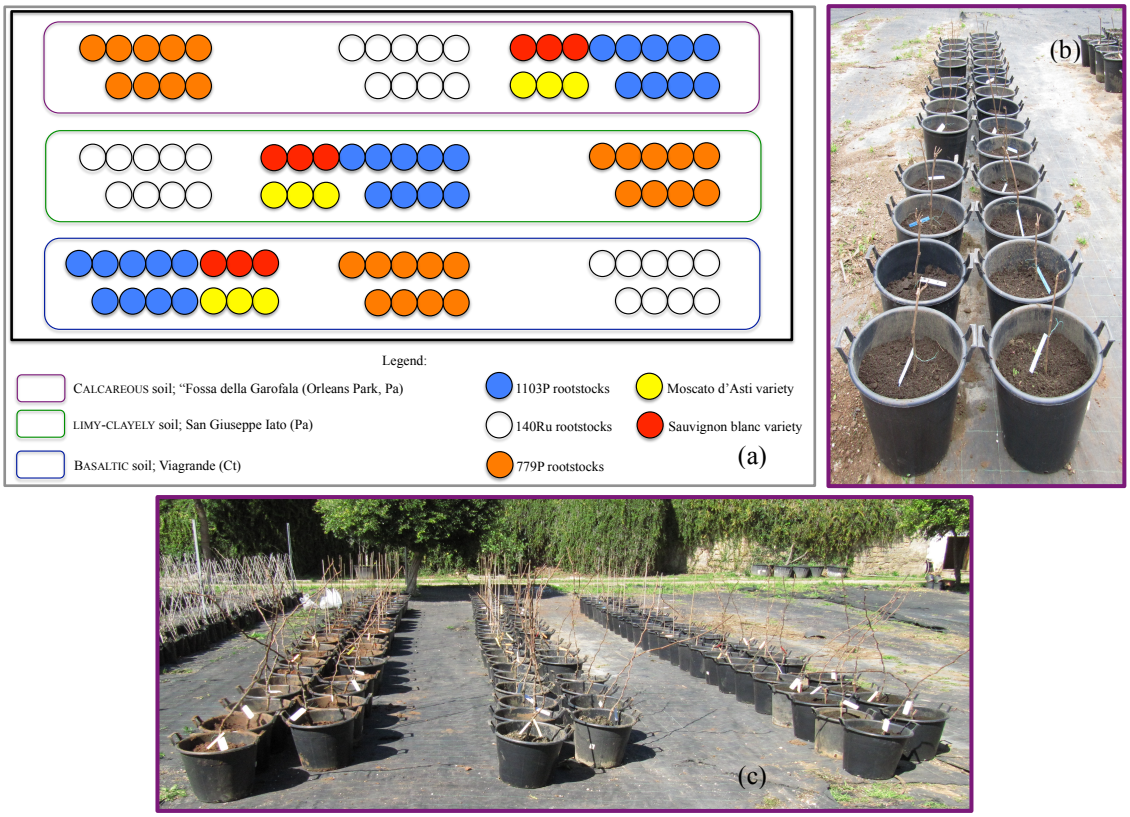
Figure 22 Soil collection point from distinct geographic areas of Sicily

After elimination of grassy layer, 1 ton of each soil was collected from 0 to 50 cm deep in order to build the experimental system. As described in section 2.2.1.1, sampling was carried out using a pre-cleaned stainless steel spade and the sampled soil was stored in polyethylene bags. An aliquot of each soil was separately stored for the subsequent determinations described in 2.2.1.2 and 2.2.1.3.

4.3 On-soil experimental system

The on-soil experimental system consisted of a set of 99 plants (81 rootstocks and 18 vine varieties), planted in 45 cm diameter pots. At the pot bottom was created a 5 Kg drainage gravel layer. Each pot was filled with a singular soil, creating a profile useful for vines development. Density soil determined different pots mass, particularly were made 39 Kg of PA-2 soil, 29 Kg of PA-1 and 27 Kg of ET-1 soil per each treatment. On the 2011-12 season 27 unrooted rootstock vines and 6 varieties plants were dedicated for each soil splitting them into three groups of nine for rootstock and two groups of three for varieties (Figure 23). To reduce the plants variability we proceeded cutting roots to 10 cm, and plant weight was determined; so 99 plants were selected with equal weight and vigor. The plants did not receive any disease spry, but only irrigation in order to avoid any stress conditions.

Figure 23 (a) On-soil experimental design, (b) a real detail and (c) the whole system



4.4 Sampling design

The rootstocks were sampled in three phases of the vegetative period. The first at 58 days after budding, the second and the third at 51 days intervals then 109 and 160 days respectively from budding (Table 25).

Table 25 Times and phases of sampling

Abbreviation (Stage)	Time from the budding (days)
St 1	+58
St 2	+109
St 3	+160

At each period, plants were wholly sampled (3 replicates), separating the most involved different organs (Table 26) and treated as reported in sections 2.2.2.1 and 2.2.2.2. About Moscato d'Asti and Sauvignon blanc varieties were sampled only clusters during harvesting time (3 replicates) and treated as reported in sections 2.2.3.1.

Table 26 List of various rootstocks parts

Hypogeal	Epigeal
Fine roots (Ø ≤ 1mm)	Stem
Middle roots (1mm ≤ Ø ≤ 2mm)	Herbaceous and lateral shoots with their apexes
Woody roots (Ø ≥ 2mm)	Leaves and petioles

4.5 Results

4.5.1 Soils

Pseudo-total fraction

Table 27 shows the REE concentrations, expressed in mg kg^{-1}, of the three investigated soils obtained by reported method in section 2.2.1.2. Total REE contents ranged from 165 – 214 mg Kg^{-1}; these results suggest a larger REE delivery from arenaceous and marly parent materials of PA-1 soil (213.64 ± 7.03 mg Kg^{-1}) with respect to calcarenitic parent materials of PA-2 soil (164.61 ± 8.23 mg Kg^{-1}).

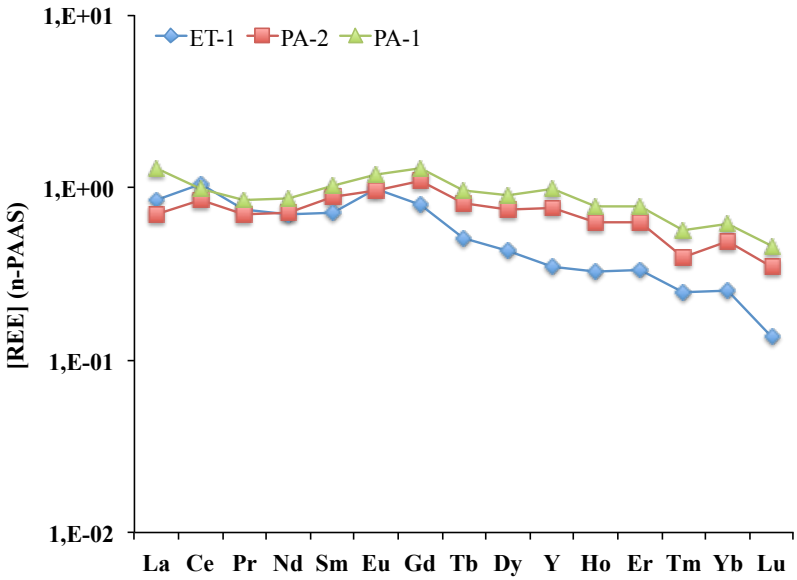
Table 27 REE contents and its standard deviation (mg Kg^{-1} ± s) measured in *pseudo-total fractions* from studied soils

	ET-1	PA-2	PA-1
Y	9.37 ± 0.64	20.52 ± 1.65	26.52 ± 0.91
La	32.63 ± 2.21	26.59 ± 2.70	49.38 ± 3.80
Ce	83.98 ± 5.20	67.97 ± 7.21	77.65 ± 5.52
Pr	6.58 ± 0.45	6.20 ± 0.61	7.52 ± 0.49
Nd	23.87 ± 1.66	24.17 ± 2.17	29.52 ± 1.82
Sm	3.93 ± 0.31	4.86 ± 0.45	5.66 ± 0.27
Eu	1.06 ± 0.07	1.04 ± 0.09	1.27 ± 0.06
Gd	3.70 ± 0.25	5.09 ± 0.47	6.04 ± 0.25
Tb	0.39 ± 0.03	0.63 ± 0.06	0.75 ± 0.03
Dy	2.00 ± 0.15	3.47 ± 0.29	4.19 ± 0.15
Ho	0.32 ± 0.03	0.62 ± 0.05	0.78 ± 0.02
Er	0.95 ± 0.08	1.77 ± 0.14	2.22 ± 0.09
Tm	0.10 ±0.02	0.16 ± 0.01	0.23 ± 0.01
Yb	0.71 ± 0.06	1.36 ± 0.12	1.72 ± 0.07
Lu	0.06 ± 0.01	0.15 ± 0.02	0.20 ± 0.01
Σ REE	**169.62 ± 5.96**	**164.61 ± 8.23**	**213.64 ± 7.03**
RSD%	**3.51**	**5.00**	**3.29**

According to natural abundance, for all studied soils the lowest concentrations were determined for Lu (0.06 ± 0.01 – 0.20 ± 0.01 mg Kg^{-1}) while Ce shows the highest concentrations (67.97 ± 7.21 - 83.98 ± 5.20 mg kg^{-1}).

Figure 24 shows the REE patterns normalised to Post Archean Australian Shale (**PAAS**) as reference (Taylor & McLennan, 1995). To evaluate and compare the REE characteristics, the elementary anomalies were calculated according to Equation 3 (Alibo & Nozaki, 1999).

Figure 24 Shale-normalised REE patterns of *pseudo-total fractions* from studied soils



Pseudo-total soil fractions showed very different features that may be justified by mineralogical peculiarity. Sedimentary PA soils are similar with symmetrical REE distributions along the series, with MREE enriched with respect to LREE and HREE. On the other hand, ET-1 soil shows similar features to PA soils from La to Eu and normalised REE path decreasing from Gd to Lu. The larger LREE partitioning in ET-1 is consistent with the more incompatible nature of these elements that usually allows them to fractionate into intraplate basic magmatic products such as those associated with the Mt. Etna volcanism (Busà et al., 2002; Viccaro et al., 2006). By contrast, the MREE fractionation in PA soils could be related to their larger Fe-oxyhydroxides contents that are enriched in MREE (McKey, 1989; Haley et al., 2004). In detail, the LREE enrichment with respect to HREE can be expressed by the Σ[LREE]/Σ[HREE] ratio equal to 3.47 per ET-1, 1.65 and 1.61 for soils PA-1 and PA-2, respectively. Moreover, since MREE and HREE form more stable complexes with organic substance

regarding LREE, they are enriched in soils with a higher organic matter content (Laveuf & Cornu, 2009; Loell et al., 2011); similar evidence can be confirmed by MREE anomaly (MREE/MREE*) ranging from 1.25 in ET-1 to 1.50 in PA-2. Additional confirmation is the mutual fractionation of Y and Ho expressed as both $Y/Ho_{(molar)}$ ratio and Y anomaly. The ET-1 soil shows chondritic Y/Ho whereas PA soils show higher Y/Ho. Similarly ET soil shows a positive Eu anomaly that is not found in PA soils. These features agree with observed chondritic Y/Ho values in basaltic soils (Thompson et al., 2013) whereas superchondritic terms are found in carbonate sediment fractions (Xu et al., 2010). Positive Eu anomalies are usually related to the larger Eu mobility in hydrothermal fluids where it usually occurs as Eu^{2+} (Bau, 1991; Bau and Moller, 1992). Therefore, these characters are typical of magmatic-hydrothermal systems and are not observed in sedimentary materials as carbonates and clays.

Bioavailable fraction

Table 28 shows the REE concentrations in bioavailable fractions, expressed in mg kg^{-1}, of the three investigated soils obtained by reported method in section 2.2.1.3.

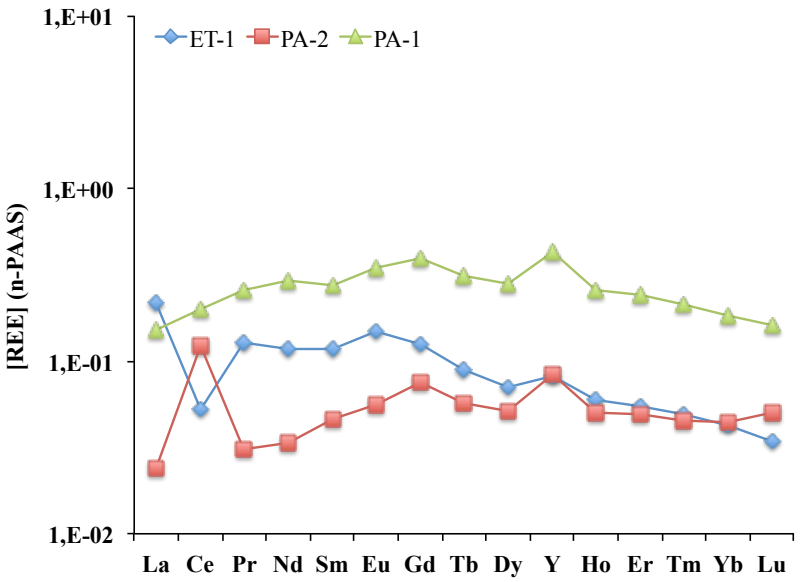
Table 28 REE contents and its standard deviation (mg Kg^{-1} ± s) measured in *bioavailable* fractions from studied soils

	ET-1	PA-2	PA-1
Y	2.21 ± 0.04	2.24 ± 0.03	11.69 ± 0.31
La	8.26 ± 0.14	0.91 ± 0.02	5.77 ± 0.20
Ce	4.17 ± 0.08	9.75 ± 0.33	15.98 ± 0.45
Pr	1.13 ± 0.01	0.27 ± 0.01	2.27 ± 0.06
Nd	3.98 ± 0.08	1.14 ± 0.03	9.93 ± 0.30
Sm	0.65 ± 0.02	0.25 ± 0.01	1.54 ± 0.04
Eu	0.160 ± 0.003	0.060 ± 0.003	0.38 ± 0.01
Gd	0.58 ± 0.02	0.35 ± 0.01	1.83 ± 0.05
Tb	0.070 ± 0.001	0.040 ± 0.002	0.24 ± 0.01
Dy	0.33 ± 0.01	0.24 ± 0.01	1.31 ± 0.03
Ho	0.060 ± 0.001	0.050 ± 0.003	0.26 ± 0.01
Er	0.160 ± 0.004	0.140 ± 0.004	0.69 ± 0.02
Tm	0.020 ± 0.001	0.020 ± 0.001	0.090 ± 0.002
Yb	0.120 ± 0.003	0.130 ± 0.004	0.51 ± 0.01
Lu	0.0100 ± 0.0003	0.020 ± 0.001	0.070 ± 0.002
Σ REE	**21.91 ± 0.19**	**15.62 ± 0.33**	**52.54 ± 0.66**
RSD%	**0.85**	**2.14**	**1.26**

REE contents ranged from 15.6 – 52.5 mg Kg^{-1}, the highest values was found in PA-1 soil (52.54 ± 0.66 mg Kg^{-1}) while PA-2 was the less concentrated (15.62 ± 0.33 mg Kg^{-1}). In addition, bioavailable soil fraction of PA-1 soil is characterised by the highest Ce and Y concentrations equal to 15.98 ± 0.45 and 11.69 ± 0.31 mg kg^{-1}, respectively. The obtained results show that the bioavailable fraction of three investigated soils reaches at most 25% of the pseudo-total content; the highest value was found in PA-1 soil (24.6%) and the lowest value (9.5%) in PA-2 soil.

The shale-normalised patterns of bioavailable soil fractions (Figure 25) further highlight the different nature of studied soils. REE patterns in bioavailable soil fractions, with respect to the pseudo-total ones, are characterised by a less LREE enrichment with respect to HREE, by Ce anomalies, positive in PA-2 and negative in ET-1 soils, and by MREE enrichment centred on Eu or Gd in PA-1. The ET-1 soil is characterised by a LREE enrichment (Σ[LREE]/Σ[HREE] equal to 2.82), while PA soils are less LREE enriched showing both ratio values equal to 1.13. A MREE enrichment occurs in PA-1 soil pattern (MREE/MREE* equal to 1.57).

Figure 25 Shale-normalised REE patterns measured in *bioavailable fractions* from studied soils



Ce and Y drift from general trends, the first in ET-1 soil reaches Ce/Ce* values equal to 0.30, while in the PA-2 soil is equal to 4.47. The $Y/Ho_{(molar)}$ ratio values were superchondritic (84.2 in PA-1 and 83.9 in PA-2 respectively) in PA soils while in ET-1 soil $Y/Ho_{(molar)}$ was equal to 69.9. This evidence is consistent with the larger Lewis acid softness of yttrium with respect to Ho (Cotton, 2006) leading to its larger mobility

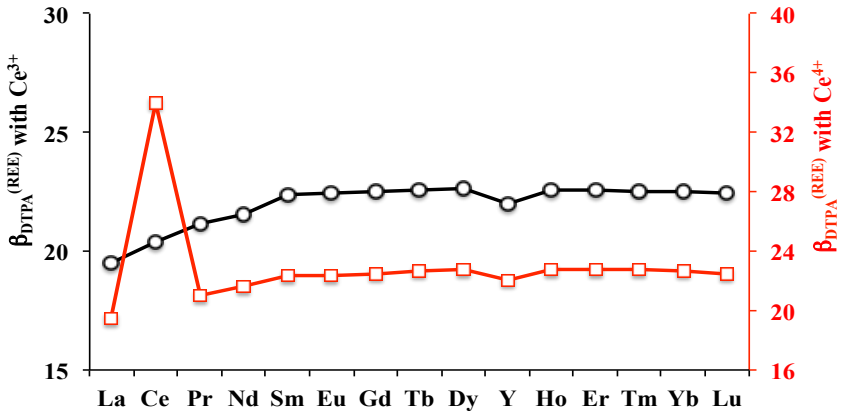
during interactions between soil and circulating solution. Higher $Y/Ho_{(molar)}$ values recognised in the bioavailable soil fraction also agree with Y removal from the surface of clay minerals where it is preferentially retained with respect to Ho (Takahashi et al., 2004).

The different REE mobility along the series agrees with the reactivity of primary minerals during the weathering (Laveuf & Cornu, 2009). On the contrary observed negative Ce anomalies can be explained with the lower mobility of this element under soil conditions when Ce, occurring as Ce(IV) is preferentially retained onto soil particles and is less bioavailable (Loell et al., 2011). On the other hand, preferential Ce(IV) release from Fe-Mn oxyhydroxides can be related to the higher $\beta_{(Ce\text{-}DTPA)}$ complexes with respect to its neighbours along the REE series (Laveuf & Cornu, 2009). Because of the limited REE incorporation in the structure of Fe-Mn oxyhydroxides these elements can be easily extracted from surfaces of Fe-Mn oxyhydroxides by DTPA. Therefore, REE distributions are strongly influenced by the competition between dissolved and surface complexations. Since REE complexation constants with the major inorganic ligands are lower than REE-DTPA constants, it is likely that the extraction equilibrium, showed in Eq. 18, is in favour of products.

$$\mathbf{REEs - substrate + H_2DTPA^{3-} \leftrightarrow substrate^{3-} + REEs - H_2DTPA} \qquad \textbf{Eq. 18}$$

If the extraction process depended only by reported equilibrium, recognised REE patterns would be similar to the sequence of stability constants for $\beta_{DTPA}^{(REE)}$ (Byrne & Li, 1995; Figure 26). Values of $\beta_{DTPA}^{(REE)}$ progressively increase along the REE series; Ce^{4+} in contrast of Ce^{3+} show a greater stability constant value shifting from trend.

Figure 26 Stability constant values for REE-DTPA complexes, $\beta_{DTPA}^{(REE)}$ both with Ce^{3+} and Ce^{4+}



Any changes from these features could be justified by specific characters of solid substratum. Comparing the REE patterns of bioavailable fractions with the sequence of stability constants for REE-DTPA complexes, the observed differences suggest that REE contents in bioavailable fractions are both related to dissolution of the Fe/Mn-oxyhydroxide component and REE fractionation induced by DTPA complexation.

4.5.2 Rootstocks

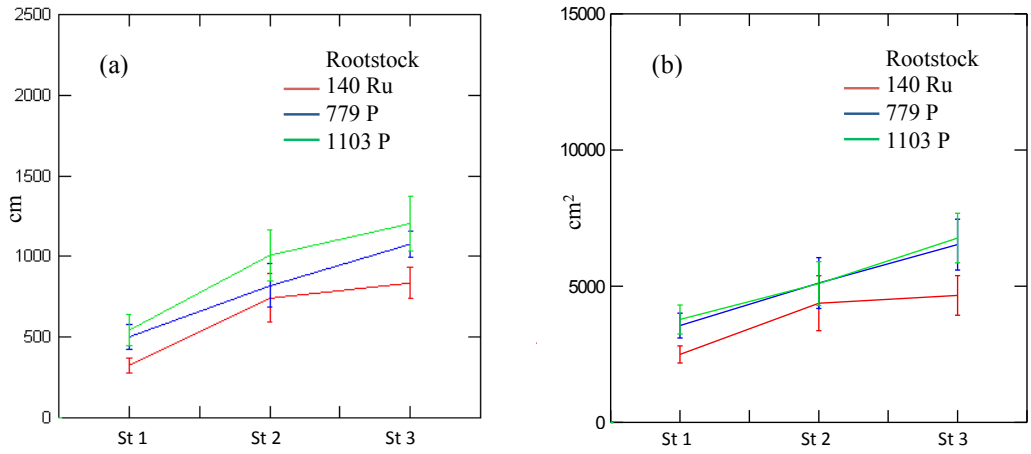
4.5.2.1 Agronomic evidence

Rootstock effect

During experimentation, the examined rootstocks have also been studied from an agronomic viewpoint (unpublished data) (Mistretta et al., 2011). Here will be briefly shows the obtained results to emphasize the biogeochemical discussion.

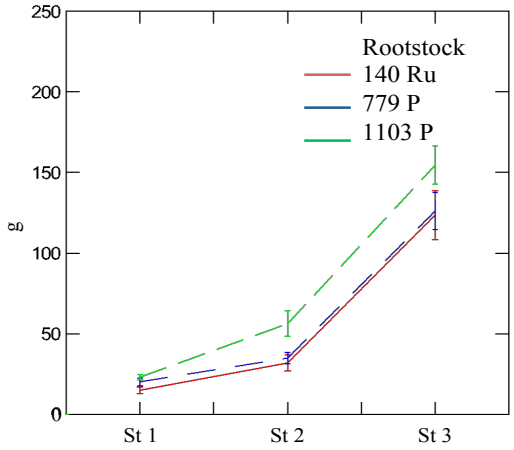
The 1103P rootstock was on average the most vigorous and with greater vegetative expression; in St 3, the differences with regard to other rootstocks have become marked, showing distinctly 3 different types of vegetative feature. In all growth stages, the Paulsen's rootstocks showed the highest values of leaf area with respect to 140Ru (Figure 27).

Figure 27 (a) Total shoots length per plant (cm) and (b) total leaf area per plant (cm^2) in the three rootstocks during the 3 growth stage



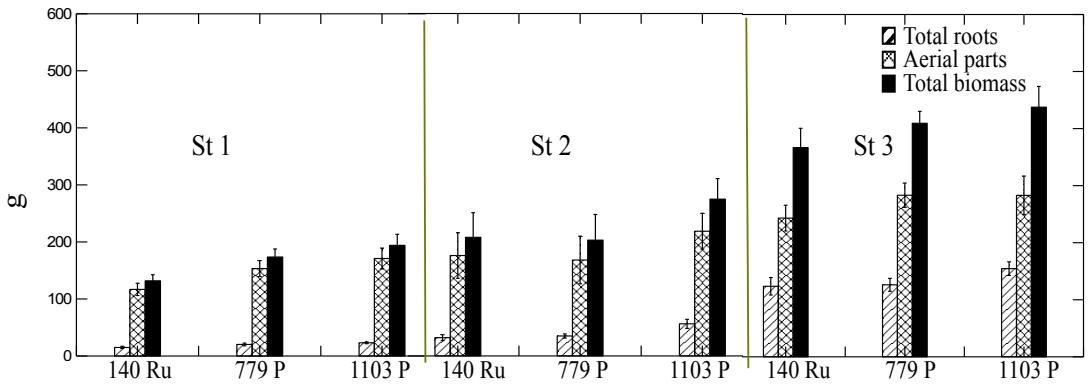
The roots weight also showed a rootstock effect during the three growth stage (Figure 28); the 1103P rootstock showed greater roots mass, with respect to other two rootstocks.

Figure 28 Fresh weight of total roots (g) in the three rootstocks during the 3 growth stage



The major contribution to total weight of roots is given by the fine roots with respect to middle and woody ones; Swanepoel & Southey (1989) reported, in no limiting water situations, similar behaviour in root system development of 1103P rootstock with respect to 140 Ru. In St 3, for all rootstocks, the fine roots were always most represented (68.9%, 65.5% and 64.3% in 779P, 140Ru and 1103P respectively) and the percentage of middle roots subsequently exceeds woody roots changing the roots arrangement respect to St 1. During growth stages the total biomass of three rootstocks increases but the contribution of aerial apparatus is lower than root apparatus (Figure 29). The 1103P rootstock has produced more total and root biomasses, followed by 779P and 140Ru and all analysed data highlight that 140 Ru rootstock lesser developed with respect to Paulsen's; the rootstock classification point out 140 Ru as very vigorous rootstock but it is true during induced vigor to graft combination and the results show that, in no limiting conditions, the 140Ru not enhances its vigor characteristics.

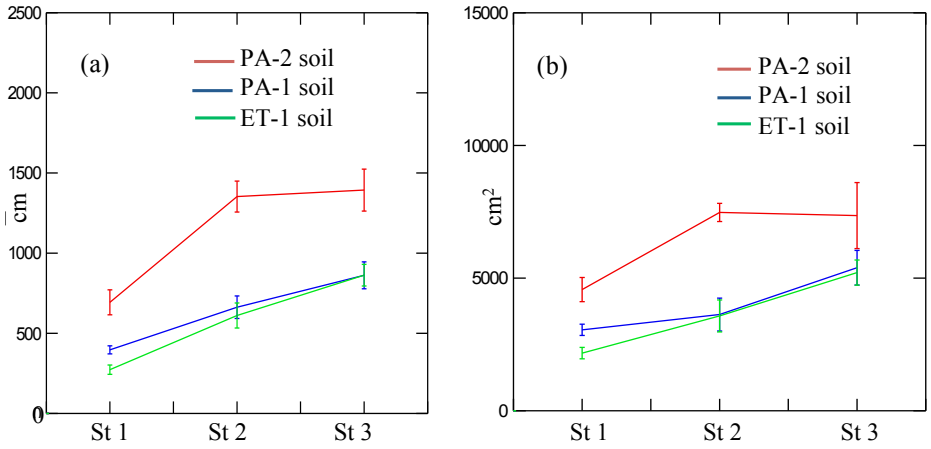
Figure 29 Total biomass (g, d.w.) per plant separating both aerial and root apparatus in the three rootstocks during the 3 growth stage



Soil effect

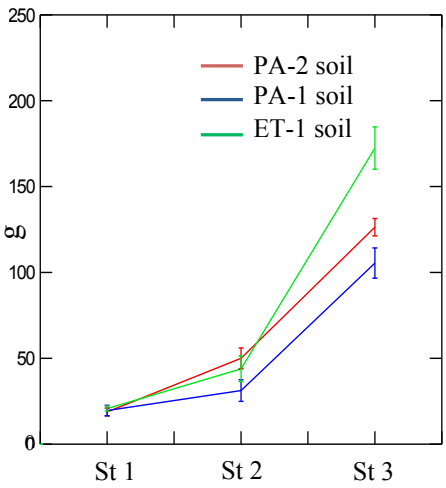
The soil has significantly affected the plants growth. During the 3 growth stages, PA-2 soil has induced more vigor to three rootstocks (Figure 30), while there were no differences on the other two soils (PA-1 and ET-1). PA-2 soil in fact, has also favoured vegetative plant expression (according to greater development of shoots) and the values of leaf area per shoot were always higher than the other two soils.

Figure 30 (a) Total shoots length per plant (cm) and (b) total leaf area per plant (cm^2) in three investigated soil during the 3 growth stage



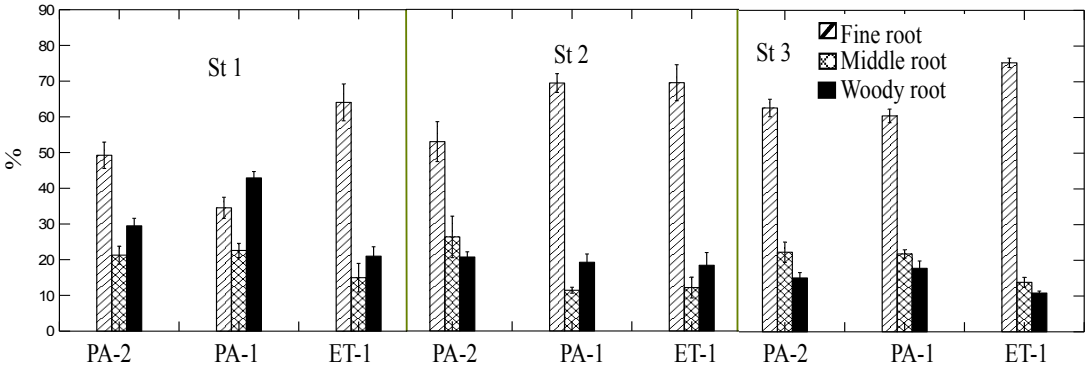
In literature root development in function of the soil composition is quite known. In agreement with Morlat & Jacquet (1993; 2003) ET-1 soil has allowed the formation of a root apparatus greater than sedimentary soils (PA-1 and PA-2) (Figure 31).

Figure 31 Fresh weight of total roots (g) in three investigated soil during the 3 growth stage



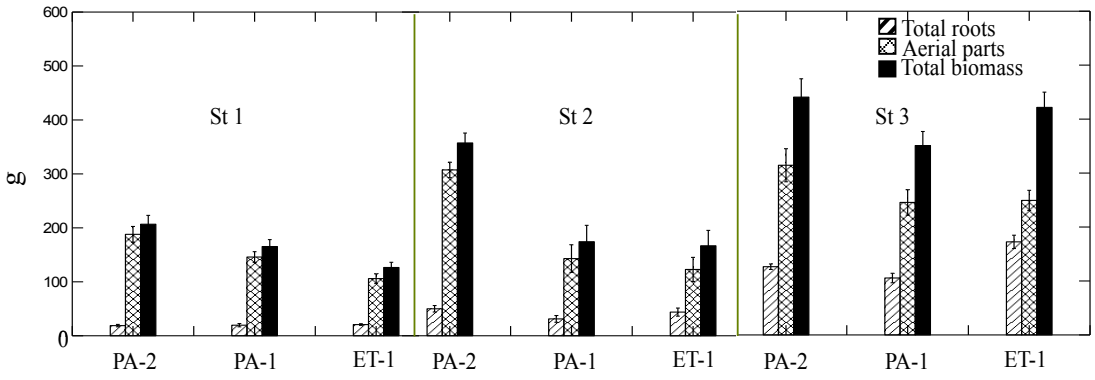
In St 3 in fact, the greater roots' weight was measured in ET-1 soil (172.4 g), followed by PA-2 (126.4 g) and PA-1 (105.5 g) respectively; moreover, the root percentage was also quite defined between three soils and in particular both PA-2 and ET-1 soil showed a greater incidence of fine roots on total distribution (Figure 32).

Figure 32 Percentage distribution of fine, middle and woody roots in three investigated soil during the 3 growth stage



During growth stages the total biomass has changed as a function of three soils. At St 1 rootstocks onto PA-2 soil showed the higher values of total biomass (206.7 g) than rootstocks onto PA-1 soil (165.4 g) and onto ET-1 soil (126.5 g) respectively; a greater contribution of aerial apparatus compared with root apparatus was observed. At St 3 rootstocks onto PA-2 and ET-1 soils showed similar total biomass (440.8 g and 421.7 g, respectively): as said, in PA-2 soil a greater aerial apparatus was observed, while in ET-1 soil the root apparatus was more developed. The PA-1 soil produced the lowest total biomass per plant (Figure 33).

Figure 33 Total biomass (g d.w.) per plant separating both aerial and root apparatus in three investigated soil during the 3 growth stage



4.5.2.2 REE content

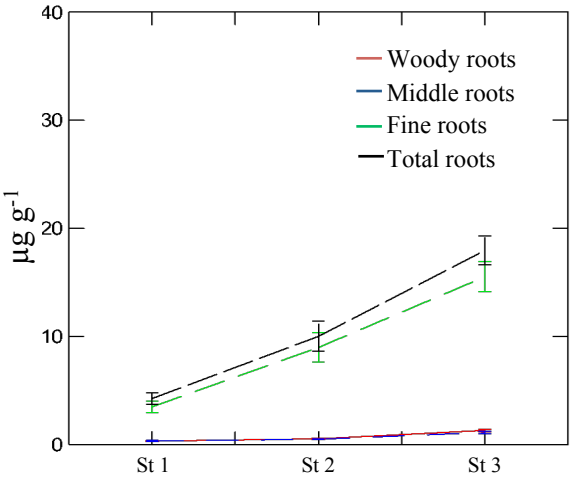
REE contents in rootstock samples (1103P, 779P and 140Ru) grown on investigated soil are reported in Supplementary material (Tables 34-36(S.M.)). Samples are reported as leaves, shoots and roots (woody, middle and fine), and are identified according to growth stage achieved (being only rootstocks, only three different periods are considered). To evaluate the REE distribution onto rootstocks for each growth stage the total REE concentrations both in the whole plants and in the plant organs were calculated. Obtained values are the average calculated from three different plants (Table 29).

Table 29 Total and REE contents per organ in the *1103P*, *779P* and *140Ru* rootstocks during the 3 growth stage

	µmol/total plant (d.w.)								
Plant organs	**ET-1**			**PA-2**			**PA-1**		
	1103P								
	St 1	St 2	St 3	St 1	St 2	St 3	St 1	St 2	St 3
Leaves	0.10	0.25	0.26	0.14	0.51	0.25	0.08	0.22	0.25
Herbaceous shoots	0.03	0.04	0.08	0.06	0.09	0.06	0.02	0.03	0.06
Woody roots	0.22	0.66	0.96	0.27	0.57	1.96	0.36	0.47	1.53
Middle roots	0.33	0.61	0.84	0.66	0.57	2.33	0.18	0.41	1.03
Fine roots	4.25	23.06	25.70	2.99	9.17	12.48	1.78	8.79	14.99
Σ REE	**4.93**	**24.63**	**27.84**	**4.12**	**10.91**	**17.09**	**2.42**	**9.93**	**17.86**
s	**0.36**	**1.71**	**3.06**	**0.33**	**1.58**	**1.49**	**0.30**	**0.77**	**0.90**
RSD %	**7.38**	**6.96**	**11.01**	**8.09**	**14.53**	**8.73**	**12.23**	**7.76**	**5.06**
	779P								
	St 1	St 2	St 3	St 1	St 2	St 3	St 1	St 2	St 3
Leaves	0.09	0.18	0.24	0.13	0.38	0.24	0.09	0.17	0.22
Herbaceous shoots	0.02	0.04	0.08	0.03	0.07	0.06	0.02	0.05	0.05
Woody roots	0.19	0.31	0.51	0.17	0.39	1.39	0.81	0.98	1.18
Middle roots	0.11	0.27	0.69	0.14	0.61	0.79	0.52	0.61	1.11
Fine roots	8.79	9.46	22.63	2.61	2.90	12.18	2.21	7.11	8.90
Σ REE	**9.19**	**10.25**	**24.16**	**3.08**	**4.35**	**14.65**	**3.63**	**8.92**	**11.46**
s	**0.23**	**0.94**	**1.43**	**0.29**	**0.13**	**0.74**	**0.36**	**0.35**	**0.71**
RSD %	**2.49**	**9.20**	**5.90**	**9.53**	**3.01**	**5.05**	**10.07**	**3.96**	**6.20**
	140Ru								
	St 1	St 2	St 3	St 1	St 2	St 3	St 1	St 2	St 3
Leaves	0.07	0.19	0.28	0.10	0.44	0.19	0.06	0.19	0.24
Herbaceous shoots	0.01	0.05	0.06	0.02	0.08	0.06	0.01	0.03	0.05
Woody roots	0.19	0.47	1.22	0.25	0.28	1.32	0.35	0.60	1.77
Middle roots	0.36	0.32	0.73	0.38	0.69	1.09	0.37	0.37	1.46
Fine roots	6.36	7.59	22.92	4.81	4.95	10.51	1.31	5.25	9.30
Σ REE	**6.99**	**8.62**	**25.21**	**5.55**	**6.45**	**13.17**	**2.10**	**6.43**	**12.82**
s	**0.82**	**0.82**	**1.70**	**0.73**	**0.63**	**1.01**	**0.25**	**0.85**	**0.92**
RSD %	**11.77**	**9.55**	**6.74**	**13.08**	**9.79**	**7.70**	**12.05**	**13.28**	**7.22**

Evolutions of REE contents during rootstocks growth are reported in Figure 34. Here these values change from 2.42 ± 0.30 to 27.84 ± 3.06 μmol/total plant (d.w.) being the highest REE contents (from 90% to 98%) found in fine roots regardless of soils.

Figure 34 REE total content in root apparatus of vines rootstocks



By contrast, REE contents in Moscato d'Asti and Sauvignon blanc berries (Table 30) are lower than those found in aerial plant parts (ranging from 6.01 ± 0.31 to 49.00 ± 0.52 μg Kg^{-1}), about one order of magnitude concerning leaves. The REE behaviour in berries is consistent with the few reference data about these materials, resulting in REE less concentrated in berries respecting other aerial parts of grapevines (Tyler, 2004; Bertoldi et al., 2009; 2011).

Table 30 REE contents and its standard deviation (μg Kg^{-1} ± s, d.w.) measured in Moscato d'Asti and Sauvignon blanc berries

	Σ REE	**s**	**RSD %**
Moscato d'Asti			
ET-1	16.79	0.34	2.0
PA-2	6.01	0.31	5.2
PA-1	6.83	0.14	2.1
Sauvignon blanc			
ET-1	49.00	0.52	1.1
PA-2	9.74	0.44	4.5
PA-1	10.32	0.47	4.5

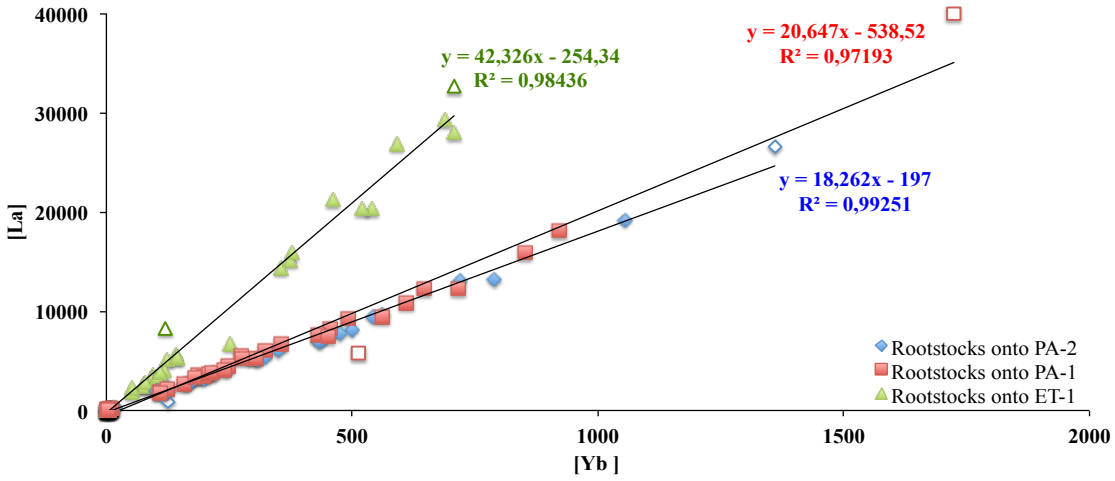
Moreover, our data show that absorption process was greater than REE translocation since the content (g d. w.) of middle and woody roots was lower than fine

root content, as expected (Tyler, 2004). The translocation process described for *off-soil* plants was also highlighted by rootstocks; during its development a concentration increase in shoots and leaves was observed. The REE percentage content in regard to total rootstock amounts ranged from 1% to 8%; the higher values are found in rootstocks onto PA-2 soil which, as mentioned, has increased the vegetative expression.

Unlikely, the soil had a significant effect on the REE absorption by roots. The ET-1 soil favoured the increased absorption in all stages, followed by PA-2 and PA-1 soils respectively. Rootstocks grown onto ET-1 showed an amount of absorbed REE (with respect to bioavailable soil fraction) ranging from 0.11% to 0.63%, whereas rootstocks grown on PA soils showed values spanning from 0.07% - 0.37% and 0.02% - 0.15% respectively. The larger REE absorption is found during the St 3. This is probably due to the characteristics of different soils and their acidic character, induced by the lower clay and carbonate contents in ET-1 soil than two other substrata. According to Tyler (2004) a significant negative correlation was obtained between REE concentration in roots and soil pH in a greenhouse study of wheat. In another greenhouse study relationship between soil solution chemistry and REE uptake was studied in *Agrostis capillaris* as influenced by soil acidity; the concentration of whole REE in the roots of the grass were inversely and linearly related to the pH of the soil solution (Tyler, 2004). Moreover, according to Laveuf & Cornu (2009), the REE absorption is controlled in addition to pH by the nature of clay minerals. Under acidic and low ionic strength conditions REE adsorption occurs with an outer-sphere mechanism onto 001 planes of clay minerals and this process allows to a weaker physiosorption related to the permanent structural charge. On the contrary, under alkaline conditions REE are adsorbed as inner-sphere complexes onto amphoteric size at the edges of particles (Takahashi et al., 2004).

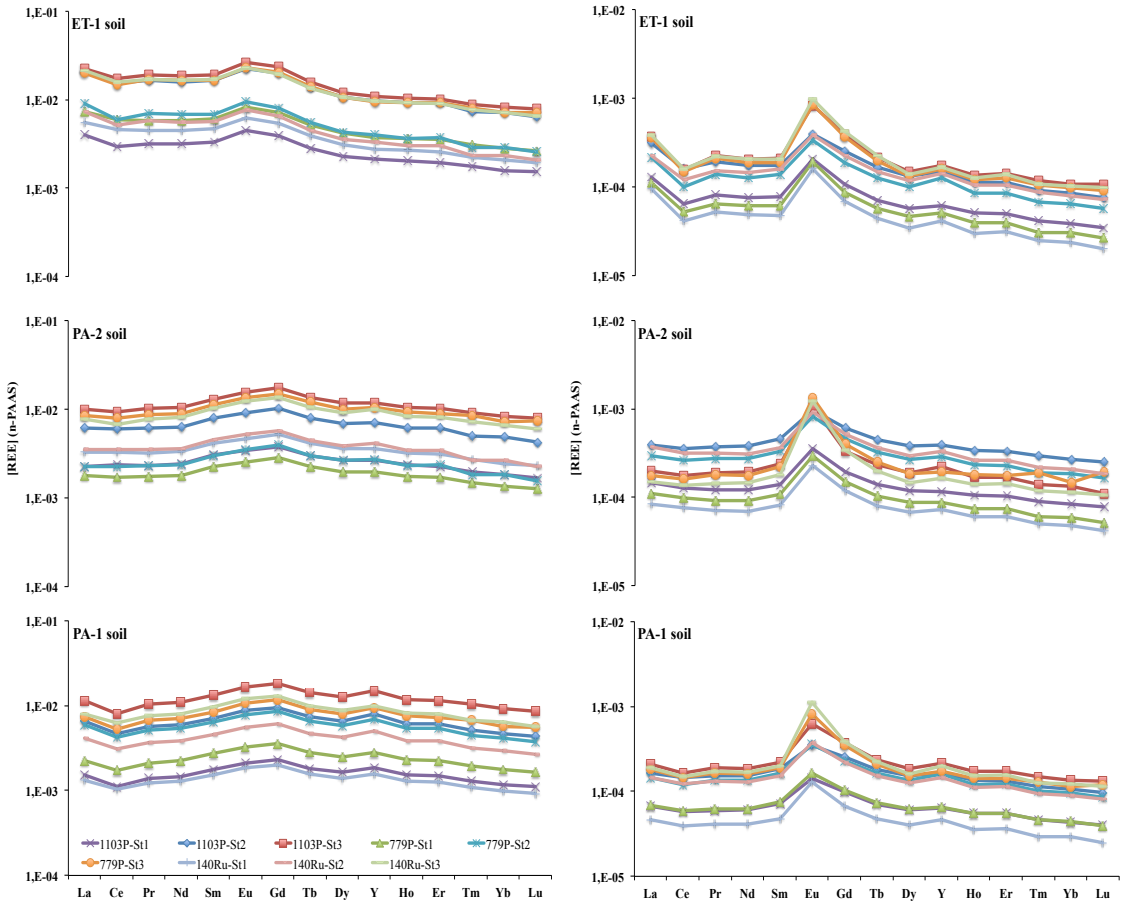
The relationship between Yb and La (Figure 35), used as indicator of LREE behaviour with respect to HREE (Semhi et al., 2009b) for every single rootstock and related soil, show that a linear correspondence occurs regardless of phenological stage. This is suggested by high R^2 coefficient equal to 0.992, 0.971 and 0.984 for PA-2, PA-1 and ET-1 systems respectively. This correspondence discriminates the soil effect highlighting extreme similarity between rootstocks grown onto PA-2 and PA-1 soils and a marked difference from rootstocks onto ET-1 soil.

Figure 35 Relationship between Yb and La (µg Kg^{-1}) measured in rootstocks (full symbols) and soil fractions (open symbols)



The PAAS-normalised patterns of rootstocks highlight the soil effect on REE absorption (Figure 36); the results will be evaluated as a function of soil and distinguishing between aerial and root apparatus.

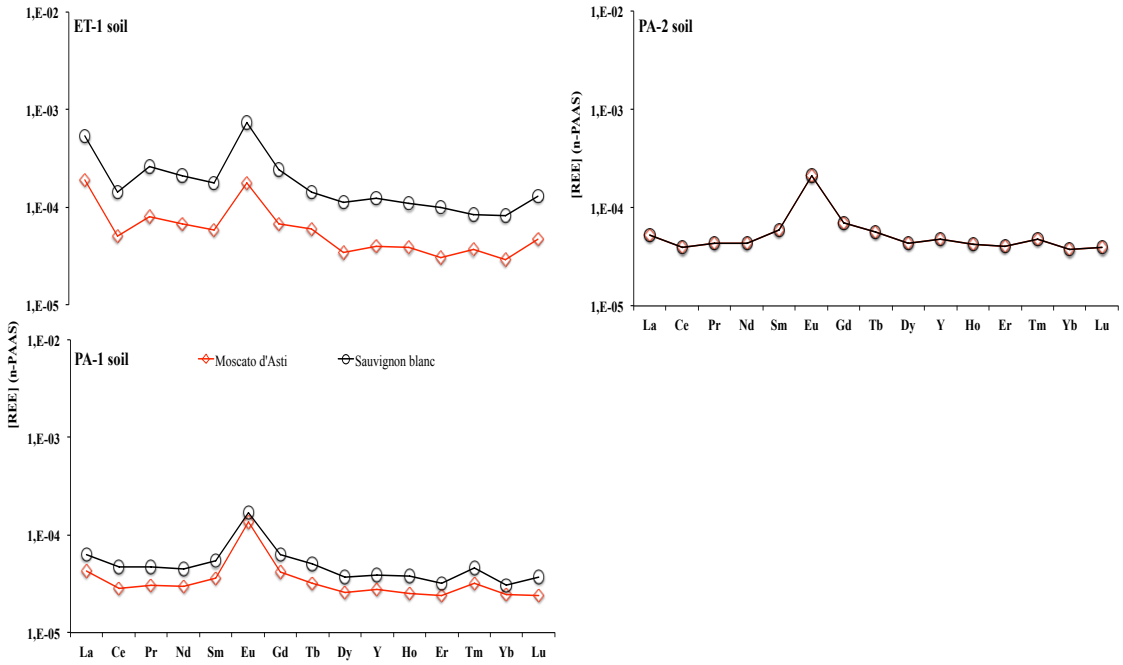
Figure 36 Shale-normalised REE patterns measured in (left) root and (right) aerial parts of different investigated rootstocks growing on different considered soils



Shale-normalised patterns were enriched in MREE and showed higher REE contents in roots, whereas aerial parts showed Eu-positive anomalies. In detail, we note larger positive Eu anomalies in shoots rather than in leaves and these Eu/Eu* values increase during plants growth. Eu/Eu* values attained to about 10 during St 3. No Y-anomalies occur hence Yttrium behaves just like the neighbour elements. In ET-1 and PA-1 soils slightly negative Ce-anomalies are observed; by contrast in PA-2 soil there are no evidence of Ce-anomalies suggesting that Cerium is absorbed as Ce(III). The strong similarities are observed among the REE patterns recognised in the studied rootstocks grown on the same soil; these data indicate that rootstocks do not influence REE behaviour during the grapevine growth.

REE-normalised patterns recognised in Sauvignon blanc and Moscato d'Asti berries (Figure 37) show similar shape of those found in other aerial plant parts, being always characterised by significant positive Eu anomalies. REE patterns of two investigated varieties grown on the same soils are characterised by the same features highlighting that, as for rootstocks, no variety effect occurs.

Figure 37 Shale-normalised REE patterns measured in Moscato d'Asti and Sauvignon blanc berries growing on different considered soils



The patterns show that rootstock and variety effects are negligible with respect to soil effect; the three rootstocks, which from the agronomic point of view showed their different characteristics of vigor depending on growth soil, are characterised by overlapping patterns. This evidence suggests that plant, despite its peculiarities, during REE uptake keeps track of growth substrate providing its fingerprint.

4.6 Discussion

The early field investigations can hardly reveal the fractionation features of REE in plants owing to the limitations of using the normalization method, which is not effective in identifying the small variations in the transfer process from soils to plants (Liang et al., 2008). In order to recognise whether a relationship exists between REE contents in plants and fractions extracted from soils, the distribution coefficients (K_d) for different REE were calculated with respect to both pseudo-total and bioavailable soil fractions (Figures 38-39). Obtained K_d values are about three orders of magnitude larger in roots than aerial portions and berries. The sequence of K_d values was similar for different employed rootstocks and vine berries grown on the same soil. All patterns are characterised by flat features along the REE series if calculated with respect to pseudo-total soil fraction. Moreover, rootstocks and berries grown onto ET-1 soil show Ce-negative anomalies; on the other hand in plants grown onto sedimentary soils Cerium behaves as its neighbour elements. The sequence of K_d values calculated with respect to bioavailable soil fractions is characterised by different Ce behaviour: specimens grown onto PA-2 and ET-1 soils are characterised by Ce-negative and Ce-positive anomalies respectively.

REE fractionations with both variable valence (Ce) and stable valence (La, Yb, Lu) can be easily observed in plants owing to the combined effects on physical, chemical and biological factors (Liang et al., 2008) giving conventional geochemical parameters as $(La/Yb)_n$ and/or $(Ce/Ce^*)_n$ useful to differentiate plants and berries coming from different substrata. Our data suggest that this differentiation can be made if REE concentrations in plant are directly compared with those in bioavailable soil fraction (Figure 40) rather than pseudo-total soil fraction or normalised concentration with respect to a material taken as references (Figure 41) (*i.e.*, UCC, PAAS, Chondrite).

Figure 38 REE patterns measured in different investigated rootstocks normalised to *pseudo-total* soil fractions

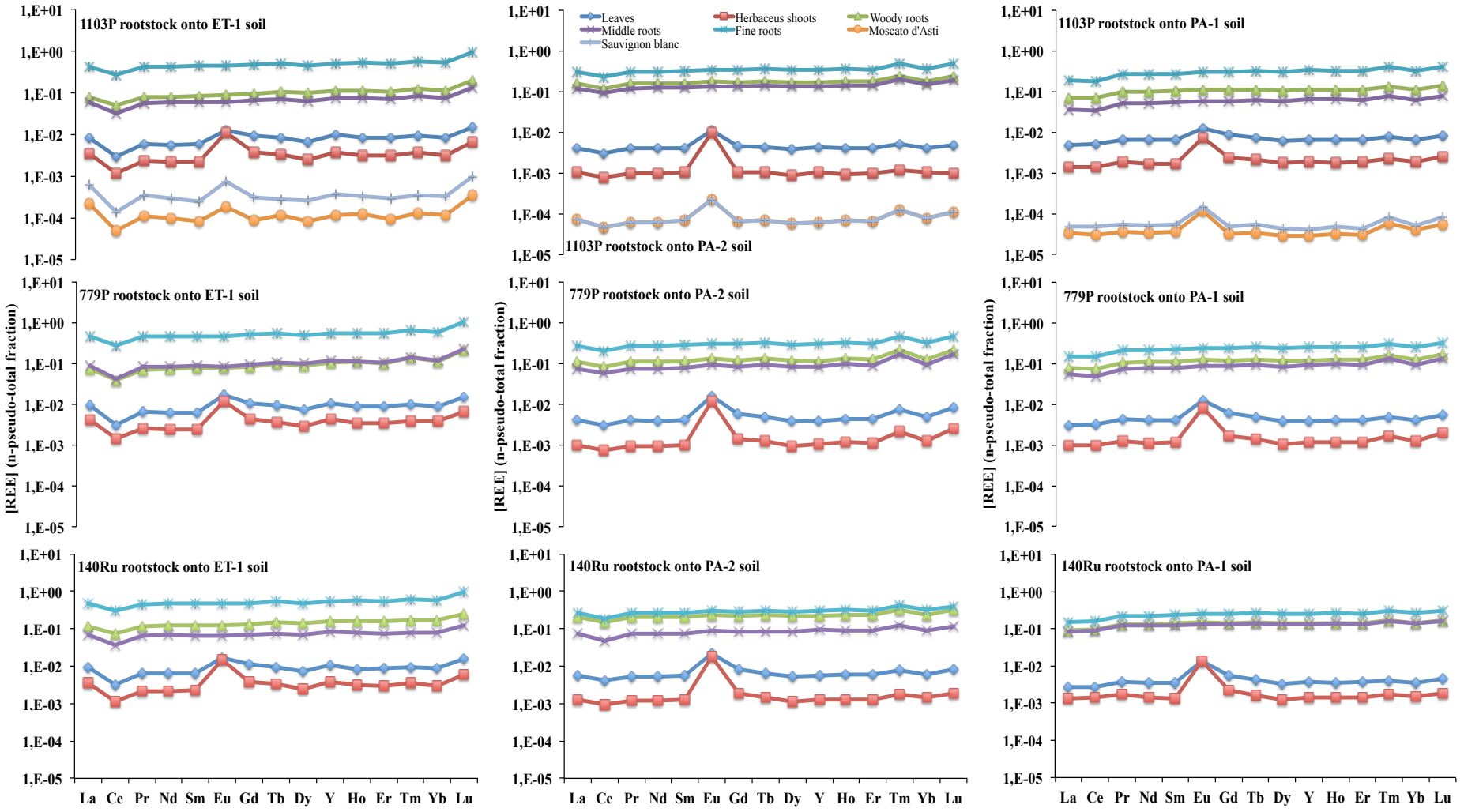


Figure 39 REE patterns measured in different investigated rootstocks normalised to *bioavailable* soil fractions

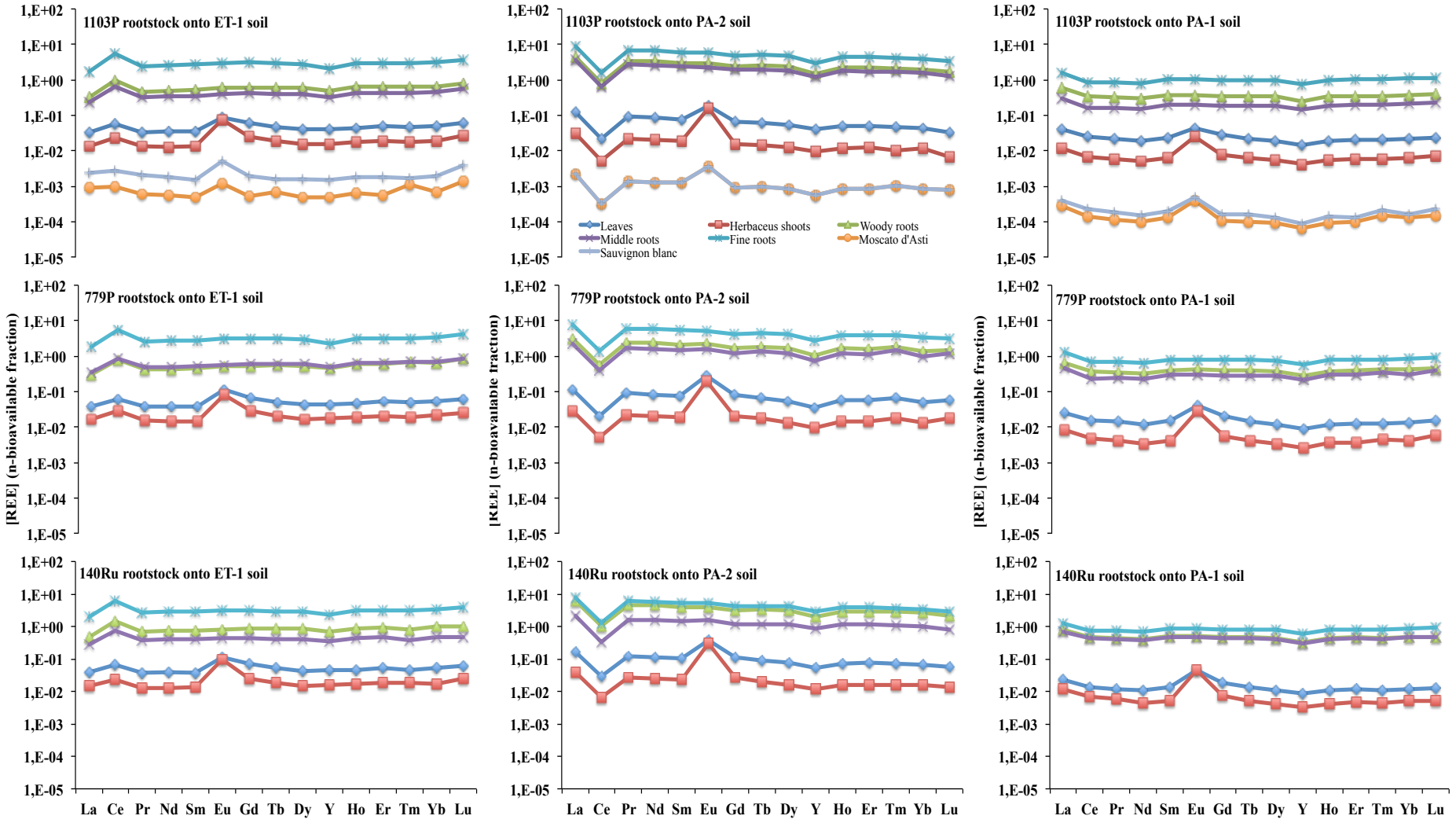


Figure 40 Relationship between La/Yb and Ce/Ce* measured in rootstocks and berries normalised to *bioavailable* soil fraction

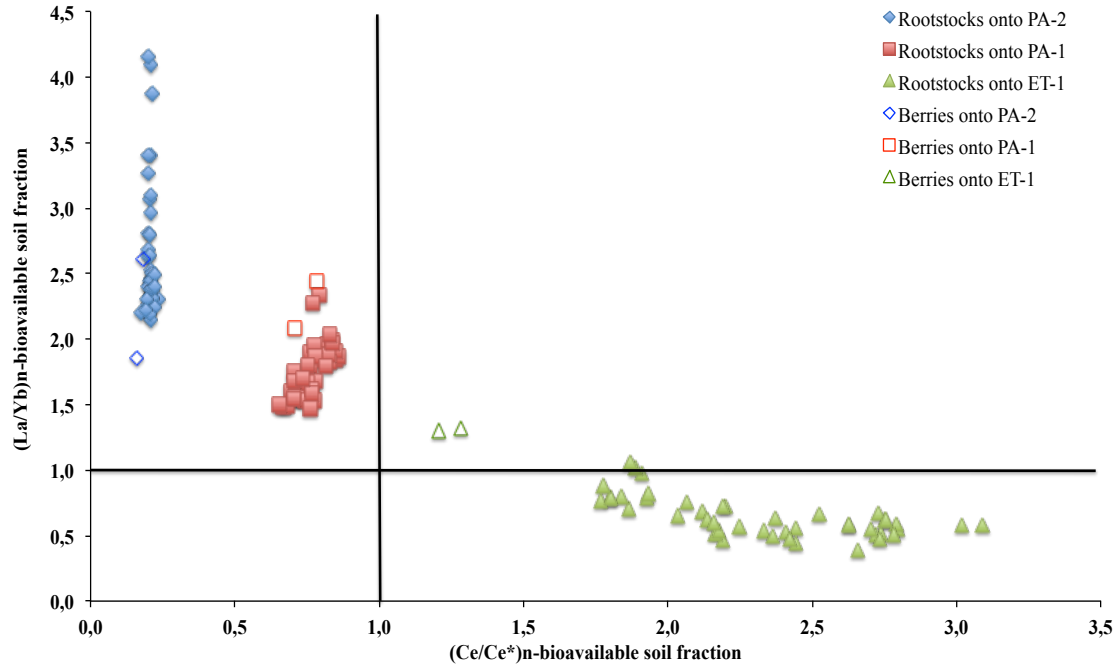
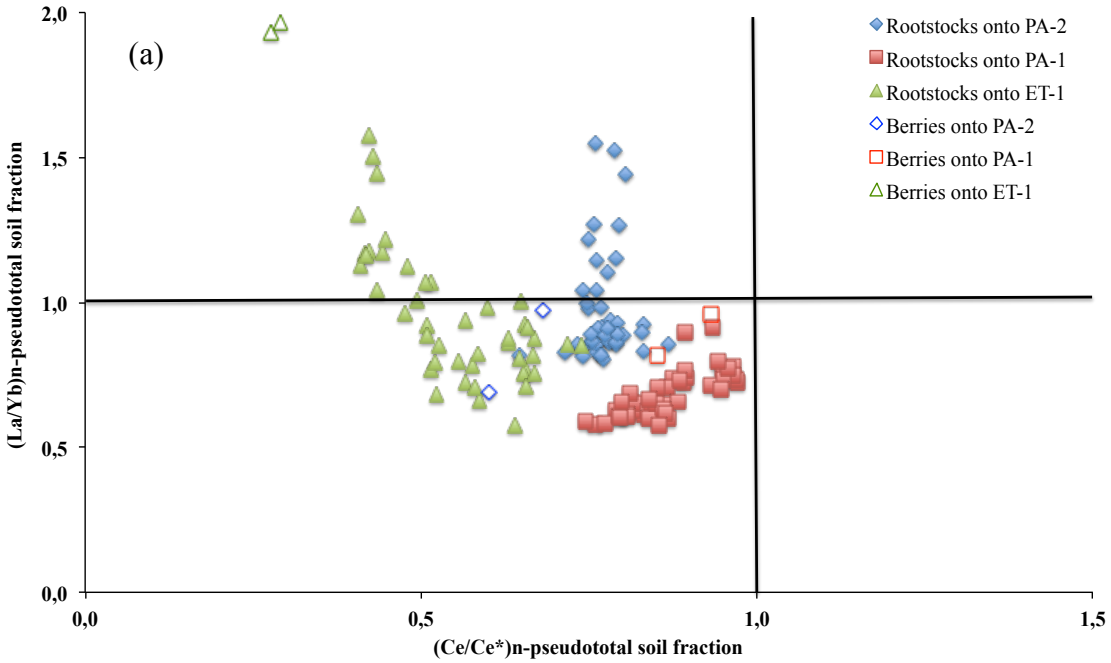
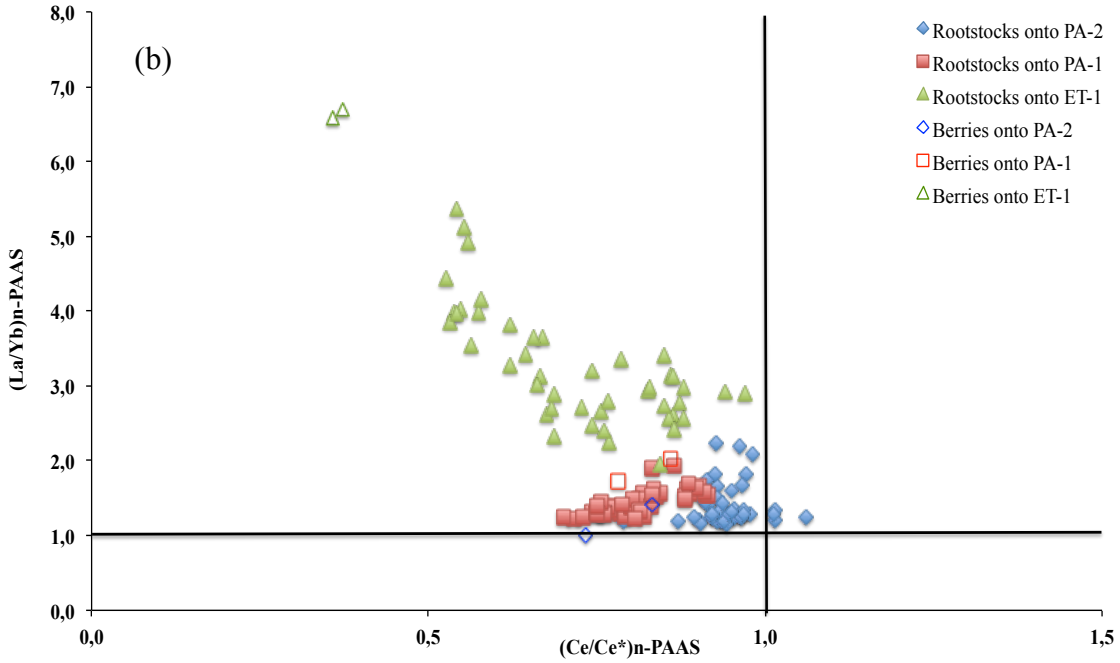


Figure 41 Relationship between La/Yb and Ce/Ce* measured in rootstocks and berries normalised (a) to *pseudo-total* soil fraction and (b) to PAAS



(Continued)


(b)
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
(La/Yb)n-PAAS
0,0
0,5
1,0
1,5
(Ce/Ce*)n-PAAS
Rootstocks onto PA-2
Rootstocks onto PA-1
Rootstocks onto ET-1
Berries onto PA-2
Berries onto PA-1
Berries onto ET-1

Field research

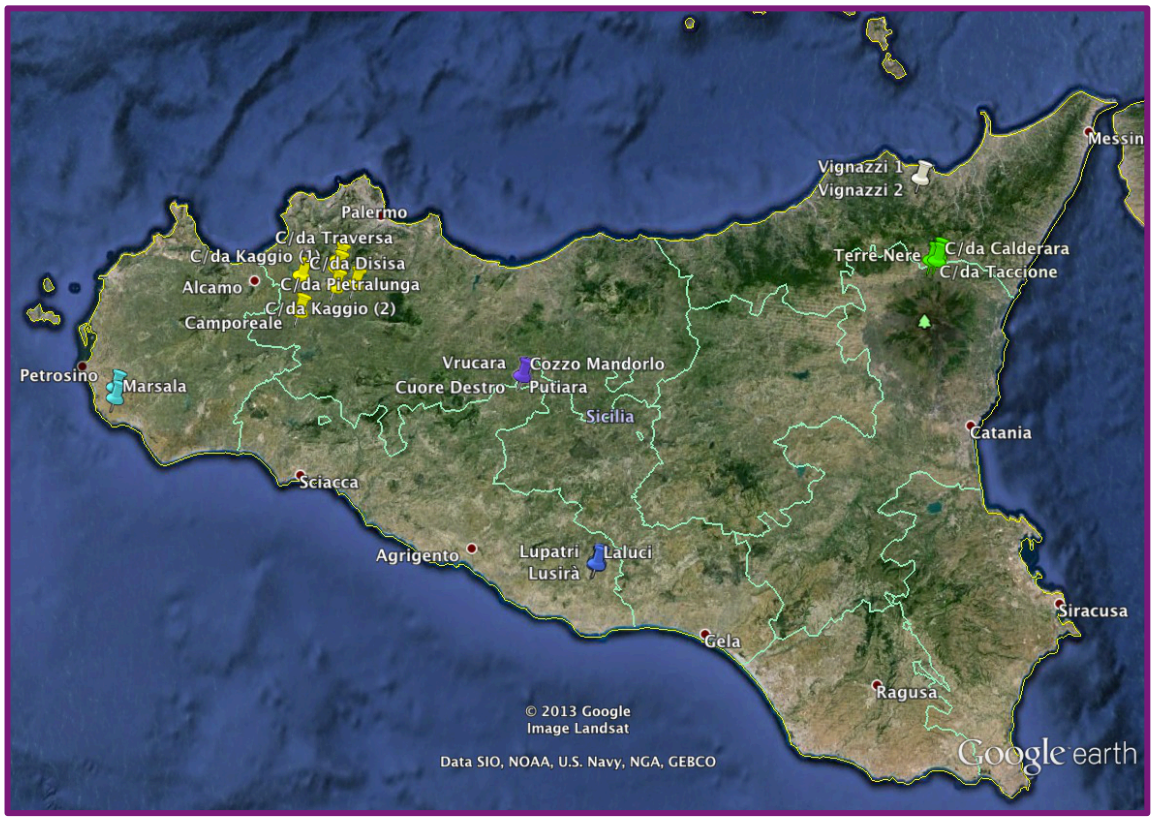
5.1 Background

After studying vine system under controlled conditions, it is necessary to investigate soil and berry relationship in field. Obtained results by *on-soil* system showed that rootstock and variety effects are negligible with respect to different soil effect onto vine system, so it was decided to investigate different soils and several fruit varieties maintaining the same rootstock as far as possible.

5.2 Field collection

The samples (soils and vine clusters, respectively) were collected from distinct geographic areas of Sicily (Figure 42) on the 2013 harvesting time.

Figure 42 Location of Sicilian wineries



- ✓ **F. Sapienza winery** located between San Giuseppe Jato and Camporeale (Pa) (**PA-3**):
  - "*Camporeale*" samples (37°51'50.81" N-13° 5'5.69" E, 322 m Sea level) (**PA-3a**) Grillo variety grafted onto 140Ru on calcareous-marly soil,
  - "*C/da Disisa*" samples (37°56'59.77" N-13° 5'48.06" E, 421 m Sea level) (**PA-3b**) Grillo variety grafted onto 140Ru on calcareous-marly soil,
  - "*C/da Pietralunga*" samples (37°55'9.25" N-13°15'21.14" E, 504 m Sea level) (**PA-3c**) Grillo variety grafted onto 140Ru on calcareous-marly soil,
  - "*C/da Kaggio (1)*" samples (37°57'41.29" N-13°12'19.92" E, 509 m Sea level) (**PA-3d**) Cataratto variety grafted onto 140Ru on calcareous-marly soil,
  - "*C/da Kaggio (2)*" samples (37°55'4.90" N-13°11'46.83" E, 361 m Sea level) (**PA-3e**) Cataratto variety grafted onto 140Ru on calcareous-marly soil,
  - "*C/da Traversa*" samples (37°58'50.36" N-13°12'39.06" E, 523 m Sea level) (**PA-3f**) Cataratto variety grafted onto 140Ru on calcareous-marly soil;
- ✓ **Terre Nere and Mangano winery** located in Randazzo (Ct) (**ET-2**):
  - "*Terre Nere*" samples (37°53'8.97" N-15°1'7.99" E, 616 m Sea level) (**ET-2a**) Nerello mascalese variety on volcanic soil,
  - "*C/da Taccione*" samples (37°52'32.89" N-14°59'55.05" E, 718 m Sea level) (**ET-2b**) Nerello mascalese variety on volcanic soil,
  - "*C/da Calderara*" samples (37°52'27.14" N-15°0'53.29" E, 698 m Sea level) (**ET-2c**) Cabernet-sauvignon variety on volcanic soil;
- ✓ **Baglio del Cristo winery** located in Campobello di Licata (Ag) (**AG-1**):
  - "*Lusirà*" samples (37°13'40.00" N-13°55'28.00" E, 258 m Sea level) (**AG-1a**) Syrah variety grafted onto 1103P on evaporitic soil,
  - "*Lupatri*" samples (37°13'22.00" N-13°55'18.00" E, 246 m Sea level) (**AG-1b**) Nero d'Avola variety grafted onto 1103P on evaporitic soil,
  - "*Laluci*" samples (37°13'15.00" N-13°55'7.00" E, 225 m Sea level) (**AG-1c**) Grillo variety grafted onto 1103P on evaporitic soil;
- ✓ **Feudo Montoni winery** located in Cammarata (Ag) (**AG-2**):
  - "*Cozzo Mandorlo*" samples (37°40'26.00" N-13°44'28.00" E, 627 m Sea level) (**AG-2a**) Catarratto variety grafted onto 140Ru on carbonatic soil,
  - "*Cuore Destro*" samples (37°40'29.00" N-13°44'30.00" E, 614 m Sea level) (**AG-2b**) Nero d'Avola variety grafted onto 140Ru on carbonatic soil,

- "*Vrucara*" samples (37°40'36.00" N-13°44'40.00" E, 558 m Sea level) (**AG-2c**) Catarratto variety on carbonatic soil,
- "*Putiara*" samples (37°40'33.00" N-13°44'23.00" E, 628 m Sea level) (**AG-2d**) Chardonay variety grafted onto 140Ru on carbonatic soil;

✓ **Experimental camps of SAF department** located near to Marsala (Tp) (**TP-1**):

- "*Marsala*" samples (37°41'2.79" N-12°31'4.14" E, 10 m Sea level) (**TP-1a**) Grillo variety grafted onto 1103P on calcarenitic soil,
- "*Petrosino*" samples (37°42'34.84" N-12°31'7.75" E, 19 m Sea level) (**TP-1b**) Grillo variety grafted onto 1103P on calcarenitic soil;

✓ **Tenuta Gatti winery** located in Librizzi (Me) (**ME-1**):

- "*Vignazzi 1*" samples (38°4'37.06" N-14°59'40.28" E, 309 m Sea level) (**ME-1a**) Nocera variety grafted onto 1103P on metamorphic soil,
- "*Vignazzi 2*" samples (38°4'38.26" N-14°59'39.55" E, 316 m Sea level) (**ME-1b**) Cabernet-sauvignon variety grafted onto 1103P on metamorphic soil.

After elimination of grassy layer, a sample of each soil was collected from 0 to 50 cm deep; as described in section 2.2.1.1, sampling was carried out using a pre-cleaned stainless steel spade and the sampled soil was stored in polyethylene bags. An aliquot of each soil was analysed as described in 2.2.1.2 and 2.2.1.3 sections. As reported in section 2.2.3.1 during harvesting time any cluster varieties were sampled using a ceramic scissors and stored in polyethylene containers; an aliquot of each sample was analysed as described in 2.2.3.2.

5.3 Results

5.3.1 Soils

Pseudo-total fraction

REE contents in pseudo-total soil fractions are reported in Supplementary material (Table 37 (S.M.)). Table 31 shows the total REE concentrations, expressed in mg kg^{-1}, of the investigated soils. Total REE contents ranged from 28 – 417 mg Kg^{-1}, the maximum values were found in ET-2 soils (about 403.28 ± 18.53 mg Kg^{-1}) while in AG-2 soils the lowest values (ranging from 27.80 ± 1.69 – 43.27 ± 1.50 mg Kg^{-1}) were found. PA-3, TP-1, ME-1 and AG-1 soils show intermediate REE amounts between 62.32 ± 3.11 and 172.29 ± 3.20 mg Kg^{-1}.

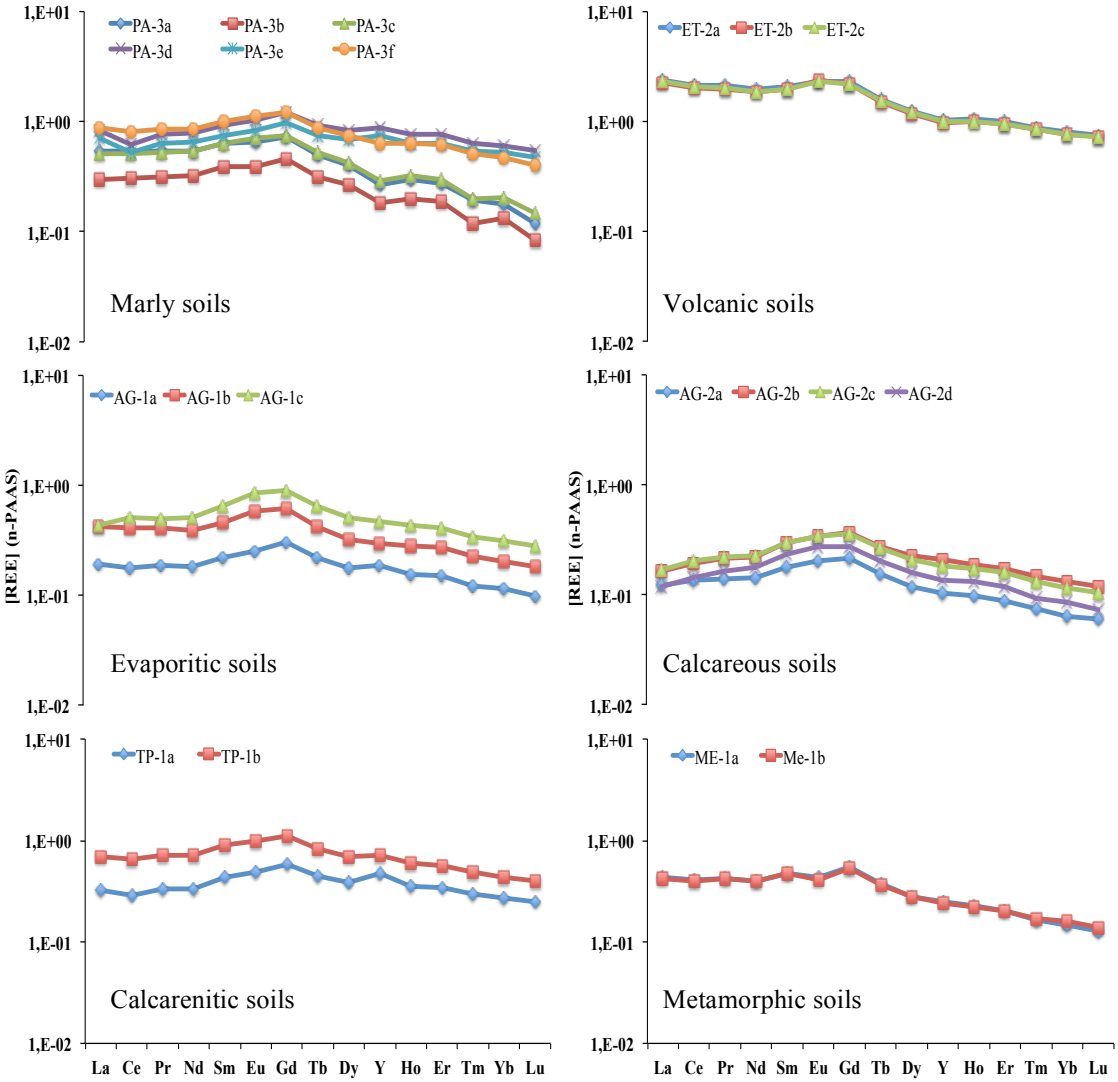
Table 31 Total REE contents and its standard deviation (mg Kg^{-1} ± s) measured in *pseudo-total* soil fractions from studied areas

		Σ REE	s	RSD%
Marly soils	PA-3a	105.20	4.71	4.5
	PA-3b	62.32	3.11	5.0
	PA-3c	103.81	4.94	4.8
	PA-3d	159.73	1.55	1.0
	PA-3e	134.39	8.34	6.2
	PA-3f	172.29	3.20	1.9
Volcanic soils	ET-2a	416.73	13.41	3.2
	ET-2b	392.79	27.22	6.9
	ET-2c	403.28	18.53	4.6
Evaporitic soils	AG-1a	39.33	1.02	2.6
	AG-1b	83.66	2.63	3.1
	AG-1c	105.86	2.79	2.6
Calcareous soils	AG-2a	27.80	1.69	6.1
	AG-2b	43.16	0.34	0.8
	AG-2c	43.27	1.50	3.5
	AG-2d	31.64	1.82	5.7
Calcarenitic soils	TP-1a	72.29	0.92	1.3
	TP-1b	147.84	3.44	2.3
Metamorphic soils	ME-1a	80.93	2.29	2.8
	ME-1b	79.43	1.12	1.4

The large variations of total REE contents in soil are highly dependent on the soil type and parent material from which they are issued (Hu et al., 2006a). The mineralogical composition control REE content in soil (Hu et al., 2006b) distinguished two major types of parent materials according to their REE content and REE contents in the soil issued from their weathering: (1) basic or acid igneous rocks, sandstones and shales (2) loess and calcareous rocks. According to Hu et al. (2006b), our data show a higher content for basaltic soils regard to calcareous soils. PAAS-normalised patterns in Figure 43 showed very different features that may be justified by mineralogical peculiarity. All patterns are similar, with REE distributions symmetrical along the series, with MREE enriched with respect to LREE and HREE. On the other hand, ET-2 soils show similar features to other soils from Tb to Lu while LREE pointed out higher values with respect to HREE. The larger LREE partitioning in ET-2 soils is consistent with the more incompatible nature of these elements as observed in ET-1 soil. By contrast, because of coordination numbers and ionic radii consideration, soil coming from parent carbonates weathering show a similar REE signature with MREE-enriched

pattern (Laveuf & Cornu, 2009 and references therein). In detail, the LREE enrichment with respect to HREE can be expressed by the Σ[LREE]/Σ[HREE] average ratio equal to 2.5 per ET-2, while for the other soils ranged between 1.1 and 1.6. Absent or negligible Ce- and Y-anomalies are observed for all soils. Y/Ho molar ratio in pseudo-total fractions, ranging from 49 to 67, suggest that Y behaves as a LREE and therefore ratio values closer to chondritic signature.

Figure 43 Shale-normalised REE patterns of *pseudo-total* soil fractions from studied areas



Bioavailable fraction

REE contents in bioavailable soil fractions are reported in Supplementary material (Table 38 (S.M.)). Table 32 shows the total REE concentrations, expressed in mg kg^{-1},

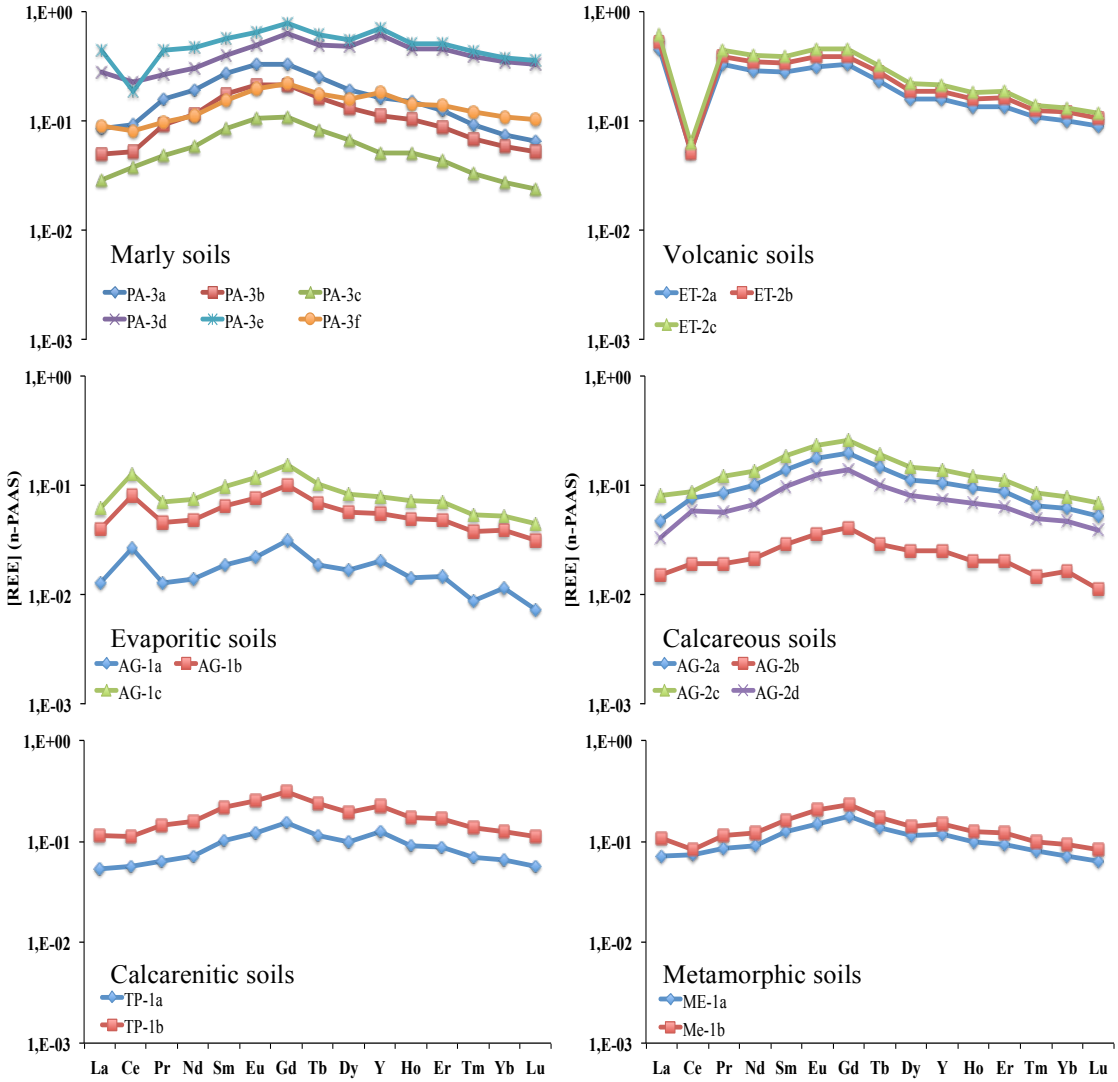
of the investigated areas; total REE contents ranged from 4.20 – 84.65 mg Kg^{-1}, the highest values was found in PA-3d,e soils while AG soils were less concentrated. Bioavailable fractions indicated that its incidence in whole soil is rather different among the investigated soils, varying from 9% to 30% in the ET-2, AG-1, TP-1 and ME-1 soils, while in PA-3d,e and AG-2a,c,d soils bioavailable soil fraction reaches about 50% with respect to pseudo-total amounts.

Table 32 Total REE contents and its standard deviation (mg Kg^{-1} ± s) measured in *bioavailable* soil fractions from studied areas

		Σ REE	**s**	**RSD%**
Marly soils	PA-3a	28.21	0.33	1.2
	PA-3b	17.35	0.16	0.9
	PA-3c	9.62	0.03	0.3
	PA-3d	69.54	0.26	0.4
	PA-3e	84.65	0.50	0.6
	PA-3f	23.45	0.02	0.1
Volcanic soils	ET-2a	43.59	0.19	0.4
	ET-2b	50.61	1.00	2.0
	ET-2c	59.11	0.27	0.4
Evaporitic soils	AG-1a	4.20	0.11	2.6
	AG-1b	13.02	0.05	0.4
	AG-1c	20.21	0.27	1.4
Calcareous soils	AG-2a	18.04	0.38	2.1
	AG-2b	4.38	0.07	1.5
	AG-2c	23.58	0.75	3.2
	AG-2d	12.98	0.22	1.7
Calcarenitic soils	TP-1a	15.41	0.09	0.6
	TP-1b	30.93	0.33	1.1
Metamorphic soils	ME-1a	18.50	0.46	2.5
	ME-1b	23.67	0.48	2.0

The shale-normalised patterns of bioavailable soil fractions (Figure 44) further highlight the different nature of studied soils. REE patterns in bioavailable soil fractions are characterised by MREE enrichment centred on Gd and giving positive Gd-anomalies (Gd/Gd*>1). This behaviour suggests that in bioavailable fraction are present Fe-oxyhydroxides, usually enriched in MREE (Bau & Dulski, 1996; Bau, 1999). The MREE excess cannot be related to phosphates presence because they are absent in the investigated soils. Furthermore, phosphates are not added by man as these vineyards are cultivated through "*organic techniques*".

Figure 44 Shale-normalised REE patterns of *bioavailable* soil fractions from studied areas

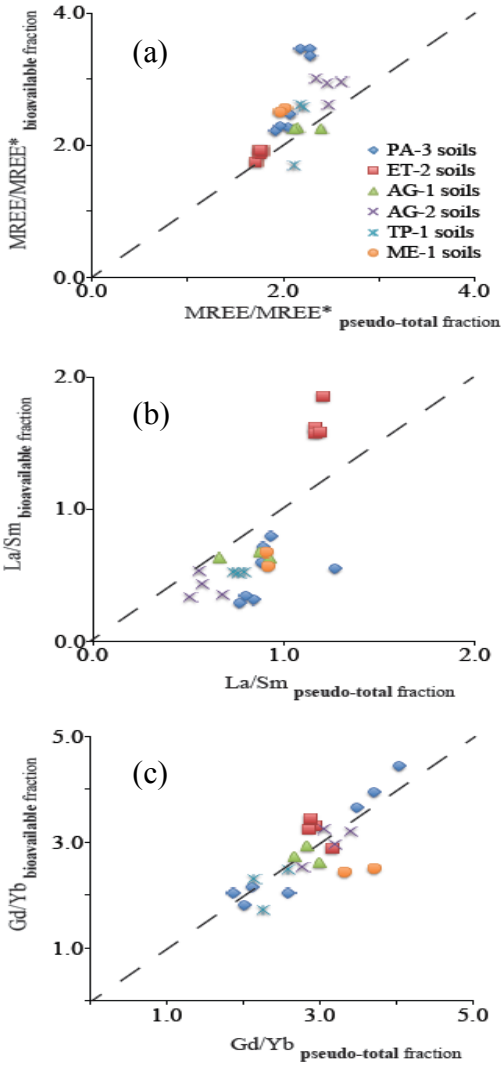


Ce-anomalies are rarely present in these samples suggesting that Cerium, even if present as Ce (IV), is preferentially bonded to the Mn-oxides co-precipitating as CeO_2 (Elderfield, 1988; Moffett, 1990). This REE behaviour is evident in all soils formed at expense of a sedimentary precursor more or less marly, from flysch to massive limestone; in these soils there are evidence of positive Ce-anomalies. By contrast if soil precursor is a calcarenitic rock, the REE patterns is symmetrical with respect to Gd giving Gd/Gd*>1. Soils with evaporitic precursors show REE patterns similar to calcarenitic ones with Gd/Gd*>1 but, unlike calcarenitic soils, show significant positive Ce-anomalies. Very similar behaviour to evaporitic soils characterises soils coming from North-Eastern Sicily, where there are extensive outcrops of metamorphic rocks. These soils are characterised by positive Gd-anomaly, symmetrical patterns with respect to Gadolinium and absence or negligible Ce-anomalies. REE behaviour is instead very

different in volcanic soils. The latter are characterised by a general enrichment in LREE compared to HREE (Σ[LREE]/Σ[HREE] near to 2.6), strong negative Ce-anomalies (Ce/Ce* = 0.12), almost flat behaviour pattern from Pr to Sm, Eu and Gd slightly enriched and progressive decrease in REE normalised concentrations up to Lu. The Y/Ho molar ratio values were slightly higher compared to chondritic value (Y/Ho≈52) for all analysed soils, suggesting a greater Yttrium mobility with respect to Holmium; the higher values were found in TP-1 and PA-3e (Y/Ho equal to 70.65 and 68.90 respectively).

The bioavailable fractions of examined soils are slightly MREE enriched with respect to pseudo-total ones. This is most evident in marly and calcareous soils whereas it is less enriched in volcanic soils (Figure 45a).

Figure 45 REE-ratios in soil. All plots show the indicated REE data manipulation in pseudo-total and bioavailable fractions, for all sites. (a) MREE/MREE* ratios, (b) La/Sm ratios, (c) Gd/Yb ratios



On the other hand, the pseudo-total fraction, exclude soils formed at expense of volcanic precursor, is LREE enriched (Figure 45b); infact, the volcanic soils show a bioavailable fraction more LREE enriched. This can be justified by presence of higher LREE content onto more soluble soil phases. Regarding Gd/Yb normalised ratio it not observed significant differences in patterns shape of the two soil fractions (Figure 45c). Calculating MREE anomaly according to Haley et al. (2004), this evidence shows that MREE and HREE consequently change in soil fractions while LREE content is responsible for observed changes in MREE anomaly amplitude values (Figure 45a).

5.3.2 Berries

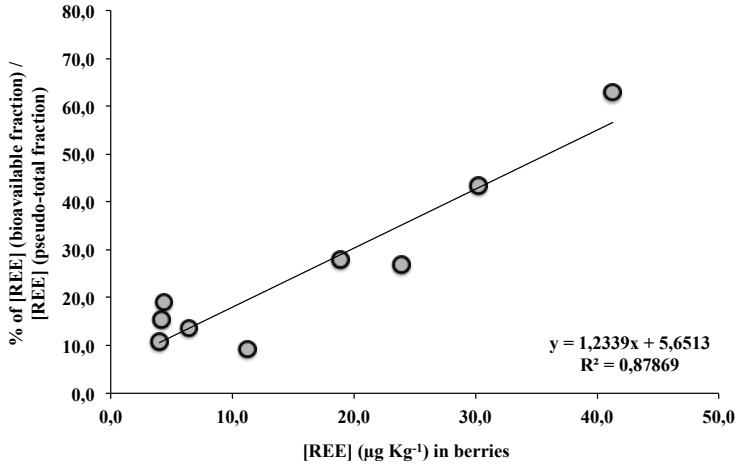
REE contents in berry samples are reported in Supplementary material (Table 39 (S.M.)). Table 33 shows the total REE concentrations, expressed in μg kg^{-1}, growing onto investigated soils; total REE contents ranged from 4.00 ± 0.11 to 46.28 ± 0.88 μg Kg^{-1}, the highest values were found in berries planted on ET-2 soils while berries coming from AG-1 soils showed lower values. The higher amounts in ET-2 berries are in agreement with the higher REE amount in the pseudo-total soil fraction.

Table 33 Total REE contents and its standard deviation (μg Kg^{-1} ± s, d.w.) measured in berry samples

	Σ REE	s	RSD%
PA-3a	23.89	0.50	2.1
PA-3b	18.93	0.62	3.3
PA-3c	11.26	0.21	1.9
PA-3d	30.24	0.27	0.9
PA-3e	41.28	0.62	1.5
PA-3f	6.45	0.05	0.7
ET-2a	46.28	0.88	1.9
ET-2b	27.21	0.26	1.0
ET-2c	44.45	0.43	1.0
AG-1a	4.00	0.11	2.7
AG-1b	4.21	0.24	5.7
AG-1c	4.42	0.08	1.7
AG-2a	23.50	0.39	1.7
AG-2b	14.21	0.38	2.7
AG-2c	9.41	0.48	5.1
AG-2d	9.76	0.55	5.7
TP-1a	15.59	0.48	3.1
TP-1b	19.19	1.79	9.3
ME-1a	8.64	0.15	1.7
ME-1b	28.53	0.43	1.5

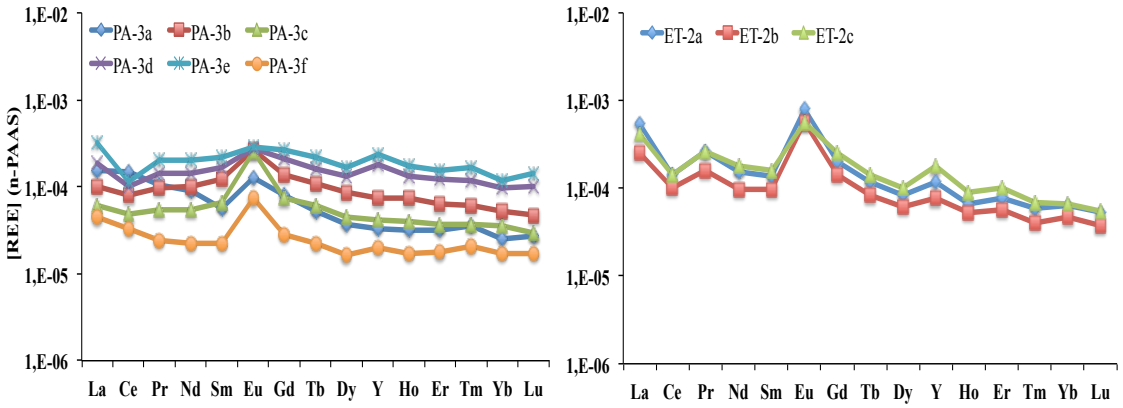
A linear correlation was found between REE amount in berries and REE percentage in bioavailable fraction with respect to the pseudo-total ones (Figure 46). This is clearly showed by AG-2 berries; these soils were characterised by lower REE contents in pseudo-total soil fractions, and at the same time showed the highest REE percentage extracted in bioavailable fractions.

Figure 46 Relationship between REE amount in berries and REE percentage in bioavailable soil fractions



Shale-normalised REE patterns recognised in sampled berries (Figure 47) show similar shape, being always characterised by significant Eu-positive anomalies ranging from 1.8 to 7.7.

Figure 47 Shale-normalised REE patterns measured in different berries growing onto considered areas



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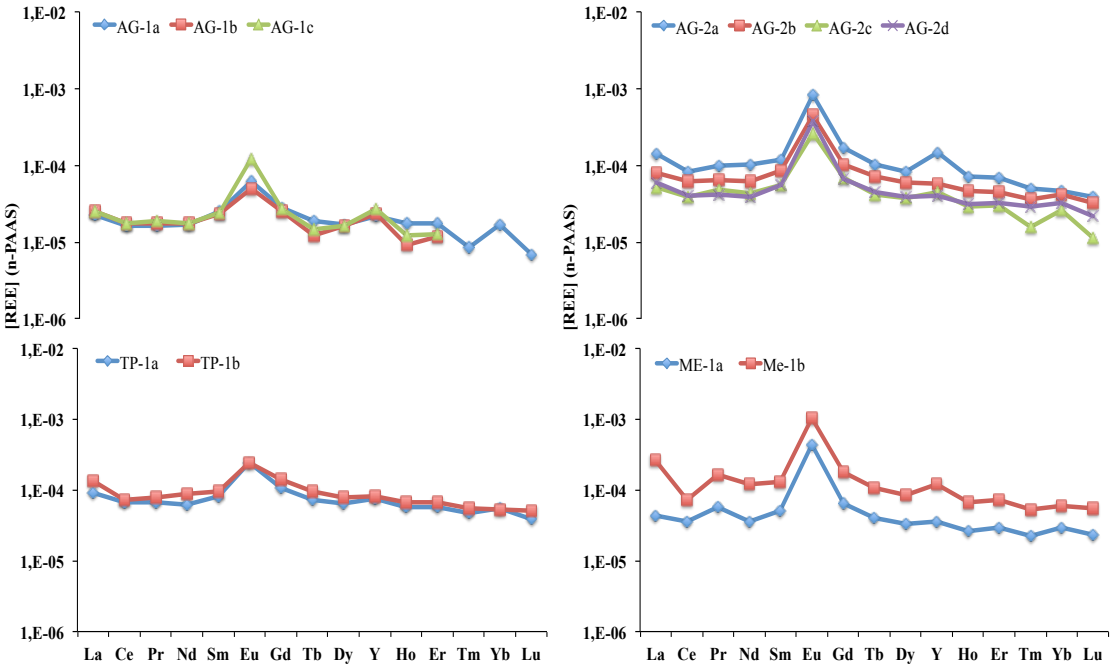


Ce anomalies allow to discriminate vine grown onto different soil; in particular ET-2 and ME-2b berries are characterised by negative Ce anomalies (Ce/Ce* near to 0.4) while other samples show Ce/Ce* slightly lower than 1. Moreover, Y similarly behaves to the bioavailable soil fraction.

Analysis of REE patterns from investigated vine varieties grown on the same soils are characterised by the same features confirming that, as for rootstocks, no variety effect occurs.

5.4 Discussion

In order to confirm the results obtained by *on-soil* investigation discussed in section 4.6, the K_d coefficients for different REE were calculated with respect to both pseudo-total and bioavailable soil fractions (Figures 48-49).

The sequence of K_d values are characterised by slight Ce-negative anomalies if calculated with regard to pseudo-total soil fractions; K_d calculated with respect to both soil fractions are characterised by Y-decoupling in regard to Ho for plants grown onto ET-2, AG-1b,c, AG-2a and ME-1b, while for the other case no deviation from trend was observed. By contrast, the K_d values calculated respecting bioavailable soil fractions are characterised by different Ce behaviour: plants grown onto AG-1 and ET-2 soils are characterised by Ce-negative and Ce-positive anomalies respectively.

Figure 48 REE patterns measured in different investigated berries normalised to *pseudo-total* soil fractions

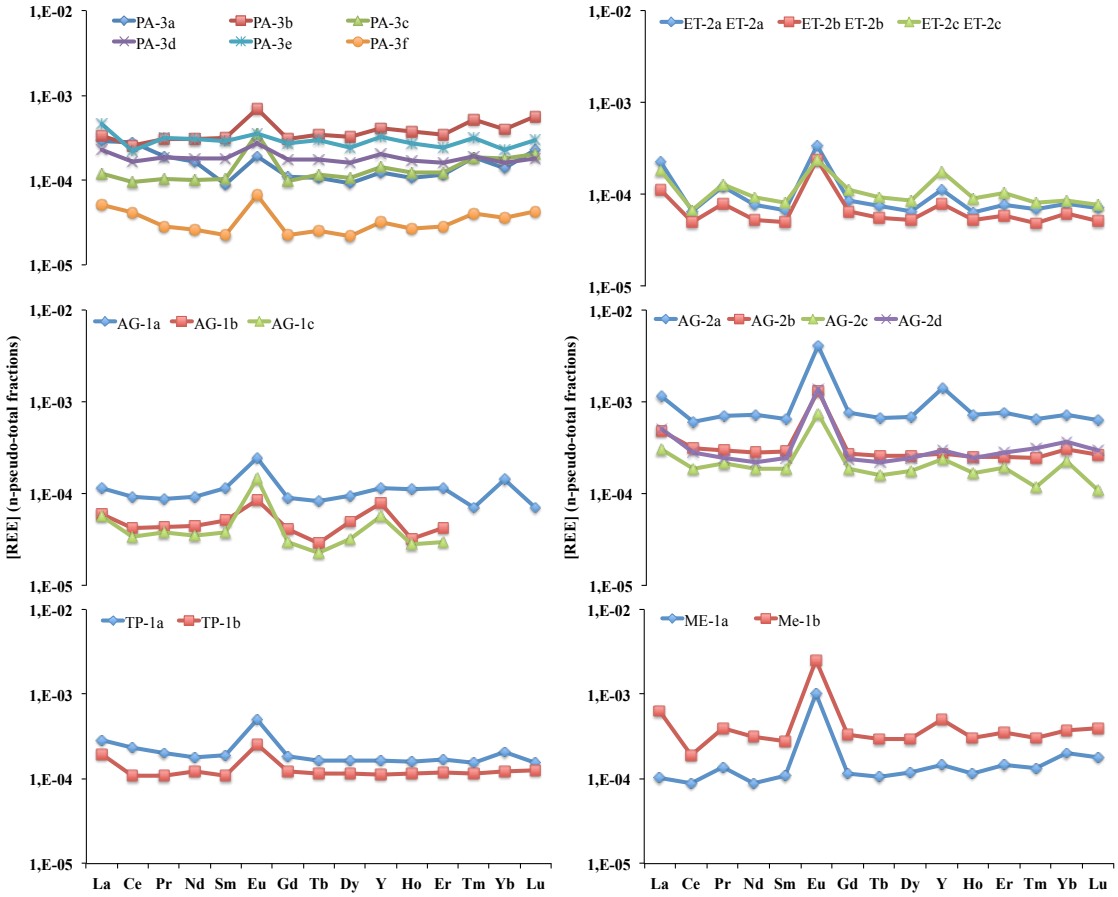
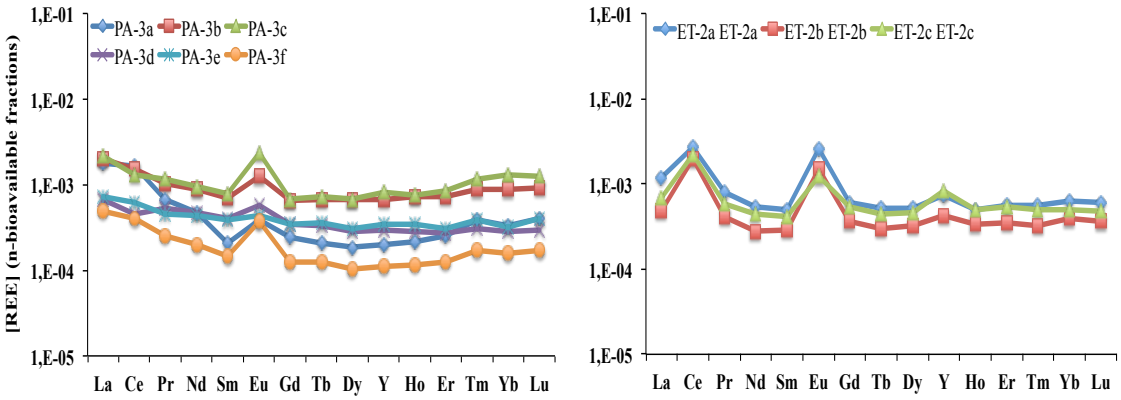
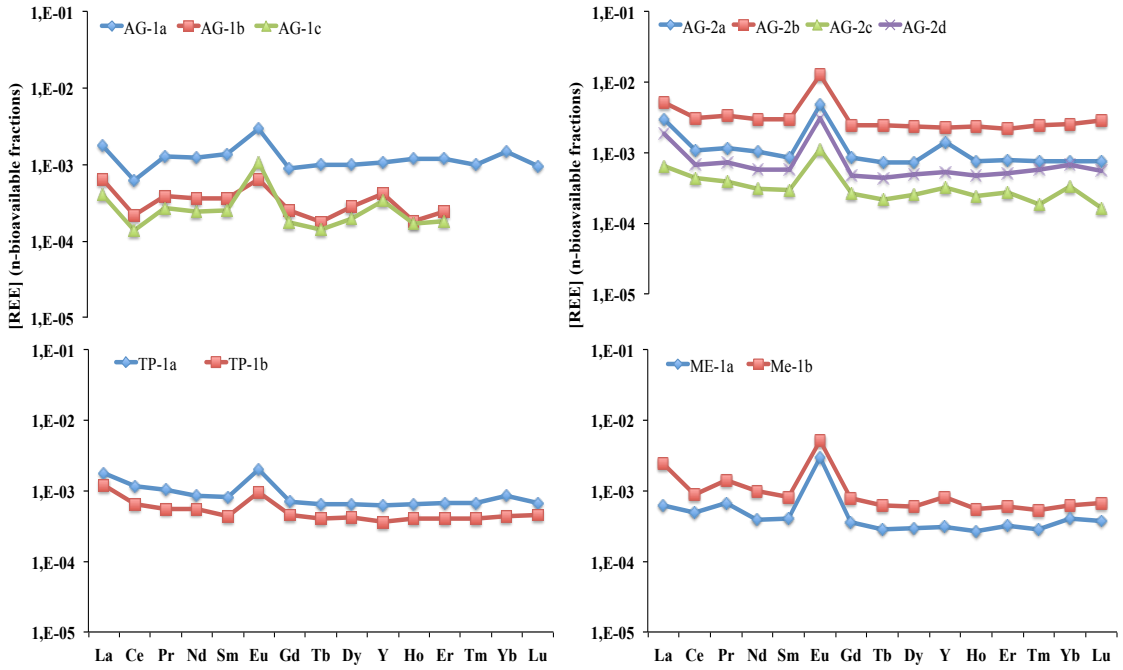


Figure 49 REE patterns measured in different investigated berries normalised to *bioavailable* soil fractions

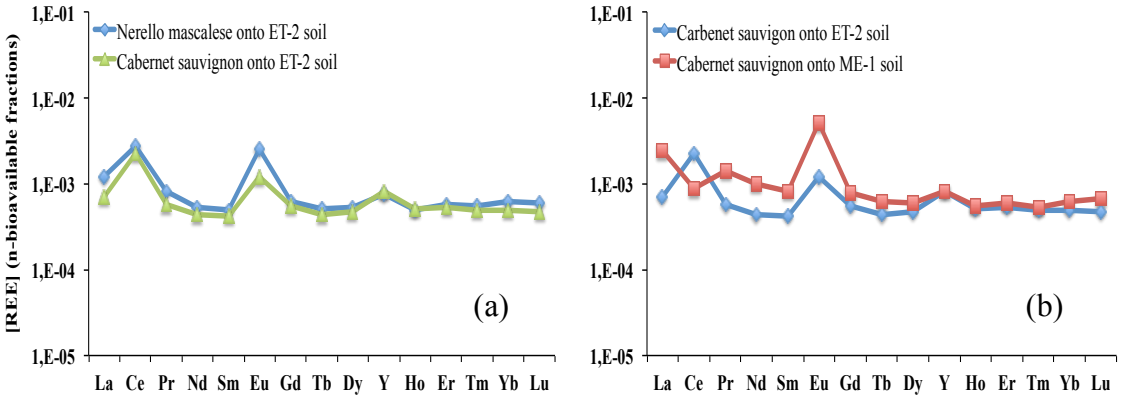


(*Continued*)



The obtained data confirm that, as for rootstocks, the fruit variety does not influence the REE absorption from growth substrate; Nerello mascalese variety and Cabernet sauvignon grown onto ET-2 soils show overlapping patterns, by contrast Cabernet sauvignon variety grown onto ET-2 and ME-1 are characterised by different trend if normalised to bioavailable soil fraction rather than pseudo-total ones (Figure 50).

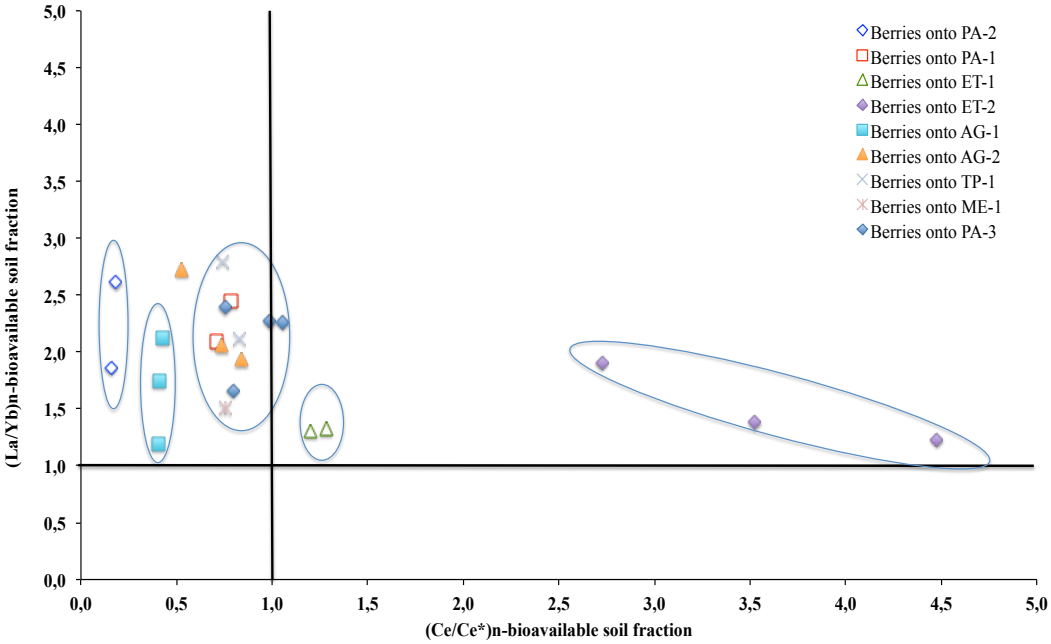
Figure 50 Comparison between (a) different varieties grown onto the same soil and (b) same varieties grown onto different soil



The geochemical parameters, Ce/Ce* and $(La/Yb)_n$, seem to be promising tools to trace the origin of products derived from vines. Our data indicate that the use of these tools allows us to differentiate vines grown on soils originated from different parent-rocks; all that can be made if REE concentrations in berries are directly compared with

those in bioavailable soil fraction (Figure 51) rather than pseudo-total soil fraction or normalised concentration with respect to a material taken as references (*i.e.*, UCC, PAAS, Chondrite).

Figure 51 Relationship between La/Yb and Ce/Ce* measured in berries normalised to *bioavailable* soil fraction

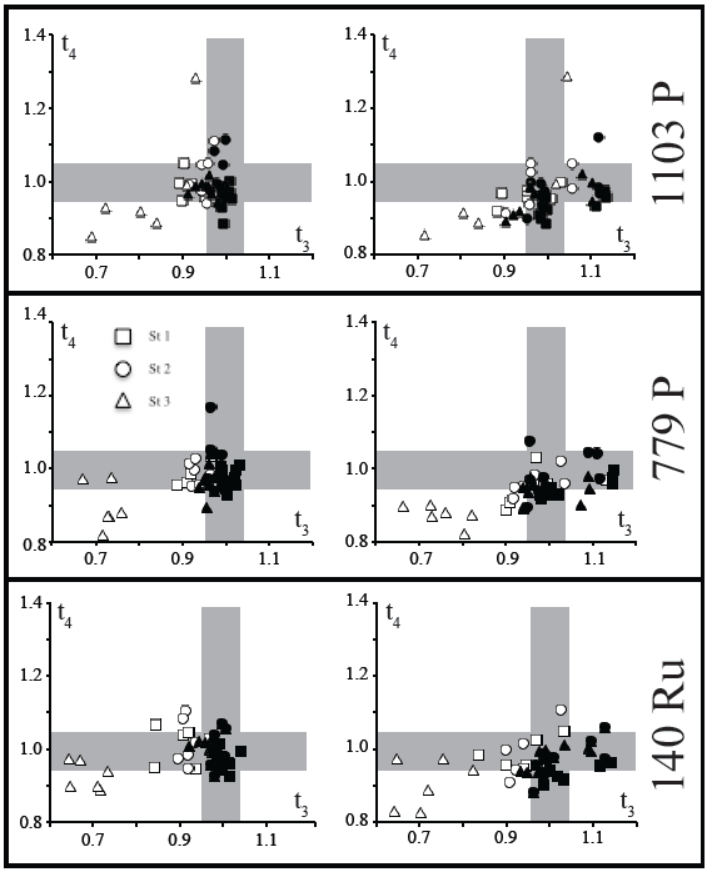


Comprehensive discussion

Plant/soil relationships: roots

Sequences of K_d values along the REE series showed significant tetrad effects in the third and fourth tetrads (Gd-Ho and Er-Lu, respectively). Their amplitudes are larger during the first and third growth stages (St 1 and St 3, respectively) where M-type tetrad effects are recognised in roots, whereas W-type tetrad effects are found in shoots and leaves, especially for K_d values calculated with respect to the bioavailable soil fraction (Figure 52).

Figure 52 Amplitudes of tetrad effect measured for 3rd (t_3) and 4th (t_4) tetrads from K_d values calculated with respect to (left) pseudo-total and (right) bioavailable soil fractions



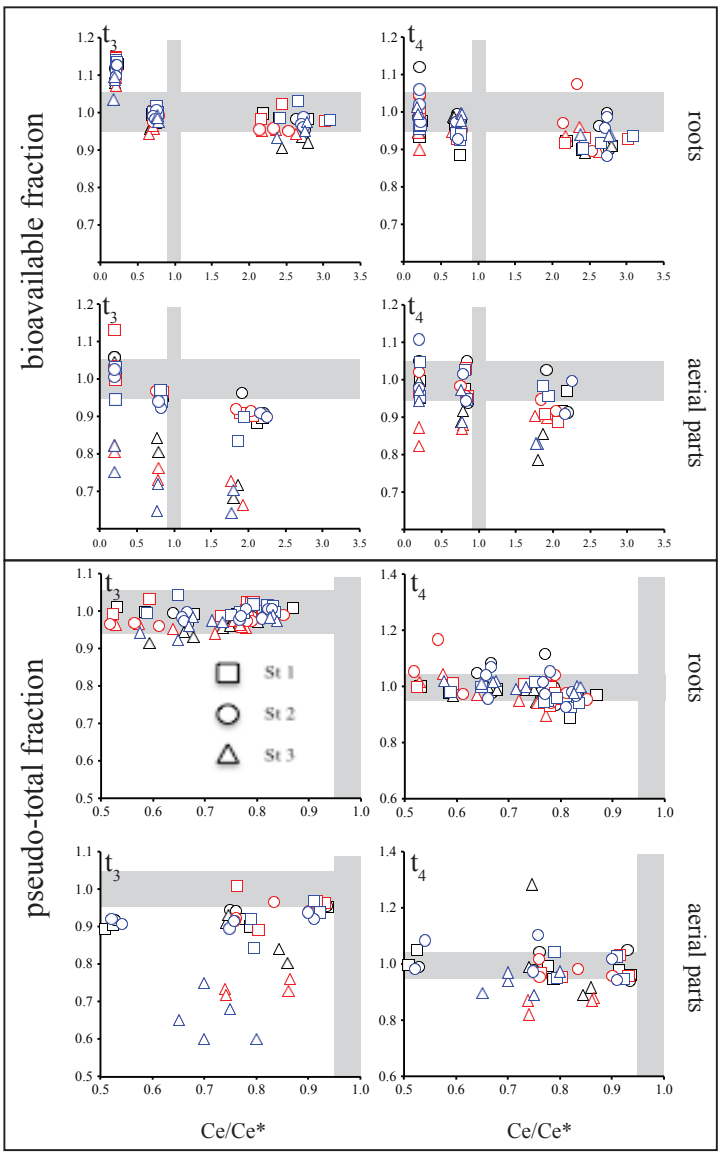
Squares, circles and triangles represent amplitudes of tetrad effects for samples of the St 1, St 2 and St 3 stage, respectively. Full symbols refer to root samples whereas open symbols represent aerial parts of *Vitis vinifera*. Dashed areas represent non-significant values.

In the aerial plant organs, significant tetrad effects mainly occur during the first and third stages (W-Type TE if $t_i \leq 0.95$; M-type TE if $t_i \geq 1.05$; not significant tetrad

effects if $0.95 < t_i < 1.05$) and are apparently not influenced by rootstock and soil types (Figure 52).

According to the theory of Tetrad Effects, Masuda et al. (1987) indicated that W-type tetrad effects imply that REE have been released from a coexisting solid and are in dissolved form. In our samples, similar features were associated with negative Ce anomalies (Ce/Ce*<1) in the aerial parts of investigated plants (Figure 53), suggesting an oxidative Ce scavenging as insoluble CeO_2 that is therefore subtracted from the dissolved pool (Bau and Koschinsky, 2009 and references therein).

Figure 53 Amplitudes of tetrad effects and related Ce/Ce* values from K_d values calculated with respect to bioavailable and pseudo-total soil fractions for roots and aerial parts of *Vitis vinifera* at different growth stages. Dashed areas represent non-significant values.



In contrast, the desultory recognition of M-type tetrad effects is limited to roots and associated with positive Ce anomalies therein, suggesting that authigenic solids are deposed in the roots and the surfaces of these solids interact with dissolved REE allowing their surface complexation. Similar evidence has been shown in wheat roots (Ding et al., 2005; 2007; Do Carmo Lima e Cunha et al., 2012). The amplitudes of tetrad effects imply that both REE dissolved complexation in aerial parts and surface complexation onto authigenic solids in roots occur with an inner-sphere mechanism that can allow the observed t_3 and t_4 values (Masuda and Ikeuki, 1979; Masuda et al., 1987; Kawabe, 1992; Bau, 1999; Monecke et al., 2002). Ding et al. (2006) suggested that phosphate deposition in wheat roots is responsible for M-type tetrad effects, whose amplitudes grow during plant growth similar to those in grapevines.

LREE partitioning observed in REE patterns in roots (Figure 36) is a typical feature of several plants (Brioschi et al., 2013) and can be explained with the well-known reduced mobility of lighter REE that are more surface-reactive than MREE and HREE (Byrne and Sholkovitz, 1996). At the same time, MREE accumulation in the finest roots agrees with the preferential accumulation of elements from Sm to Ho in the outer membranes during the early stages of cell growth (Gao et al., 2003; Dong et al., 2009). This process could be consistent with phosphate crystallisations in plants invoked by Tyler (2004) and Ding et al. (2005), since these compounds are usually enriched in MREE (Hannigan and Sholkovitz, 2001). In any case, the observed REE depletion in aerial parts of the grapevines and the REE fractionations in their roots is suggestive of a large-scale REE fractionation that could be an effect of REE interactions with biological membranes, as the Casparian strip occurring in the youngest roots, which can induce a geochemical decoupling of the element pair usually characterised by similar geochemical behaviour (Sparks et al., 2011).

Aerial portions

As previously mentioned, the REE migration from roots to the aerial portions in the studied plants involves REE depletions, positive Eu anomalies and associated W-type Tetrad Effects in aerial parts. Due to the presence of REE in the dissolved phase (Masuda et al., 1987), this evidence is suggestive of REE release from root tissues to the plant fluids where Eu is preferentially complexed in xylem fluids. Ding et al. (2006) reported similar Eu anomalies in wheat leaves and explained them via a Eu-enriched phosphate crystallisation in several plant organs. However, this hypothesis does not

seem convincing due to the lack of knowledge about these Eu-rich phosphates (Brioschi et al., 2013). On the contrary, the strong similarity between Ca^{2+} and Eu^{3+} makes it seem more reasonable that a Eu-Ca substitution can be induced during physiological processes occurring under favourable Eu/Ca ratios in substrata (Zeng et al., 2003). Observed positive Eu-anomalies also align with the greater stability of organic-REE complexes with respect to Ca complexes (Sastri et al., 2003), which can be explained by the stronger Eu^{3+} bond with proteins with respect to Ca^{2+} (Kruk et al., 2003). Evidences of promotion of biological activities played by Eu^{3+} are reported in *Lathyrus sativus L.* roots (Tian et al., 2003). Here, Eu^{3+} reduction to Eu^{2+} was demonstrated to influence the electron capture and transport of metals by binding proteins. In hydrothermal systems, Eu^{2+} has a lower affinity for sorption and its mobility is higher than Eu^{3+} and relative to its REE neighbours (Bau, 1991; Bau and Moller, 1992). Therefore, the possibility that observed positive Eu anomalies resulted from increased Eu mobility in the reduced Eu^{2+} form cannot be ruled out. Furthermore, positive Eu anomalies can result in enhanced Eu mobility and this process is related to the increasing nutrient transportation during metabolic processes in plants (Tian et al., 2003), which is corroborated by the recognition of positive Eu anomalies in the sap of xylem from several plants. This evidence suggests that a unique mechanism is responsible for transporting nutrients as well as trace elements and REE (Brioschi et al., 2013).

The role played during REE exploitation in agricultural practices carried out in China is justified by the capability of these elements to favour some metabolic processes in plants. The latter lead to increases the plant productivity according to several mechanisms (Schroeder et al., 2001). Some of the above-mentioned studies evidence the Eu^{3+} capability to intervene on the outward K^+ channels of *Vicia* guard cells and consequently regulating the cell water contents (Xue & Yang, 2009). These binding sites have been demonstrated as powerful ligands favouring REE migrations in a wide range of physical-chemical conditions (Sonke & Salters, 2006; Xiong, 2011; Loges et al., 2012). Cations involved in migration processes through the xylem of grapevines are complexed with organic acids by means of polar bindings (Taiz & Zeiger, 2006) occurring between O-donor groups and REE^{3+}, as demonstrated during hydroponic growth (Ding et al., 2006).

The behaviour of REE in berries (Figures 37, 47) is consistent with the few data about trace element distributions in these materials, resulting in REE being less concentrated in berries with respect to other aerial parts of grapevines (Tyler, 2004;

Bertoldi et al., 2009; 2011). Only positive Eu anomalies remain to testify to a greater Eu mobility in plant fluids with respect to other REE. As regards berries grown in field, our data show that the analysis on geochemical behaviour of REE discriminate the grapes grown on volcanic soils in comparison with the grapes coming from vineyard planted onto sedimentary soils. On the other hand, this discrimination cannot involve the grapes coming from soils originated at the expense of different nature sedimentary lithotypes and the ones grown on substrates originated by crystalline rocks.

Only La/Yb ratio and Ce anomaly normalised to bioavailable soil fractions allow a distinction of the grapes grown on soils of different nature (Figures 40, 41, 51) and this is fully demonstrated particularly for the grapes coming from volcanic soils. The reason of this difference is connected to the specific properties that distinguish REE behaviour both during CHARAC and non-CHARAC processes (Bau, 1996). During the CHARAC process the REE differentiate on the basis of charge/radius ratio (Shannon, 1976), that is by lanthanide contraction (Taylor & Mc Lennan, 1995). On the contrary, during the non-CHARAC process the REE behaviour changes exclusively by progressive filling of the *4f* orbital (Byrne & Sholkovitz, 1996). It seems that this change is not able to determine a significant discrimination among the REE distribution in grapes coming from vineyards planted on sedimentary or metamorphic soils.

Conclusion

Our research, that has been conducted for three years, has allowed to investigate the effects of soil-plant and roots-shoot-aerial parts interactions through REE migration dynamic. The off-soil growth system has permitted us to establish that the plant absorbs REE on the basis of processes respecting the substrate composition. This could be interesting in order to use the geochemical distribution of REE as an investigating instrument about geographic origin of grapes.

REE behaviour during the plant growth on different types of soils has been analysed and it has finally confirmed the results obtained in off-soil growth experiments. The migration process of REE from rhizosphere to root apparatus seems to be dominated by REE adsorption or complexation onto biological membrane surfaces of roots, rather than by REE migration as a result of dissolved complexation processes. These data, resulting from the analysis conducted in the light of Tetrad Effect Theory, are the best evidence of the "added value" that REE geochemical behaviour analysis in biological systems can offer in comparison with a research simply directed to evaluate absolute concentrations of these elements in vegetable tissues. The further application of Tetrad Effect Theory to the study on the REE distribution into plants' aerial parts has instead allowed to establish that elements migration analysed through xylem is a process in which the REE complexation plays an important role. Besides the generalised presence of positive Eu anomalies into plants' aerial parts indicates a major Eu mobility with respect to the other elements of series and it suggests its possible presence as in +2 valance state onto metabolic fluids.

The fructification period of greenhouse and field grown plants made it possible to examine the occurring relationship between REE composition in grapes and in respective growth soils. This last aspect has completed the research confirming that grapes chemism reflects in general soils composition so as to distinguish basaltic soils from those soils originated at sedimentary lithotypes' expense. This composing memory of origin soils shows up through HREE/LREE fractionations and in terms of Ce anomalies. On the contrary, Eu anomalies have no meaning as an identification character of origin soils being the result of metabolic processes of plants.

On the other hand, the comparison among collected data concerning grapes and respective soils highlights limits of only use the REE geochemical behaviour as an

instrument to investigate the origin of grapes coming from sedimentary or crystalline soils. The simple change of REE characteristics referring to complexation, that is the process characterising REE migration from soil to plant and inside the plant itself, is not sufficient to distinguish grapes grown in soils originated by sedimentary or metamorphic lithotypes because crystallization of authigenic phases in diagenetic environment and the same metamorphic blastese do not induce significative REE fractionations. While, lanthanide contraction governing REE behaviour during CHARAC processes is able to discriminate LREE and HREE according to the "*geochemical compatibility*" logic. Therefore, REE behaviour can allow to differentiate grapes grown on soils formed at the expense of volcanic lithotypes from those grown somewhere else.

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Supplementary material

Table 34(S.M.) REE contents and Y/Ho values measured in *1103P* rootstock growth on-soil during the 3 growth stage

Plant organs	Soil and Stage	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu	Σ REE	Y/Ho
		Average µmol/total plant (d.w.)																
Leaves	ET-1; St 1	0.0278	0.0289	0.0040	0.0143	0.0023	0.0009	0.0026	0.0003	0.0013	0.0148	0.0002	0.0007	0.0001	0.0005	0.0001	0.0988	60.61
Herbaceous shoots		0.0079	0.0080	0.0010	0.0035	0.0006	0.0006	0.0007	0.0001	0.0003	0.0038	0.0001	0.0002	0.00002	0.0001	0.00002	0.0270	62.58
Woody roots		0.0516	0.0649	0.0097	0.0365	0.0063	0.0015	0.0060	0.0007	0.0037	0.0379	0.0007	0.0019	0.0002	0.0014	0.0002	0.2233	55.31
Middle roots		0.0784	0.1033	0.0148	0.0548	0.0090	0.0023	0.0083	0.0010	0.0048	0.0483	0.0009	0.0025	0.0003	0.0019	0.0003	0.3308	54.07
Fine roots		0.9746	1.5073	0.1762	0.6536	0.1079	0.0282	0.1008	0.0121	0.0577	0.5652	0.0106	0.0291	0.0037	0.0223	0.0033	4.2526	53.26
Leaves	ET-1; St 2	0.0707	0.0777	0.0104	0.0356	0.0056	0.0020	0.0065	0.0007	0.0033	0.0387	0.0006	0.0017	0.0002	0.0012	0.0002	0.2550	65.06
Herbaceous shoots		0.0148	0.0124	0.0016	0.0055	0.0009	0.0008	0.0010	0.0001	0.0005	0.0065	0.0001	0.0003	0.00003	0.0002	0.00002	0.0448	75.06
Woody roots		0.1459	0.2305	0.0284	0.1043	0.0174	0.0043	0.0170	0.0018	0.0095	0.0935	0.0016	0.0047	0.0004	0.0036	0.0003	0.6632	58.91
Middle roots		0.1387	0.2084	0.0266	0.0972	0.0158	0.0041	0.0154	0.0017	0.0083	0.0808	0.0014	0.0042	0.0004	0.0031	0.0003	0.6063	57.60
Fine roots		5.4749	8.3029	0.9825	3.5725	0.5768	0.1535	0.5563	0.0635	0.2908	2.7461	0.0523	0.1482	0.0170	0.1102	0.0152	23.0627	52.47
Leaves	ET-1; St 3	0.0766	0.0690	0.0108	0.0377	0.0060	0.0035	0.0090	0.0008	0.0033	0.0414	0.0006	0.0019	0.0002	0.0014	0.0002	0.2624	65.46
Herbaceous shoots		0.0250	0.0206	0.0032	0.0107	0.0018	0.0024	0.0027	0.0002	0.0009	0.0119	0.0002	0.0005	0.0001	0.0004	0.0001	0.0806	63.28
Woody roots		0.2117	0.3318	0.0412	0.1505	0.0250	0.0068	0.0253	0.0029	0.0136	0.1351	0.0025	0.0069	0.0009	0.0053	0.0008	0.9602	53.59
Middle roots		0.1992	0.2756	0.0374	0.1372	0.0218	0.0061	0.0220	0.0025	0.0114	0.1147	0.0021	0.0059	0.0007	0.0046	0.0007	0.8419	53.41
Fine roots		5.8549	9.3180	1.1275	4.1098	0.6556	0.1765	0.6441	0.0718	0.3213	3.0342	0.0586	0.1624	0.0198	0.1244	0.0180	25.6969	51.79
Leaves	PA-2; St 1	0.0267	0.0488	0.0053	0.0198	0.0037	0.0015	0.0041	0.0005	0.0024	0.0256	0.0005	0.0013	0.0002	0.0010	0.0001	0.1412	56.02
Herbaceous shoots		0.0136	0.0237	0.0024	0.0088	0.0015	0.0010	0.0017	0.0002	0.0009	0.0098	0.0002	0.0005	0.0001	0.0004	0.0001	0.0647	57.08
Woody roots		0.0455	0.0899	0.0105	0.0405	0.0078	0.0017	0.0080	0.0010	0.0054	0.0567	0.0010	0.0027	0.0003	0.0021	0.0003	0.2734	57.87
Middle roots		0.1074	0.2142	0.0251	0.0980	0.0194	0.0042	0.0193	0.0025	0.0131	0.1383	0.0024	0.0068	0.0008	0.0051	0.0007	0.6575	56.85
Fine roots		0.4696	1.0428	0.1099	0.4295	0.0848	0.0184	0.0858	0.0111	0.0578	0.6074	0.0107	0.0295	0.0036	0.0226	0.0032	2.9866	56.66
Leaves	PA-2; St 2	0.0903	0.1682	0.0199	0.0753	0.0144	0.0044	0.0155	0.0019	0.0095	0.1030	0.0018	0.0049	0.0006	0.0038	0.0005	0.5141	57.84
Herbaceous shoots		0.0173	0.0317	0.0036	0.0133	0.0025	0.0025	0.0026	0.0003	0.0015	0.0155	0.0003	0.0007	0.0001	0.0006	0.0001	0.0926	59.09
Woody roots		0.0957	0.1857	0.0217	0.0837	0.0162	0.0037	0.0167	0.0020	0.0111	0.1182	0.0019	0.0056	0.0005	0.0043	0.0004	0.5673	62.64
Middle roots		0.0962	0.1858	0.0220	0.0846	0.0165	0.0036	0.0170	0.0021	0.0112	0.1196	0.0020	0.0058	0.0006	0.0043	0.0005	0.5720	59.51
Fine roots		1.5163	3.0528	0.3423	1.3217	0.2615	0.0583	0.2695	0.0349	0.1784	1.9146	0.0333	0.0927	0.0110	0.0703	0.0097	9.1674	57.47

Plant organs	Soil and Stage	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu	Σ REE	Y/Ho
Leaves	PA-2; St 3	0.0432	0.0795	0.0096	0.0361	0.0070	0.0041	0.0079	0.0009	0.0044	0.0552	0.0008	0.0023	0.0003	0.0017	0.0002	0.2533	67.81
Herbaceous shoots		0.0111	0.0200	0.0023	0.0089	0.0018	0.0036	0.0019	0.0002	0.0010	0.0128	0.0002	0.0006	0.0001	0.0004	0.00005	0.0650	69.16
Woody roots		0.3256	0.6222	0.0749	0.2905	0.0560	0.0131	0.0602	0.0077	0.0395	0.4198	0.0074	0.0202	0.0025	0.0155	0.0023	1.9576	56.42
Middle roots		0.3868	0.7447	0.0879	0.3420	0.0662	0.0155	0.0711	0.0091	0.0467	0.5041	0.0090	0.0242	0.0031	0.0192	0.0027	2.3324	56.15
Fine roots		2.0582	3.9756	0.4738	1.8363	0.3598	0.0821	0.3829	0.0495	0.2505	2.6986	0.0472	0.1304	0.0165	0.1026	0.0146	12.4788	57.14
Leaves	PA-1; St 1	0.0148	0.0265	0.0030	0.0114	0.0021	0.0007	0.0024	0.0003	0.0014	0.0157	0.0003	0.0008	0.0001	0.0006	0.0001	0.0801	58.39
Herbaceous shoots		0.0037	0.0063	0.0007	0.0027	0.0005	0.0003	0.0005	0.0001	0.0003	0.0037	0.0001	0.0002	0.00002	0.0001	0.00002	0.0193	60.23
Woody roots		0.0645	0.1037	0.0139	0.0546	0.0104	0.0023	0.0108	0.0014	0.0074	0.0854	0.0014	0.0039	0.0005	0.0029	0.0004	0.3635	60.18
Middle roots		0.0319	0.0490	0.0068	0.0266	0.0051	0.0011	0.0052	0.0007	0.0036	0.0438	0.0007	0.0019	0.0002	0.0014	0.0002	0.1782	64.22
Fine roots		0.3273	0.4772	0.0671	0.2647	0.0496	0.0113	0.0521	0.0067	0.0361	0.4381	0.0070	0.0194	0.0024	0.0146	0.0021	1.7755	62.91
Leaves	PA-1; St 2	0.0390	0.0706	0.0083	0.0313	0.0059	0.0018	0.0065	0.0008	0.0038	0.0426	0.0007	0.0020	0.0002	0.0015	0.0002	0.2153	59.73
Herbaceous shoots		0.0066	0.0114	0.0013	0.0049	0.0009	0.0007	0.0010	0.0001	0.0006	0.0068	0.0001	0.0003	0.00002	0.0002	0.00003	0.0348	65.41
Woody roots		0.0882	0.1348	0.0183	0.0710	0.0132	0.0030	0.0140	0.0017	0.0093	0.1097	0.0017	0.0049	0.0005	0.0036	0.0004	0.4743	64.25
Middle roots		0.0740	0.1203	0.0154	0.0596	0.0112	0.0026	0.0119	0.0015	0.0079	0.0943	0.0015	0.0043	0.0005	0.0032	0.0004	0.4086	62.35
Fine roots		1.6312	2.4042	0.3195	1.2524	0.2375	0.0560	0.2566	0.0329	0.1728	2.2115	0.0337	0.0946	0.0112	0.0694	0.0098	8.7934	65.53
Leaves	PA-1; St 3	0.0459	0.0768	0.0096	0.0358	0.0067	0.0030	0.0091	0.0009	0.0044	0.0519	0.0008	0.0024	0.0003	0.0018	0.0003	0.2497	61.72
Herbaceous shoots		0.0110	0.0174	0.0022	0.0077	0.0014	0.0014	0.0020	0.0002	0.0010	0.0127	0.0002	0.0006	0.0001	0.0004	0.0001	0.0583	66.23
Woody roots		0.2764	0.4306	0.0584	0.2276	0.0433	0.0101	0.0462	0.0058	0.0302	0.3622	0.0059	0.0163	0.0020	0.0126	0.0018	1.5296	61.28
Middle roots		0.1846	0.2612	0.0384	0.1527	0.0287	0.0071	0.0318	0.0040	0.0214	0.2732	0.0043	0.0117	0.0015	0.0089	0.0013	1.0308	64.07
Fine roots		2.6728	3.8554	0.5585	2.2014	0.4153	0.1012	0.4614	0.0588	0.3080	3.9591	0.0611	0.1693	0.0213	0.1285	0.0181	14.9903	64.76

Table 35(S.M.) REE contents and Y/Ho values measured in *779P* rootstock growth on-soil during the 3 growth stage

Plant organs	Soil and Stage	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu	Σ REE	Y/Ho
		Average μmol/total plant (d.w.)																
Leaves	ET-1; St 1	0.0251	0.0249	0.0035	0.0122	0.0019	0.0010	0.0022	0.0002	0.0011	0.0133	0.0002	0.0006	0.0001	0.0004	0.0001	0.0867	65.01
Herbaceous shoots		0.0056	0.0046	0.0006	0.0021	0.0003	0.0004	0.0004	0.00004	0.0002	0.0024	0.00003	0.0001	0.00001	0.0001	0.00001	0.0171	71.47
Woody roots		0.0412	0.0600	0.0081	0.0301	0.0054	0.0013	0.0052	0.0007	0.0033	0.0334	0.0006	0.0017	0.0002	0.0013	0.0002	0.1925	55.52
Middle roots		0.0259	0.0312	0.0047	0.0176	0.0029	0.0007	0.0028	0.0003	0.0017	0.0174	0.0003	0.0008	0.0001	0.0006	0.0001	0.1072	57.46
Fine roots		1.9574	3.3090	0.3558	1.3187	0.2183	0.0565	0.2054	0.0241	0.1158	1.0889	0.0209	0.0580	0.0071	0.0442	0.0063	8.7865	52.06
Leaves	ET-1; St 2	0.0474	0.0476	0.0071	0.0249	0.0042	0.0018	0.0046	0.0005	0.0024	0.0324	0.0004	0.0012	0.0001	0.0009	0.0001	0.1757	74.68
Herbaceous shoots		0.0122	0.0098	0.0015	0.0052	0.0009	0.0006	0.0010	0.0001	0.0005	0.0061	0.0001	0.0002	0.00003	0.0002	0.00002	0.0383	72.41
Woody roots		0.0724	0.0962	0.0132	0.0489	0.0080	0.0019	0.0079	0.0008	0.0044	0.0471	0.0007	0.0022	0.0002	0.0017	0.0001	0.3058	65.91
Middle roots		0.0712	0.0803	0.0125	0.0459	0.0073	0.0019	0.0070	0.0007	0.0037	0.0391	0.0006	0.0019	0.0001	0.0014	0.0001	0.2737	64.15
Fine roots		2.3606	3.2459	0.4147	1.5027	0.2400	0.0641	0.2254	0.0254	0.1167	1.1281	0.0208	0.0591	0.0066	0.0441	0.0061	9.4601	54.29
Leaves	ET-1; St 3	0.0740	0.0620	0.0100	0.0342	0.0054	0.0039	0.0082	0.0007	0.0030	0.0369	0.0006	0.0017	0.0002	0.0012	0.0002	0.2422	66.03
Herbaceous shoots		0.0249	0.0225	0.0031	0.0100	0.0016	0.0021	0.0026	0.0002	0.0009	0.0113	0.0002	0.0005	0.0001	0.0004	0.0001	0.0804	66.02
Woody roots		0.1204	0.1611	0.0228	0.0832	0.0137	0.0037	0.0136	0.0017	0.0076	0.0758	0.0015	0.0039	0.0006	0.0031	0.0005	0.5132	51.10
Middle roots		0.1703	0.2066	0.0317	0.1160	0.0188	0.0049	0.0183	0.0022	0.0098	0.0992	0.0018	0.0050	0.0007	0.0039	0.0006	0.6898	54.53
Fine roots		5.2515	7.9487	1.0139	3.6729	0.5882	0.1572	0.5693	0.0650	0.2886	2.7286	0.0527	0.1485	0.0182	0.1111	0.0165	22.6310	51.78
Leaves	PA-2; St 1	0.0259	0.0483	0.0049	0.0182	0.0033	0.0014	0.0036	0.0004	0.0019	0.0206	0.0004	0.0010	0.0001	0.0007	0.0001	0.1308	58.38
Herbaceous shoots		0.0045	0.0080	0.0009	0.0034	0.0007	0.0007	0.0009	0.0001	0.0006	0.0060	0.0001	0.0003	0.0000	0.0002	0.00003	0.0265	61.90
Woody roots		0.0285	0.0565	0.0066	0.0256	0.0050	0.0011	0.0050	0.0006	0.0034	0.0359	0.0006	0.0017	0.0002	0.0014	0.0002	0.1725	57.08
Middle roots		0.0224	0.0450	0.0052	0.0203	0.0040	0.0009	0.0040	0.0005	0.0027	0.0290	0.0005	0.0014	0.0002	0.0011	0.0002	0.1373	57.70
Fine roots		0.4410	0.8680	0.0976	0.3810	0.0749	0.0162	0.0748	0.0098	0.0506	0.5343	0.0094	0.0260	0.0032	0.0195	0.0028	2.6091	57.01
Leaves	PA-2; St 2	0.0658	0.1244	0.0145	0.0546	0.0106	0.0042	0.0117	0.0014	0.0067	0.0760	0.0012	0.0034	0.0004	0.0026	0.0004	0.3778	61.88
Herbaceous shoots		0.0148	0.0248	0.0025	0.0092	0.0017	0.0016	0.0019	0.0002	0.0010	0.0114	0.0002	0.0005	0.0001	0.0004	0.00005	0.0704	63.90
Woody roots		0.0657	0.1289	0.0148	0.0569	0.0110	0.0025	0.0114	0.0013	0.0074	0.0802	0.0013	0.0039	0.0003	0.0029	0.0003	0.3888	63.04
Middle roots		0.0994	0.1982	0.0228	0.0882	0.0174	0.0039	0.0181	0.0022	0.0119	0.1301	0.0021	0.0062	0.0006	0.0047	0.0005	0.6063	61.05
Fine roots		0.4611	0.9738	0.1069	0.4152	0.0829	0.0186	0.0867	0.0111	0.0575	0.6204	0.0106	0.0300	0.0035	0.0227	0.0030	2.9040	58.45

Plant organs	Soil and Stage	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu	Σ REE	Y/Ho
Leaves	PA-2; St 3	0.0397	0.0744	0.0092	0.0340	0.0066	0.0057	0.0096	0.0010	0.0043	0.0473	0.0009	0.0024	0.0004	0.0019	0.0004	0.2375	54.88
Herbaceous shoots		0.0093	0.0170	0.0020	0.0076	0.0016	0.0038	0.0021	0.0002	0.0010	0.0114	0.0002	0.0006	0.0001	0.0005	0.0001	0.0576	53.17
Woody roots		0.2357	0.4470	0.0533	0.2040	0.0402	0.0100	0.0425	0.0059	0.0275	0.2932	0.0056	0.0144	0.0023	0.0112	0.0021	1.3948	52.41
Middle roots		0.1294	0.2527	0.0298	0.1142	0.0225	0.0056	0.0243	0.0034	0.0158	0.1694	0.0033	0.0085	0.0014	0.0066	0.0013	0.7883	50.74
Fine roots		1.9735	3.8608	0.4584	1.7760	0.3556	0.0817	0.3777	0.0491	0.2446	2.6927	0.0471	0.1281	0.0170	0.0992	0.0150	12.1766	57.17
Leaves	PA-1; St 1	0.0156	0.0282	0.0033	0.0124	0.0023	0.0008	0.0026	0.0003	0.0015	0.0164	0.0003	0.0008	0.0001	0.0006	0.0001	0.0852	59.61
Herbaceous shoots		0.0033	0.0057	0.0006	0.0024	0.0005	0.0004	0.0005	0.0001	0.0003	0.0036	0.0001	0.0002	0.0000	0.0001	0.00002	0.0177	64.31
Woody roots		0.1435	0.2297	0.0309	0.1216	0.0233	0.0052	0.0244	0.0031	0.0167	0.1900	0.0032	0.0087	0.0010	0.0065	0.0009	0.8086	60.32
Middle roots		0.0916	0.1402	0.0196	0.0778	0.0149	0.0034	0.0156	0.0020	0.0108	0.1268	0.0021	0.0057	0.0007	0.0042	0.0006	0.5160	60.53
Fine roots		0.3856	0.6205	0.0819	0.3259	0.0618	0.0143	0.0647	0.0084	0.0446	0.5415	0.0086	0.0239	0.0029	0.0178	0.0025	2.2051	62.65
Leaves	PA-1; St 2	0.0296	0.0523	0.0064	0.0240	0.0046	0.0017	0.0051	0.0006	0.0029	0.0361	0.0005	0.0016	0.0002	0.0012	0.0002	0.1670	66.16
Herbaceous shoots		0.0100	0.0160	0.0021	0.0078	0.0015	0.0008	0.0017	0.0002	0.0010	0.0127	0.0002	0.0005	0.0001	0.0004	0.0001	0.0550	70.70
Woody roots		0.1785	0.2801	0.0375	0.1453	0.0273	0.0063	0.0293	0.0036	0.0193	0.2265	0.0036	0.0103	0.0011	0.0077	0.0010	0.9773	62.61
Middle roots		0.1108	0.1817	0.0232	0.0896	0.0169	0.0039	0.0180	0.0022	0.0119	0.1409	0.0023	0.0064	0.0007	0.0047	0.0006	0.6138	62.58
Fine roots		1.3463	1.9483	0.2615	1.0236	0.1931	0.0451	0.2080	0.0262	0.1378	1.7472	0.0268	0.0749	0.0088	0.0549	0.0076	7.1101	65.31
Leaves	PA-1; St 3	0.0399	0.0671	0.0084	0.0311	0.0058	0.0038	0.0086	0.0008	0.0036	0.0425	0.0007	0.0020	0.0002	0.0015	0.0002	0.2164	61.43
Herbaceous shoots		0.0094	0.0150	0.0018	0.0064	0.0012	0.0019	0.0018	0.0002	0.0007	0.0095	0.0002	0.0004	0.0001	0.0003	0.0001	0.0490	61.00
Woody roots		0.2137	0.3229	0.0452	0.1747	0.0332	0.0082	0.0361	0.0047	0.0234	0.2856	0.0047	0.0128	0.0018	0.0098	0.0015	1.1785	60.16
Middle roots		0.2005	0.2776	0.0419	0.1643	0.0313	0.0078	0.0346	0.0046	0.0229	0.2951	0.0048	0.0128	0.0019	0.0096	0.0017	1.1114	60.97
Fine roots		1.6009	2.3771	0.3354	1.3124	0.2470	0.0601	0.2740	0.0348	0.1802	2.2518	0.0356	0.0985	0.0124	0.0735	0.0106	8.9044	63.26

Table 36(S.M.) REE contents and Y/Ho values measured in *140Ru* rootstock growth on-soil during the 3 growth stage

Plant organs	Soil and Stage	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu	Σ REE	Y/Ho
		Average μmol/total plant (d.w.)																
Leaves	ET-1; St 1	0.0221	0.0199	0.0029	0.0099	0.0015	0.0008	0.0018	0.0002	0.0009	0.0111	0.0002	0.0005	0.0001	0.0003	0.00004	0.0720	70.28
Herbaceous shoots		0.0043	0.0034	0.0004	0.0015	0.0002	0.0004	0.0003	0.0000	0.0001	0.0016	0.0000	0.0001	0.00001	0.0001	0.00001	0.0124	68.40
Woody roots		0.0428	0.0596	0.0082	0.0306	0.0051	0.0013	0.0050	0.0006	0.0030	0.0306	0.0006	0.0015	0.0002	0.0012	0.0002	0.1903	54.88
Middle roots		0.0735	0.1171	0.0149	0.0560	0.0098	0.0023	0.0095	0.0012	0.0059	0.0581	0.0011	0.0030	0.0004	0.0022	0.0003	0.3551	53.75
Fine roots		1.4021	2.4489	0.2620	0.9638	0.1583	0.0410	0.1460	0.0171	0.0804	0.7435	0.0144	0.0397	0.0048	0.0306	0.0043	6.3569	51.49
Leaves	ET-1; St 2	0.0498	0.0539	0.0077	0.0272	0.0046	0.0019	0.0053	0.0006	0.0027	0.0340	0.0005	0.0014	0.0002	0.0010	0.0001	0.1911	68.48
Herbaceous shoots		0.0132	0.0145	0.0019	0.0069	0.0012	0.0008	0.0014	0.0001	0.0007	0.0088	0.0001	0.0004	0.00004	0.0003	0.00004	0.0504	68.51
Woody roots		0.1002	0.1604	0.0200	0.0739	0.0126	0.0031	0.0121	0.0013	0.0070	0.0739	0.0012	0.0036	0.0003	0.0026	0.0003	0.4725	61.79
Middle roots		0.0721	0.1069	0.0137	0.0498	0.0082	0.0021	0.0078	0.0009	0.0043	0.0448	0.0008	0.0022	0.0002	0.0016	0.0002	0.3155	59.75
Fine roots		1.8543	2.7077	0.3275	1.1803	0.1877	0.0501	0.1742	0.0198	0.0913	0.8886	0.0163	0.0459	0.0052	0.0340	0.0047	7.5875	54.67
Leaves	ET-1; St 3	0.0862	0.0738	0.0115	0.0399	0.0062	0.0047	0.0103	0.0009	0.0033	0.0418	0.0006	0.0019	0.0002	0.0014	0.0002	0.2829	66.77
Herbaceous shoots		0.0199	0.0163	0.0024	0.0083	0.0014	0.0023	0.0022	0.0002	0.0007	0.0094	0.0001	0.0004	0.00005	0.0003	0.00005	0.0640	67.18
Woody roots		0.2657	0.4268	0.0523	0.1921	0.0318	0.0083	0.0304	0.0035	0.0171	0.1676	0.0031	0.0087	0.0009	0.0066	0.0008	1.2157	54.79
Middle roots		0.1855	0.2285	0.0337	0.1232	0.0191	0.0051	0.0181	0.0019	0.0094	0.0967	0.0016	0.0047	0.0005	0.0036	0.0004	0.7319	59.79
Fine roots		5.4322	8.2848	0.9896	3.6089	0.5721	0.1521	0.5280	0.0601	0.2804	2.6786	0.0507	0.1399	0.0170	0.1074	0.0150	22.9168	52.88
Leaves	PA-2; St 1	0.0194	0.0355	0.0037	0.0135	0.0024	0.0011	0.0029	0.0003	0.0016	0.0182	0.0003	0.0009	0.0001	0.0006	0.0001	0.1006	60.54
Herbaceous shoots		0.0038	0.0072	0.0008	0.0029	0.0005	0.0006	0.0006	0.0001	0.0003	0.0038	0.0001	0.0002	0.00002	0.0001	0.00002	0.0209	63.54
Woody roots		0.0408	0.0805	0.0095	0.0369	0.0072	0.0016	0.0072	0.0009	0.0049	0.0504	0.0009	0.0025	0.0003	0.0019	0.0003	0.2458	54.72
Middle roots		0.0632	0.1274	0.0146	0.0565	0.0110	0.0024	0.0109	0.0014	0.0074	0.0772	0.0014	0.0038	0.0005	0.0029	0.0004	0.3811	55.99
Fine roots		0.7977	1.6537	0.1787	0.6983	0.1361	0.0292	0.1367	0.0178	0.0911	0.9575	0.0169	0.0466	0.0058	0.0352	0.0051	4.8064	56.63
Leaves	PA-2; St 2	0.0849	0.1491	0.0167	0.0613	0.0114	0.0043	0.0130	0.0015	0.0072	0.0859	0.0013	0.0038	0.0005	0.0029	0.0004	0.4441	64.07
Herbaceous shoots		0.0169	0.0289	0.0030	0.0110	0.0019	0.0023	0.0023	0.0003	0.0012	0.0144	0.0002	0.0006	0.0001	0.0005	0.0001	0.0837	64.40
Woody roots		0.0479	0.0919	0.0107	0.0412	0.0079	0.0018	0.0080	0.0009	0.0052	0.0587	0.0009	0.0026	0.0002	0.0020	0.0002	0.2801	67.56
Middle roots		0.1137	0.2234	0.0257	0.0991	0.0195	0.0042	0.0198	0.0023	0.0131	0.1497	0.0022	0.0068	0.0005	0.0051	0.0004	0.6857	68.28
Fine roots		0.7983	1.6776	0.1839	0.7060	0.1403	0.0309	0.1410	0.0183	0.0943	1.0480	0.0175	0.0487	0.0057	0.0366	0.0050	4.9521	59.85

Plant organs	Soil and Stage	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu	Σ REE	Y/Ho
Leaves	PA-2; St 3	0.0314	0.0600	0.0070	0.0267	0.0052	0.0043	0.0077	0.0008	0.0034	0.0394	0.0006	0.0019	0.0002	0.0014	0.0002	0.1903	60.71
Herbaceous shoots		0.0094	0.0172	0.0020	0.0075	0.0015	0.0044	0.0022	0.0002	0.0009	0.0110	0.0002	0.0005	0.0001	0.0004	0.0001	0.0576	61.09
Woody roots		0.2223	0.4069	0.0505	0.1963	0.0383	0.0091	0.0409	0.0052	0.0262	0.2910	0.0050	0.0137	0.0018	0.0105	0.0015	1.3192	58.48
Middle roots		0.1855	0.2984	0.0406	0.1603	0.0310	0.0079	0.0342	0.0043	0.0225	0.2766	0.0044	0.0122	0.0015	0.0093	0.0013	1.0897	62.99
Fine roots		1.7409	3.0928	0.3958	1.5501	0.3058	0.0713	0.3282	0.0420	0.2166	2.4978	0.0410	0.1137	0.0139	0.0868	0.0120	10.5087	60.93
Leaves	PA-1; St 1	0.0109	0.0191	0.0022	0.0082	0.0015	0.0006	0.0017	0.0002	0.0010	0.0116	0.0002	0.0005	0.0001	0.0004	0.0001	0.0582	64.43
Herbaceous shoots		0.0020	0.0032	0.0004	0.0014	0.0003	0.0003	0.0003	0.00003	0.0002	0.0023	0.00003	0.0001	0.00001	0.0001	0.00001	0.0106	66.51
Woody roots		0.0642	0.1042	0.0137	0.0535	0.0100	0.0023	0.0103	0.0013	0.0071	0.0791	0.0013	0.0036	0.0004	0.0027	0.0004	0.3542	60.25
Middle roots		0.0661	0.1006	0.0141	0.0557	0.0106	0.0024	0.0110	0.0014	0.0075	0.0899	0.0014	0.0040	0.0005	0.0030	0.0004	0.3687	62.20
Fine roots		0.2310	0.3899	0.0489	0.1926	0.0363	0.0084	0.0377	0.0049	0.0258	0.3020	0.0050	0.0137	0.0017	0.0102	0.0015	1.3096	60.57
Leaves	PA-1; St 2	0.0349	0.0608	0.0072	0.0264	0.0050	0.0018	0.0057	0.0007	0.0032	0.0388	0.0006	0.0017	0.0002	0.0013	0.0002	0.1883	65.51
Herbaceous shoots		0.0055	0.0089	0.0010	0.0037	0.0007	0.0008	0.0008	0.0001	0.0004	0.0057	0.0001	0.0002	0.00003	0.0002	0.00002	0.0282	70.70
Woody roots		0.1113	0.1765	0.0232	0.0889	0.0167	0.0038	0.0171	0.0021	0.0114	0.1356	0.0021	0.0060	0.0007	0.0044	0.0006	0.6005	64.14
Middle roots		0.0675	0.1001	0.0140	0.0544	0.0103	0.0024	0.0108	0.0013	0.0073	0.0912	0.0014	0.0039	0.0004	0.0029	0.0004	0.3682	66.54
Fine roots		0.9662	1.4667	0.1921	0.7515	0.1422	0.0334	0.1511	0.0192	0.1021	1.2942	0.0198	0.0552	0.0065	0.0404	0.0056	5.2463	65.22
Leaves	PA-1; St 3	0.0439	0.0719	0.0091	0.0340	0.0064	0.0052	0.0098	0.0009	0.0040	0.0513	0.0008	0.0023	0.0003	0.0017	0.0002	0.2418	65.22
Herbaceous shoots		0.0086	0.0136	0.0017	0.0057	0.0010	0.0026	0.0017	0.0002	0.0006	0.0087	0.0001	0.0004	0.00004	0.0003	0.00004	0.0453	66.43
Woody roots		0.3177	0.5130	0.0684	0.2668	0.0501	0.0119	0.0538	0.0067	0.0350	0.4038	0.0066	0.0185	0.0022	0.0141	0.0019	1.7705	60.93
Middle roots		0.2530	0.4327	0.0558	0.2165	0.0411	0.0098	0.0445	0.0055	0.0289	0.3361	0.0055	0.0154	0.0018	0.0118	0.0016	1.4599	61.15
Fine roots		1.6040	2.6304	0.3507	1.3800	0.2676	0.0633	0.2873	0.0365	0.1895	2.2538	0.0367	0.1011	0.0122	0.0774	0.0105	9.3006	61.40

Table 37(S.M.) REE contents (mg Kg^{-1}) and Y/Ho values measured in *pseudo-total* soil fractions from studied areas during *field research*

Type of soil	Soil	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu	Σ REE	Y/Ho
	Average values																	
Marly soils	PA-3a	20.49	42.60	4.83	18.56	3.50	0.71	3.38	0.38	1.87	7.16	0.30	0.78	0.08	0.51	0.05	105.20	44.51
	PA-3b	11.44	24.61	2.80	11.06	2.15	0.42	2.13	0.25	1.25	5.02	0.20	0.54	0.05	0.37	0.04	62.32	47.57
	PA-3c	19.64	41.26	4.69	18.22	3.56	0.77	3.50	0.40	1.99	7.90	0.32	0.85	0.08	0.57	0.06	103.81	45.79
	PA-3d	31.55	49.00	6.80	27.00	5.11	1.11	5.65	0.71	3.91	23.75	0.77	2.19	0.26	1.69	0.24	159.73	57.33
	PA-3e	26.84	41.55	5.66	22.43	4.16	0.90	4.60	0.57	3.25	20.08	0.63	1.82	0.22	1.49	0.21	134.39	59.05
	PA-3f	33.64	63.95	7.54	29.29	5.52	1.22	5.71	0.68	3.54	17.09	0.63	1.77	0.20	1.33	0.17	172.29	50.37
Volcanic soils	ET-2a	92.33	171.42	18.82	67.50	11.56	2.54	10.93	1.25	5.84	27.69	1.04	2.87	0.35	2.25	0.33	416.73	49.34
	ET-2b	86.94	161.73	17.59	63.03	10.85	2.57	10.25	1.18	5.56	26.51	0.99	2.74	0.34	2.17	0.32	392.79	49.56
	ET-2c	89.75	167.13	17.96	64.23	11.01	2.54	10.38	1.20	5.63	26.89	1.00	2.74	0.34	2.18	0.31	403.28	49.73
Evaporitic soils	AG-1a	7.45	14.00	1.66	6.25	1.23	0.27	1.44	0.17	0.82	5.03	0.15	0.44	0.05	0.33	0.04	39.33	61.26
	AG-1b	16.02	33.14	3.61	13.21	2.51	0.62	2.87	0.33	1.50	8.04	0.28	0.78	0.09	0.58	0.08	83.66	53.88
	AG-1c	16.42	41.03	4.37	17.16	3.57	0.92	4.15	0.50	2.37	12.62	0.43	1.19	0.14	0.88	0.12	105.86	54.38
Calcareous soils	AG-2a	4.67	10.74	1.24	4.85	0.99	0.22	1.01	0.12	0.56	2.80	0.10	0.25	0.03	0.18	0.03	27.80	52.77
	AG-2b	6.35	15.47	1.89	7.58	1.65	0.37	1.73	0.21	1.05	5.69	0.19	0.50	0.06	0.38	0.05	43.16	56.46
	AG-2c	6.45	16.21	1.93	7.76	1.63	0.37	1.67	0.20	0.99	5.00	0.17	0.45	0.05	0.33	0.05	43.27	53.68
	AG-2d	4.56	11.44	1.46	5.95	1.29	0.29	1.28	0.16	0.76	3.67	0.13	0.33	0.04	0.24	0.03	31.64	52.61
Calcarenitic soils	TP-1a	12.38	22.80	2.94	11.41	2.42	0.53	2.70	0.35	1.82	12.64	0.35	0.98	0.12	0.76	0.11	72.29	67.22
	TP-1b	26.24	52.78	6.33	24.39	4.96	1.05	5.24	0.64	3.20	19.19	0.59	1.62	0.20	1.23	0.17	147.84	60.44
Metamorphic soils	ME-1a	16.40	32.19	3.68	13.43	2.59	0.47	2.53	0.29	1.32	6.70	0.22	0.58	0.07	0.41	0.06	80.93	55.91
	ME-1b	16.21	31.21	3.67	13.32	2.59	0.44	2.45	0.28	1.30	6.57	0.22	0.58	0.07	0.44	0.06	79.43	55.64

Table 38(S.M.) REE contents (mg Kg^{-1}) and Y/Ho values measured in *bioavailable* soil fractions from studied areas during *field research*

Type of soil	Soil	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu	Σ REE	Y/Ho
		Average values																
Marly soils	PA-3a	3.27	7.29	1.40	6.53	1.52	0.36	1.54	0.19	0.91	4.42	0.15	0.35	0.04	0.21	0.03	28.21	55.51
	PA-3b	1.91	4.17	0.81	3.91	0.97	0.23	1.00	0.13	0.61	3.05	0.10	0.25	0.03	0.16	0.02	17.35	56.09
	PA-3c	1.10	2.99	0.43	1.97	0.47	0.12	0.51	0.06	0.31	1.40	0.05	0.12	0.01	0.08	0.01	9.62	50.70
	PA-3d	10.85	18.07	2.37	10.42	2.22	0.53	2.90	0.39	2.23	16.53	0.45	1.29	0.16	0.97	0.14	69.54	67.43
	PA-3e	17.14	14.82	3.89	16.06	3.12	0.71	3.61	0.47	2.57	18.91	0.51	1.43	0.17	1.06	0.15	84.65	68.90
	PA-3f	3.45	6.48	0.86	3.84	0.86	0.21	1.03	0.14	0.74	4.91	0.14	0.39	0.05	0.31	0.04	23.45	63.74
Volcanic soils	ET-2a	17.22	4.15	2.88	9.84	1.55	0.34	1.54	0.18	0.74	4.25	0.13	0.39	0.04	0.28	0.04	43.59	58.75
	ET-2b	20.12	4.04	3.40	11.74	1.86	0.42	1.83	0.22	0.88	5.05	0.16	0.47	0.05	0.34	0.05	50.61	59.80
	ET-2c	23.44	5.06	3.93	13.60	2.16	0.49	2.13	0.25	1.02	5.84	0.18	0.53	0.06	0.38	0.05	59.11	59.81
Evaporitic soils	AG-1a	0.49	2.11	0.11	0.47	0.11	0.02	0.15	0.01	0.08	0.55	0.01	0.04	0.004	0.03	0.003	4.20	71.98
	AG-1b	1.54	6.38	0.40	1.63	0.36	0.08	0.47	0.05	0.27	1.51	0.05	0.14	0.02	0.11	0.01	13.02	56.36
	AG-1c	2.36	10.30	0.62	2.51	0.54	0.13	0.72	0.08	0.39	2.11	0.07	0.20	0.02	0.15	0.02	20.21	54.45
Calcareous soils	AG-2a	1.82	6.17	0.75	3.37	0.77	0.19	0.91	0.11	0.53	2.85	0.09	0.25	0.03	0.17	0.02	18.04	56.06
	AG-2b	0.58	1.55	0.17	0.72	0.16	0.04	0.19	0.02	0.12	0.69	0.02	0.06	0.01	0.05	0.005	4.38	63.19
	AG-2c	3.06	7.03	1.08	4.64	1.04	0.25	1.19	0.15	0.68	3.73	0.12	0.32	0.03	0.22	0.03	23.58	57.66
	AG-2d	1.25	4.70	0.50	2.29	0.55	0.13	0.65	0.08	0.38	2.02	0.07	0.18	0.02	0.13	0.02	12.98	55.23
Calcarenitic soils	TP-1a	2.01	4.54	0.57	2.41	0.56	0.13	0.71	0.09	0.46	3.37	0.09	0.25	0.03	0.19	0.02	15.41	70.85
	TP-1b	4.33	8.88	1.27	5.35	1.21	0.28	1.44	0.18	0.90	5.99	0.17	0.47	0.06	0.35	0.05	30.93	65.48
Metamorphic soils	ME-1a	2.73	5.82	0.76	3.08	0.70	0.16	0.84	0.11	0.53	3.16	0.10	0.27	0.03	0.20	0.03	18.50	60.59
	ME-1b	4.11	6.66	1.01	4.11	0.89	0.22	1.07	0.13	0.65	4.00	0.12	0.34	0.04	0.27	0.04	23.67	60.81

Table 39(S.M.) REE contents (ng Kg^{-1}, d.w.) and Y/Ho values measured in berries from studied areas during *field research*

Soil	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Y	Ho	Er	Tm	Yb	Lu	Σ REE	Y/Ho
	Average values																
PA-3a	5882.66	11893.24	922.82	3076.65	309.87	135.75	367.64	40.65	169.86	874.52	31.62	90.06	14.48	69.88	11.60	23891.29	51.30
PA-3b	3829.87	6272.06	846.93	3390.90	681.79	290.62	652.55	84.54	400.10	2032.57	72.61	182.67	24.99	145.55	20.53	18928.26	51.93
PA-3c	2374.18	3917.40	486.99	1845.88	369.98	272.16	344.31	46.43	211.54	1120.84	38.86	105.39	15.18	101.47	12.75	11263.37	53.50
PA-3d	7276.98	8168.23	1255.33	4929.85	910.97	301.35	986.97	126.51	628.63	4815.36	131.15	347.36	48.40	272.37	43.02	30242.48	68.11
PA-3e	12244.83	9109.97	1767.20	6883.84	1191.80	313.71	1249.36	169.27	781.35	6494.57	173.07	438.36	68.61	336.67	61.62	41284.25	69.61
PA-3f	1721.68	2641.90	216.70	770.64	124.10	79.97	129.07	17.02	76.38	537.96	16.70	50.09	8.39	47.73	7.48	6445.80	59.76
ET-2a	20682.85	11325.55	2313.24	5243.07	773.48	873.13	939.97	92.41	386.07	3144.23	66.44	219.43	24.27	177.51	23.16	46284.81	87.78
ET-2b	9772.12	8105.23	1392.55	3281.55	538.87	618.67	664.35	65.08	289.35	2106.16	52.00	160.50	16.63	133.59	16.28	27212.91	75.13
ET-2c	16189.82	11306.81	2280.20	6032.06	891.64	600.09	1169.72	111.08	476.19	4781.84	90.65	282.78	27.97	187.68	23.79	44452.33	97.85
AG-1a	849.52	1292.80	146.46	579.87	141.66	67.92	129.06	14.40	78.61	581.50	17.10	49.64	3.50	47.26	3.01	4002.31	63.07
AG-1b	978.23	1388.85	157.19	584.27	129.94	53.46	117.69	9.42	75.50	637.20	9.07	33.16	< LOQ	32.71	< LOQ	4205.22	130.30
AG-1c	948.92	1419.68	166.62	598.50	135.09	133.38	125.28	11.26	76.28	719.64	12.02	35.71	0.94	34.39	1.04	4418.75	111.02
AG-2a	5415.36	6529.71	872.45	3480.25	654.00	902.81	783.25	80.77	387.01	3963.93	71.52	196.14	19.95	129.66	16.63	23503.44	102.81
AG-2b	3029.89	4860.47	566.85	2124.65	477.37	479.03	472.88	55.44	275.13	1553.45	46.79	126.40	14.42	116.00	13.86	14212.62	61.59
AG-2c	1981.72	3062.24	417.97	1442.10	308.51	279.18	316.76	32.40	174.92	1195.97	29.02	87.29	6.30	74.33	4.93	9413.63	76.44
AG-2d	2325.10	3190.21	361.90	1315.71	311.79	411.75	310.01	34.50	184.76	1081.64	31.46	93.30	11.85	90.73	9.35	9764.07	63.77
TP-1a	3566.23	5312.99	592.62	2069.40	459.94	262.28	500.38	56.83	302.25	2055.48	56.28	164.55	18.88	156.59	16.67	15591.37	67.75
TP-1b	5198.78	5773.10	694.11	2993.37	537.61	264.58	648.48	73.93	370.49	2178.83	67.24	192.87	22.65	151.79	21.80	19189.63	60.10
ME-1a	1686.87	2822.00	502.27	1204.74	279.59	477.12	295.83	30.66	156.90	966.67	25.91	85.07	8.96	83.10	9.94	8635.63	69.21
ME-1b	10158.20	5862.06	1442.75	4131.17	719.89	1133.45	823.22	83.69	389.58	3302.64	66.86	208.46	21.31	165.57	23.90	28532.74	91.62

Reviewers

Università degli Studi di Palermo

DIPARTIMENTO DI SCIENZE DELLA TERRA E DEL MARE (DiSTeM)

We would be grateful if you could rewiew the enclosed paper, which is the PhD thesis in Geochemistry by **Nicola Tuzzolino, PhD Student.** Please complete the sheet below with your comments. If you desire, you can add separately, more detailed considerations and suggestions to improve the paper. Please take into account that we asked the candidate to produce a short English condensed version, which could satisfactory illustrate the main results obtained throughout the last three years. Please send back before the 10th of December 2013. Thank you for the cooperation.

The Coordinator of the PhD course in GEOCHEMISTRY
(Prof. F. Parello)

Reviewer: BERTOLDI DANIELA Date: 09/12/2013

1. Are the results presented new and original?	Yes
2. Is the paper clearly presented?	Yes
3. Are the structure and organization satisfactory?	Yes
4. Are the interpretations and conclusions sound and justified by data?	Yes
5. Are the illustrations, tables and references all adequate or all necessary?	Yes
6. Does the title of this paper clearly reflect its content?	Yes

7. GENERAL COMMENT ON THE WHOLE PAPER:

This study explores rare earth elements (REE) content and transfer in the soil – *Vitis vinifera* system. Few other studies, often reported only in conference proceedings or PhD thesis, have recently described some of these topics, as well as REE content in wine. However, this thesis reports on a detailed investigation of new aspects. The most interesting novelty is the separation of the study into three parts (off-soil system, on-soil system and in field system) that allowed a better understanding of the mechanism involved in REE transfer and fractionation, through a biogeochemical approach. The experimental plan and the whole study are accurately designed. The candidate presented an extensive analysis, where he evaluated a considerable multiplicity of aspects (geochemistry, analytical chemistry, plant physiology, agronomy, viticulture….), which proves the acquisition of an in-depth knowledge of this subject.

Signature of the reviewer Daniela Bertoldi

Università degli Studi di Palermo

DIPARTIMENTO DI SCIENZE DELLA TERRA E DEL MARE (DiSTeM)

We would be grateful if you could rewiew the enclosed paper, which is the PhD thesis in Geochemistry by **Nicola Tuzzolino, PhD Student.** Please complete the sheet below with your comments. If you desire, you can add separately, more detailed considerations and suggestions to improve the paper. Please take into account that we asked the candidate to produce a short English condensed version, which could satisfactory illustrate the main results obtained throughout the last three years. Please send back before the 10th of December 2013. Thank you for the cooperation.

The Coordinator of the PhD course in GEOCHEMISTRY
(Prof. F. Parello)

Reviewer: Riccardo Petrini Date: 29.11.2013

1. Are the results presented new and original? Yes

2. Is the paper clearly presented? Yes

3. Are the structure and organization satisfactory? Yes

4. Are the interpretations and conclusions sound and justified by data? Yes

5. Are the illustrations, tables and references all adequate or all necessary? Yes

6. Does the title of this paper clearly reflect its content? Yes

7. GENERAL COMMENT ON THE WHOLE PAPER:

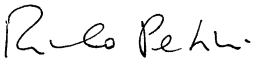
This work is dealing with the REE partitioning and migration in the soil-plant system and in the different part of the plant, with special reference on Vitis vinifera. This topic is of the uppermost importance, also for the implications on food-safety and traceability, which has become a priority among consumers.

The Thesis is well organized and well written; analytical data are of high quality and the correct emphasis has been given to the experimental procedures. The results are of interest for a wide audience, and the conclusions properly supported by the discussion.

I particularly appreciated this work.

As a marginal comment, I wonder is there might be some effect of the different pruning method on REE migration in plant; this part seems to me not addressed in the Thesis.

Signature of the reviewer



Università degli Studi di Palermo

DIPARTIMENTO DI SCIENZE DELLA TERRA E DEL MARE (DiSTeM)

We would be grateful if you could review the enclosed paper, which is the PhD thesis in Geochemistry by **Nicola Tuzzolino, PhD Student.** Please complete the sheet below with your comments. If you desire, you can add separately, more detailed considerations and suggestions to improve the paper. Please take into account that we asked the candidate to produce a short English condensed version, which could satisfactory illustrate the main results obtained throughout the last three years. Please send back before the 10th of December 2013. Thank you for the cooperation.

The Coordinator of the PhD course in GEOCHEMISTRY
(Prof. F. Parello)

Reviewer: Yoshio Takahashi Date: Dec. 27, 2014

1. Are the results presented new and original?	Yes	No
2. Is the paper clearly presented?	Yes	No
3. Are the structure and organization satisfactory?	Yes	No
4. Are the interpretations and conclusions sound and justified by data?	Yes	No
5. Are the illustrations, tables and references all adequate or all necessary?	Yes	No
6. Does the title of this paper clearly reflect its content?	Yes	No

7. GENERAL COMMENT ON THE WHOLE PAPER:

This thesis aims to extract information of transportation process of rare earth elements (REE) in the *Vitis Viniveral* - soil system based on the physic-chemical characteristics of REE. The results obtained in this study include various implications. First, this approach allows us to understand biogeochemical behavior of REE in plants based on the variation of REE pattern including Ce anomaly, Eu anomaly, and tetrad effect, since they can be affected by complexation reactions, redox reactions, deposition of minerals etc in plants. Second, it is suggested that the variation of REE pattern can give information on the lithotype where the plants have grown. Using REE pattern for the identification of production area is a unique idea that can be extended not only to other plants, but other biological materials such as shellfish in ocean etc. Identification of area of such products (wine, other foodstuffs etc) has been an important field for isotope geochemistry, but this thesis has opened such field also to REE geochemistry.

The approach of this thesis is very sound, that is, the experiments and discussion were designed from "off-soil growth", "on-soil growth", and "field research", which means that the author constructed the thesis from simple system to more complex systems. In addition, very comprehensive introduction (Chapter 1) and thorough description of methods (Chapter 2) must be highly evaluated.

Based on these reasons, the thesis is worthy of conferral of PhD degree to Mr. Nicola Tuzzolino.

Signature of the reviewer

Yoshio TAKAHASHI

Acknowledgements

This experience of life has involved several people who deserve at least a sense gratitude for everything they have given me at human level and for all the important things they have taught me.

To the tutor, Prof. P. Censi, for the "warm and vibrant" exchanges of opinion because, as he always says, failure is not expected!

To the co-tutor, Dr. F. Saiano, for his contribution.

To the SAF department, in particular to Dr. A. Pisciotta for the friendly and instructive suggestions that he gave me and for the "initiation" he allowed me about viticulture world; to the greenhouse staff for the support and the practical realisation of the research work in field.

Thanks to the kind cooperation of several Sicilian wineries the last phase of thesis was performed. So, I would also thank the wineries represented by Dr. F. Sapienza, Dr. C. Statella, Dr. S. Mangano and Dr. N. Gatti Russo and in particular Dr. C. Bonetta and Dr. F. Sireci for their confidence, their interest and for the opportunity they gave me to apply my intuitions directly in their wineries. They welcomed my project and they were very interested in it as a future chance for their work too.

Last but not least, massive thank you to my family in particular to Maria, and my friends for their support and encouragement (especially during darkest moments). I want also to thank my aunt Rena for her help and her precise and patient support during the correction of this manuscript.

"Everybody is a genius. But if you judge a fish by its ability to climb a tree, it will live its whole life believing that it is a stupid".

A. Einstein