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Behaviour of Rare Earth Elements in the Soil/*Vitis Vinifera L.* system. Geochemical Approach for Food Traceability

PhD Thesis

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Abstract

The geographic traceability of food products through the use of chemical markers is an important challenge to ensure quality and authenticity of food. In recent years, the behaviour of Rare Earth Elements (REE) has been identified as possible tool for food geographical identification based on their known capability of tracing pedo-genetic and petro-genetic processes. In this thesis, the behaviour of REE in the Soil/*Vitis vinifera L.* system has been explored using a geochemical approach. The goal is to understand if the normalized pattern of REE (REE*) can be a useful tool to trace the geographical origin of food. REE may be accumulated in plants keeping their distribution in passing from soil to leaves or fruits. However, the mechanism of soil/plant REE transfer is poorly known, even if leaves may incorporate metals leached from atmospheric dust particles in particular environmental conditions. We focused on plants grown in both greenhouse and field using REE enriched and non-enriched substrates wondering if REE soil enrichments influence the growth of *Vitis vinifera L.* and the REE accumulation in plant organs testing the use of REE* as discriminator of small amounts of REE in the soil. We, also, have evaluated the role of xylem-sap in the transfer of REE transfer and the possible physiological impact in *Vitis vinifera L.*

We found that the stress generated by REE enriched soil does not influence neither the plant mass nor the REE accumulation in leaves and demonstrated that the REE* in plant organs traces enriched soil substrates discriminating plants from different soils of growth.

This work allows to propose that REE* as potential marker for identifying the substrate where *Vitis vinifera L.* grows. This work yields, also, important consequences from environmental perspective: since the REE amount in the substrates does not influence the amount accumulated in leaves REE polluted soils should not influence the amount of REE found in *Vitis vinifera L* food-products. Finally, discrimination of substrate enrichments suggests that REE* is a potential tool for quality and safety of other ecosystems. Our experimental investigation improves our knowledge on REE uptake in soil-*Vitis vinifera L.* system, highlighting the potential use of REE as biogeochemical tracers of environmental conditions.

Résumé

La traçabilité géographique des produits alimentaires à l'aide de marqueurs chimiques est un défi important pour garantir la qualité et l'authenticité des aliments. Ces dernières années, le comportement des Eléments de Terres Rares (REE) a été identifié comme un possible outil pour l'identification géographique des aliments, sur la base de leur propriété de tracer les processus pédo-génétiques et pétro-génétiques. Dans cette thèse, le comportement des REE dans le système Sol*Vitis vinifera L* a été exploré en utilisant une approche géochimique. L'objectif est de comprendre si le spectre normalisé des REE (REE*) peut être un outil pour retracer l'origine géographique des aliments. Les REE peuvent être accumulés dans les plantes maintenant leur distribution lors du passage du sol aux feuilles ou aux fruits. Le mécanisme de transfert des REE entre le sol et la plante est cependant mal connu, même si les feuilles peuvent incorporer des métaux lessivés des particules de poussière atmosphérique dans des conditions environnementales particulières. Nous nous sommes concentrés sur des plantes cultivées à la fois en serre et en champ en utilisant des substrats enrichis et non enrichis en REE, en nous demandant si les enrichissements en REE du sol influencent la croissance de *Vitis vinifera L* et l'accumulation des REE dans les organes de la plante. Nous avons testé l'utilisation des REE* comme discriminateur des petites quantités de REE dans le sol. Nous avons également évalué le rôle de la Sève lors du transfert des REE et leur possible impact physiologique sur *Vitis vinifera L*.

Nous avons trouvé que le stress généré par un sol enrichi en REE n'influence ni la masse de la plante ni l'accumulation de REE dans les feuilles. Nous démontrons que les REE* des organes de la plante sont capables de tracer les conditions d'enrichissement du sol en discriminant les conditions environnementales de la croissance de *Vitis vinifera L*. Puisque les REE* peuvent être utilisés pour différencier les plantes de différents sols de croissance, nous proposons que les REE* sont un marqueur potentiel pour identifier le substrat de croissance de *Vitis vinifera L*. Ce travail a également des conséquences importantes du point de vue environnemental. En étant la quantité initiale des REE dans les substrats est indépendante de la quantité accumulée dans les feuilles, les sols éventuellement pollués par les REE ne devraient pas influencer de manière significative la quantité des REE trouvée dans le produit alimentaire de *Vitis vinifera L*. Enfin, en raison de la discrimination des enrichissements de substrat, les REE sont un outil potentiel pour évaluer la qualité et la sécurité d'autres écosystèmes. Cette étude expérimentale améliore nos connaissances sur la capture des REE dans le système Sol/*Vitis vinifera L*. et confirme l'utilisation des REE comme traceurs biogéochimiques des conditions environnementales.

Riassunto

La tracciabilità geografica dei prodotti alimentari, attraverso l'uso di traccianti chimici, è una sfida importante per garantire la qualità e l'autenticità degli alimenti. Negli ultimi anni, il comportamento degli Elementi delle Terre Rare (REE) è stato identificato come possibile strumento per l'identificazione geografica degli alimenti, sulla base della nota proprietà di tracciare i processi pedo-genetici e petro-genetici. In questa tesi, il comportamento dei REE nel sistema Suolo/*Vitis vinifera L.* è stato esplorato utilizzando un approccio geochimico. L'obiettivo è capire se il modello normalizzato di REE (REE*) può essere uno strumento utile per tracciare l'origine geografica degli alimenti. Le REE possono essere accumulate nelle piante mantenendo la loro distribuzione nel passaggio dal suolo alle foglie o ai frutti, anche se le foglie possono incorporare i metalli lisciviati dalle particelle di polvere atmosferica in particolari condizioni ambientali. Tuttavia, il meccanismo di trasferimento di REE dal suolo alle piante è poco conosciuto. Ci siamo concentrati su piante cresciute sia in serra che in campo usando substrati arricchiti e non arricchiti di REE chiedendoci se gli arricchimenti del suolo di REE influenzassero la crescita di *Vitis vinifera L.* e l'accumulo di REE negli organi della pianta, testando l'uso di REE* come discriminatore di piccole quantità di REE nel suolo. Inoltre, abbiamo valutato il ruolo giocato dallo xylema nel trasferimento di REE e il possibile impatto fisiologico nella *Vitis vinifera L.*

Abbiamo trovato che lo stress generato dal suolo arricchito di REE non influenza né la massa della pianta né l'accumulo di REE nelle foglie e abbiamo dimostrato che le REE* negli organi della pianta sono in grado di tracciare le condizioni del suolo arricchito discriminando le condizioni ambientali di crescita della *Vitis vinifera L.* Poiché REE* può essere usato per differenziare le piante da diversi terreni di crescita, proponiamo che l'uso di REE* sia un potenziale marcatore per identificare il substrato di crescita di *Vitis vinifera L.* Dal nostro lavoro si possono dedurre importanti implicazioni dal punto di vista ambientale. Poiché la quantità iniziale di REE nei substrati non influenza la quantità accumulata nelle foglie, eventuali suoli inquinati da REE non dovrebbero influenzare significativamente la quantità di REE trovata nei prodotti alimentari di *Vitis vinifera L.* Infine, la capacità di discriminare degli arricchimenti del substrato suggerisce che REE* può essere uno strumento potenziale per valutare la qualità e la sicurezza di altri ecosistemi. La nostra indagine sperimentale migliora le nostre conoscenze sull'assorbimento di REE nel sistema Suolo/*Vitis vinifera L.* evidenziando il potenziale uso di REE come traccianti biogeochimici delle condizioni ambientali.

Chapter 1: Introduction

Vitis vinifera L. is one of the most important botanic species exploited for alimentary purposes. Developed for both fruits and wine in different soils and climates, *Vitis vinifera L.* cultivations extend over a total area of 7.15 million hectares (Zheng et al., 2020) and the specific origin of food is crucial for quality and authenticity of the food today (Marchionni et al., 2016; Richter et al., 2019; Zhao et al., 2016). The challenge of this thesis work is to understand if Rare Earths Elements (REE) can be a useful tool for tracing geographical origin of this food product based on the known utility of REE for tracing pedo-genetic and petro-genetic processes (Aide and Aide, 2012; Migaszewski et al., 2015). The overall spectra of REE may reflect complex reaction mechanisms responsible for the soil-plant transfer (Bertoldi et al., 2011; Brioschi et al., 2013; Censi et al., 2014; Pisciotta et al., 2017; Punturo et al., 2018; Sasmaz et al., 2018; Tyler, 2004). Several works reported that the REE distribution pattern, in *Vitis vinifera L*, as well as in other plants, may depend on the elemental composition of the soil (Censi et al., 2014; Punturo et al., 2018; Pisciotta et al., 2017). However, leaves can trap metals transported in the atmospheric dust particles (especially in Mediterranean conditions) (Bargagli, 1998; Censi et al., 2011; 2017; Pallardy, 2008; Tomašević et al., 2004).

The behaviour of REE in plants was investigated for understanding the bioaccumulation processes of these elements (Reimann and De Caritat, 2000, 2005; Reimann et al., 2015; Sucharovà et al., 2012; Tyler, 2004) with low focusing on the mechanism of soil/plant REE transfer (Censi et al., 2014; Miao et al., 2011; Yu et al., 2007). Most of the works consist of experiments of REE soil adsorption under highly specialized and specific conditions as

controlled simplified surfaces and using a single REE sorbate (Dinali et al., 2019). These works evaluated the dose-response under hydroponic experimental conditions which facilitate the uptake of metal-ions in roots while they are not necessarily representative of real-world situations (Thomas et al., 2014; Ye et al., 2018; Yuan et al., 2017). To ensure significance of REE pattern as a fingerprint for real provenance of food products, we want to test if REE content and distribution in plants are related to soil metal migration or if the content in leaves or grapes can be influenced by atmospheric fallout carrying out investigations of REE mobility from soil to plant evaluating the different accumulation points: substrates, roots and leaves, as well as the xylem-sap (Fig. 1.1).

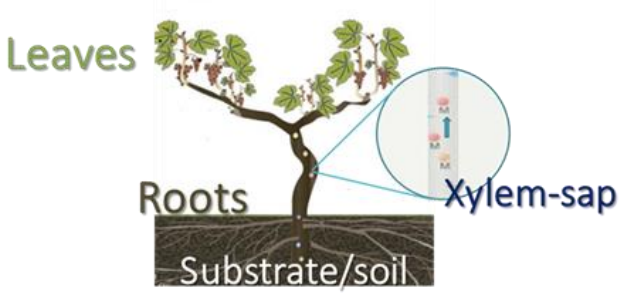

Fig. 1.1. Soil/*Vitis vinifera L.* system

In *Vitis vinifera L.*, the root apparatus carries out anchoring function, hormone synthesis, water absorption and accumulation of reserve substances while leaves have the function of accumulating trace elements and nutrients redistributing the products of photosynthesis (Angelini, 2007). The xylem-sap represents the main conduit where water, dissolved nutrients and other metabolic products in *Vitis vinifera L.* are transported (Diaz-Espejo and Hernandez-Santana, 2017; Pratt and Jacobsen, 2018; Kant, 2017).

1.1. REE behaviour in the natural environment

Chemically, REE are a group of 14 unique elements from ^{58}Ce to ^{71}Lu (Lanthanide series) which is often associated with Yttrium. Two subgroups of REE can be distinguished according to their mass number: the first, Light Rare Earth Elements (LREE) from La to Gd are more basic and more soluble (therefore mobile) compared to the second group, called Heavy Rare Earth Elements (HREE) from Tb to Lu plus Y (Fig. 1.2).

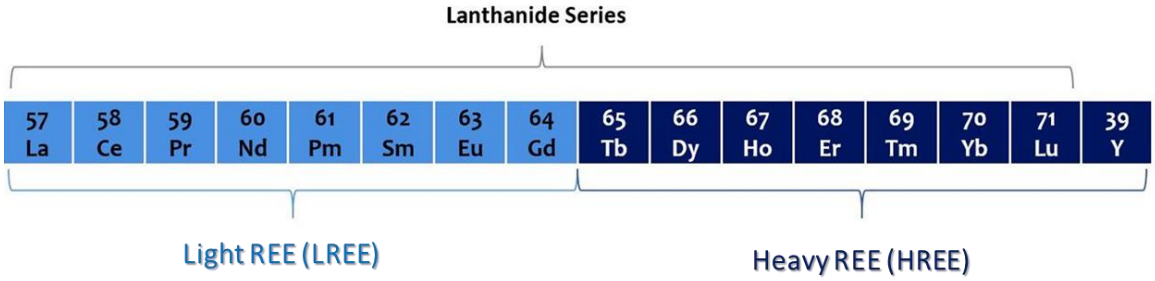

Fig. 1.2. Rare Earth Elements Series

The lanthanide group is characterized by the progressive filling of the 4f orbitals. Since 4f subshell lies inside the already filled 5s and 5p subshells, 4f electrons are not involved in chemical bonds, allowing elements from ^{58}Ce to ^{71}Lu to have the same outer electronic configuration. This state corresponds to $[Xe]6s^2 5d^1$ (Shannon, 1976). However, inefficient shielding of the increased nuclear charge, by the extra 4f electrons, causes the 5s and 5p bonding electrons to contract in towards the nucleus. The *ionic radii* of the REE cations consequently decrease as Z increases, producing the known effect called "lanthanide contraction" (Aide and Aide, 2012). The progressive decrease of ionic dimensions produces slight changes in the

charge /radius ratio influencing the reactivity, especially in aqueous solutions. In natural environments, most REE occur as trivalent ions [REE^{3+}] except for cerium (Ce) that may be also tetravalent (Ce^{4+}) under oxidizing conditions (>300 mV at pH 7). Eu and Yb may, also, reach the +2 state under extremely reducing conditions (Li et al., 2012). Another important chemical characteristic of REE, determining their specific behaviour, is that they can assume different coordination numbers, in function of the atomic number and oxidation state (Aide and Aide, 2012), determining a specific reactivity.

REE are not rare in a true sense as the term indicate as they are widely distributed in the Earth's crust (Kabata-Pendias, 2011). The average concentration of REE in the Earth's upper crust is relatively high (0.015% of the Earth's crust). Cerium is the 25th most abundant element by mass surpassing even Cu and Pb. REE are more common than Ag or Hg and the rarest of this group (thulium) is more abundant than Cd or Se (Taylor and McLennan, 1988). Typical concentrations in soils are a few tens of mg/kg for Nd and a few hundreds of mg/kg for the sum of the REE (Laveuf and Cornu, 2009; Loell et al., 2011; Tyler, 2004; Kabata-Pendias, 2011). Figure 1.3 reports the REE spectra of the Upper Continental Crust (UCC).

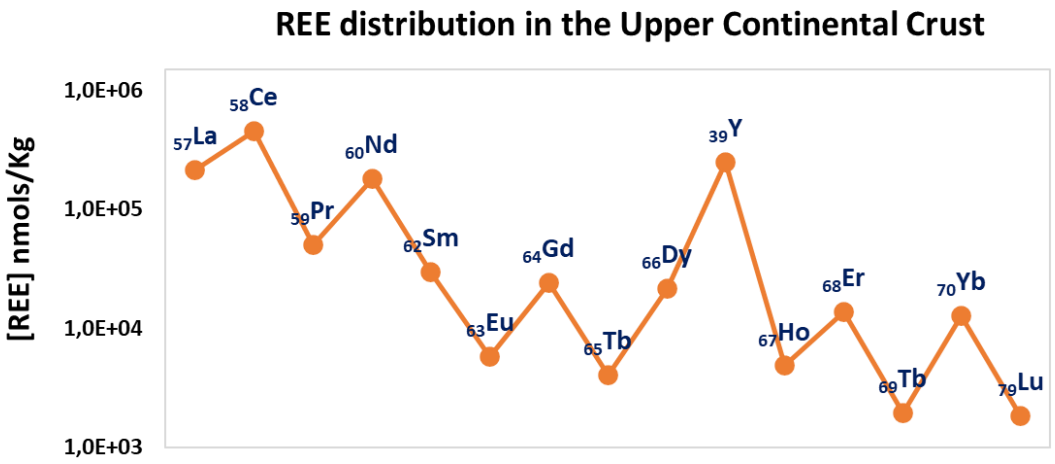

Fig. 1.3. Upper Continental Crust (UCC) distribution

This natural spectrum shows that REE concentration ranges by three orders of magnitude in the Earth Crust with a global abundance decreasing from La to Lu with a 'zig zag' shape. REE with even atomic number are generally more abundant than REE with odd atomic numbers reflecting the Oddo-Harkins rule (Migaszewski et al., 2015). The normalisation is a useful tool for compensating element-to-element irregularities and allowing to identify the possible presence of relative variations on the REE abundance as result of particular reaction mechanisms (Aide and Aide, 2012; Sojka et al 2021; Vermeire et al., 2016). In this work, we use the REE spectra as a possible tool to trace the geographical origin of *Vitis vinifera L.*, verifying the correspondence between the elemental profile of the *Vitis vinifera L.* organs with those of the substrates/soil. We calculate the REE distribution pattern by the ratio between the REE relative abundance in different compartments (substate, roots, xylem-sap and leaves) and the lithological reference (UCC) as reported in the follow equations (Laveuf and Cornu, 2009):

:

$$[REE^*]_{compartments} = \frac{REE_{compartments}}{REE_{UCC}} \quad 1.1$$

$$[REE^*]_{vitis\ vinfera} = \frac{REE_{Vitis\ vinifera}}{REE_{UCC}} \quad 1.2$$

In the following, the REE distribution pattern normalized to UCC will be called REE*. The advantage of normalization is to minimize differences in the relative abundance of REE allowing to distinguish small variations that highlight specific behaviour.

1.2. REE dynamics in soil/*Vitis vinifera L.* system

In natural soils, REE concentrations mainly depend on rock composition, weathering conditions, and the predominance of LREE over HREE (Migaszewski et al., 2015; Tyler, 2004). The REE concentrations of biomasses are, at least, by one order of magnitude lower than soils, varying over several orders of magnitude as a function of plant species and soil concentrations (Brioschi et al., 2013; Kabata-Pendias, 2011). Concentrations of the REE in plants vary within a broad range, from below 1 to above 15,000 μg/kg and accumulate in roots gradually decreasing in leaves, stem, flowers and fruits (Brioshi et al 2013; Kabata-Pendias, 2011; Tayler, 2004).

The mobility of REE from soil to roots is mainly dominated by complexation reactions where REE are transported by inorganic and organic ligands. Specifically, the main inorganic ligands are carbonate, hydroxide, chloride, sulphate, fluoride ions while organic ligands are humic and fulvic acids (Arbuzov et al.2018; Aysha et al., 2017; Brioshi et al 2013; Borrego et

al., 2012; Cao et al 2001; Gupta et a., 2018; Mihajlovicet al., 2018; Tyler, 2004). The adsorption of trace elements on the root surface occurs in cationic form over negatively charged cell walls made of cellulose, pectins, and glycoproteins working as specific ion exchangers (Arif et al., 2016). Cations are available at the root surface from the dissociation of their complex ions that accumulate into the root apoplast (Krzesłowska, 2011). Cations are retained in the root cells or translocated radially to the root stele, once accumulated into root apoplast, and subsequently loaded into the xylem and phloem tissues. These ways of transport correspond to the apoplastic/passive transportation (xylem-sap) and symplastic/active transportation (phloem) (Fig. 1.4) (Hossain et al., 2012; Shan et al., 2003).

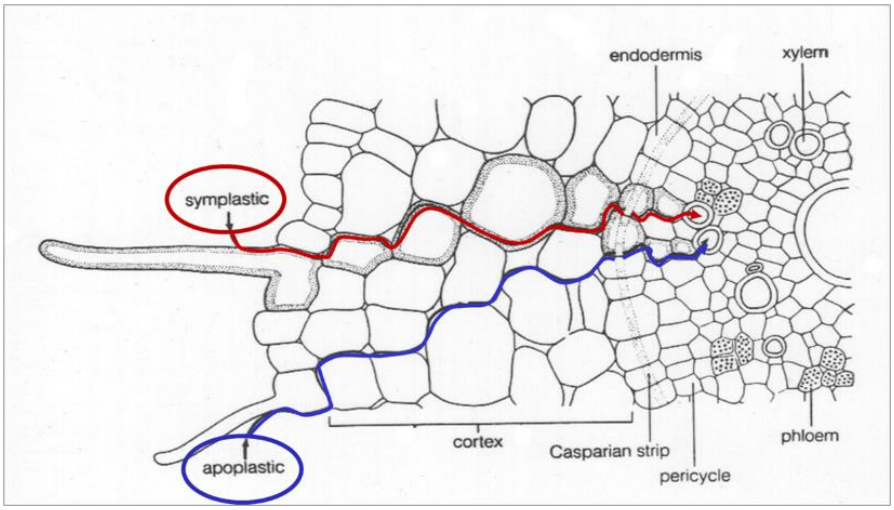

Fig. 1.4. Xylem-sap and phloem pathways

Xylem-sap and phloem are hydraulically interconnected, but their role in the transport of nutrients and trace elements is different. The phloem mediates the transport of compounds from sites of synthesis or storage (green tissues or storage tissues) to sites of consumption

(developing or non-green tissues), whereas xylem-sap transports the components from roots to photo-synthetically active leaves (Buhtz, 2004). Since this research focuses on the dynamic transport of REE from soil to aerial part, we have chosen to analyse the xylem-sap pathway.

Recently, because of new technologies, green energies and medical devices a fast increase in demand and production of REE has been observed (Alves et al., 2020; Alonso et al. 2012; Balaram et al., 2019; Long et al., 2010). However, the very low recyclability of these elements (Ciacci et al., 2015), their spreading as fertilizers in agriculture and their use as food additives for livestock and poultry (Redling et al., 2006) have generated high concentrations in soils (Zhenggui et al., 2001; Yuan et al., 2017) leading REE to be emerging pollutants (Alonso et al. 2012; Goodenough et al., 2017; Kegl et al., 2020; USGS, 2020; Turra, 2018; Yan et al., 2020). REE are considered non-essential trace elements entering passively into plant tissues because of their similarity with essential ions (Kabata-Pendias and Pendias 2001; Wang et al., 2008, 2017; Yuan et al., 2017). Since plants can take up non-essential trivalent elements, REE may enter the food chains and have health consequences for consumers. Therefore, we have investigated the potentiality of the *Vitis vinifera L.* to accumulate REE in their organs when exposed to a mixture of REE supplied at equimolar concentration in the substrates of growth.

Thesis outlook

To plan this thesis work, the following questions have been identified:

- Does REE enrichment in substrate influences the plant growth and REE accumulation in roots, xylem-sap and leaves?
- Is the uptake of REE selective in *Vitis vinifera L*?
- Is REE* useful to discriminate small differences in REE amount?
- Does REE* fractionate in the xylem-sap?
- Is *Vitis vinifera L.* physiology sensitive to the REE transport?

Chapter 2: Experimental planning

Globally, we planned two experiments in which we studied the transfer of REE from soil to *Vitis vinifera L* into two substrates of growth: control and spiked. In the first experiment, *Vitis vinifera L.* grew on control substrate with an extremely low REE content (lower than MQL, see Appendix A 1.4) and spiked substrate enriched by 3 orders of magnitude REE compared to the control. In the second experiment we used a control substrate poor in REE but with an REE profile similar to natural soil and a spiked substrate enriched by 2 times compared to the control. We choose to test also small REE relative abundance variations, to evaluate the applicability of the distribution patterns to natural systems. Indeed, the variations in terms of relative abundances in natural soils are small (Calabrese et al., 2011; Censi et al., 2017; Scalenghe et al., 2016) even if REE are emerging contaminants resulting from the increasing technological uses and agricultural practices (Balarm et al., 2018; Jowitt et al., 2018; Tommasi et al., 2020). We choose substrates mainly composed of peat, which is constituted by high concentrations of organic substances (humic acid), that can be able to complex REE (Arbuzov et al., 2018; Cao et al., 2001). Both substrates were spiked with an equimolar solution of REE. We used an equimolar solution of REE to perturb the natural differences in the relative abundance of each REE, minimizing the mass effect of every REE. This allows emphasizing the eventual behaviour of a single REE during the transfer from soil to plant.

2.1. Experimental set-up

The semi-natural experiments were carried out in the experimental field at the Department of Agricultural, Food and Forestry Sciences (University of Palermo, Italy) using *Vitis vinifera L*. plants. The first year the plants grew in a native soil located in the southwest of Sicily (Italy), constituted by clayey sandy sediments. Pristine plants, one year in age, were put in off-soil polyurethane pots with peat/gravel and peat/cocoa fibre substrate for the first and second experiment respectively. In the first experiment, pots are in green-house whereas in the second one pots are in contact with atmospheric agents. For both experiments, no disease spray was used and a micro-irrigation system was used according to deficit irrigation strategy (Di Lorenzo et al., 2005). Before planting, the root length was uniformed to 10 cm to enssure an equivalent development for both experimental conditions. Multi-REE working standard solutions (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) were prepared by diluting the multi-REE standard stock solution (1000 mg/L; pH<1) to 0.05 mM of each element in high purity water (pH ~5.5), obtaining an initial working solution of pH close to 5.6 allowing to mainly consider REE as free-ions (De Boer et al., 1996). Multi-REE working standard solutions were added one time to spiked substrates after planting the pristine plants.

2.1.1. First experiment

Thirty pristine *Vitis vinifera L*. individual plants (Moscato d'Asti, rootstock 1103 P) of one year in age from native soils were put into polyurethane pots with a homemade substrate with a peat-gravel ratio of 2:3 w/w. We used the same amount (5 kg) of substrate for both experimental conditions. Two different growth conditions were investigated: one using peat-

gravel substrate (control experiment) and another using spiked substrate enriched by 3 orders of magnitude REE compared to the control (spiked experiment) (Fig. 2.1).

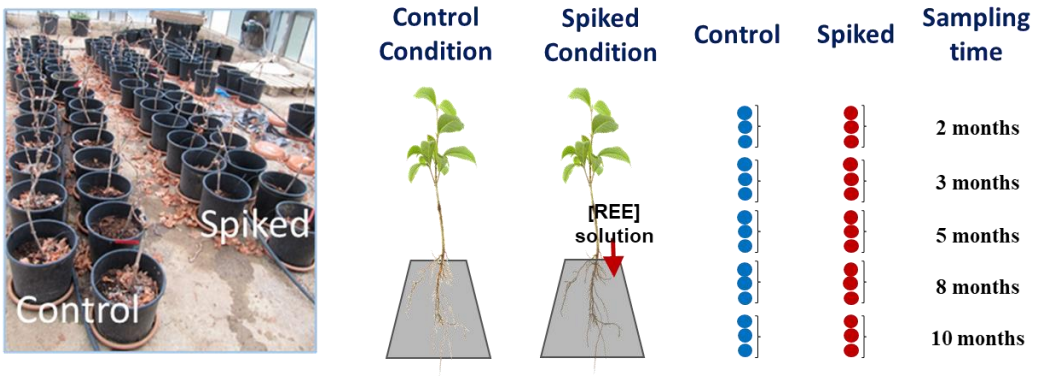

Fig. 2.1. The off-soil experimental system (first experiment)

Sampling was carried out at five different sampling times selected according to the different plant-life periods: budding-flowering (2 months), fruit-setting (3 months), veraison (5 months), harvest (8 months), post-harvest (10 months). Each sampling was replicated three times and, for every sampling time, plants were separated into roots and aerial parts. Roots were in turn separated in woody ($Ø \geq 2mm$), middle ($1mm \leq Ø \leq 2mm$) and fine-roots ($Ø \leq 1mm$), while aerial parts consist of leaves and herbaceous shoot, petioles, wood shoot (one-year-old), wood shoot (two-years-old) (Fig. 2.2).

Fig. 2.2. *Vitis vinifera L* organs collected in the first experiment

Each sampling was replicated three times and the REE determination was performed, for every sampled part, by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

2.1.2 Second experiment

Pristine plants of one year in age from native soils were put into polyurethane pots with a homemade substrate with a peat-cocoa fibre ratio of 2:3 w/w. We used the same amount (1 kg) of substrate for both experimental conditions. Here, two different growth conditions were investigated: one using the homemade substrate (control experiment) and another adopting the same substrate spiked two times respects the control one (spiked experiment). The whole experimental system consisted of 24 plants of which 12 controls and 12 spiked. Sampling was carried out at 4 different times (3, 4, 5, 7 months of growth) collecting both control and spike samples (Fig. 2.3).

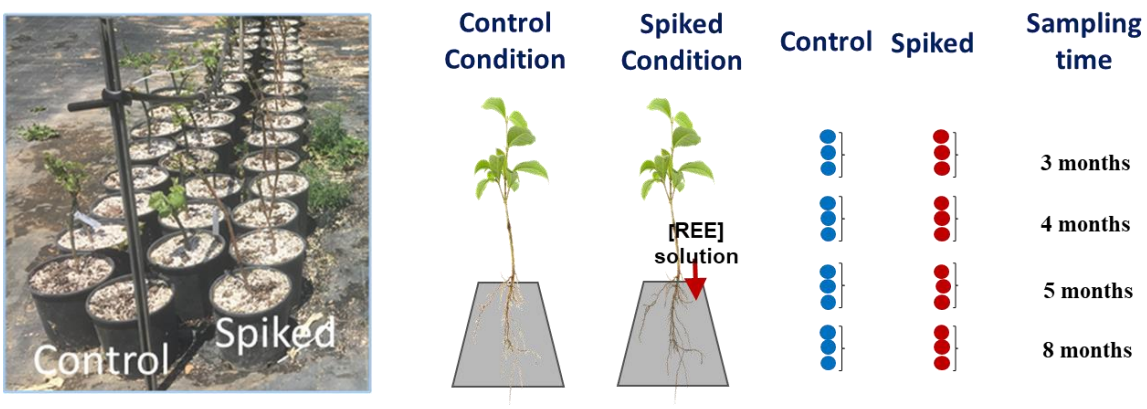

Fig. 2.3. The off-soil experimental system (second experiment)

Here, we concentrated the sampling in the period in wich the greatest variations in terms of REE distribution were observed in the first experiment. For every sampling time, plants were separated into roots and leaves and xylem-sap was also extracted (Fig. 2.4). Substrates were collected one time, after the equimolar addition of REE.

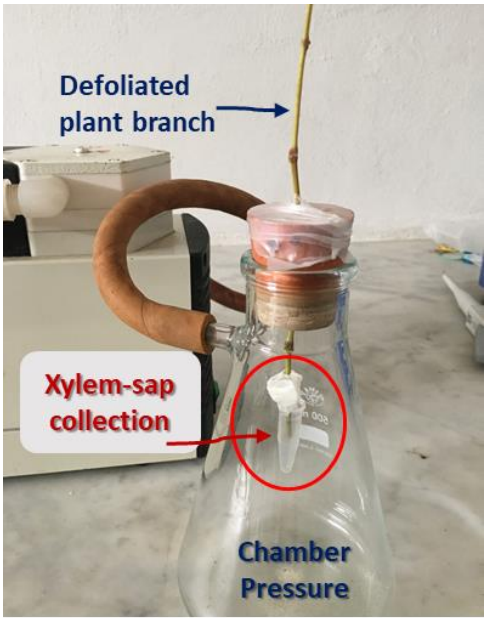

Fig. 2.4. Xylems-sap extraction

Substrates, leaves and roots were replicated three times, whereas is not possible to make replicates for xylem-sap due to the low amount extracted (each sample is related to the instant (t) in which the xylem-sap was collected). The REE determination was performed for every sampled part. Xylem-sap was also characterized in terms of macronutrients, micronutrients and organic acids.

Table 2.1 summarizes the environmental conditions of the experiments

	First Experiment	Second Experiment
Control Substrate	$[REE]_{Control} < MQL$	$[REE]_{Control} < [REE]_{native\ soil}$
Spiked Substrate	$[REE]_{Spiked} > [REE]_{Control}$ 3 order of magnitude	$[REE]_{Spiked} > [REE]_{Control}$ 2 time
Investigation time	10 months	7 months
Selected organs	Roots, Leaves, Herbaceous shoot Petioles, Wood shoot	Roots, xylem-sap, Leaves

Tab. 2.1. Summary of *Vitis vinifera L.* growth conditions for the first and second experiment. REE concentrations [REE], Method Quantification Limit (MLQ)

Chapter 3: Materials and Method

This chapter describes the materials used for chemical analysis, the chemical methods employed and the related analytical problems, as well as the control strategies adopted to control or minimize them.

3.1. Working standard solutions

Multi-element (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb Dy, Y, Ho, Er, Tm, Yb, Lu) and internal standard solutions (Re) were daily prepared by stepwise dilution of the stock standard solutions DBH, Merck or CPI International (1000 ± 5 mg/L) in HNO_3 1 % (w/w) medium. We used ultrapure grade reagent, nitric acid (65%), hydrogen peroxide (30%), analyte standard solutions of REE (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb Dy, Y, Ho, Er, Tm, Yb, Lu), Micronutrients (MIN) (Fe, Mn, Cu, Zn, Ba) and Macronutrients (MAN) (Mg, K, Ca) and internal standard solutions (Re) each 1000 ± 5 mg/L, were purchased from BDH, Merck and CPI International (Italy) while ultrapure water 18.2 MΩ cm^{-1}, was produced by an EASYpureII (Thermo, Italy).

3.1.1 Certified Reference Material

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

3.2. Sample preparation

After sampling, leaves and roots were weighed, chopped, dried (105°C for 24h), ground in an agate mortar and stored in a PE vessel. Substrate samples were dried in an oven at 105°C, gently crushed, sieved (Ø 0.5 mm) and homogenized. Aliquots of 0.500 g (DW, dried weight) of leaves, of 0.250 g (DW) of rootsand of 0.250 g (DW) of both substrates were transferred in a Teflon^{TM} vessel with 4.5 ml of 2:1 v/v mixture of HNO_3:H_2O_2 and put in a microwave oven. All samples were digested in a closed microwave system (MarsXpress, CEM, Milan Italy) with an increasing temperature from room temperature to 200°C in 10 minutes. The final temperature was maintained for 50 min. Power was 1600 Watt, while pressure was not controlled. After digestion, the leaves and roots extracts were quantitatively transferred into graduate polypropylene test tubes and diluted with ultrapure water to 10 ml. The substrates extracts were diluted with ultrapure water to 100 ml. Every analytical sequence included a procedural blank: ultrapure water digested as a sample.

3.2.1 Xylem-sap extraction

Xylem-sap was collected using the pressure chambers method. This approach involves cutting the plant stem or root and putting the fragment inside a chamber pressurized lower than ambient pressure, used to force the sap out of the cut surface (Netzer al., 2017). However, it is important to highlight that xylem-sap extraction from roots does not allow selecting the xylem-sap from the radical exudates. Therefore, to avoid contamination by radical exudates we chose to extract the xylem-sap from the branch, even if this choice involves the extraction of a smaller quantity of sap. Another source of xylem-sap contamination can be the phloem. The phloem

circulates on the external channels while the xylem-sap is deep inside the branch, therefore to minimize possible phloem contamination branch cuticle was cut before extraction. To extract the xylem-sap, *Vitis vinifera L*. branches were rapidly defoliated and cut from the plant. A ring of bark was removed and the cut surface rinsed with distilled water. The branch tip was inserted through a rubber stopper in a plastic vial, inside a Buchner flask while extraction was performed applying negative pressure. The pressure in the chamber was gradually decreased to 1.4 MPa until xylem-sap sap flowed out from the excised tip (Oddo et. al., 2014). At the end of the extraction, about 250 µl of sap was collected. 100 µl of the extracted xylem-sap were collected for organic acids determination through liquid chromatography coupled to mass spectrometry (HPLC-MS/MS) and 100 µl was quantitatively transferred to a polypropylene graduate tube and diluted with HNO3 in ultrapure-water (0.1%) to 3 mL before Inductively Coupled Plasma Mass Spectrometry (ICP-MS) determinations.

3.3. Chemical analysis: organic acids determination in xylem-sap

Organic acids determination was carried out by HPLC-MS/MS system (Waters Alliance- Micromass Quattro Micro, Waters, USA). Synergy Hydro-RP column (250 × 4.6 mm with 4 µm particles from Phenomenex Torrance, CA, USA) was employed to separate citric, lactic, malic and succinic acids in isocratic conditions using a mobile water/methanol (95:5) phase with 0.15% formic acid. Instrumental conditions were: injection volume: 20 µL; flow rate: 0.4 ml/min; column temperature: 30°C; MS/MS—Ionization mode: negative-ion electrospray ionization (ESI-); capillary voltage: 2.5kV; source temperature: 120°C; desolvation temperature: 400°C; cone gas flow: 50 L/h; desolvation gas flow: 800 L/h; collision

cell pressure: $3.5x10^{-3}$ mbar; dwell time: 0.1 s for all analytes. The Multiple Reaction Monitoring (MRM) mode was used for data acquisition. The mass transitions are reported in Table 3.1. Analyte quantification was obtained by external calibration curves in the range 0.1 - 1 mg/L.

Analyte	Quantification transition (m/z)	Qualification transition (m/z)	Cone voltage (CV)	Collision Energy (CE)
Citric acid	191 > 87	191 > 111	20	15
Lactic acid	89 > 43		15	10
Malic acid	133 > 115	133 > 71	15	10
Succinic acid	117 > 73	117 > 99	15	10

Tab. 3.1. Summary of the experimental mass spectrometry conditions

3.4 Chemical analysis: REE determination

REE concentration was measured in both substrates, all plants organs and roots for both first and second experiments. Xylem-sap was also characterized in terms of MAN: (Mg, K, Ca) and MIN (Fe, Mn, Cu, Zn, Ba). All analyses were performed by ICP-MS instrument.

ICP-MS parameters were daily optimized monitoring ^{7}Li, ^{89}Y, ^{140}Ce, and ^{205}Tl masses with a minimal precision of 2%, with ^{187}Re used as internal standard. Each solution was measured three times, and ICP-MS analyses were carried out with a classical external calibration approach. Such approach involved investigating a range of concentrations for each element between 2.5 and 500 pg/mL and using ^{187}Re (1000 pg/mL) as internal standard to compensate for any signal instability or sensitivity changes during the analysis. Operating conditions are shown in Table 3.2.

RF power	1550 W
Sample uptake rate	0.400 mL/min
Plasma gas flow 15 L/min	15 L/min
Auxiliary gas flow 0.85 L/min	0.85 L/min
Make-up argon flow rate	0.25 L/min
Nebulizer gas flow 1.00 L/min	1.00 L/min
Number of scans	3
Ion lens settings	Adjusted daily to obtain max. signal intensity
Washing time	1 min (5% v/v HNO_3)
Oxide $^{156}CeO^+/^{140}Ce^+$ ratio	< 0.5 %
Double charged $^{70}Ce^{++}/^{140}Ce^+$ ratio	< 0.5%
Measured REE Isotope :	^{89}Y, ^{139}La, ^{140}Ce, ^{141}Pr, ^{143}Nd, ^{147}Sm, ^{151}Eu, ^{158}Gd, ^{159}Tb,^{161}Dy, ^{165}Ho, ^{167}Er, ^{171}Yb, ^{175}Lu
Measured macronutrients (MAN) Isotope	^{26}Mg, ^{39}K, ^{43}Ca
Measured micronutrients (MIN) Isotope :	^{56}Fe, ^{55}Mn, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{135}Ba
Internal standard	^{187}Re 1000 pg/mL

Tab. 3.2. ICP-MS operating conditions and measurement parameters

To ensure long term stability during the ICP-MS analysis the internal standard (^{187}Re 1000 pg/mL) was adopted. The use of the internal standard has allowed controlling the calibration standards, the variation in plasma ionization, corrosion of cone apertures, the possible fluctuations in instrument operating conditions ("drift") as well to compensate for any signal instability. The internal standard solution was added automatically into the sample line using a T-piece, fitted-in peristaltic pump. Trace elements results were calculated using the ratio of the analyte and internal standard signal.

3.4.1. Spectral Interference

Spectral interferences are one of the main problems in quadrupole ICP-MS determination. There are three types of interference (isobaric, double-charged ions, and polyatomic ions) and they must be managed differently. The isobaric interferences occur when two isotopes of different elements have the same mass. For example, both calcium and argon have isotopes at a mass of 40 amu, therefore, Ar will interfere with the measurement of Ca at this isotope. This kind of interference cannot be eliminated and, in these cases, it is necessary to select, if it exists, a different isotope that does not suffer an isobaric overlap. The second type is the doubly charged problem. Most elements form singly charged ions in the ICP, however, some elements possess the second ionization potential sufficiently low to allow the formation of doubly charged ions in the argon plasma. These ions will interfere with analytes at half of their actual mass. This kind of interference can be managed or with an accurate isotope selection or minimizing doubly charged ions formation in the plasma through a fine regulation of plasma parameters (power, depth, Ar flows) keeping the percentage of double-charged ions (% of Ce^{2+}/Ce^{+} or Ba^{2+}/Ba^{+} ratios) less than 2–3% (Pupyshev et al., 2001). The most problematic type of interference is the isobaric one from polyatomic ions. Polyatomic ions form in the plasma or from interactions in the interface region, either due to incomplete atomisation or from recombination reactions with reagents used for sample preparation (for example HCl), plasma gases (argon) or entrained atmospheric gases (oxygen, carbon dioxide, nitrogen) (Templeton et al 1994). An example of this is the interference of isotopes of barium oxide ($^{135}Ba^{16}O^{+}$, $^{137}Ba^{16}O^{+}$) with rare earth elements such as samarium, gadolinium and europium, or isotopes of Argon oxide ($^{40}Ar^{16}O^{+}$) with Fe. Management strategies for polyatomic interferences include

controlling the presence of the interfering components in the sample, analyte isotope selection, mathematical correction, and the use of collision cell. The collision cell technique uses a non-reactive gas, such as helium, to reduce the kinetic energy of the larger (larger cross-section) polyatomic ions within the cell. A simple positive voltage gradient at the exit of the cell discriminates against the low-energy polyatomic and transmits the higher-energy monoatomic ions using a process called kinetic energy discrimination (KED). KED is effectively able to reduce almost all the polyatomic interferences simultaneously, and for this reason, is the preferred technique for multi-elemental determinations. Also, a convenient method to minimize oxide species formation in the plasma is measuring the signal intensity ratio of cerium oxide to cerium (CeO^+/ Ce^+). Cerium is chosen since has a particularly high affinity for oxygen and cerium oxide has a strong bond that is not easily dissociated in the plasma (CeO bond enthalpy = 795 kJ mol^{-1}). From what has been said it is clear that spectral interferences in the measurement by ICP-MS have to be carefully considered to obtain correct results for REE quantification. Major spectral interferences that can affect the measurement in our experimental conditions and the management strategies adopted are reported in the following scheme (Fig. 3.1):

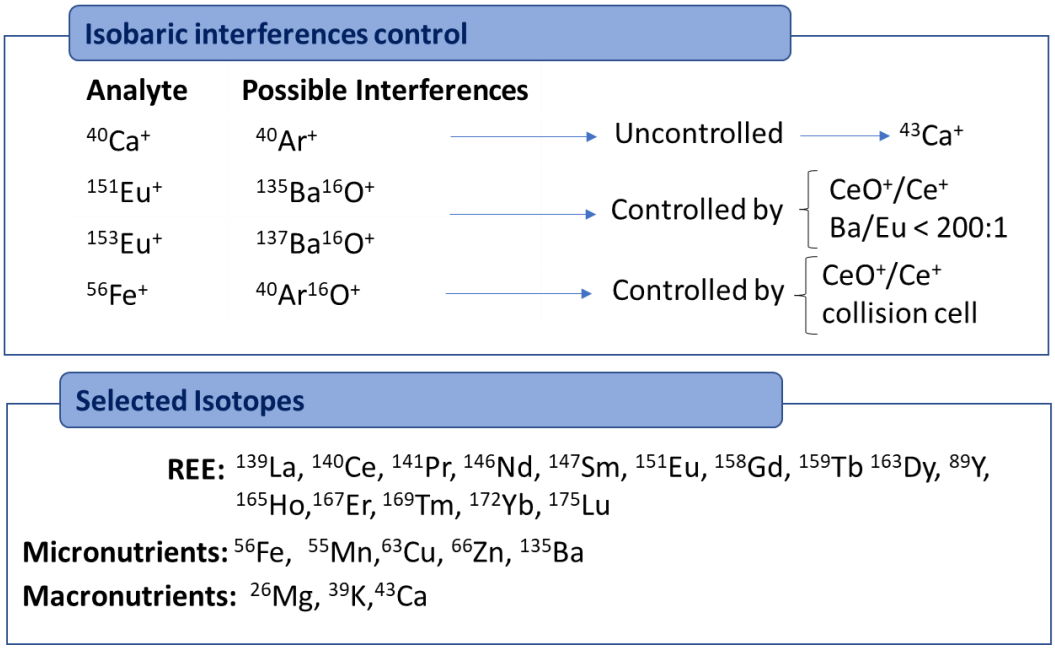

Fig. 3.1. Isobaric interference and selected isotopes

Possible isobaric interferences related to europium isotopes (^{151}Eu, ^{153}Eu) by polyatomic barium oxide ions ($^{135}Ba^{16}O^{+}$, $^{137}Ba^{16}O^{+}$) were estimated using the certified INCT-OBTL-5 Oriental Basma Tobacco Leaves standard and measuring the Ba concentration. Whereas the possible isobaric interferences related to Iron isotopes (^{56}Fe) by polyatomic argon oxide ions ($^{40}Ar^{16}O^{+}$) was minimized by the use of the collision cell.

3.5. Data analysis

Overall development of the plant was evaluated by the variation of the mass during growth. The preferential accumulation of REE in the organ plants was evaluated by the concentration detected in the roots and the aerial part of the plants. The REE transfer was evaluated by Translocation Factors (TF), corresponding to the ratio between the REE concentration in shoots and roots (Krzciuk and Gałuszka, 2015). TF was determined through the following equation:

$$TF = \frac{[REE]_{Shoot}}{[REE]_{Root}} \qquad 3.1$$

where: $[REE]_{Shoot}$ and $[REE]_{Root}$ are REE concentrations in µmol/g in the aerial parts and roots, respectively. If TF>1, metals accumulate preferentially in shoots compared to roots, while, if TF<1 metals accumulate preferentially in roots. The capacity of substrate to retain REE were evaluated by the REE amount (µmol/Kg) in both control and spiked substrates. The REE distribution in *Vitis vinifera L.* was evaluated in all compartments studied (native soils, substrates, *Vitis vinifera L.* organs) normalizing the relative abundance of REE in roots, xylem-sap and leaves to UCC (Upper Continental Crust), a standard lithological reference (Eq.1 and 2). REE distribution pattern allows, promptly, to evaluate an enrichment or depletion of a group or an individual REE relative to the others (Laveuf and Cornu, 2009; Pisciotta et al., 2017). These relative differences are called, respectively, "fractionations" or ''anomalies", whose intensities are further expressed by ratios (Laveuf and Cornu, 2009). The possible REE anomalies (Censi et al., 2014) have been estimated by the following equations:

$$\left[\frac{REE}{REE^*}\right]_{shoot/root} = \frac{\left(\frac{REE_{shoot}}{REE_{roots}}\right)_i^2}{\left[\left(\frac{REE_{shoot}}{REE_{root}}\right)_{i+1}\left(\frac{REE_{shoot}}{REE_{root}}\right)_{i-1}\right]} \qquad 3.2$$

$$\left[\frac{REE}{REE^*}\right]_{shoot/UCC} = \frac{\left(\frac{REE_{shoot}}{REE_{UCC}}\right)_i^2}{\left[\left(\frac{REE_{shoot}}{REE_{UCC}}\right)_{i+1}\left(\frac{REE_{shoot}}{REE_{UCC}}\right)_{i-1}\right]} \qquad 3.3$$

where the subscript "UCC", "shoot", "root" correspond to the REE concentration in the lithological reference, in aerial parts and roots respectively. The subscript "i" indicates every REE of the series, whereas "(i − 1)" and "(i + 1)" are its immediate neighbour, before and after within the series (Censi et al., 2014; Laveuf and Cornu, 2009).

Chapter 4: Data Validation and data quality assurance

In this thesis, we focus on method validation and data quality assurance for REE determination in the different matrices (soil/substrates, roots, leaves and xylem-sap). The method uncertainty was calculated using a metrological approach. This approach allows to consider the most important contributions which may affect the uncertainty (Magnusson, 2014; Pryseley et al., 2010). The performance parameters evaluated during this validation study were: working range, Instrumental Detection and Quantification Limit (IDL, IQL), Precision, Trueness, Method Detection Limit and Quantification Limit (MDL, MQL) (Specific detail to calculate these parameters are reported in Appendix A). First of all, the ICP-MS performances and instrumental response were evaluated, defining the working range, IDL and IQL, then we used a specific strategy to assure the validity of the data in different matrices (substrates, roots, leaves and xylem-sap).

***Leaves*:** precision was evaluated by the repeatability, performing independent replicated measurements both from *Tobacco Leaves* Certified Reference Material (CRM) and real leaves samples. Trueness was estimated by the recovery from CRM, the MDL and MQL were evaluated on measurements of a real samples of leaves.

Soil and roots: precision was evaluated by the repeatability, performing independent replicated measurements in reals samples of soil/substrate and roots. Trueness were estimated by the recovery on real samples and the MDL and MQL detection and quantification limit were evaluated on measurements of soil and roots reals samples.

Xylem-sap: precision was evaluated by the repeatability, performing independent replicated measurements on REE spiked water after proving that the matrix effects were negligible. Recovery was not kept into consideration because the analysis is performed in direct injection. MDL and MQL coincide with the IDL and IQL.

For all determinations, based on the validation data, data quality assurance was carried out through a double test (sample reply twice in the same analytical batch) and control charts (calibration point or certified material analysed in different batches) (see Appendix A3). Both validation strategy and data quality assurance, adopted for leaves and xylem-sap, are discussed below.

4.1. Instrumental parameters

Working range: was chosen in the interval in which the instrumental response is in a linear relation with analyte concentrations (Araujo, 2009; De Souza et al., 2005). Linearity of the calibration curve was assessed by Mandel's test (see Appendix A1.1) and the working range was determined in in the range of 1-500 pg/mL for REE.

For all elements, in the working range 1-500 pg/mL, the Mandel's test was positive and the residual was lower than ± 20% indicating a normal distribution.

Instrumental detection and quantification* limit *(IDL e IQL): IDL and IQL were determined analysing ten different aliquots of standard solution at REE concentration near the lower point of the working range (1 ng/l) (see Appendix A1.2 and A1.3). Results are reported in Table 4.1.

Elements	$\bar{x}_{St.sol.}$	IDL	IQL
Y	1.05	0.33	1.11
La	1.02	3.09	10.30
Ce	1.10	4.48	14.93
Pr	1.03	0.12	0.40
Nd	1.03	3.17	10.58
Sm	1.15	1.05	3.51
Eu	1.02	0.10	0.32
Gd	1.10	0.87	2.88
Tb	1.29	0.72	2.41
Dy	1.02	0.06	0.20
Ho	1.05	0.32	1.06
Er	1.23	0.33	1.10
Tm	1.06	0.25	0.83
Yb	1.03	0.18	0.60
Lu	1.08	0.40	1.32

Tab. 4.1. REE experimental mean value of standard solutions ($\bar{x}_{St.\ sol.}$) at 1 ng/L, Instrumental Detection and Quantification Limit value (IDL and IQL)

We found that instrumental quantification limit ranged between 0.3 and 14 ng/l indicating a very good instrumental sensibility.

4.2. REE measurements validation in leaves

In this section, we report the results of the validation strategy adopted for leaves. After the definition of working range and instrumental detection and quantification limit, we evaluate the system variability calculating precision and recovery and the method sensibility performance.

4.2.1. Precision

Precision was evaluated in terms of method repeatability (see Appendix A1.5). Repeatability was evaluated by eight independent replicated measurements both from CRM and leaves samples. Huber's tests and Shapiro-Wilks's were applied to control the presence of outliers and normal distribution of the data, respectively. After normal distribution and outlier presence tests, a measure of the dispersion of a set of n values (standard deviation, σr) and repeatability limit (r) was calculated (see Appendix A1.5). The repeatability results from CRM and leaves samples are reported in Table 4.2 and 4.3 respectively.

Elements	$\overline{x}_{CRM}$	**n**	**r**	**RSD%**
Y	1057	8	9.6	2.6
La	1637	8	15.3	4.2
Ce	2866	8	18.8	5.2
Pr	336	8	16.2	4.4
Nd	1286	8	13.9	3.8
Sm	256	8	9.9	2.8
Eu	62.0	8	5.3	1.5
Gd	270	8	8.1	2.2
Tb	33.8	8	4.3	1.2
Dy	188.1	8	4.3	1.2
Ho	35.9	8	5.1	1.4
Er	102	8	7.1	1.7
Tm	13.3	8	8.2	1.9
Yb	112	8	16.2	4.8
Lu	12.0	8	9.1	2.3

Tab. 4.2. REE experimental mean value of CRM $(\overline{x}_{CRM})$, replicas number (n), repeatability limit (r), Relative Standard Deviation (RSD%) in µg Kg^{-1}

Table 4.2 show that the CRM Relative Standard Deviation percentage (RSD%) for all investigated elements was very low, between 1-5% in the performed test.

Elements	$\overline{x}_{leaves}$	n	r	RSD %
Y	361.78	8	14.29	3.93
La	705.82	8	14.37	3.95
Ce	81.53	8	20.47	5.63
Pr	299.07	8	19.30	5.31
Nd	56.94	8	20.87	5.74
Sm	17.11	8	30.05	8.27
Eu	52.59	8	25.47	7.01
Gd	7.52	8	45.19	12.43
Tb	33.51	8	25.93	7.13
Dy	431.64	8	25.77	7.09
Ho	6.78	8	29.18	8.03
Er	16.20	8	28.88	7.94
Tm	2.77	8	35.60	9.79
Yb	12.29	8	28.61	7.87
Lu	2.44	8	34.46	9.48

Tab. 4.3. REE experimental mean value of Leaves ($\overline{x}_{leaves}$), replicas number (n), repeatability limit (r), Relative Standard Deviation (RSD%) in µg Kg^{-1}

Table 4.3 shows that the Leaves Relative Standard Deviation Percentage (RSD%) for all investigated elements was between 3-12%, in the performed test.

As expected, the RSD% determined by leaves samples is higher than the value obtained from Reference Certified Material. Indeed, RSD% for real samples is affected by both the variability of the sampling and heterogeneity of the samples themselves. Therefore, precision assessment is more realistic when calculated from actual samples rather than certified material.

4.2.2. Trueness

Trueness was evaluated in terms of recovery values, comparing the experimental data to the Certified Reference Material value (see Appendix A1.6.and A1.6.1). Results are reported in Table 4.4.

Elements	$\overline{x}_{CRM}$	C_{CRM}	Rec%
Y	963	1057	110
La	1690	1637	97
Ce	2990	2866	96
Pr	321	336	105
Nd	1330	1286	97
Sm	264	256	97
Eu	60.2	62.0	103
Gd	243	270	111
Tb	34.7	33.8	97
Dy	184.0	188.1	102
Ho	34.5	35.9	104
Er	101	102	101
Tm	13.6	13.3	98
Yb	115	112	97
Lu	16.7	12.0	72

Tab. 4.4. REE experimental mean value of CRM $(\overline{x}_{CRM})$, certified concentration value of CRM (C_{CRM}), and recovery % (REC%) in µg Kg^{-1}

Table 4.4. shows that recovery value ranging from 97 to 111%; only Lu shows a recovery of 72%. For most of the elements tested the recovery is satisfactory, meaning that the analytes were completely extracted by the samples.

4.2.3. Method Detection and Quantification Limit MDL, MQL

MDL and MQL were evaluated by ten different measurements of real leaves samples (see Appendix A1.4). MDL and MQL were determined at REE concentration near the IDL and IQL (Table 4.1). To obtain this condition, after digestion, leaves samples were diluted and measured, the results are reported in Table 4.5.

Element	$\bar{x}_{leaves}$	MDL	MQL
Y	32.98	9.87	32.89
La	29.05	14.70	49.00
Ce	56.00	27.69	92.31
Pr	5.96	3.14	10.46
Nd	22.06	10.95	36.50
Sm	4.22	1.92	6.41
Eu	1.51	0.33	1.11
Gd	3.94	1.77	5.89
Tb	0.52	0.21	0.68
Dy	2.50	0.92	3.06
Ho	4.84	1.75	5.84
Er	1.22	0.43	1.42
Tm	1.86	0.58	1.95
Yb	0.92	0.28	0.94
Lu	1.65	0.52	1.75

Tab. 4.5. REE experimental mean value of Leaves $(\overline{x_{leaves}})$, (ng/kg) Method Detection and Quantification Limit values (IDL and IQL)

Figure 4.1 shows the ratio between Instrumental and Method Quantification Limit.

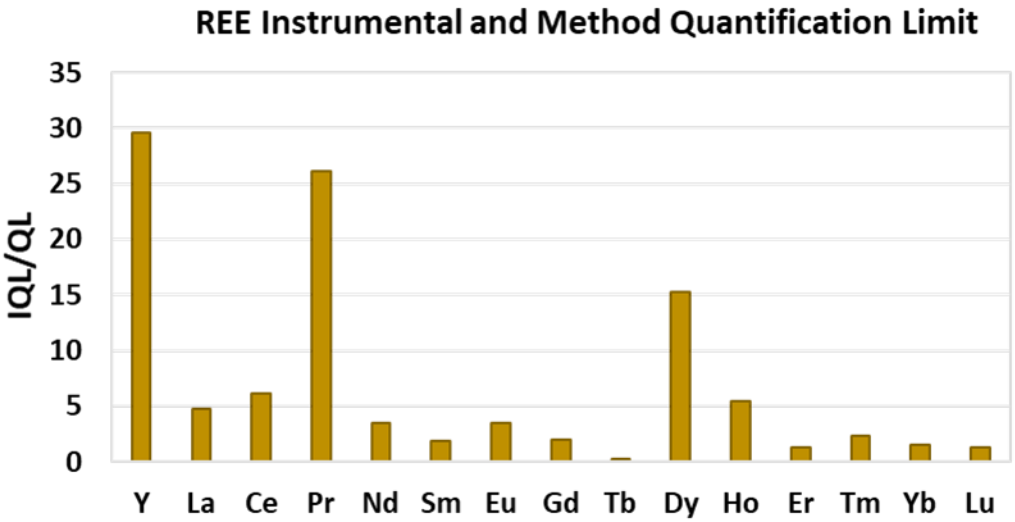

Fig. 4.1. Ratio between Instrumental Quantification Limit (IQL) and Method Quantification Limit (MQL)

We found that MQL was higher than IQL, because MQL keeps into consideration the matrix effect. However, results demonstrate the very good performance of ICP-MS even considering the matrix effect. In particular, for all elements the MDL and MQL values were comparable or lower than the mean concentration estimated in real samples, indicating that the methods developed both for extraction and determination of the REE are adequate.

4.3. Uncertainty

Measurement uncertainty (U)" is formally defined as a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand (Ellison et al., 2012; Guide JCGM 100:2008).

Uncertainty from metrological approaches is calculated taking into account all sources contributing to the results, through applying the law of propagation of errors (see Appendix A2). Examining the input variables and their contributions the sources of uncertainty that must be taken into account were identified (Specific detail to calculate these parameters are reported in Appendix A Tab. A1):

- Repeatability Uncertainty (U_{rep})
- Recovery Uncertainty (U_{rec})
- Reference Material Uncertainty (U_{ref})

4.3.1. Repeatability contribution

After verification of the normal distribution and absence of outliers, the Repeatability Uncertainty Contribution (U_{rep}) was calculated, both from CRM (Table 4.6) and real leaves samples (Table 4.7) (see Appendix A Tab. A1).

Table 4.6 reports the mean value by six replicas of the CRM ($\bar{x}_{CRM}$), analysed as described in section 3.2, and the Repeatability Uncertainty (U_{rep}).

Elements	$\bar{x}_{CRM}$	U_{rep}	U_{rep}%
Y	1057	0.001	0.13
La	1637	0.001	0.14
Ce	2866	0.001	0.09
Pr	336	0.007	0.70
Nd	1286	0.002	0.16
Sm	256	0.006	0.56
Eu	62	0.012	1.23
Gd	270	0.004	0.43
Tb	33.8	0.018	1.84
Dy	188.1	0.003	0.33
Ho	35.9	0.020	2.04
Er	102	0.010	1.01
Tm	13.3	0.089	8.90
Yb	112	0.021	2.09
Lu	12	0.109	10.93

Tab. 4.6. REE experimental mean value of CRM ($\overline{x_{CRM}}$), repeatability uncertainty (U_{rip})

Table 4.7 reports the mean experimental value by six replicas of reals leaves samples ($\bar{x}_{leaves}$), analysed as described in section 3.2, and the Repeatability Uncertainty (U_{rep}).

Elements	$\bar{x}_{leaves}$	**U_{rep}**	**U_{rep}%**
Y	361.78	0.17	17.49
La	705.82	0.19	19.24
Ce	81.53	0.26	3.62
Pr	299.07	0.10	0.93
Nd	56.94	0.23	5.29
Sm	17.11	0.16	25.34
Eu	52.59	0.14	6.99
Gd	7.52	0.21	86.74
Tb	33.51	0.26	11.17
Dy	431.64	0.17	17.49
Ho	6.78	0.21	20.99
Er	16.20	0.24	24.49
Tm	2.77	0.26	26.24
Yb	12.29	0.35	34.98
Lu	2.44	0.33	33.23

Tab. 4.7. REE experimental mean values of Leaves ($\overline{x_{leaves}}$), repeatability uncertain (U_{rep})

We found that the repeatability contribution uncertainty, calculated from reals samples, is much higher than the reference material because real sample replicas are affected by sampling variability. Consequently, the contribution of repeatability of real samples will be taken into account in the calculation of the combined uncertainty.

4.3.2. Recovery contribution

The Recovery Contribution ($U_{rec)}$ was calculated from CRM (Table 4.8) (see Appendix A Tab. A1). Table 4.8 reports the certified concentration values of the CRM (C_{CRM}) (expected value), the mean experimental value by six replicas of the CRM ($\overline{x}_{CRM}$), analysed as described in section 3.2, and the Recovery Uncertainty (U_{rec}).

Elements	C_{CRM}	$\overline{x}_{CRM}$	U_{rec} %
Y	963	1057	2.34
La	1690	1637	3.17
Ce	2990	2866	3.67
Pr	321	336	3.61
Nd	1330	1286	4.42
Sm	264	256	2.88
Eu	60.2	62	3.54
Gd	243	270	8.28
Tb	34.7	33.8	3.49
Dy	184	188.1	27.18
Ho	34.5	35.9	7.27
Er	101	102	3.08
Tm	13.6	13.3	2.39
Yb	115	112	10.59
Lu	16.5	12	40.01

Tab. 4.8. Certified concentration values of CRM (C_{CRM}), REE experimental mean value of CRM ($\overline{x}_{CRM}$), Uncertain of the recovery % (U_{rec}%)

4.3.3 Reference materials contribution

To evaluate Reference Materials Contribution (Uref), the uncertainty of the standard material (declared by the supplier company) and of the uncertainty of the dilutions (U_{dil}) made to prepare the calibration standards must be taken into account (Appendix A Tab. A1).

Table 4.9 reports the uncertainty calculated for the stock solution of REE ($u_{stock\ sol}$) used to prepare the calibration standards.

	Unit	**Quantity**	**declared material uncertain**	**$u_{stock\ sol.}$**
Stock solution	(µg/mL)	1000	±5	0.0029

Tab. 4.9. Reference uncertainty for stock standard REE solution ($u_{stock\ sol}$)

The subsequent step was to evaluate the contribution of the uncertainty of the dilutions (U_{dil}) made to prepare the calibration standards solutions. Calibration standards were daily prepared in the range 1– 500 pg/mL by stepwise dilution of the multi-element stock standard solution. The dilution process is summarized in Table 4.10.

	Initial concentration	**Volumes**	**declared material uncertainty**	**dilution factor**	**Final concentration**	**u_{dil}**
I dilution	1000 (mg/L)	Flask class A (50 ml)	0.06	1000	1 (mg/L)	0.0007
		Pipette (0.05 ml)	0.0003			0.0034
II dilution	1 (mg/L)	Flask class A (50 ml)	0.06	1000	1 (µg/L)	0.0007
		Pipette (0.05 ml)	0.0003			0.0034
III dilution	1 (µg/L)	Flask class A (50 ml)	0.06	1000	1 (ng/L)	0.0007
		Pipette (0.05 ml)	0.0003			0.0034

Tab. 4.10. Dilution uncertainty to prepare the calibration standards solutions (u_{dil})

4.4. Combined Uncertainty

The combined uncertainty was calculated considering the contributions of the repeatability (U_{rep}), recovery (U_{rec}) and reference materials (U_{ref}). Combined uncertainty (U_c) was calculated both from repeatability of CRM (Table 4.11) and real leaves samples (Table 4.12), applying the law of propagation of the errors (see Appendix A2) (Skoog et al., 1988; McCormick, 2016).

Elements	**U_{rec}**	**$(U_{rep})_{CRM}$**	**U_{ref}**	**U_c**
Y	2.34	0.13	0.67	2.44
La	3.17	0.14	0.67	3.24
Ce	3.67	0.09	0.67	3.74
Pr	3.61	0.70	0.67	3.74
Nd	4.42	0.16	0.67	4.47
Sm	2.88	0.56	0.67	3.01
Eu	3.54	1.23	0.67	3.81
Gd	8.28	0.43	0.67	8.32
Tb	3.49	1.84	0.67	4.00
Dy	27.18	0.33	0.67	27.19
Ho	7.27	2.04	0.67	7.58
Er	3.08	1.01	0.67	3.31
Tm	2.39	8.90	0.67	9.24
Yb	10.59	2.09	0.67	10.82
Lu	40.01	10.93	0.67	41.48

Tab. 4.11. Recovery Uncertainty (U_{rec}), Repeatability Uncertainty from CRM $(U_{rep})_{CRM}$, Reference Material Uncertainty (U_{ref}), Combined Uncertainty (Uc), All contribute are expresses as percentages

Elements	U_{rec} %	$(U_{rep})_{Leaves}$	U_{ref}%	U_c%
Y	2.34	17.49	0.67	17.66
La	3.17	19.24	0.67	19.51
Ce	3.67	3.62	0.67	5.21
Pr	3.61	0.93	0.67	3.79
Nd	4.42	5.29	0.67	6.93
Sm	2.88	25.34	0.67	25.52
Eu	3.54	6.99	0.67	7.86
Gd	8.28	86.74	0.67	87.13
Tb	3.49	11.17	0.67	11.72
Dy	27.18	17.49	0.67	32.33
Ho	7.27	20.99	0.67	22.22
Er	3.08	24.49	0.67	24.69
Tm	2.39	26.24	0.67	26.35
Yb	10.59	34.98	0.67	36.56
Lu	40.01	33.23	0.67	52.02

Tab. 4.12. Recovery Uncertainty (U_{rec}), Repeatability Uncertainty from leaves $(U_{rep})_{leaves}$, Reference Material Uncertainty (U_{ref}), Combined Uncertainty (Uc), All contribute are expresses as percentages

Figure 4.2. shows the Combined Uncertainty (Uc) calculated considering the uncertainty contribution form leaves repeatability (U-Leaves) and from CRM (U-CRM).

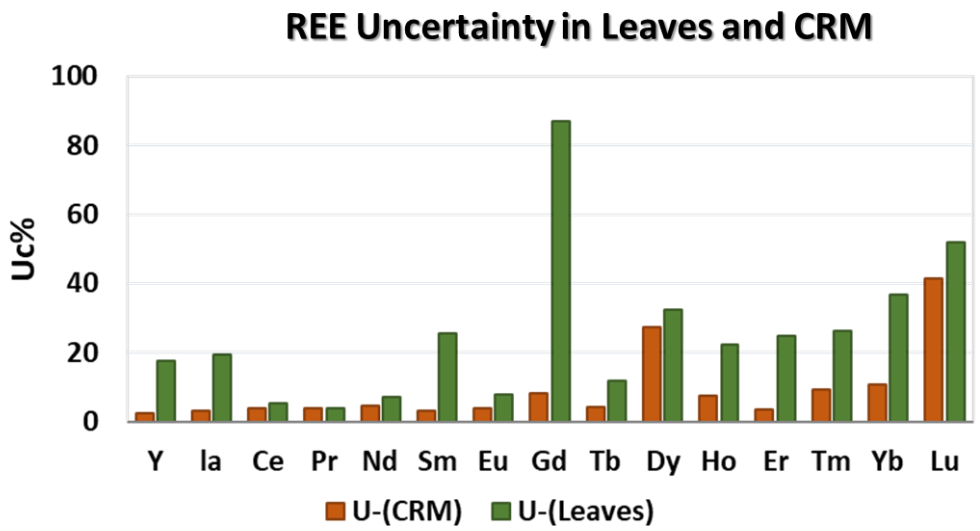

Fig. 4.2. Percentage of Combined Uncertainty (Uc%) calculated by the repeatability uncertainty from leaves (U-Leaves) and CRM (U-CRM)

We found that for all elements the uncertainty calculated from real samples is higher than uncertainty from CRM. Even if the uncertainty calculation from the CRM is a correct way to estimate the validity of the measurements (Ellison et al., 2012), we found that in our case is not representative to real variability of the system. Indeed, if the variability from real samples was not been tested, we had greatly underestimated the intrinsic variability of the system. This should make us reflect on the importance of validation planning and the strategies adopted to obtain a realistic estimate of the uncertainty.

The combined uncertainty has also been calculated both for soils and roots by real samples, we do not show the data for avoiding redundancies. However, the highest values were obtained for the leaves probably because the concentration values are lower.

4.5. REE measurements validation in xylem-sap

This section reports the strategies adopted to estimate the uncertainty of REE measurements in the xylem-sap. To our knowledge, neither official data nor certified materials of REE content in xylem-sap are available, probably due to the difficulty of determining trace elements from a very low amount of fluid and at the sub µmolar level. Also, the low amount of sap extracted implies that replicates cannot be performed and preconcentration techniques cannot be applied. Since a validation study needs official data or replicates of real samples, we need to ask how to implement a procedure to control the quality of the data. Therefore, to assess the variability of the REE determination in xylem-sap we developed a data evaluation strategy from the following considerations:

Strengths points

- Xylem-sap analysis can be performed by direct injection
- No sample pre-treatment is required
- Xylem-sap matrix effect should be negligible

Drawbacks

- Very low xylem-sap amount extract (c ~200 µl)
- REE values near the instrumental quantification limit

4.5.1 Matrix effect evaluation

Due to the difficulty of performing replicas of xylem-sap we verified the matrix effect. Indeed, if the matrix effect is negligible the validation tests can be carried out directly in water (Ellison et al., 2012). To assess if two systems are comparable, we need to prove that the spread of two datasets belongs to the same population; in other words, if the precisions are similar or dissimilar. This can be performed by comparing the variance of the two systems using the F Test (Appendix A1.7).

In this work, we compared a series of replicate measurements between xylem-sap and water. We prepared in-house reference material of the xylem-sap by mixing sap extracted from five *Vitis vinifera L.* plants. We choose to mix the extracts to have a sufficient amount of sap to perform replicates. The choice of mixing the extracts may not be shared, as it could be objected that sampling variability is not taken into account. Actually, to assess the matrix effect, all factors that are not directly related to the nature of the matrix itself should be minimized or eliminated, therefore the sampling variability should be made negligible. Furthermore, reference sample must be as homogeneous as possible since it has been compared to a homogenous system, such as the water matrix. The xylem-sap REE in-house reference material was analysed as reported in section 3.4. REE concentrations were calculated and five aliquots of water sample were prepared by adding an amount of REE corresponding to the value detected in xylem sap, thus two comparable systems were obtained.

Table 4.13 reports the mean concentrations of REE, in xylem-sap ($\bar{x}_{Xy}$) and in water ($\bar{x}_{Sw}$), the standard deviations ($\sigma_{(Sw)}$ and $\sigma_{(Xy)}$), the variances ($S^2_{(Sw)}$ and $S^2_{(Xy)}$) for both matrix and the results of the F test.

	Spiked Water (Sw)				Xylem-sap matrix (Xy)					
Elements	$\bar{x}_{Sw}$	**σ (Sw)**	**S^2 (Sw)**	**RSD%(Sw)**	$\bar{x}_{Xy}$	**σ (Xy)**	**S^2 (Xy)**	**RSD%(Xy)**	**F_{calc}**	**F_{th}**
La	15.12	1.23	1.51	8.11	15.43	2.58	6.66	16.72	4.42	5.05
Ce	25.23	2.03	4.13	8.06	25.07	4.33	18.75	17.27	4.54	5.05
Pr	2.00	0.40	0.16	19.81	1.83	0.28	0.08	15.07	2.06	5.05
Nd	8.49	0.61	0.37	7.16	6.15	1.15	1.32	18.65	3.56	5.05
Sm	1.91	0.21	0.04	11.03	1.98	0.42	0.18	21.44	4.05	5.05
Eu	1.22	0.12	0.01	9.85	0.94	0.23	0.05	24.52	3.67	5.05
Gd	2.17	0.20	0.04	9.37	1.72	0.47	0.22	27.16	5.27	5.05
Tb	1.09	0.27	0.07	24.75	0.59	0.25	0.06	41.97	1.20	5.05
Y	5.16	0.91	0.83	17.66	6.39	1.96	3.85	30.71	4.64	5.05
Dy	1.21	0.15	0.02	12.46	1.90	0.39	0.15	20.26	6.59	5.05
Ho	1.15	0.11	0.01	9.22	0.82	0.26	0.07	32.11	6.27	5.05
Er	1.03	0.16	0.02	15.19	0.93	0.34	0.11	36.42	4.70	5.05
Tm	0.96	0.12	0.02	12.80	0.60	0.27	0.07	44.20	4.69	5.05
Yb	1.32	0.21	0.04	15.73	1.15	0.44	0.19	37.90	4.40	5.05
Lu	1.19	0.13	0.02	11.13	0.72	0.26	0.07	36.52	3.97	5.05

Tab. 4.13. REE mean value in Spiked Water **($\bar{x}_{Sw}$),** REE mean value in xylem-sap **($\bar{x}_{Xy}$),** standard deviation (σ_r), variance (S^2), relative standard deviation (RSD%), in ng/L and F_{cal} and F_{th}

The results show that the relation $F_{calc} < F_{th}$ is verified for most of the elements considered meaning that there is no significant variation between the two systems (Appendix A1.7). Only Ga, Dy and Ho show a slight deviation, probably, due to the value lower than the IQL for Ga and Dy and the value very close to the IQL for the Ho. This result allows to bypass the first drawback point (very low xylem-sap amount extract) providing a way to evaluate the contributes of uncertainty in water.

4.5.2. Combined Uncertainty

To plan the validation studies, we must keep into account that the contribution of the recovery can be considered negligible because measurements are performed in direct injection. Therefore, to estimate the combined uncertainty, only the contributions of repeatability (U_{rep}) and reference materials (U_{ref}) were considered. To evaluate the repeatability uncertainty (U_{rep}), we considered that the critical point is that the REE amount in xylem-sap is very close to the IQL. Therefore, repeatability uncertainty was performed by ten independent replicated measurements on water samples spiked with REE amounts near the IQL and at 10 ng/l.

Table 4.14 reports the mean value of REE measurements in spiked water ($\bar{x}_{Sw}$), the standard deviation (σ_{Sw}), the repeatability limit (r) and Repeatability Uncertainty contribution (U_{rep}) calculated at REE values near the IQL.

Elements	$\bar{x}_{Sw}$	σ_{Sw}	RSD%	r	(U_{rep})	U_{rep}%
Y	1.02	0.01	0.88	3.21	0.35	35.16
La	9.90	1.29	13.00	47.28	0.53	53.42
Ce	15.40	0.43	2.77	10.07	0.07	7.31
Pr	1.20	0.02	1.60	5.83	0.54	54.35
Nd	9.78	0.59	6.02	21.87	0.25	25.00
Sm	5.45	0.45	8.25	30.01	0.62	61.56
Eu	1.23	0.02	1.98	7.21	0.66	65.50
Gd	3.43	0.10	2.99	10.87	0.35	35.43
Tb	2.40	0.13	5.51	20.04	0.93	93.34
Dy	1.35	0.03	2.36	8.56	0.71	71.12
Ho	1.21	0.03	2.65	9.63	0.89	88.97
Er	1.48	0.02	1.14	4.13	0.31	31.25
Tm	0.91	0.01	0.91	3.31	0.41	40.90
Yb	1.26	0.04	2.98	10.83	0.96	96.43
Lu	1.56	0.06	3.53	12.83	0.92	91.94

Tab. 4.14. REE experimental mean value of Spiked Water $(\bar{x}_{Sw})$, standard deviation (σ_{Sw}), Relative Standard Deviation (RSD%) repeatability limit (r) and Repeatability Uncertainty (U_{rep}) of the ten replicas (n) obtained by spiked water at REE values near the IQL

We found a very high values of uncertainty for most elements investigated. In order to evaluate how much the concentration value is critical in results evaluation, we calculate the Repeatability Uncertainty contribution at values about an order of magnitude greater than the IQL. We perform ten independent replicated measurements on water samples, spiked with 10 ng/L of each REE.

Table 4.15 reports the mean value of REE measurements in spiked water ($\bar{x}_{Sw}$), the standard deviation (σ_{Sw}), the repeatability limit (r) and Repeatability Uncertainty contribution (U_{rep}) calculated at 10 ng/L.

Elements	$\bar{x}_{Sw}$	**σr**	**RSD%**	**r**	(U_{rep})	**U_{rep}%**
Y	10.43	0.40	3.84	13.97	0.15	14.98
La	9.50	1.19	12.50	45.44	0.54	53.50
Ce	9.79	0.33	3.40	12.35	0.14	14.09
Pr	9.98	0.06	0.59	2.16	0.02	2.42
Nd	10.49	0.61	5.80	21.08	0.22	22.45
Sm	10.08	0.55	5.46	19.83	0.22	22.00
Eu	10.15	0.54	5.36	19.47	0.21	21.44
Gd	10.06	0.10	1.02	3.71	0.04	4.12
Tb	9.95	0.15	1.53	5.56	0.06	6.25
Dy	9.95	0.05	0.52	1.89	0.02	2.12
Ho	9.91	0.07	0.73	2.64	0.03	2.98
Er	9.88	0.17	1.70	6.18	0.07	7.00
Tm	9.51	0.83	8.68	31.55	0.37	37.12
Yb	9.86	0.37	3.79	13.80	0.16	15.65
Lu	10.16	0.11	1.03	3.76	0.04	4.14

Tab. 4.15. REE experimental mean value of Spiked Water **($\overline{x_{Sw}}$),** standard deviation (σ_{Sw}), Relative Standard Deviation (RSD%) repeatability limit (r) and Repeatability Uncertainty (U_{rep}) of the ten replicas (n) obtained by spiked water at REE concentration 10 ng/l

We found that the increase of one order of magnitude in REE concentration leads to a huge variation in repeatability estimation. For example, RSD% for Lutetium is 4% at 10 ng/l while is 90% at 1 ng/l, highlighting how important is the choice of the specific conditions in repeatability studies.

The other uncertain contributions to keep in consideration were the uncertainty of the reference materials and the sample dilution. For reference material contribution (U_{ref}) we can refer to the value calculated for leaves validation (Table 4.9 and 4.10). Whereas, sample dilution contribution was reported in Table 4.16 (See Appendix A Tab. 1A).

	Volumes	**declared material uncertainty**	**U_{dil}**
Sample dilution	Pipette (3 ml)	0.05	0.0096
	Pipette (0.1 ml)	0.0004	0.0023

Tab. 4.16 Sample Dilution Uncertainty U_{dil}

Applying the law of propagation of the errors (Appendix A Tab. A1) reference material (U_{ref}), sample dilution (U_{dil}), repeatability contributes (U_{rep}) were included in the final formula for combined uncertainty (U_c).

Combined Uncertainty (Uc) was calculated from repeatability tests at 10ng/L (Table 4.17) and at REE concentration near the IQL (Table 4.18).

Elements	**(U_{rep})**	**U_{ref}**	**U_{dil}**	**U_c**
Y	14.98	0.67	0.99	15.02
la	53.50	0.67	0.99	53.52
Ce	14.09	0.67	0.99	14.15
Pr	2.42	0.67	0.99	2.70
Nd	22.45	0.67	0.99	22.49
Sm	22.00	0.67	0.99	22.03
Eu	21.44	0.67	0.99	21.47
Gd	4.12	0.67	0.99	4.29
Tb	6.25	0.67	0.99	6.37
Dy	2.12	0.67	0.99	2.44
Ho	2.98	0.67	0.99	3.21
Er	7.00	0.67	0.99	7.10
Tm	37.12	0.67	0.99	37.13
Yb	15.65	0.67	0.99	15.70
Lu	4.14	0.67	0.99	4.31

Tab. 4.17. Repeatability Uncertainty from spiked waters (U_{rep}) at 10ng/L, Reference Material Uncertainty (U_{ref}), sample Dilution Uncertainty (U_{dil}), Combined Uncertainty (U_c). All contributes are expressed as percentage

Elements	$(U_{rep})_{sw}$	U_{ref}	U_{dil}	U_c
Y	35.16	0.67	0.99	35.18
la	53.42	0.67	0.99	53.43
Ce	7.31	0.67	0.99	7.41
Pr	54.35	0.67	0.99	54.37
Nd	25.00	0.67	0.99	25.03
Sm	61.56	0.67	0.99	61.57
Eu	65.50	0.67	0.99	65.52
Gd	35.43	0.67	0.99	35.45
Tb	93.34	0.67	0.99	93.35
Dy	71.12	0.67	0.99	71.13
Ho	88.97	0.67	0.99	88.97
Er	31.25	0.67	0.99	31.28
Tm	40.90	0.67	0.99	40.92
Yb	96.43	0.67	0.99	96.44
Lu	91.94	0.67	0.99	91.95

Tab. 4.18. Repeatability Uncertainty from spiked waters (U_{rep}) at 1ng/L, Reference Material Uncertainty (U_{ref}), sample dilution Uncertainty (U_{dil}), Combined Uncertainty (U_c). All contributes are are expressed as percentage

Figure 4.3 shows the combined uncertainty calculated by the contribution of the repeatability uncertainty from water fortified with REE at 1 and 10 ng/l concentration values.

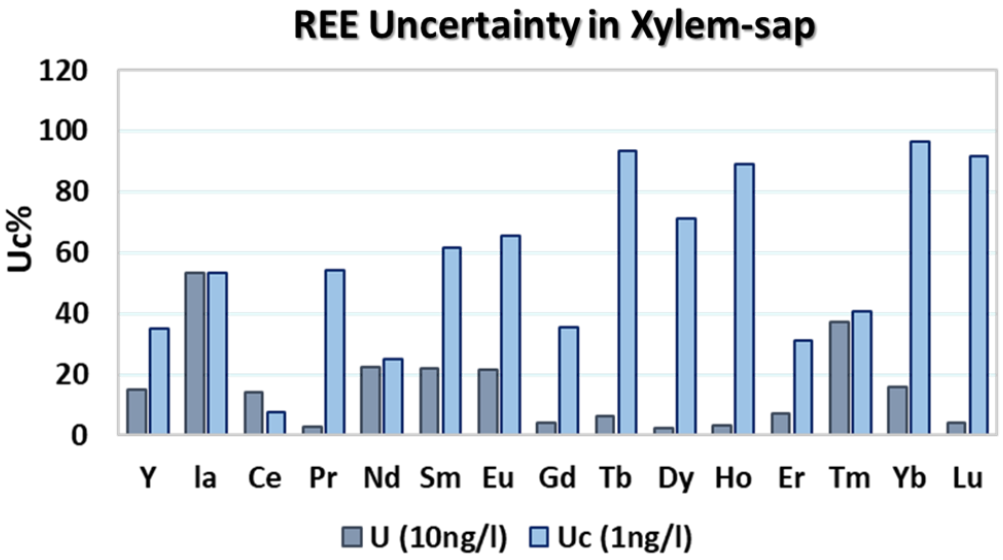

Fig. 4.3. Percentage of Combined Uncertainty (Uc%) calculated by the repeatability of spiked water at 1 and 10 ng/l.

Results clearly show that both the contribution of sample preparation and dilution is not significant. It is possible to observe that the uncertainty, basically, depends on the concentration. Therefore, in this work the uncertainty is attributed in function of the REE concentration detected in xylem-sap.

4.6. Quality control

In this section, we report the activities carried out intending to ensure that errors in the analytical data are of a magnitude appropriate for use. We planned the Quality Control (QC) in the routine analysis to verify that the method and the system performance were coherent with the validation parameters, focusing on the most critical performance. The main critical points in leaves, roots and soil REE determination are the repeatability of real samples and the REE extraction efficiency. Whereas, the critical point for the REE determination in xylem-sap is hight variability at REE values near the IQL. Checks results of the Quality Control (QC) were plotted in X-chart (detail to draft the X-Chart is reported in Appendix A3). Control charts were performed for all elements measured while X-chart was drawn in function of dispersion measurements of a set of values as described in Appendix A3. The precision range was calculated during the validation study. Figure 4.4 shows the X-cart of gadolinium obtained by different measurements of CRM while Figure 4.5 shows the X-chart of lanthanum obtained by calibration near the IOQ.

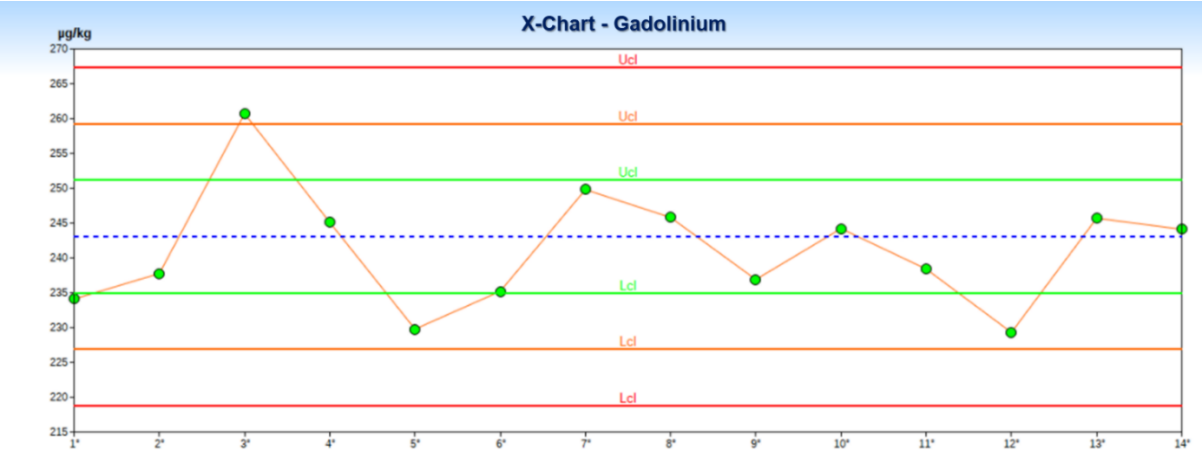

Fig. 4.4. X-Chart of Gadolinium from CRM.

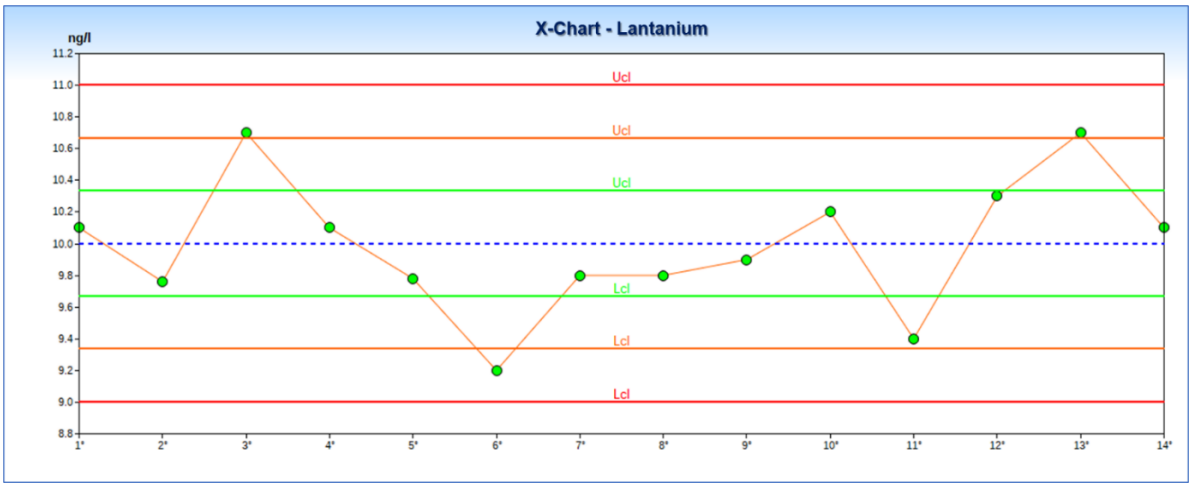

Fig. 4.5. X-Chart of Lanthanum from calibration solution (10ng/L)

The X-chart of Figures 4.4 and 4.5 show that the process is under control during the time of measurements meaning that Variability is coherent with the value obtained, in terms of the efficiency of extraction and sensibility. The within-batch precision was obtained analysing two samples independently prepared twice in the same batch (duplicate analysis). Results were accepted if X_1-X_2<r, where X_1 and X_2 were concentration values of independent replicas of the same sample and r is the repeatability limit determined during the validation studies. All data

used in this thesis were under control from X-charts and duplicate analyses. When results were not under control the batch was eliminated or repeated.

Chapter 5: Results and Discussions

In this chapter are reported the results obtained investigating the transfer dynamics of REE in the soil/*Vitis vinifera L*. system. Our results are related to two experimental investigations:

- The first was carried out in a greenhouse, here *Vitis vinifera L.* grew on a control substrate with low REE content and the spiked substrate was artificially enriched in REE by three orders of magnitude respect the soil substrate.
- The second was carried out in field: here we used a control substrate poor in REE having a similar profile to natural soil and a spiked substrate artificially enriched 2-times in REE compared to the control substrate.

Studying the physiological response of *Vitis vinifera L*. to stress generated by contaminated soil, we have tried to answer the questions set to plan the experiments. Those are below recalled:

- Does REE enrichment in substrate influences the plant growth and REE accumulation in roots, xylem-sap and leaves?
- Is the uptake of REE selective in *Vitis vinifera L.*?
- Is REE* useful to discriminate small differences in REE amount?
- Does REE* fractionate in the xylem-sap?
- Is *Vitis vinifera L.* physiology sensitive to the REE transport?

The main results of these investigations are presented in the form of articles.

5.1. Does REE enrichment in the substrate influence plant growth and REE accumulation in roots, xylem-sap and leaves? Is the uptake of REE selective in *Vitis vinifera L.*?

In this article we have evaluated the overall REE transport by the total REE accumulation (∑REE) in plant organs during plant development, questioning if the plant growth is influenced by the REE-enriched substrate and if REE transport in *Vitis vinifera L.* depends on the level of REE in the substrate of growth. We evaluated the variation in REE relative abundance by full normalised spectra in the different compartments in function of the different amounts of REE present in the substrates. The correspondence between the elemental profile of the *Vitis vinifera L.* organs with the substrates/soil was verified. We wondered if the uptake of REE is selective in *Vitis vinifera L.* and if the normalized distribution patterns (REE*) are useful to discriminate the artificial addition of REE in the substrates.

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The distribution of Rare Earth Elements discriminates the growth substrate of *Vitis vinifera L.*

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Highlights

- Rare Earth Element pattern in *Vitis vinifera L.* plants discriminate natural from contaminated soils.
- Roots primary accumulate REE from contaminated soils.
- The mass of *Vitis vinifera L* plant is not affected by REE contaminated soils.

Abstract

Sustainable agricultural, food-related strategies and geographic traceability require understanding of the plant physiological response to stress potentially generated by contaminated soils. Here, we have investigated the effect of contaminated substrate on growth of *Vitis vinifera L.* plants analysing the distribution of full Rare Earth Elements (REE) spectra in different parts of the plant. Experiments were carried out using pristine plants growing in a handmade substrate (blank experiment) and in REE artificially-enriched soil (spiked experiment). Our results show that both plant mass and REE amount in leaves are not influenced by the substrate enrichment while roots are by one-order of magnitude enriched for three-orders of magnitude enhancement of the soil substrate. This clearly indicates that soil contamination does not significantly influence the REE amount in the aerial parts. However, the spectra of REE normalized changes when the soil is enriched. We found that Light-REE (from La to Gd) are by more than one order of magnitude enriched compared to Heavy-REE (from Tb to Lu plus Y) in spiked experiment showing the specific response of *Vitis vinifera L*. to the stress generated by soil contamination. We propose that REE distribution spectra is a marker of *Vitis vinifera L.* substrate of growth and providing a new tool for tracing the geographical origin of agri-food products.

1. Introduction

The identification of foodstuff provenance is of significant importance for quality control, food safety and adulteration pushing consumers and legislators to develop tools for clear identification of food geographical origin (Richter et al., 2019). The social and economic extent questioned also the European Union estimating that cost of food fraud is by 8–12 billion euro per year (European Commission, 2019) advocating thus the urgency for product quality and food authenticity (Brunner et al., 2010; Drivelos and Georgiou, 2012; Marchionni et al., 2016). We focused on *Vitis vinifera L.* growth because grapevine cultivated worldwide for fruits, wine and juice (Vivier et al., 2002; Laucou et al., 2018) adapts in different soils and climates (Soneji et al., 2011). We questioned the plant physiological response to Rare Earth Elements (REE) contaminated soil evaluating the transport from soil to plant in view of discriminating polluted soil from the 'natural' one. REE consist of 14 unique elements from ^{58}Ce to ^{71}Lu (Lanthanoids series) that, associated with Y and La, are characterised by the progressive filling of the 4f orbitals. REE have similar chemical and geochemical behaviour in solids while are governed by interface processes in liquid-surface reactions with both inorganic and organic materials (Censi et al., 2015; Vermeire et al., 2016). The overall REE spectra may also reflect complex reaction mechanisms of soil-plant transfer (Tyler, 2004; Liang et al., 2008; Brioschi et al., 2013; Sasmaz et al., 2018) making then potential tracers for food-product quality (Bettinelli et al., 2005; Bertoldi et al., 2009, 2011; Oddone et al., 2009; Aceto et al., 2013; Censi et al., 2014; Pepi et. al 2016, 2017, 2018; Pisciotta et al., 2017; Punturo et al., 2018).

The distribution of REE is of potential interest for identifying the geographical origin of agri-food products as these trace metals are emerging contaminants resulting from increasing

technological uses and agricultural practices (Miao et al., 2011; Li et al., 2013; Pagano et al., 2015a). A relation between REE content in plant and human body transfer has not been completed established while the toxic effect of REE was identified in bacteria, animals and humans (Feng et al., 2006; Censi et al., 2011; Jiang et al., 2012; Durães et al., 2014; Pagano et al., 2015b; Wang et al., 2017; Turra et al., 2018; Krzciuk and Gałuszka, 2019).

REE behaviour plant was previously investigated in dose-response studies during hydroponic growth making critical the extension to real-growth situations (Thomas et al., 2014). For this purpose, we investigated the REE distribution in the different parts of plant during one-year-long experiments using two different substrates: one with a handmade substrate (blank experiment) and another with the same substrate artificially enriched in REE (spiked experiment). We questioned if during growth an enriched substrate could influence the mass of *Vitis vinifera L.* and metal accumulation in the different plant organs. Evaluating the REE transport from roots to the aboveground organs using the full REE distribution spectra, we want to assess if the different soil of growth can be discriminated by the analysis of the final product. Pioneer works on *Vitis vinifera L.* adopted a multivariate statistic approach (Bertoldi et al., 2011; Aceto et al., 2013) while recently the REE properties were proposed as a possible tracer of soil-grape reactions (Censi et al., 2014; Pisciotta et al., 2017). We carried out controlled experimental plant growth and report evidence of REE fractionation between native soil and plant organs establishing possible discrimination of the growth conditions of *Vitis vinifera L.*

2. Materials and methods

2.1. Experimental set-up

Thirty pristine *Vitis vinifera L*. individual plants (Moscato d'Asti, rootstock 1103 P) of one year in age from native soils were used in an off-soil experiment at the Department of Agricultural Food and Forest Sciences of the University of Palermo (Italy). The choice of experimental substrate is fundamental because REE mobility depends on complexation reactions with the main substrate components and root exudates. We selected a gravel and peat substrate constituted by humic acids having very low REE content. This REE amount is lower than the Limit of Quantification, LOQ, as reported in supplementary materials. We assessed the plant physiological response to contaminated soil (spiked experiment) enriching by 3 orders of magnitude the experimental substrate of growth (blank experiment). REE determined in the experimental substrate are lower than 0.9 nmol/kg (maximum value of the quantitation limit). Therefore, the addition of 2.5 µmol per Kg of each REE corresponds to an enrichment of at least 3 orders of magnitude. Pristine plants were put into polyurethane pots with a homemade substrate with a peat-gravel ratio of 2:3 w/w. We used the same amount (5 Kg) of substrate for both experimental conditions. Before planting, the root length was uniformed to 10 cm to assure an equivalent development. Multi-REE working standard solution (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) was prepared by diluting the standard stock solution (1000 ppm; pH<1) to 0.05 mM of each element in high purity water (pH ~ 5.5). Here, REE are considered under the form of free aqueous-ions (De Boher et al., 1996). *Vitis vinifera L*. was planted in experimental pots with 5 Kg of peat-gravel substrate and 250 ml of multi-REE solution (0.05 mM). We choose 250 ml as this volume allows homogenising the spiked

substrate without solution leaks. We measured pH in both solution substrates before and after the addition of multi-REE solution and did not observe significative variation between starting and final experimental solutions. Two different growth conditions were investigated: one using the homemade substrate (blank experiment) and another adopting the same substrate spiked with 2.5 µmol per Kg of each REE (spiked experiment) (Fig.1).

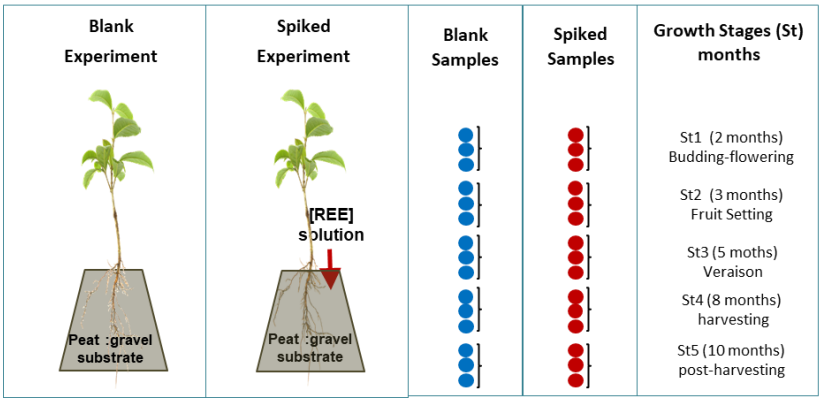

Figure 1. The off-soil experimental system

In both experimental conditions, plants were homogeneously irrigated avoiding material loss by leaching and stress while no disease spray was used. Sampling was carried out at five different growth stages (St) selected according to the different plant-life periods: budding-flowering (2 months), fruit-setting (3 months), veraison (5 months), harvesting (8 months), post-harvesting (10 months). Each sampling was replicated three times and for every sampling time, plants were separated into roots and aerial parts. Roots were in turn separated in woody ($Ø \geq 2mm$), middle ($1mm \leq Ø \leq 2mm$) and fine-roots ($Ø \leq 1mm$), while aerial parts in leaves and herbaceous shoot, petioles, wood shoot (one-year-old), wood shoot (two years old). The REE determination was performed for every sampled part. The experiment was conducted in a

greenhouse, therefore the potential influence of air particulate contamination was considered negligible (Bargagli, 1998). In leaves, the influence of atmospheric fallout was found in areas of high anthropogenic impact (Censi et. al., 2017).

2.2. Analysis

2.2.1. Sample preparation

Ultrapure grade reagent, nitric acid (65%), hydrogen peroxide (30%) and standard solutions (yttrium, lanthanides and rhenium, each 1000 ± 5 mg/L), were purchased from BDH, Merck and CPI International (Italy) while ultrapure water 18.2 $M\Omega cm^{-1}$, produced with an EASYpureII (Thermo, Italy), was used. Every part of the plant was weighed, chopped, dried (105°C for 24h), grounded in an agate mortar and stored in a PE vessel. Substrate sample and native soil were dried in an oven at 105°C, gently crushed, sieved (Ø 0.5 mm) and homogenized. 0.500 g (DW, dried weight) of organ's plant and 0.250 g (DW) of both substrates and native soil were transferred in a Teflon vessel with 4.5 ml of 2:1 v/v mixture of HNO_3:H_2O_2 and put in a microwave oven. All samples were digested in a closed microwave system (MarsXpress, CEM, Milan Italy) with an increasing temperature from room temperature to 200°C in 10 minutes. The final temperature was maintained for 50 min. Power was 1600 Watt, while pressure was not controlled. After digestion, the extract plant organs were quantitatively transferred into graduate polypropylene test tubes and diluted with ultrapure water to 10 mL, whereas substrates and native soil extract were diluted with ultrapure water to 100 mL. Every analytical sequence included a procedural blank: ultrapure water digested as a sample.

2.2.2. Chemical measurements

REE concentration was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using the Agilent Technologies 7500ce Series Spectrometer at the following operative conditions: power 1550 W; nebulizer gas flow 1.00 L/min; auxiliary gas flow 0.85 L/min and plasma gas flow 15 L/min. Acquisition time was 180 s and three replicates were performed. Analyses were carried out with an external calibration method in the range between 1.0 and 10.000 pg/mL, for every investigated element (^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{151}Eu, ^{158}Gd, ^{159}Tb ^{163}Dy, ^{89}Y, ^{165}Ho, ^{167}Er, ^{169}Tm, ^{172}Yb, ^{175}Lu) while ^{187}Re (1000 pg/mL) was used as internal standard. Stability test was performed before each analysis monitoring ^{7}Li, ^{59}Co, ^{89}Y, ^{140}Ce, and ^{205}Tl masses and adopting at least 2% of precision. The oxide and doubly charged ion interferences were controlled verifying that CeO^+/Ce^+ and Ce^{2+}/Ce^+ ratios were less than 0.5%. Possible isobaric interferences related to europium isotopes (^{151}Eu, ^{153}Eu) by polyatomic barium oxide ions ($^{135}Ba^{16}O^+$, $^{137}Ba^{16}O^+$) were estimated using certified standard INCT-OBTL-5 Oriental Basma Tobacco Leaves and the Ba concentrations. We did not find any interference as the (Ba/Eu) ratio was less than 200 (De Boer et al., 1996; Cao et al., 2001).

2.2.3. Data quality assurance

The quality of the REE analytical determination, linearity, precision, sensitivity and recovery of the method was also evaluated. Specifically, linearity (regression coefficient and dynamic range) was estimated by a duplicate of eight standard solutions and using correlation coefficient higher than 0.99 for all REE in the 0.001 - 10 µg/L working range. The precision (repeatability), expressed as relative standard deviation percentage (RSD%), was determined by repeating six

times each calibration level and obtaining RSD% lower than 5% for all measurements. Limit of Detection (LOD) as well Limit of Quantification (LOQ) were determined by six calibration blank solutions (1% ultra-pure HNO_3) through the following relations:

$$LOD = \mu b + 3\ \sigma b \qquad 1$$

$$LOQ = \mu b + 10\ \sigma b \qquad 2$$

where μb is the blank signal and σb is the deviations standard of the blank. The recovery was estimated by the INCT-OBTL-5 Oriental Basma Tobacco Leaves certified standard materials (Samczynski et al., 2012) considering values higher than QL. The recovery percentage ranged between 85% and 111% (data reported in supplementary materials) while the error was estimated by the propagation formula (Ambrus et al., 2004). The uncertainty (uδ) corresponding to laboratory bias was estimated by different aliquots of certified material (INCT-OBTL-5 Oriental Basma Tobacco Leaves) measured in 10 not-consecutive days according to ISO/IEC 11352:2012 guideline. Results are reported in supplementary materials.

2.3.Data processing

The overall plant development was evaluated by the variation of dried mass during growth. Possible accumulation of REE in organs was estimated by concentrations in roots and aerial parts. The Translocation Factors (TF), corresponding to the ratio between the REE concentration in aerial parts and roots (Krzciuk and Gałuszka, 2015), was evaluated according to Eq.4:

$$TF = \frac{[REE]_{aerial\ part}}{[REE]_{roots}} \qquad 4$$

Where: $[REE]_{aerial\ part}$ and $[REE]_{roots}$ are the REE concentration (µmol/g) in both aerial part and root respectively. When TF<1, metal accumulate preferentially in roots, when TF>1, metal accumulate preferentially in aerial part, while when TF < 0.1, the plants tend to exclude the metal (Wang et al 2012).

The variation of REE abundance in different parts of the plant was calculated by the ratio between the sum of light REE (ΣLREE) and the sum of heavy REE (ΣHREE) through the following Eqns.:

$$\left[\frac{\sum LREE^*}{\sum HREE^*}\right]_{aerial\ part/UCC} = \frac{\frac{\sum LREE_{aerial\ part}}{\sum HREE_{aerial\ part}}}{\frac{\sum LREE_{UCC}}{\sum HREE_{UCC}}} \qquad 5$$

$$\left[\frac{\sum LREE^*}{\sum HREE^*}\right]_{Roots/UCC} = \frac{\frac{\sum LREE_{Roots}}{\sum HREE_{Roots}}}{\frac{\sum LREE_{UCC}}{\sum HREE_{UCC}}} \qquad 6$$

where ∑LREE is the sum concentration from La to Gd, and ∑HREE is the sum concentration from Yb to Lu plus Y. The subscript "UCC" (Upper Continental Crust) corresponds to the average lithospheric reference. "Aerial part" and "Roots", correspond to REE concentration (nmol/kg) in aerial part and roots respectively. For every sample, the distribution pattern of REE was calculated normalizing the relative abundance of REE in different plant organs to the lithological reference (UCC) (Laveuf and Cornu, 2009; Pisciotta et al., 2017).

$$[REE^*]_{native\ soil} = \frac{REE_{native\ soil}}{REE_{UCC}} \quad 7$$

$$[REE^*]_{roots} = \frac{REE_{roots}}{REE_{UCC}} \quad 8$$

$$[REE^*]_{aerial\ part} = \frac{REE_{aerial\ part}}{REE_{UCC}} \quad 9$$

The REE distribution pattern allows promptly evaluates an enrichment or depletion of a group or an individual REE relative to the others. These relative differences are called respectively fractionations or ‘‘anomalies”, whose intensities are further expressed by ratios (Laveuf and Cornu, 2009). The possible REE anomalies (Censi et al., 2014) have been estimated by the following Eqns. 10 and 11:

$$\left[\frac{REE}{REE^*}\right]_{shoots/roots} = \frac{\left(\frac{REE_{shoots}}{REE_{roots}}\right)_i^2}{\left[\left(\frac{REE_{shoots}}{REE_{roots}}\right)_{i+1}\left(\frac{REE_{shoots}}{REE_{roots}}\right)_{i-1}\right]} \quad 10$$

$$\left[\frac{REE}{REE^*}\right]_{shoots/UCC} = \frac{\left(\frac{REE_{shoots}}{REE_{UCC}}\right)_i^2}{\left[\left(\frac{REE_{shoots}}{REE_{UCC}}\right)_{i+1}\left(\frac{REE_{shoots}}{REE_{UCC}}\right)_{i-1}\right]} \quad 11$$

where the subscript “UCC”, “aerial part”, “roots” correspond to the REE concentration in the reference material, in aerial part and roots respectively. The subscript “i” indicates every REE of the series, whereas “(i − 1)” and “(i + 1)” are immediate neighbours, before and after within the series (Laveuf and Cornu, 2009; Censi et al., 2014).

3. Results and Discussion

3.1. Overall transport of REE in Vitis vinifera L.

We have initially evaluated the plant mass variation along the 10 months of growth. Fig. 2 shows that in the first eight months, plant weight progressively increased by 4 times in both spiked and blank experiments, while later decreased reflecting the period of rest corresponding to leave's fall. During the time of investigation, we did not find either plant mass changing between spiked and blank experiments or visible disease such as growth accidents, indicating that the substrate enrichment does not significantly influence the growth of *Vitis vinifera L.*

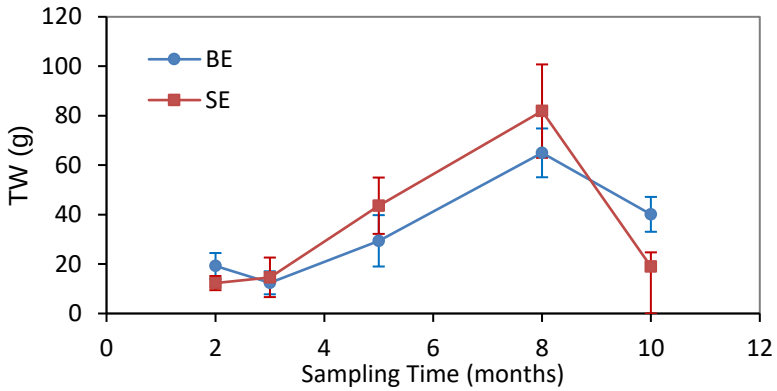

Figure 2. Total Weight (TW) of the plants in Blank and Spiked Experiment (BE and SE respectively) as a function of the time of growth

Our results are in agreement with that observed in *Arabidopsis thaliana*, corn (*Zea mays*) and *mungbean Vigna* growing from soils enriched in Ce and La (He and Loh, 2000; Diatloff et al., 2008). However, we found that in roots the total REE concentration in the spiked experiment is by one order of magnitude higher compared to the blank suggesting the possible filter role of plant roots (Brioschi et al., 2013).

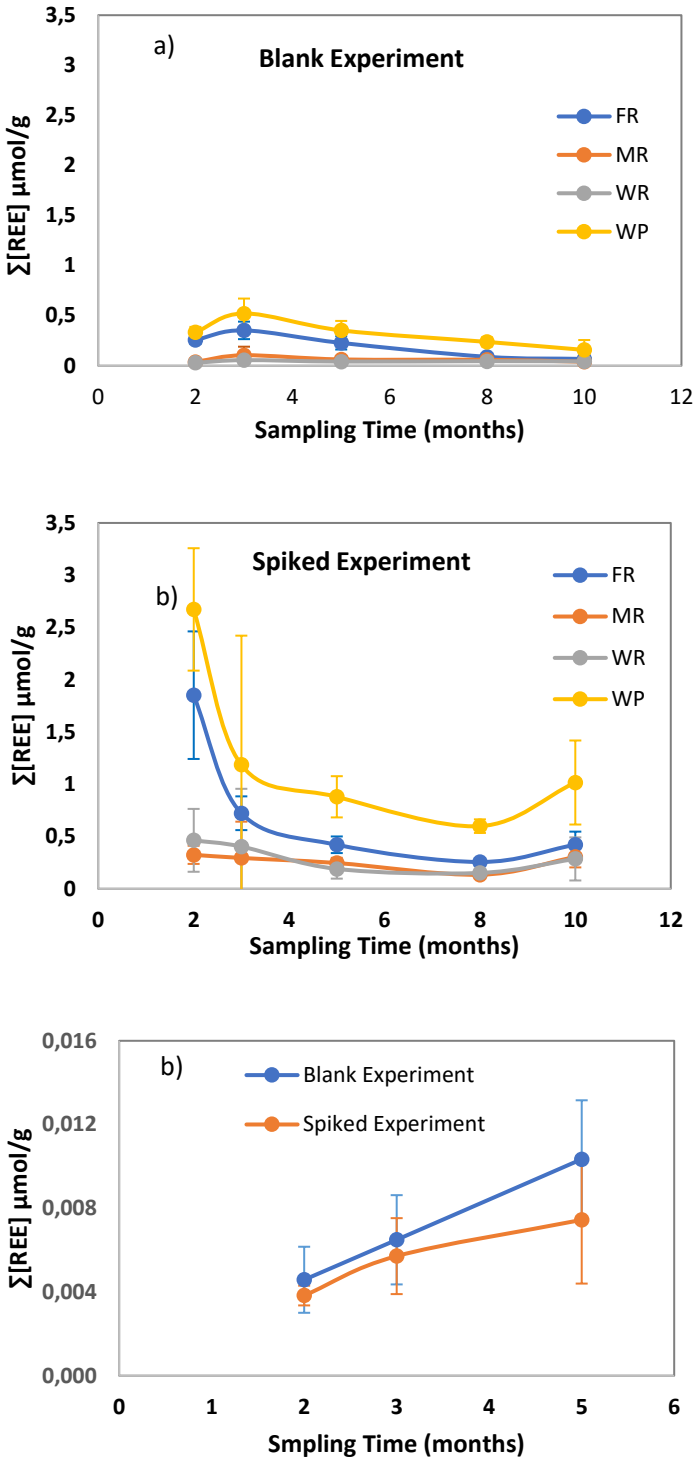

Figure 3-a) Blank experiment. b) Spiked experiment. (FR)Fine Roots, (MR) Middle Roots, (WR) Woody Roots and (WP) Whole plant. c) ∑REE concentration in leaves in blank and spiked experiments

Fig.s 3 (a and b) show that the highest REE concentration in whole roots is found in the initial stage St2 at 0.33 ± 0.04 and 2.67 ± 0.88 μmol/g for blank and spiked conditions, respectively, while from St2 to St5 the REE concentration decreases by 2 times in both experimental conditions. The higher value found in the first stage of growth has been found in fine roots more than middle or woody roots. The REE preferential enrichment in roots observed in our experiment is in agreement with fine root enrichment in U and Nd found in Scots pine spruce, beech and oak growing from polluted soils (Thiry et al., 2005; Brioschi et al., 2013) and interpreted as the greater ability of fine roots in producing radical exudates compared to medium and large ones (Walker et al., 2003). It is likely that through exudation, roots may regulate soil microbial community influencing the chemical and physical soil properties that in turn affect trace element mobilization from soil to plant (Antoniadis et al., 2017). In leaves, the REE concentration increases linearly during plant's growth spanning from 0.004 (± 0.001) to 0.010 (± 0.002) μmol/g for blank experiment and from 0.0038 (± 0.0004) to 0.007 (0.004) μmol/g for spiked experiment (Fig. 3c). These values are, however, significantly lower compared to those found in roots. Highest amounts of REE were found after eight months of growth (St4) corresponding to the last stage before leaves fall. REE concentrations are on the same order of magnitude in the two examined conditions, despite REE concentration in roots being by one order of magnitude higher in the spiked experiment. Therefore, it appears that *Vitis vinifera L.* limits the transfer of REE maintaining their levels low in the aerial parts. REE transfer efficiency in the aerial part of *Vitis vinifera L.* has been evaluated for every specific REE at every stage of the growth in the different plants' organs. We found that TF calculated for both

experimental conditions (Fig. 4a, b) was < 1 for all elements meaning that REE accumulation is greater in roots compared to aerial parts.

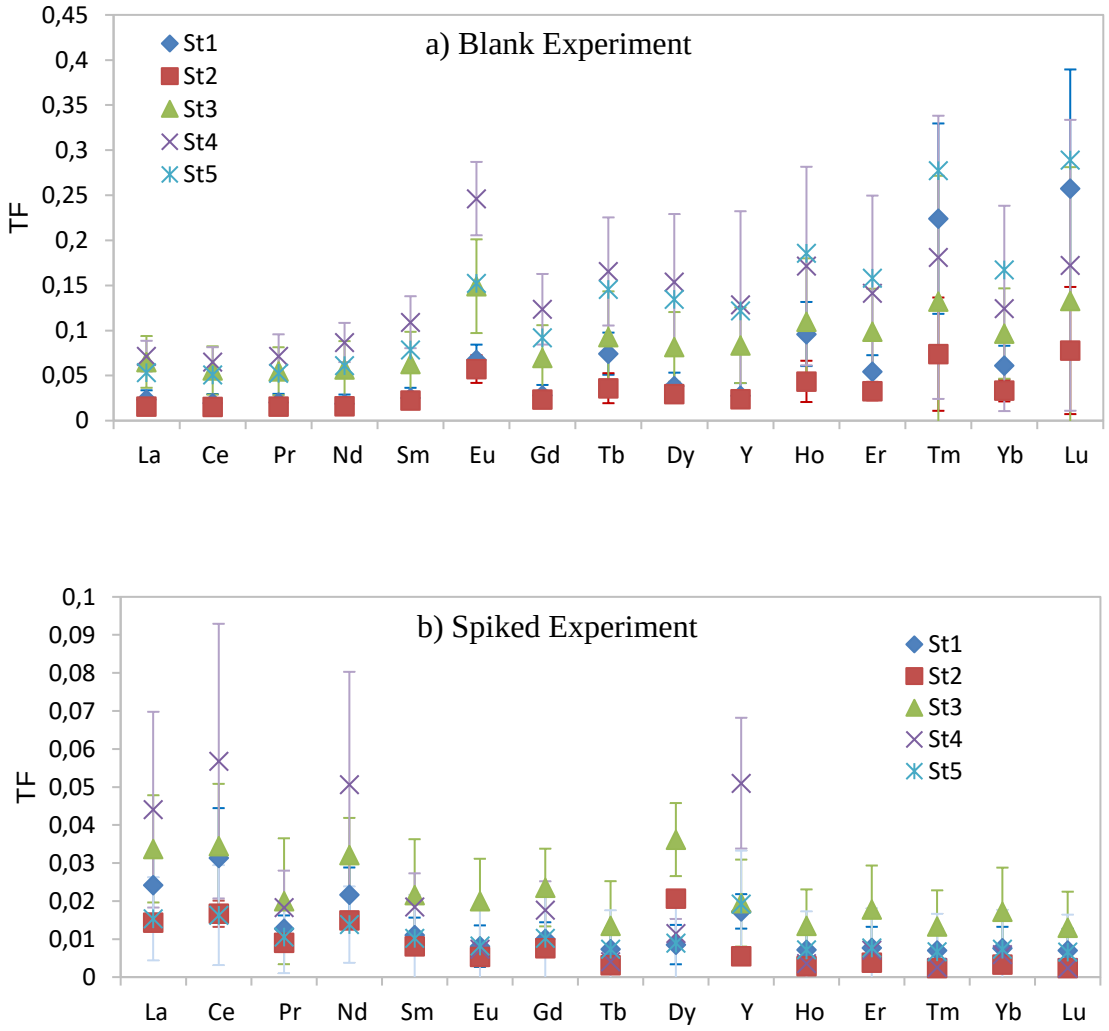

Figure 4. Translocation Factor (TF). a) ratio between REE concentration (µmols/g) in aerial part and roots at each Sampling Time (St1, St2, St3, St4, St5) in the blank experiment. b) ratio between REE concentration in aerial part and roots at each Sampling Time (St1, St2, St3, St4, St5) in the spiked experiment.

Specifically, TF is by 3 to 15 times lower in spiked experiment compared to the blank one. It is important to highlight that in spiked conditions the higher REE amount uptake from roots produces a lower transfer capacity to aerial parts. This may correspond to a possible detoxification mechanism developed by *Vitis vinifera L* to answer to non-essential toxic trace element exposures (Pourrut et al., 2011). REE are considered non-essential trace elements entering into plant tissues passively because of their similarity with essential ions (Kabata-Pendias and Pendias 2001; Wang et al., 2008; Yuan et al., 2017). This detoxification process by endodermis cells should correspond to the earlier proposed for Pb behaviour in different species of plants (Pourrut et al., 2011). *Vitis vinifera L.* can transfer even lower amounts of trace elements as Cu, Zn, Pb or nutrients (N) into berries than in leaves (Araujo et al., 1995; Kabata-Pendias and Pendias 2001; Kment et al., 2005; Chopin et al. 2008). Globally, even if the REE amount transferred is different between the two experimental conditions, our experimental investigation shows that REE enrichment of substrate does not influence the plant physiological response to environmental stress. *Vitis vinifera L*. preferably accumulates REE in fine roots with a likely strategy governed by processes of exclusion compatible to the mass development.

3.2.REE fractionation in Vitis vinifera L.

The transfer of REE in the overall plant has been initially estimated by the relative fractionation of REE group using the ratio between Light-REE and Heavy-REE (∑LREE*/∑HREE*). Fig. 5 shows that the (∑LREE*/∑HREE*) ratio remains constant for every experimental condition and single lines can be parametrised for every condition.

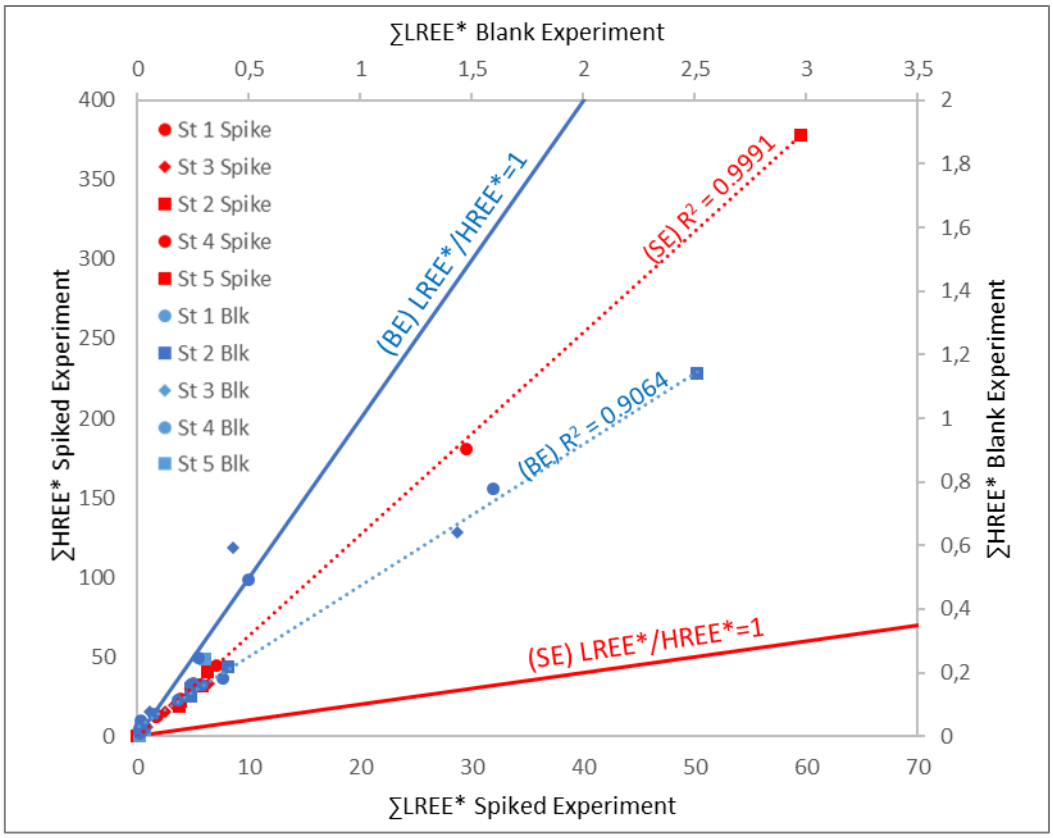

Figure 5. Normalized (∑LREE*/∑HREE*) in plants organs during different growing stages. (BE) Blank Experiment, (SE) Spiked experiment

We found that the (∑LREE*/∑HREE*) ratio in spiked experiment is by 1 order of magnitude higher compared to the blank indicating the plant answer to the soil REE enrichment. If the enrichment may be explained by a possible enhancement of complexation reaction of REE ions added (Byrne et al., 1995), the (∑LREE*/∑HREE*) ratio of the entire plant does not appear to give operative information discriminating the different growth conditions. We, thus, focused on the content in the different organs. Figs. 6 show the distribution of REE normalised pattern in roots and aerial part for the two different experimental conditions.

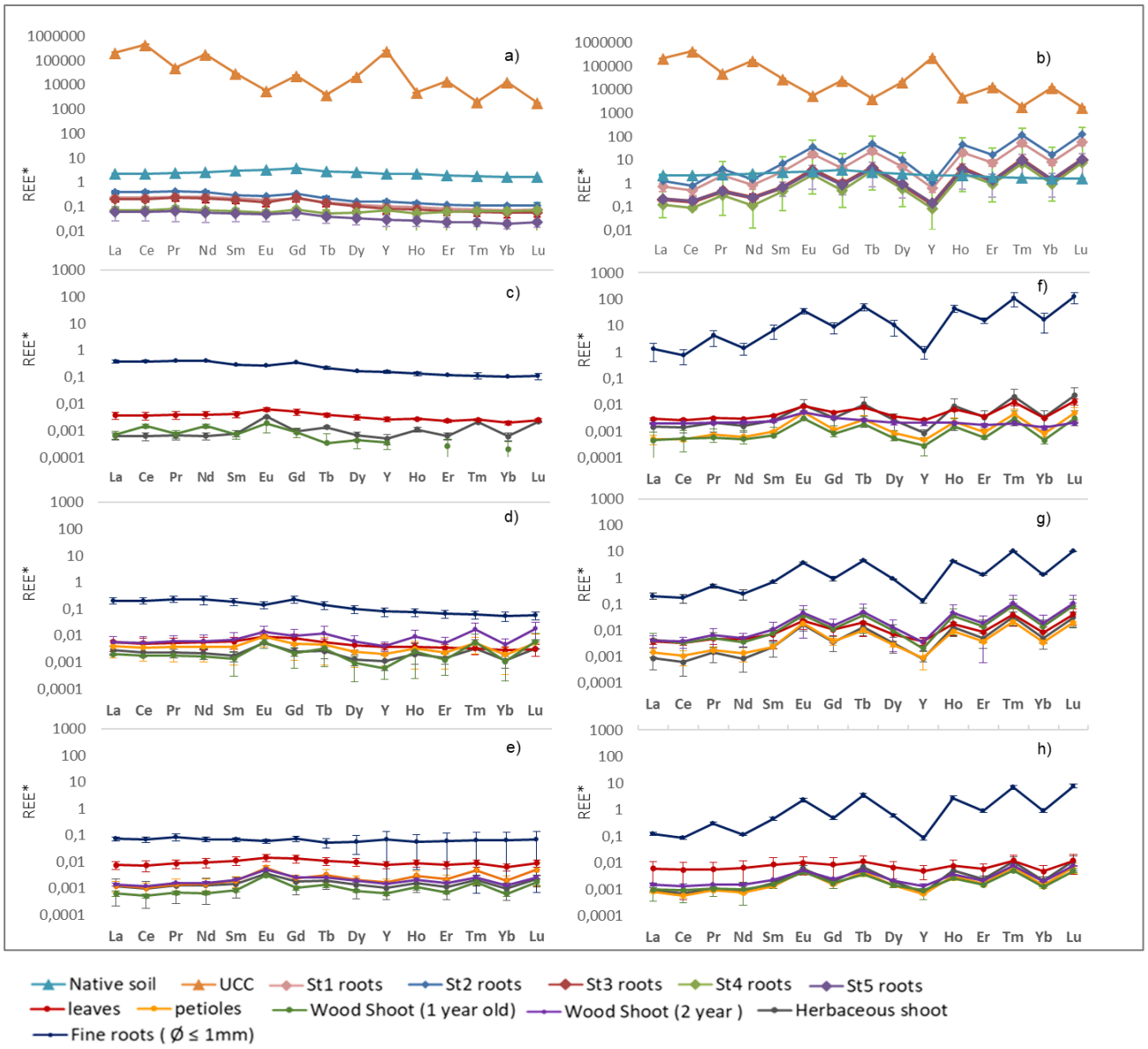

Figure 6. Blank experiment: a,b,c,d; spiked experiment; e,f,g,h. a) UCC distribution and REE* of native soil and roots (St1-St5) blank experiment. b) and REE* of native soil and roots (St1-St5) spiked experiment. c,d,e) REE* aerial part and fine roots (St2-St3-St4) in the blank experiment. f,g,h) REE* aerial part and fine roots (St2-St3-St4) in the spiked experiment.

We found that in blank experiment (Fig. 6a) the REE pattern is characterised by a constant decreasing from LREE to HREE reflecting the pristine native soil as peat-gravel is an inert

substrate. The enhanced LREE mobility observed in our study may reflect the ability of humic substance complexation that are present in the soil (Coppin, 2002; Pourret et al., 2007). Differently, in spike experiment (Fig. 6b), the REE pattern of roots increases from LREE to HREE with a 'zig-zag' shape. Here, the root pattern is symmetrical to the UCC distribution reflecting the equimolar plant absorption of REE and does not correspond to native soil. Our results indicate that the REE transport in *Vitis vinifera L.* is not selective and depends on the amount of REE present in the substrate of growth. Roots reflect native soil in blank experiment while they mirror UCC in spiked experiment. The aerial part of plant reflects the behaviour of REE root in both blank (and spiked experiments as displayed in figures (Figs 6 c,d,e,f,g,h). The aerial part *Vitis vinifera L*. shows also a positive Eu anomaly in the blank experiment (Table 1) corresponding to the possible physiological Eu–Ca substitution during metabolic reactions as previously observed by Brioschi et al., (2013) and Pisciotta et al., (2017).

Eu anomaly	***St1***	***St2***	***St3***	***St4***	***St5***
$\left[\frac{Eu}{Eu^*}\right]_{roots/UCC}$	0.72	0.74	0.73	0.66	0.79
$\left[\frac{Eu}{Eu^*}\right]_{aerial\ part\ /UCC}$	1.32	1.33	1.46	1.19	1.13
$\left[\frac{Eu}{Eu^*}\right]_{aerial\ part\ /roots}$	6.42	6.23	5.09	4.49	3.21

Table 1. REE anomalies calculated by Eqns. 10 and 11 in the blank experiment, at each sampling time (St1, St2, St3, St4, St5).

Irrespective of the spiked conditions, the REE pattern of aerial parts remains unchanged during the plant's growth indicating that *Vitis vinifera L.* does not significantly fractionate REE during the complex reaction mechanism of metal uptakes and transfer. The different normalised REE

patterns observed in our study are thus related to substrate variability. Since in blank conditions the REE distribution reflects REE pattern of native soils and in spiked conditions reflects UCC distribution, we propose that the REE full normalized spectra are a useful tool for discriminating soil conditions during plant growth.

Implication and conclusion

Despite the weak toxicity of REE, new applications and developing technologies in agricultural automotive and telecommunication sectors may increase the environmental levels of REE in soils and, in turn, the levels of plant exposition. These emerging new sources of pollution are still associated to mining conventional pollution and leaching of REE waste minerals in several areas of the world. The results of our experimental investigation revealed that, in the case of *Vitis vinifera L.*, the transfer efficiency of REE from roots to leaves is lower in polluted substrates compared to unpolluted ones, although the roots of polluted substrates are by one order of magnitude enriched in REE. We found that possible REE pollution in soil should not significantly influence the amount of REE transferred to the aerial parts indicating that grapevine products obtained from *Vitis vinifera L.* cultivations, should have a very low amount of REE. The results obtained in this experimental simulation, do not show clear evidence of REE possible competition with calcium in biological mediated calcium-processes, which could account for some of the plant toxicity.

Evaluating the plant physiological response to REE contaminated soil, we found that REE transport in *Vitis vinifera L.* is probably not selective, and mainly depending on the level of

REE in the substrate of growth. REE distribution pattern keeps unaltered in plant organs: in the unpolluted substrate (blank experiment), roots and aerial part reflect native soil, while in polluted soils (spiked experiment) the UCC. We finally propose that the use of the full REE normalized spectra is a promising helpful tool able to discriminate the substrate of *Vitis vinifera L.* growth meaning that REE may provide an estimation of the geographical origin of this kind of food.

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Reference

Aceto, M., Robotti, E., Oddone, M., Baldizzone, M., Bonifacino, G., Bezzo, G., 2013. A traceability study on the Moscato wine chain. Food. Chem. 138, 1914–1922.

Ambrus, A., 2004. Reliability of measurements of pesticide residues in food. Accred. Qual. Assur. 9, 288-304.

Antoniadis, V., Levizou, E., Shaheen, S.M., Ok, Y.S., Sebastian, A., Baum, C., Rinklebe, J., 2017. Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation–A review. Earth Sci. Rev. 171, 621-645.

Araujo, F., Williams, L.E., Matthews, M.A., 1995. A comparative study of young 'Thompson Seedless' grapevines (Vitis vinifera L.) under drip and furrow irrigation. II. Growth, water use efficiency and nitrogen partitioning. Sci. Hortic. 60(3-4), 251-265.

Bargagli, R., 1998. Trace Elements in Terrestrial Plants: an Ecophysiological Approach to Biomonitoring and Biorecovery. Berlin, Springer-Verlag, p. 324.

Bertoldi, D., Larcher, R., Bertamini, M., Otto, S., Concheri, G., Nicolini, G., 2011. Accumulation and distribution pattern of macro- and microelements and trace elements in Vitis vinifera L. cv. chardonnay berries. Journal of Agriculture and Food Chem. 59, 7224–7236.

Bertoldi, D., Larcher, R., Nicolini, G., Bertamini, M., Concheri, G., 2009. Distribution of rare earth elements in Vitis vinifera L. 'Chardonnay' berries. Vitis J. Grapevine Res. 48, 49–51.

Bettinelli, M., Spezia, S., Baffi, C., Beone, G.M., Rocchetta, R., Nassisi, A., 2005. ICP-MS determination of REE in tomato plants and related products: a new analytical tool to verify traceability. At. Spectrosc. 26, 41-50.

Brioschi, L., Steinmann, M., Lucot, E., Pierret, M.C., Stille, P., Prunier, J., 2013. Transfer of rare earth elements (REE) from natural soil to plant systems: implications for the environmental availability of anthropogenic REE. Plant Soil. 366(1-2), 143-163.

Brunner, M., Katona, R., Stefánka, Z., Prohaska, T., 2010. Determination of the geographical origin of processed spice using multielement and isotopic pattern on the example of Szegedi paprika. Eur. Food Res. Technol. 231(4), 623-634.

Byrne, R.H., Li, B., 1995. Comparative complexation behaviour of the rare earths. Geochim. Cosmochim. Acta. 59, 4575 - 4589.

Cao, X., Yin, M., Wang, X., 2001. Elimination of the spectral interference from polyatomic ions with rare earth elements in inductively coupled plasma mass spectrometry by combining algebraic correction with chromatographic separation. Spectrochim. Acta B. 56, 431-441.

Censi, P., Cibella, F., Falcone, E. E., Cuttitta, G., Saiano, F., Inguaggiato, C., Latteo, V., 2017. Rare earths and trace elements contents in leaves: A new indicator of the composition of atmospheric dust. Chemosphere. 169, 342-350.

Censi, P., Cangemi, M., Brusca, L., Madonia, P., Saiano, F., Zuddas, P., 2015. The behaviour of rare-earth elements, Zr and Hf during biologically-mediated deposition of silica-stromatolites and carbonate-rich microbial mats. Gondwana Res. 27, 209-215.

Censi, P., Saiano, F., Pisciotta, A., Tuzzolino, N., 2014. Geochemical behaviour of rare earths in Vitis Vinifera grafted onto different rootstocks and growing on several soils. Sci. of Total. Environ. 473, 597-608.

Censi, P., Tamburo, E., Speziale, S., Zuddas, P., Randazzo, L.A., Punturo, R., Cuttitta, A., Aricò, P., 2011. Yttrium and lanthanides in human lung fluids, probing the exposure to atmospheric fallout. J. Hazard. Mater. 186, 1103–1110.

Chopin, E.I.B., Marin, B., Mkoungafoko, R., Rigaux, A., Hopgood, M.J., Delannoy, E., Laurain, M., 2008. Factors affecting distribution and mobility of trace elements (Cu, Pb, Zn) in a perennial grapevine (Vitis vinifera L.) in the Champagne region of France. Environ. Pollut. 156, 1092-1098.

Coppin, F., Berger, G., Bauer, A., Castet, S., Loubet, M., 2002. Sorption of lanthanides on smectite and kaolinite. Chem. Geol. 182, 57–68.

De Boer, J.L.M., Verweij, W., Van Der Velde-Koerts, T., Mennes, W., 1996. Levels of rare earth elements in Dutch drinking water and its sources. Determination by inductively coupled plasma mass spectrometry and toxicological implications. A pilot study. Water Res. 30, 190-198.

Diatloff, E., Smith, F.W., Asher, C.J., 2008. Effects of lanthanum and cerium on the growth and mineral nutrition of corn and mungbean. Ann. Bot. 101, 971– 982.

Drivelos, S.A., Georgiou, C.A. 2012. Multi-element and multi-isotope ratio analysis to determine the geographical origin of foods in the European Union. Trend. Anal. Chem. 40, 38–51.

Durães, N., da Silva, E.F., Bobos, I., Ávila, P., 2014. Rare Earth Elements fractionation in native vegetation from the Moncorvo iron mines, NE Portugal. Procedia Earth Planet. Sci. 10, 376-382.

European Commission. 2019. Knowledge centre for food fraud and quality. Retrieved January 13, 2019, https://ec.europa.eu/jrc/en/food-fraud-and-quality.

Feng, L., Xiao, H., He, X., Li, Z., Li, F., Liu, N., Zhao, Y., Huang, Y., Zhang, Z., Chai, Z., 2006. Neurotoxicological consequence of longterm exposure to lanthanum. Toxicol. Lett. 165, 112–120.

He, Y.W., Loh, C.S., 2000. Cerium and lanthanum promote floral initiation and reproductive growth of Arabidopsis thaliana. Plant Sci. 159, 117–124.

ISO/IEC 11352:2012 (2012). Water quality, Estimation of measurement uncertainty based on validation and quality control data, International Organization for Standardization/International Electrotechnical Commission (IEC), Geneva.

Jiang, D.G., Yang, J., Zhang, S., Yang, D.J., 2012. A survey of 16 rare earth elements in the major foods in China. Biomed. Environ. Sci. 25:267–271.

Kabata-Pendias, A., Pendias, H., 2001. Trace elements in soils and plants–CRC Press. Boca Raton.

Kment, P., Mihaljevič, M., Ettler, V., Šebek, O., Strnad, L., Rohlová, L., 2005. Differentiation of Czech wines using multielement composition–A comparison with vineyard soil. Food Chem..91, 157-165.

Krzciuk, K., Gałuszka, A., 2019. Seasonal changes in concentrations of trace elements and rare earth elements in shoot samples of Juncus effusus L. collected from natural habitats in the Holy Cross Mountains, south-central Poland. Chemosphere. 219, 954-960.

Krzciuk, K., Gałuszka, A., 2015. Prospecting for hyperaccumulators of trace elements: a review. Crit. Rev. in Biotechnol. 35, 522-532.

Laucou, V., Launay, A., Bacilieri, R., Lacombe, T., Adam-Blondon, A.F., Berard, A., Le Paslier, M.C., 2018. Extended diversity analysis of cultivated grapevine Vitis vinifera with 10K genome-wide SNPs. PLoS One, 13(2), e0192540.

Laveuf, C., Cornu, S., 2009. A review on the potentiality of rare earth elements to trace pedogenetic processes. Geoderma. 154, 1–12.

Li, X., Chen, Z., Chen, Z., Zhang, Y., 2013. A human health risk assessment of rare earth elements in soil and vegetables from a mining area in Fujian Province, Southeast China. Chemosphere, 93 1240-1246.

Liang, T., Ding, S., Song, W., Chong, Z., Zhang, C., Li, H., 2008. A review of fractionations of rare earth elements in plant. J. Rare Earths, 26, 7–15.

Marchionni, S., Buccianti, A., Bollati, A., Braschi, E., Cifelli, F., Molin, P., Parott, M., mattei, M., Tommasini, S., Conticelli, S., 2016. Conservation of $^{87}Sr/^{86}Sr$ isotopic ratios during the winemaking processes of 'Red'wines to validate their use as geographic tracer. Food chem. 190, 777-785.

Miao, L., Ma, Y., Xu, R., Yan, W., 2011. Environmental biogeochemical characteristics of rare earth elements in soil and soil-grown plants of the Hetai goldfield, Guangdong Province, China. Environ. Earth Sci. 63, 501–511.

Oddone, M., Aceto, M., Baldizzone, M., Musso, D., Osella, D., 2009. Authentication and traceability study of hazelnuts from Piedmont, Italy. J. of Agricult. Food Chem. 579 3404-3408.

Pagano, G., Guida, M., Tommasi, F., Oral, R., 2015a. Health effects and toxicity mechanisms of rare earth elements – Knowledge gaps and research prospects. Ecotoxicol. Environ. Saf. 115, 40–48.

Pagano, G., Aliberti, F., Guida, M., Oral, R., Siciliano, A., Trifuoggi, M., Tommasi, F., 2015b. Rare earth elements in human and animal health: state of art and research priorities. Environ. Res. 142, 215-220.

Pepi, S., Grisenti, P., Sansone, L., Chicca, M., Vaccaro, C., 2018. Chemical elements as fingerprints of geographical origin in cultivars of Vitis vinifera L. raised on the same SO4 rootstock. Environ. Sci. Pollut. Res. 25, 490-506.

Pepi, S., Sansone, L., Chicca, M., Vaccaro, C., 2017. Relationship among geochemical elements in soil and grapes as terroir fingerprintings in Vitis vinifera L. cv."Glera". Chem. Erde. 77,121-130.

Pepi, S., Coletta, A., Crupi, P., Leis, M., Russo, S., Sansone, L., 2016. Geochemical characterization of elements in Vitis Vinifera cv. Negroamaro grape berries grown under different soil managements. Environ. Monit. Assess. 188, 211.

Pisciotta A., Tutone L., Saiano F., 2017. Distribution of YLOID in soil-grapevine system (Vitis vinifera L.) as tool for geographical characterization of agro-food products. A two years case study on different grafting combinations. Food Chem. 221, 1214-1220.

Pourret, O., Davranche, M., Gruau., G., Dia, A., 2007. Rare earth elements complexation with humic acid. Chem. Geol. 243,128 – 141.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., Pinelli, E., 2011. Lead uptake, toxicity, and detoxification in plants. In Reviews of Environmental Contamination and Toxicology Volume 213, 113-136. Springer, New York, NY.

Punturo, R., D'Antone, C., Pepi, S., Vaccaro, C., 2018. Rare earth elements absorption patterns in grapevine "Vitis vinifera L." cultivated in carbonate terrains (south-eastern Sicily, Italy). Environ. Earth Sci. 77, 801.

Richter, B., Gurk, S., Wagner, D., Bockmayr, M., Fischer, M., 2019. Food authentication: Multi-elemental analysis of white asparagus for provenance discrimination. Food Chem. 286, 475-482.

Samczynski, Z., Dybczynski, R. S., Polkowska-Motrenko, H., Chajduk, E., Pyszynska, M., Danko, B., Czerska, E., Kulisa, K., Doner, K., Kalbarczyk, P., 2012. Two new reference materials based on tobacco leaves: Certification for over a dozen of toxic and essential elements. Sci. World J. 2012, 16.

Sasmaz, M., Obek, E., Sasmaz, A., 2018. The accumulation of La, Ce and Y by Lemna minor and Lemna gibba in the Keban gallery water, E lazig T urkey. Water Environ. J. 32, 75-83.

Soneji, J.R., Nageswara-Rao, M., 2011. Basic Botany of the Species. Wild Crop Relatives: Genomic and Breeding Resources: Temperate Fruits. 6, 223. Springer, New York, NY.

Thiry, Y., Schmidt, Pò., Van Hees, Mò., Wannijn, J., Van Bree, P., Rufyikiri, G., Vandenhove, H., 2005. Uranium distribution and cycling in Scots pine (Pinus sylvestris L.) growing on a revegetated U-mining heap. J. Environ. Radioact. 81, 201– 219.

Thomas, P.J., Carpenter, D., Boutin, C., Allison, J.E., 2014. Rare earth elements (REEs): effects on germination and growth of selected crop and native plant species. Chemosphere, 96, 57-66.

Turra, C., 2018. Sustainability of rare earth elements chain: from production to food–a review. Int. J. of Environ. Health Res. 28, 23-42.

Tyler, G., 2004. Rare earth elements in soil and plant systems — a review. Plant Soil. 267, 191–206.

US Environmental Protection Agency, 2012. Rare Earth Elements: A Review of Production, Processing, Recycling, and Associated Environmental Issues. EPA 600/R-12/572. (www.epa.gov/ord).

Vermeire, M.L., Cornu, S., Fekiacova, Z., Detienne, M., Delvaux, B., Cornélis, J.T., 2016. Rare earth elements dynamics along pedogenesis in a chronosequence of podzolic soils. Chemical Geology. 446, 163-174.

Vivier, M.A., Pretorius, I.S.,2002. Genetically tailored grapevines for the wine industry. Trends Biotechnol. 20, 472-478.

Walker, T.S., Bais, H.P., Grotewold, E., Vivanco, J.M., 2003. Root exudation and rhizosphere biology. Plant Physiol. 132, 44-51.

Wang, L., He, J., Xia, A., Cheng, M., Yang, Q., Du, C., Zhou, Q., 2017. Toxic effects of environmental rare earth elements on delayed outward potassium channels and their mechanisms from a microscopic perspective. Chemosphere. 181, 690-698.

Wang, Y., Yan, A., Dai, J., Wang, N., Wu, D., 2012. Accumulation and tolerance characteristics of cadmium in Chlorophytum comosum: a popular ornamental plant and potential Cd hyperaccumulator. Environ Monit. Assess, 184, 929–37.

Wang, L., Huang, X., Zhou, Q., 2008. Effects of rare earth elements on the distribution of mineral elements and heavy metals in horseradish. Chemosphere. 73(3), 314-319.

Yuan, M., Guo, M.N., Liu, W.S., Liu, C., van der Ent, A., Morel, J.L., Huot, H., Zhao, W.Y., Wei, X.G., Qiu, R.L., Tang, Y.T., 2017. The accumulation and fractionation of Rare Earth Elements in hydroponically grown Phytolacca americana L. Plant Soil. 421, 67-82.

Supplementary material

REE	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
LOQ ng/L	0,9	0,9	0,9	0,3	0,6	0,2	0,2	0,2	0,1	0,2	0,1	0,2	0,1	0,1	0,2
Rec %	109,8	96,9	95,9	104,7	96,7	97,0	103,0	111,1	97,4	102,2	104,1	101,0	97,8	97,4	72,7
U %	2,3	3,2	3,7	3,6	4,4	2,9	3,5	8,3	3,5	27,2	7,3	3,1	2,4	10,6	40,0

LOQ (REE Quantification limits), Rec % (recovery), Uδ (Uncertain bias)

Table

Table 1. REE anomalies calculated by Eqns. 10 and 11 in the blank experiment, at each sampling time (St1, St2, St3, St4, St5).

Figure captions

Figure 1. The off-soil experimental system

Figure 2. Total Weight (TW) of the plants in Blank and Spiked Experiment (BE and SE respectively) as a function of the time of growth.

Figure 3-a) Blank experiment. b) Spiked experiment. (FR) Fine Roots, (MR) Middle Roots, (WR) Woody Roots and (WP) Whole plant. c) ∑REE concentration in leaves in blank and spiked experiments.

Figure 4. Translocation Factor (TF). a) ratio between REE concentration (µmols/g) in aerial part and roots at each Sampling Time (St1, St2, St3, St4, St5) in the blank experiment. b) ratio between REE concentration in aerial part and roots at each Sampling Time (St1, St2, St3, St4, St5) in the spiked experiment.

Figure 5. Normalized (∑LREE*/∑HREE*) in plants organs during different growing stages. (BE) Blank Experiment, (SE) Spiked experiment

Figure 6. Blank experiment: a,b,c,d; spiked experiment; e,f,g,h. a) UCC distribution and REE* of native soil and roots (St1-St5) blank experiment. b) and REE* of native soil and roots (St1-St5) spiked experiment. c,d,e) REE* aerial part and fine roots (St2-St3-St4) in the blank experiment. f,g,h) REE* aerial part and fine roots (St2-St3-St4) in the spiked experiment.

5.2 Is REE* useful to discriminate small differences in REE amount? Does REE* fractionate in the xylem-sap?

In this article, to evaluate if the normalized distribution patterns (REE*) are useful to discriminate small differences in REE amount, we investigated the REE behaviour during the transfer from soil to leaves when the spiked substrate is poorly enriched in REE. We evaluated the variation in REE, normalizing the REE relative amount in roots xylem-sap, leaves, native soil and both substrates to the lithological reference (UCC). Also, we explored the role of xylem-sap in the REE transfer. We found that, despite the REE transfer is influenced by the plant growth REE* in xylem-sap clearly indicates that transfer takes place without REE fractionation, suggesting a conservative transport of xylem-sap.

The conservative behaviour of REE in *Vitis vinifera L*: Implication for Food Traceability

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Abstract

The identification of food-products geographical origin using REE as geographical markers requires to understand if REE content and distribution in plants are mainly related to metal ion migration from soil or if the content in leaves can be influenced by atmospheric fallout. Here, we investigated the REE mobility in soil-*Vitis vinifera L.* system. We wondered if the normalized distribution patterns (REE*) are useful to discriminate even small differences in REE amount in the substrates of growth and which is the role played by the of xylem-sap in REE transfer. We focused on plants grown in field using REE enriched and non-enriched substrates. We found that transfer takes place without REE fractionation, suggesting a conservative transport of xylem-sap. Our results show that REE* in plant organs are able to trace the soil enriched conditions discriminating the environmental conditions of *Vitis vinifera L.* growth. Since REE* can be used to differentiate plants from different soils of growth, we propose that the use of REE* is a potential marker for identifying the substrate of *Vitis vinifera L.* growth.

1. Introduction

The increasing demand for food quality and distinctiveness involve authenticity and sustainability of the product as well geographical determinations of eatable agricultural foods (Brunner et al., 2010; Drivelos and Georgiou, 2012; Marchionni et al., 2016; Richter et al 2019). The identification and classification of geographical origins requires development of reliable tools able to establish proper trade legislation worldwide as recommended by national and supranational institutions (Ilhan et al., 2021; Zhao et al 2016). In recent years, research focused on Rare Earth Elements (REE) full normalized spectra as a tool for identifying the geographical origin of food, based on the known utility on tracing pedo-genetic and petro-genetic processes (Aide and Aide, 2012; Migaszewski et al., 2015). Investigations (Bettinelli et al., 2005; Bertoldi et al., 2009, 2011; Censi et al., 2014; Pepi et. al 2016, 2017, 2018; Pisciotta et al., 2017; Punturo et al., 2018; Oddone et al., 2009) have suggested that REE keep their distribution during the transfer from soil to leaves (or fruits) proposing the REE distribution as possible geographical marker. REE distribution in *Vitis vinifera L.* may reflect soil composition (Aceto et al., 2013; Barbera et a.,2021, Censi et al., 2014; Pisciotta et al., 2017; Punturo et al., 2018) even if leaves may trap metals leached from atmospheric dust particles (Censi et al.,2017; Pallardy, 2008; Tomašević et al., 2014;). However, there is no information about atmospheric influence on REE as earlier investigations were focused on bioaccumulation processes during soil/plant REE transfers (Censi et al., 2014; Miao et al., 2011; Reimann et al., 2015; Reimann and De Caritat, 2000, 2005; Sucharovà et al., 2012; Tyler, 2004; Yu et al., 2007). On the other hand, previous experimental investigations analysed dose-response in hydroponic conducive conditions

eluding real-complexity of the rhizospheric environment (Brioschi et al., 2013; Thomas et al., 2014; Yuan et al 2017). To assure significance of REE pattern as a fingerprint of food product real provenance, we tested if REE content and distribution in plants are mainly related to metal ion migration from soil or if the content in leaves can be influenced by atmospheric fallout.

We thus have investigated the REE mobility in the different metal accumulation points: Soil substrates, Roots, Leaves and xylem-sap. We focus on the role of the xylem-sap because although it is the water channel that transports nutrients from roots to leaves (Buhtz, 2004) no information is reported on REE transport in the xylem-sap of *Vitis vinifera L.* We wondered if REE transport from soil to plant through xylem-sap may fractionate as function of different environmental conditions of growth. Analysed the REE distribution during seven months of growth using two different semi-natural soil conditions: one with a 'natural' substrate (Control) and another with the same substrate weakly enriched in REE (Spiked). To evaluate the applicability of the distribution patterns to natural systems we choose to test small REE relative abundance variations. Indeed, even if REE are emerging contaminants resulting from the increasing technological uses and agricultural practices (Balarm et al., 2018; Jowitt et al., 2018; Tommasi et al., 2020; Yan et al., 2020) the variations in terms of relative abundances in natural soils are small (Calabrese et al., 2011; Censi et al., 2017; Scalenghe et al., 2016). We carried out controlled experimental plant growth and we found that transfer takes place without REE fractionation, suggesting a conservative transport of xylem-sap.

2. Material and methods

2.1.Experimental system

Experiments were carried out in the field-laboratory of the Department of Agricultural, Food and Forestry Sciences (University of Palermo Italy) using *Vitis vinifera L*. plants. We selected one-year plants grown up in clay-sandy soils located in the southwest of Sicily (Italy). Pristine plants were put in off-soil polyurethane pots with peat/cocoa fibre substrate having low amount of REE and high level of organic substances (humic acid) (Arbuzov et al., 2018). In our field laboratory, pots were in contact with atmospheric agents (i.e; rain, wind), a micro-irrigation system was used to avoid irrigation deficit (Di Lorenzo et al., 2005) while no disease spray was used. Two different growth conditions were chosen for the pot off-soil experimental growth: one using 1 kg of a homogeneous substrate (Control Substrate), and another adopting the same homogeneous substrate artificially spiked by 12.5 μmol per Kg of every single REE (Spiked Substrate). The multi-REE spiked solution (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) was prepared by diluting standard stock solution (1000 mg/l, pH<1) to 0.05 mM of each element by high purity water (pH ~5.5) obtaining an initial working solution of pH close to 5.6 allowing to consider REE as free-ions mainly (De Boer et al., 1996). The whole experimental system consists of 24 plants of which 12 controls and 12 spiked. Both control and spiked plants were sampled at 4 different times (3, 4,5 and 7 months of growth). For every sampling time, we collected control and spiked growing plants and analysed Roots, xylem-sap and Leaves. The substrate was analysed at the beginning of the experiment and after 3 months in the spiked substrate condition.

2.2.Sample preparation

Ultrapure nitric acid (65%) and hydrogen peroxide (30%) reagents (Merck, Milan Italy) were used during all the stages of the work. Solutions containing REE and internal rhenium standard of 1000 ± 5mg/L purchased from BDH and CPI International and ultrapure water 18.2 MΩ cm^{-1}, was produced by an EASYpureII (Thermo, Milan Italy). Native soils and substrates were dried at 105°C, gently crushed, sieved (Ø 0.5 mm) and aliquots of 250 mg were transferred in PFA microwave vessels. Leaves and roots were chopped, dried at 105°C for 24h and grounded in an agate mortar, aliquots of 500 mg of leaves and 250 mg of roots and substrates were transferred in PFA microwave vessels. All samples were digested in a closed MarsXpress microwave (CEM, Bergamo Italy), with 4.5 ml of 2:1 v/v mixture of HNO_3:H_2O_2 adopting an increase of temperature from 20 to 200°C in the first minutes while the power of 1600 Watt was maintained for 50 minutes. After digestion, samples were quantitatively transferred into graduate polypropylene test tubes and diluted with ultrapure water. Dilution was to 10 ml for leaves analysis and 100 ml for native soils, substrates and roots. Blank samples were carried out using 4.5 ml of 2:1 v/v HNO_3-H_2O_2 solutions.

2.3.Xylem-sap extraction

The xylem-sap was collected using the pressure chamber method involving stem or root cutting in a depressurized chamber to force the sap out from the cut surface (Netzer et al., 2017). Because of the presence of root exudates, xylem-sap extraction was carried out on branches exclusively after rapid razor-blade defoliation, bark-ring removing and cut-surface rinsing with distilled water. The extraction of xylem-sap was performed inserting the tip branch through a

rubber stopper in a plastic vial located inside a Buchner flask. In the chamber, the applied negative pressure was gradually increased until 1.4 MPa to extract the majority of available sap. Generally, we collected 200 - 250 μl of sap from every extraction: half was used for organic acids determinations through Liquid chromatography coupled to mass spectrometry (HPLC-MS/MS) while the remaining was transferred in a polypropylene graduate tube, diluted with ultrapure-water to 3 ml for Inductively Coupled Plasma Mass Spectrometry (ICP-MS) REE determinations.

2.4.Chemical measurements

The Agilent Technologies 7500ce Series Spectrometer by ICP-MS was used for REE determinations using the following operative conditions: power 1550 W; nebulizer gas flow 1.00 L/min; auxiliary gas flow 0.85 L/min and plasma gas flow 15 L/min. Time of acquisition was 180 s for every determination and three replicates were performed by the ^{7}Li, ^{59}Co, ^{89}Y, ^{140}Ce, and ^{205}Tl isotopic masse determinations with a minimal precision of 2%. REE determinations were later performed measuring ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{151}Eu, ^{158}Gd, ^{159}Tb ^{163}Dy, ^{89}Y, ^{165}Ho, ^{167}Er, ^{169}Tm, ^{172}Yb, ^{175}Lu isotopic masses and using ^{187}Re as internal standard. Possible formation of oxide and doubly charged ion interferences were verified by the CeO^{+}/Ce^{+} and Ce^{2+}/Ce^{+} ratios that were always found inferior to 0.5%. Isobaric interferences, related to europium isotopes (^{151}Eu, ^{153}Eu) were evaluated by polyatomic barium oxide ions ($^{135}Ba^{16}O^{+}$, $^{137}Ba^{16}O^{+}$) using the certified INCT-OBTL-5 Oriental Basma Tobacco Leaves standard and Ba concentration. Barium interferences on europium determination were not found as the (Ba/Eu) ratio was less than 200 (De Boer et al., 1996).

2.5.Data processing

The capacity of substrate to retain REE were evaluated by the REE amount (µmol/Kg) in both control and spiked substrates. The REE distribution, REE*, was evaluated in both soil and substrate and the 3 different organs: Roots, xylem-sap and Leaves, normalizing the relative abundance of REE to UCC (Upper Continental Crust) (Laveuf and Cornu, 2009; Pisciotta et al., 2017) by:

$$[REE^*]_{substrate} = \frac{REE_{substrate}}{REE_{UCC}} \quad 1$$

$$[REE^*]_{organs} = \frac{REE_{organs}}{REE_{UCC}} \quad 2$$

The enrichment or depletion of a group or an individual REE relative to the others also called fractionation or anomalies was estimated by the following Eqn3:

$$\left[\frac{REE^*}{REE^*}\right]_{organs/UCC} = \frac{\left(\frac{REE_{organs}}{REE_{UCC}}\right)_i^2}{\left[\left(\frac{REE_{organs}}{REE_{UCC}}\right)_{i+1}\left(\frac{REE_{organs}}{REE_{UCC}}\right)_{i-1}\right]} \quad 3$$

3. Results and discussion

The system consists of native soil, substrates of growth and three different parts of the plant: roots, leaves and xylem-sap. Figure 1 shows the amount of each REE in both control and spiked substrates.

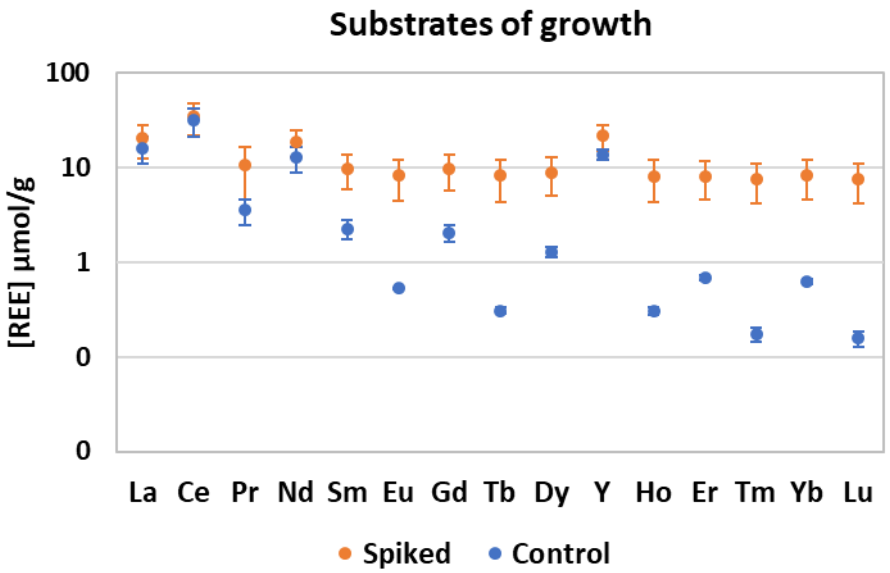

Fig. 1. REE amounts (µmol/kg) in control and spiked substates

We found that in spiked conditions REE is globally constant reflecting the equimolar addition of REE, while in the control experiment decreases by 2 orders of magnitude as a function of the atomic mass from La to Lu reflecting the variability of REE abundance of a 'natural soil' (Laveuf et al., 2009; Loell et al., 2011; Tyler, 2004). Reporting the normalised distribution REE* for the native soil and for the different substrates of growth (Fig.2), we found that both native and control substrates have a similar continuous spectrum characterised by the decreasing abundance from LREE to HREE, while in spiked substrate REE* increases from LREE to HREE following a particular 'zig-zag' shape. The artificial addition of REE discriminates the amount of REE incorporated in soil as well as differentiate the spectral distribution from the control substrate to the artificially enriched one.

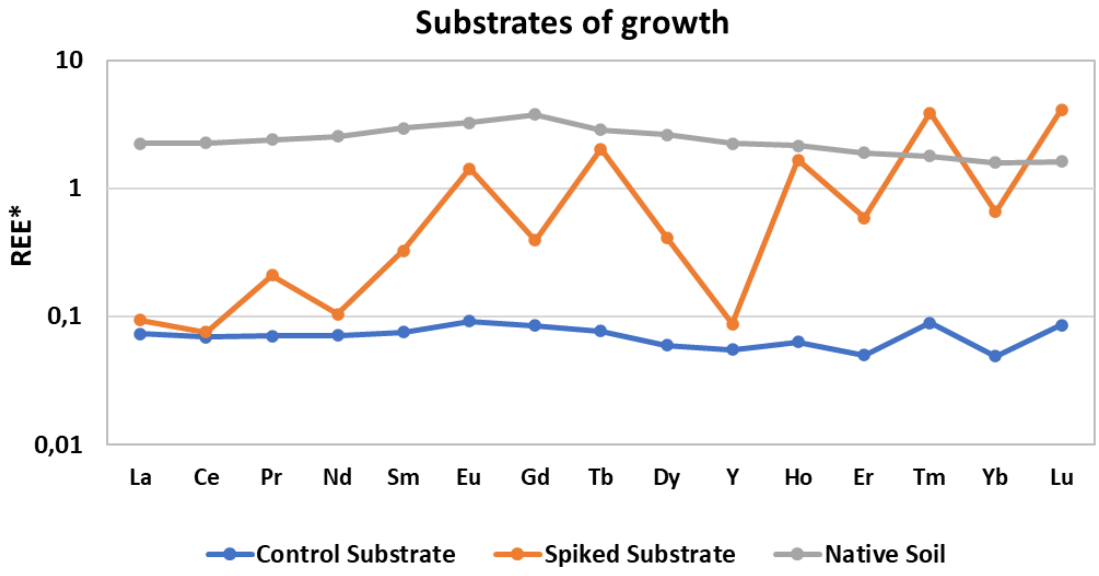

Fig. 2. REE distribution in native soil, control and spiked substrate

Figs 3 show the REE* in roots compared to the substrates of growth for both experimental conditions during plant growth. We found that in control conditions, REE* decreases from LREE to HREE in roots reflecting the higher LREE amount present in the control substrate (Fig. 3a and c). In spiked conditions, REE* of roots increases from LREE to HREE with the characteristic zig-zag shape indicating that root patterns discriminate the substrate of growth during all the times of growth. This shows that root REE* reflects the REE substrates spectra for both experimental conditions up-taking REE proportionally to the amount of REE present in the substrate. This result confirms previous observations on REE accumulation in *Vitis vinifera L.* indicating that REE* within plants may depend on the composition of the underlying soil (Aceto et al., 2013; Barbera et al., 2021; Censi et al., 2014; Pisciotta et al., 2017; Punturo et al., 2018).

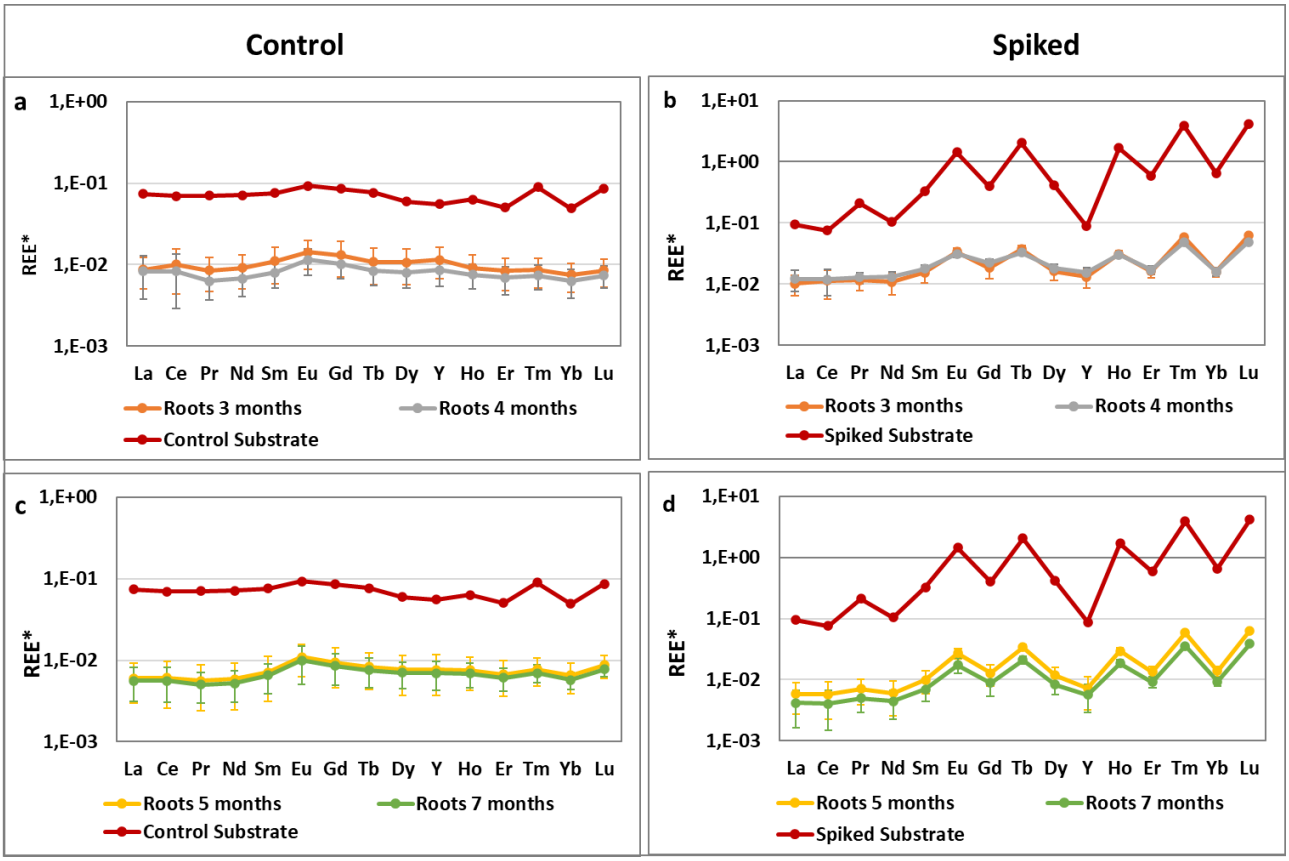

Fig. 3. Roots spectra at 3rd and 4th month of growth in control (a) and spiked (b) conditions and roots spectra at 5rd and 7th month of growth in control (c) and spiked (d) conditions, compared to substrates spectra (red line).

Figs 4 show the distribution REE* in xylem-sap compared to Roots and UCC distribution. We found that, in the first months of growth xylem-sap spectra does not discriminate between control and spiked substrates as REE* is constantly symmetrical to the UCC (Fig.s 4a and b). Whereas, after 5 months of growth (Figs 4c and d) we found that REE* in xylem-sap mimics the root REE* in both experimental conditions.

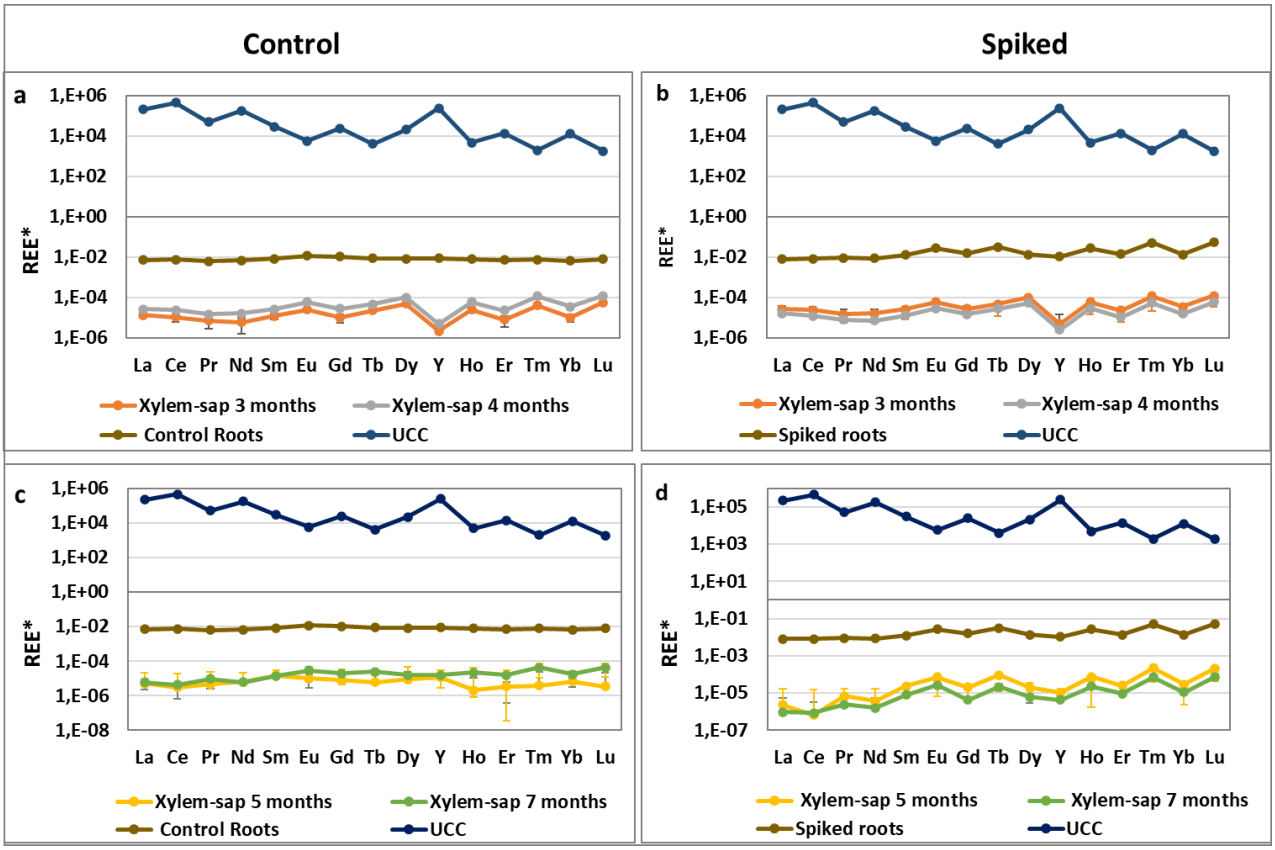

Fig. 4. Xylem-sap spectra at 3rd and 4th month of growth in control (a) and spiked (b) conditions and xylem-sap spectra at 5rd and 7th month of growth in control (c) and spiked (d) conditions, compared to UCC distribution (blue line) and roots spectra (brown line).

Figs 5 show the distribution REE* in leaves compared to roots and xylem-sap for both experimental conditions during plant growth. We found that REE* in leaves are characterized by a slight enrichment of LREE respect HREE in control conditions reflecting the roots pattern (Figs 5a, c), whereas in spiked conditions, REE* behave differently as a function of the growth time: at 3 and 4 months, REE* is significantly different of the roots and xylem-sap (Fig 5b) whereas at 5 and 7 months of growth REE* shows the 'zig-zag' shape as xylem-sap and roots (Fig 5d) suggesting that leaves reflect the xylem-sap behaviour.

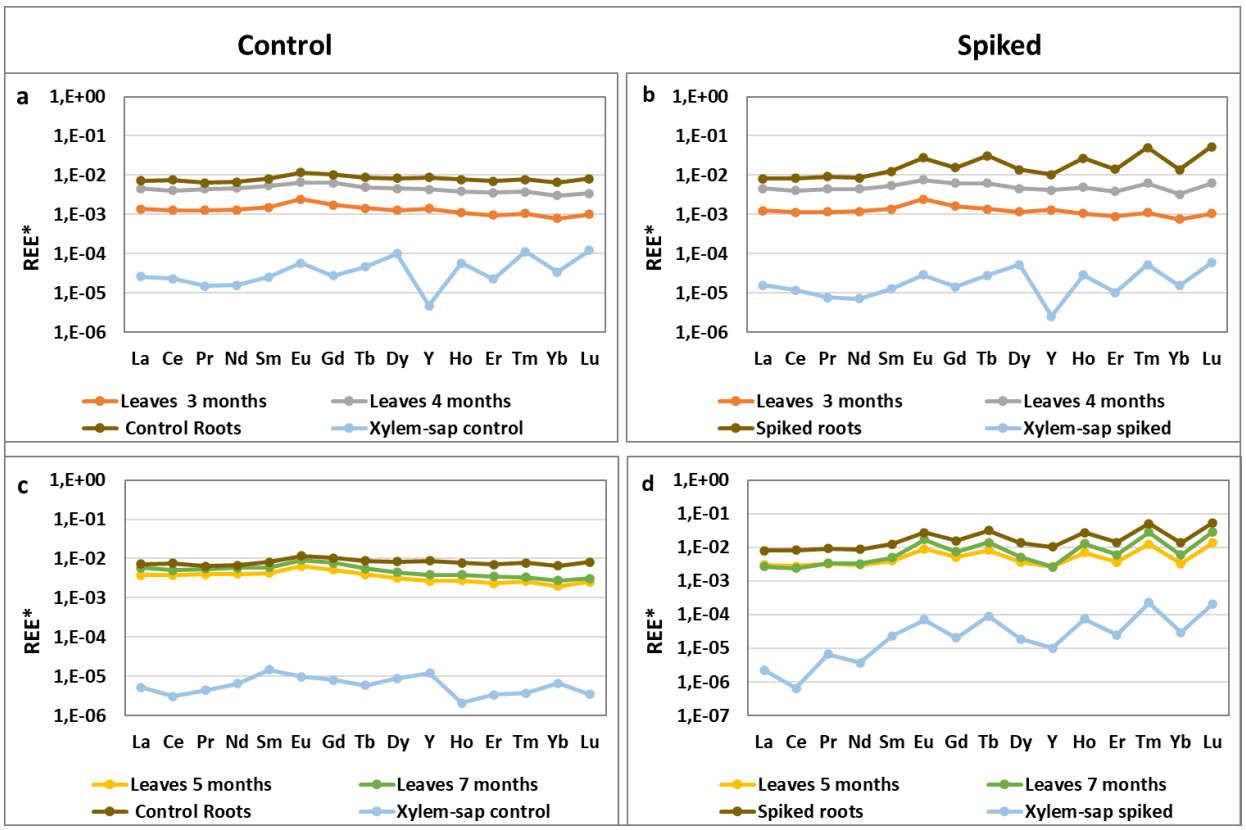

Fig. 5. Leaves spectra at 3rd and 4th month of growth in control (a) and spiked (b) conditions and leaves spectra at 5rd and 7th month of growth in control (c) and spiked (d) conditions, compared to xylem-sap distribution (light-blue line) and roots spectra (brown line).

This implies that the transfer from roots to leaves through the xylem-sap do not fractionate reflecting a coherent REE-group behaviour, suggesting a conservative transport of REE in *Vitis vinifera L.* This result confirms the apoplastic/passive transportation proposed for the transport of the nutrients and metabolic products in xylem–sap (Shan et al., 2003; Hossain et al., 2012). Figs 5, also, show that irrespective of the substrate conditions, leaves have a positive Eu anomaly and not in xylem-sap and roots (Fig 5a,b,c) and could be attributed to possible Eu–Ca substitution during physiological processes (Brioschi et al., 2013; Ding et al., 2007; Gao et al., 2003; Krzciuk et al., 2019; Liang et al., 2008) as well the stronger stability of organic-Eu

complexes (e.g. proteins) compared to Ca complexes (Gao et al., 2003; Götzke et al 2019). However, Eu anomaly may also be related to different oxidation state of Europium as in leaves, photosynthesis generates intermediates negative redox potentials (Baier and Dietz, 2005; Blankenship, 2021) enhancing a Europium bivalent oxidation state. Under these conditions, the presence of Eu^{2+} produces a greater radius enhancing europium mobility compared to other REE.

The REE equimolar addition to the substrate of growth allowed us to highlight the capacity of the substrate to retain REE and exclude possible competition phenomena between each REE during the transfer mechanisms in soil-*Vitis vinifera L*. system. Our experimental investigation reveals that the main organs of *Vitis vinifera L.* are able to register weak relative variations of REE in soil, discriminating the environmental conditions. Our results show that the full-normalized spectra of REE of roots reflect the soil enrichment from the first months of growth while xylem-sap and leaves trace this enrichment from the 5th month only. This means that xylem-sap does not modify the REE transfer highlighting the *Vitis vinifera L*. ability to keep unaltered the REE pattern during transport, suggesting that the REE* can be used to differentiate plants coming from different soil of growth in natural conditions.

Implication and Conclusion

The geographic traceability of food products is crucial to ensure the quality and authenticity of food. However, finding a chemical tracer that univocally links a specific production area with a food product is still a challenge. Our experimental investigation show that REE* in plant organs is significantly similar to the REE* of the substrates for both investigate systems. This

indicates that REE transfer is not selective but depends on the relative abundance of REE in the substrates of growth. The absence of significative anomalies in the xylem-sap corroborating with a conservative REE transport in *Vitis vinifera L.* Also, our results show that leaves follow the behaviour of the xylem-sap. This result represents an important starting point to demonstrate that REE content and distribution in plants are mainly related to metal ion migration from soil to leaves and that the influence of the atmospheric fallout in the REE distribution may be neglected. Our investigation, also, reveals that despite the REE transfer being influenced by the plant growth, REE distribution pattern in *Vitis vinifera L*. organs discriminates the environmental conditions of growth, even when small differences in terms of relative abundance of REE are present in the substrates. Since REE* can be used to differentiate plants from different soils of growth, we propose that the use of REE* is a potential marker for identifying the substrate of *Vitis vinifera L*. growth. Our work yielded also important consequences from an environmental perspective. Indeed, we found that *Vitis vinifera L*. organs trace the artificial enrichments of the substrate of growth, suggesting that REE spectra can be a useful tool to assess the quality and safety of ecosystems, discriminating possible REE pollution of the soil. Globally the results of this work highlight the potential use of REE as biogeochemical tracers of environmental conditions. These results push to advance with studies to better understand the effect of native/anthropogenic soil REE contents upon living systems, with particular attention on factors controlling the release dynamics of REE in different kinds of soils.

References

Aceto, M., Robotti, E., Oddone, M., Baldizzone, M., Bonifacino, G., Bezzo, G., 2013. A traceability study on the Moscato wine chain. Food. Chem. 138, 1914–1922.

Aide M.T., Aide C., (2012). Rare earth elements: their importance in understanding soil genesis. International Scholarly Research Notices, 2012.

Arbuzov S.I., Maslov S.G., Finkelman R.B., Mezhibor A.M., Ilenok, S.S., Blokhin, M.G., et al., (2018). Modes of occurrence of rare earth elements in peat from Western Siberia. Journal of Geochemical Exploration, 184, 40-48.

Baier, M., Dietz, K. J. (2005). Chloroplasts as source and target of cellular redox regulation: a discussion on chloroplast redox signals in the context of plant physiology. Journal of experimental botany, 56(416), 1449-1462.

Balaram, V. (2019). Rare earth elements: A review of applications, occurrence, exploration, analysis, recycling, and environmental impact. Geoscience Frontiers, 10(4), 1285-1303.

Barbera, M., Zuddas, P., Palazzolo, E., Saiano, F. (2021). The distribution of Rare Earth Elements discriminates the growth substrate of Vitis vinifera L. Chemosphere, 266, 128993.

Bertoldi D., Larcher R., Bertamini M., Otto S., Concheri G., Nicolini, G., (2011). Accumulation and distribution pattern of macro- and microelements and trace elements in Vitis vinifera L. cv. chardonnay berries. Journal of Agriculture and Food Chem. 59, 7224–7236.

Bertoldi D., Larcher R., Nicolini G., Bertamini M., Concheri G., (2009). Distribution of rare earth elements in Vitis vinifera L. 'Chardonnay' berries. Vitis J. Grapevine Res. 48, 49–51.

Bettinelli M., Spezia S., Baffi C., Beone G.M., Rocchetta R., Nassisi A., (2005). ICP-MS determination of REE in tomato plants and related products: a new analytical tool to verify traceability. At. Spectrosc. 26, 41-50.

Blankenship, R. E. (2021). Molecular mechanisms of photosynthesis. John Wiley & Sons.

Brioschi L., Steinmann M., Lucot E., Pierret M.C., Stille P., Prunier, J., (2013). Transfer of rare earth elements (REE) from natural soil to plant systems: implications for the environmental availability of anthropogenic REE. Plant Soil. 366(1-2), 143-163.

Brunner M., Katona R., Stefánka Z., Prohaska, T., (2010). Determination of the geographical origin of processed spice using multielement and isotopic pattern on the example of Szegedi paprika. European Food Research and Technology, 231(4), 623-634.

Buhtz A., Kolasa A., Arlt K., Walz C., Kehr J., (2004). Xylem sap protein composition is conserved among different plant species. Planta, 219(4), 610-618.

Calabrese S., Aiuppa A., Allard P., Bagnato E., Bellomo S., Brusca L., et al., (2011). Atmospheric sources and sinks of volcanogenic elements in a basaltic volcano (Etna, Italy). Geochim. Cosmochim. Acta 75, 7401-7425.

Censi P., Cibella F., Falcone E.E., Cuttitta G., Saiano F., Inguaggiato C., et al., (2017). Rare earths and trace elements contents in leaves: A new indicator of the composition of atmospheric dust. Chemosphere. 169, 342-350.

Censi P., Saiano F., Pisciotta A., Tuzzolino N., (2014). Geochemical behaviour of rare earths in Vitis Vinifera grafted onto different rootstocks and growing on several soils. Sci. of Total. Environ. 473, 597-608.

De Boer J.L.M., Verweij W., Van Der Velde-Koerts T., Mennes W., (1996). Levels of rare earth elements in Dutch drinking water and its sources. Determination by inductively coupled plasma mass spectrometry and toxicological implications. A pilot study. Water Res. 30, 190-198.

Di Lorenzo R., Barbagallo M.G., Costanza P., Gugliotta E., Lino T., (2005). Pre dawn, stem and leaf water potential evolution in Vitis vinifera L. cv Nero d'Avola/1103 P. under different water regime. In Proceeding for the fourteenth international conference GESCO, Geisenheim, Germany pp. 493–498.

Ding SM, Liang T, Yan JC et al (2007) Fractionations of rare earth elements in plants and their conceptive model. Sci China Ser C Life Sci 50:47–55

Drivelos S.A., Georgiou C.A., (2012). Multi-element and multi-isotope ratio analysis to determine the geographical origin of foods in the European Union. Trend. Anal. Chem. 40, 38–51.

Gao Y., Zeng F., Yi A., Ping S., Jing L., (2003). Research of the entry of rare earth elements Eu3+ and La3+ into plant cell. Biological trace element research, 91(3), 253-265.

Götzke, L., Schaper, G., März, J., Kaden, P., Huittinen, N., Stumpf, T., Weigand, J. J. (2019). Coordination chemistry of f-block metal ions with ligands bearing bio-relevant functional groups. Coordination Chemistry Reviews, 386, 267-309.

Hossain M.A., Piyatida P., da Silva J.A.T., Fujita M., (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. Journal of Botany, 2012.

İlhan, Ö. (2021) Characteristics of EU Registered Geographical Indications of Food and Agricultural Products and Investigation of Their Relationship with the Geographical Area. Aydın Gastronomy, 5(2), 117-129.

Jowitt S.M., Werner T.T., Weng Z., Mudd G.M., (2018). Recycling of the rare earth elements. Curr. Opin. Green Sustain. Chem. https://doi.org/10.1016/j.cogsc.2018.02.008.

Krzciuk K., Gałuszka A., (2019). Seasonal changes in concentrations of trace elements and rare earth elements in shoot samples of Juncus effusus L. collected from natural habitats in the Holy Cross Mountains, south-central Poland. Chemosphere. 219, 954-960.

Laveuf, C., Cornu, S., 2009. A review on the potentiality of rare earth elements to trace pedogenetic processes. Geoderma. 154, 1–12.

Liang, T., Ding, S., Song, W., Chong, Z., Zhang, C., Li, H., 2008. A review of fractionations of rare earth elements in plant. J. Rare Earths, 26, 7–15.

Loell M., Albrecht C., Felix-Henningsen P., (2011). Rare earth elements and relation between their potential bioavailability and soil properties, Nidda catchment (Central Germany). Plant Soil 349:303–317.

Marchionni S., Buccianti A., Bollati A., Braschi E., Cifelli F., Molin P., et al., (2016). Conservation of 87Sr/86Sr isotopic ratios during the winemaking processes of 'Red'wines to validate their use as geographic tracer. Food chemistry, 190, 777-785.

Miao, L., Ma, Y., Xu, R., Yan, W., 2011. Environmental biogeochemical characteristics of rare earth elements in soil and soil-grown plants of the Hetai goldfield, Guangdong Province, China. Environ. Earth Sci. 63, 501–511.

Migaszewski Z.M., Gałuszka A., (2015). The characteristics, occurrence, and geochemical behavior of rare earth elements in the environment: a review. Critical reviews in environmental science and technology, 45(5), 429-471

Netzer F., Schmid C., Herschbach C., Rennenberg H., (2017). Phosphorus-nutrition of European beech (Fagus sylvatica L.) during annual growth depends on tree age and P-availability in the soil. Environ Exp Bot 137:194–207.

Oddone M., Aceto M., Baldizzone M., Musso D., Osella D., (2009). Authentication and traceability study of hazelnuts from Piedmont, Italy. Journal of agricultural and food chemistry, 57(9), 3404-3408.

Pallardy S., (2008). Physiology of Woody Plants, third ed. Elsevier Inc.

Pepi S., Coletta A., Crupi P., Leis M., Russo S., Sansone L., (2016). Geochemical characterization of elements in Vitis Vinifera cv. Negroamaro grape berries grown under different soil managements. Environ. Monit. Assess. 188, 211.

Pepi S., Grisenti P., Sansone L., Chicca M., Vaccaro C., (2018). Chemical elements as fingerprints of geographical origin in cultivars of Vitis vinifera L. raised on the same SO4 rootstock. Environmental Science and Pollution Research, 25(1), 490-506.

Pepi S., Sansone L., Chicca M., Vaccaro C., (2017). Relationship among geochemical elements in soil and grapes as terroir fingerprintings in Vitis vinifera L. cv."Glera". Chemie der Erde, 77(1), 121-130.

Pisciotta A., Tutone L., Saiano F., (2017). Distribution of YLOID in soil-grapevine system (Vitis vinifera L.) as tool for geographical characterization of agro-food products. A two years case study on different grafting combinations. Food chemistry, 221, 1214-1220.

Punturo R., D'Antone C., Pepi S., Vaccaro C., (2018). Rare earth elements absorption patterns in grapevine "Vitis vinifera L." cultivated in carbonate terrains (south-eastern Sicily, Italy). Environmental earth sciences, 77(24), 801.

Reimann C., De Caritat P., (2000). Intrinsic flaws of element enrichment factors (EFs) in environmental geochemistry. Environ. Sci. Technol. 34, 5084e5091.

Reimann C., De Caritat P., (2005). Distinguishing between natural and anthropogenic sources for elements in the environment: regional geochemical surveys versus enrichment factors. Sci. Total Environ. 337, 91e107.

Reimann C., Englmaier P., Fabian K., Gough L., Lamothe P., Smith D., (2015). Biogeochemical plant-soil interaction: variable element composition in leaves of four plant species collected along a south-north transect at the southern tip of Norway. Sci. Total Environ. 506-507, 480-495.

Richter B., Gurk S., Wagner D., Bockmayr M., Fischer, M., (2019). Food authentication: Multi-elemental analysis of white asparagus for provenance discrimination. Food Chem. 286, 475-482.

Scalenghe R., Territo C., Petit S., Terribile F., Righi D., (2016). The role of pedogenic overprinting in the obliteration of parent material in some polygenetic landscapes of Sicily (Italy). Geoderma Reg. 7, 49-58.

Shan X., Wang H., Zhang S., Zhou H., Zheng Y., Yu H., et al., (2003). Accumulation and uptake of light rare earth elements in a hyperaccumulator Dicropteris dichotoma. Plant Science, 165(6), 1343-1353.

Sucharovà J., Suchara I., Hola M., Marikova S., Reimann C., Boyd R., et al., (2012). Top-/bottom-soil ratios and enrichment factors: what do they really show?. Applied Geochemistry, 27(1), 138-145.

Thomas, P.J., Carpenter, D., Boutin, C., Allison, J.E., 2014. Rare earth elements (REEs): effects on germination and growth of selected crop and native plant species. Chemosphere, 96, 57-66.

Tomašević M., Rajšić S., Đorđević D., Tasić M., Krstić J., Novaković V., (2004). Heavy metals accumulation in tree leaves from urban areas. Environmental Chemistry Letters, 2(3), 151-154.

Tommasi F., Thomas P.J., Pagano G., Perono G.A., Oral, R., Lyons, D.M., et al., (2020). Review of rare earth elements as fertilizers and feed additives: a knowledge gap analysis. Archives of Environmental Contamination and Toxicology, 1-10.

Tyler, G., 2004. Rare earth elements in soil and plant systems — a review. Plant Soil. 267, 191–206.

US Environmental Protection Agency, 2012. Rare Earth Elements: A Review of Production, Processing, Recycling, and Associated Environmental Issues. EPA 600/R-12/572. (www.epa.gov/ord).

Yan D., Ro S., Sunam O., Kim S., (2020). On the Global Rare Earth Elements Utilization and Its Supply-Demand in the Future. In IOP Conference Series: Earth and Environmental Science. (IOP Publishing) Vol. 508, No. 1, p. 012084.

Yu K.F., Kamber B.S., Lawrence M.G., Greig A., Zhao J.X., (2007). High-precision analysis on annual variations of heavy metals, lead isotopes and rare earth elements in mangrove tree rings by inductively coupled plasma mass spectrometry. Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. Atoms 255, 399-408.

Yuan, M., Guo, M.N., Liu, W.S., Liu, C., van der Ent, A., Morel, J.L., Huot, H., Zhao, W.Y., Wei, X.G., Qiu, R.L., Tang, Y.T., 2017. The accumulation and fractionation of Rare Earth Elements in hydroponically grown Phytolacca americana L. Plant Soil. 421, 67-82.

Zhao J., Du X., Cheng N., Chen L., Xue X., Zhao J., et al., (2016). Identification of monofloral honeys using HPLC-ECD and chemometrics. Food Chemistry, 194, 167–174.

Figure captions

Fig. 1. REE amounts (µmol/kg) in control and spiked substates

Fig. 2. REE distribution in native soil, control and spiked substrate

Fig. 3. Roots spectra at 3rd and 4th month of growth in control (a) and spiked (b) conditions and roots spectra at 5rd and 7th month of growth in control (c) and spiked (d) conditions, compared to substrates spectra (red line)

Fig. 4. Xylem-sap spectra at 3rd and 4th month of growth in control (a) and spiked (b) conditions and xylem-sap spectra at 5rd and 7th month of growth in control (c) and spiked (d) conditions, compared to UCC distribution (blue line) and Roots spectra (brown line)

Fig. 5. Leaves spectra at 3rd and 4th month of growth in control (a) and spiked (b) conditions and leaves spectra at 5rd and 7th month of growth in control (c) and spiked (d) conditions, compared to compared to xylem-sap distribution (light-blue line) and roots spectra (brown line)

5.3. Is *Vitis vinifera L.* physiology sensitive to the REE transport?

To assess if REE uptake impacts the physiology of *Vitis vinifera L.*, we have determined the concentration of the Micronutrient (MIN): Fe, Mn, Cu, Zn, as well Macronutrient (MAN): Mg, K, Ca as well the main organic acids (citric, lactic, malic and succinic acid) in the xylem-sap. We choose to monitor metal nutrients because xylem-sap hydraulic conductance varies in response to changes in sap ions content (Cochard et al., 2010; Nardini et al., 2011, 2012) and monitoring organic acid contents because cations (Fe, Al, Cd, Ni, As,) are transported in the xylem-sap by complexation with the organic ligands (Verma nee Juneja and Prakash, 2008; Ding et al., 2007; Gupta et al., 2018). We have initially determined the total ∑REE amount in xylem-sap in both experimental conditions (Fig 5.1). We found that the overall REE amount in xylem-sap is constant: ∑REE accumulated in xylem-sap does not change significantly either between the two environmental conditions or with plant growth.

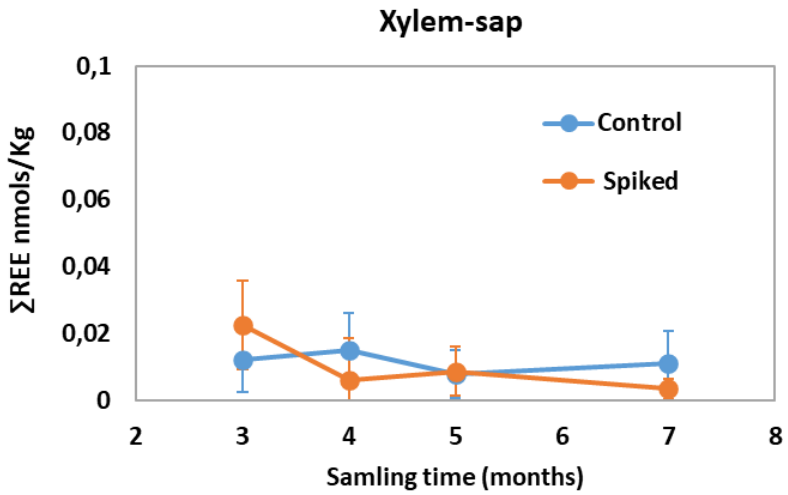

Fig 5.1. ∑REE amount in xylem-sap for control and spiked conditions during plant growth

Reporting the normalised distribution REE* for the xylem-sap respect to the substrates of growth (Fig. 5.2) we found that, initially in xylem-sap the shape of REE* pattern does not change between control and spiked conditions and REE* is significantly different of the pattern of the substrate (Fig. 5.2 a, b). Whereas, after 5 months of growth REE* pattern mimic growth substrate characteristics for both experimental conditions (Fig. 5.2 c,d) meaning that xylem-sap registers the enrichment of the substrate. We found that, despite the total accumulation significantly does not change REE spectra discriminate the experimental conditions of growth.

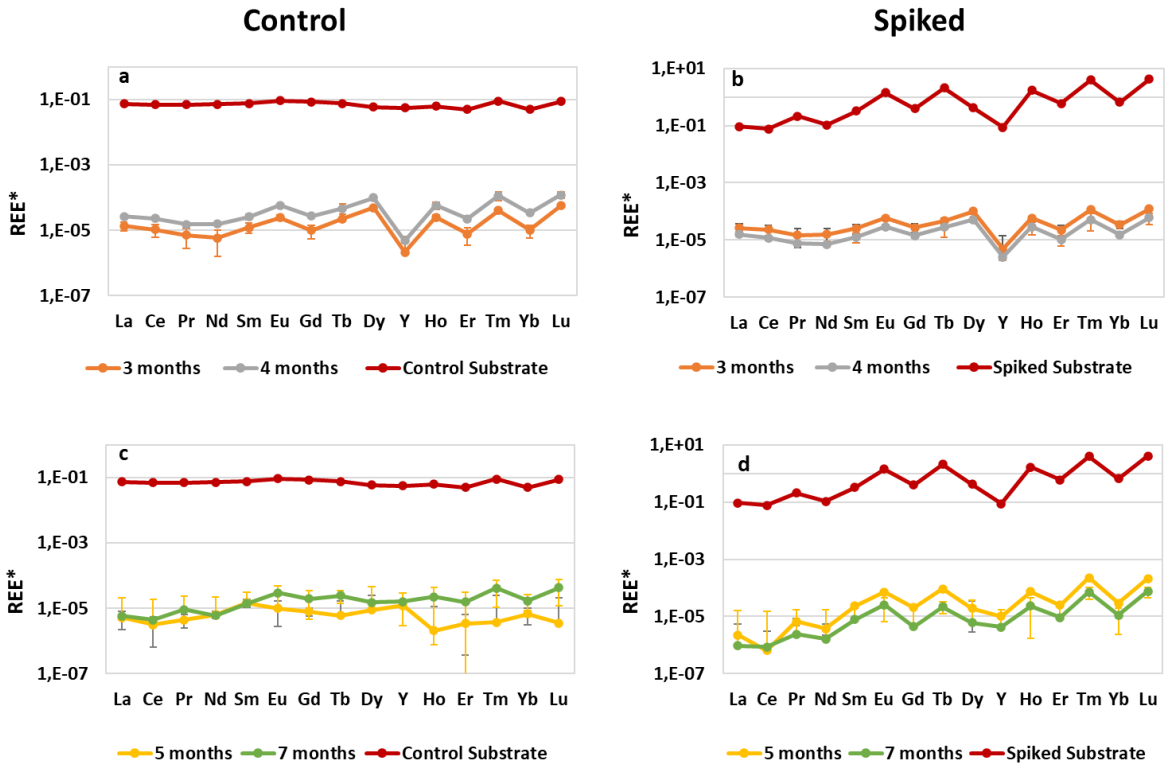

Fig. 5.2. Xylem-sap spectra at 3 and 4 months of growth in control (a) and spiked (b) conditions and at 5 and 7 months of growth in control (c) and spiked (d) conditions, compared to substrates spectra (red line).

Since the REE distribution is influenced by the growth of the plant, we have concentred our investigation on the period in which the greatest variations in terms of REE distribution were observed (4 and 7 months of growth). To evaluate if the REE amount transported in xylem-sap influence the possible interaction between REE and organic ligands, we have determined some of the major organic acids (citric, lactic, malic and succinic acid) in xylem-sap. We found that the most abundant organic acid is citric followed by malic acid. The concentration of lactic and succinic acid was less than 100 µg/L. The citric acid concentration spanning from 252.3 ± 41.5 to 392.28 ± 274.4 µg/L for control samples, and from 533.1 ± 135.5 to 367.7 ± 190.6 µg/L for spiked samples, and the malic acid concentration ranging from 208.8 ± 30.1 to 190.4 ± 67.1 µg/L for control samples, and from 247.46 ± 170.4 to 293.5 ± 186.3 µg/L for spiked samples. Figure 5.3 shows the ratio between citric and malic acid concentration in xylem-sap in control and spiked conditions at 4 and 7 months of growth.

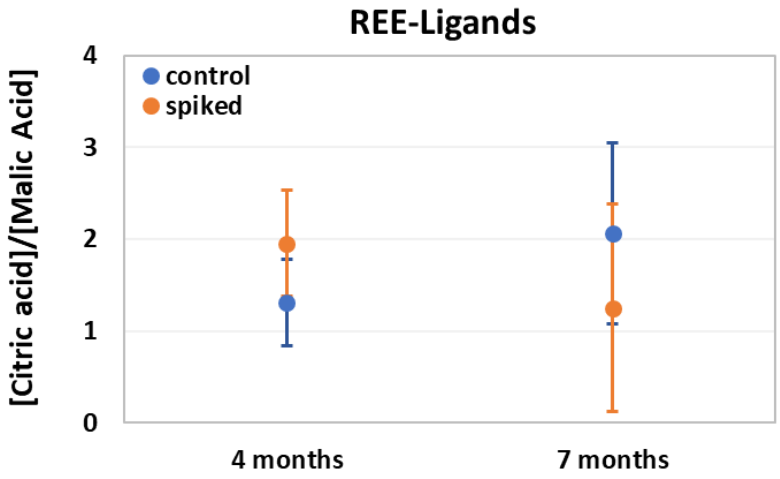

Fig. 5.3. Ratio between citric and malic acid concentration in xylem-sap in control and spiked conditions at 4 and 7 months of growth

We found that the amount of citric/malic ratio does not change between the two experimental conditions, meaning that REE enrichment does not influence the concentration of the ligands. Ligand concentration is more than 200 times higher compared to the REE concentration, suggesting that, in our system, all REE should be complexed by organic ligands (Sursyakova et al., 2016). Therefore, even if it is true that the stability constants of REE with citrate slightly increase from LREE to HREE (Byrne and Li, 1995; Götzke et al., 2019), in our system, the high ligand/analyte ratio justifies a not selective REE transport, in agreement with the absence of the REE anomaly detected by the distribution pattern. To control *Vitis vinifera L.* physiological processes we have monitored the Micronutrient (MIN): Fe, Mn, Cu, Zn, as well Macronutrient (MAN): Mg, K, Ca) amount in xylem-sap both in control and spiked conditions in the second experiment (Fig. 5.4.4). Figure 5.4. shows the MAN and MIN amount detected in xylem-sap in control and spiked conditions at 4 and 7 months of growth.

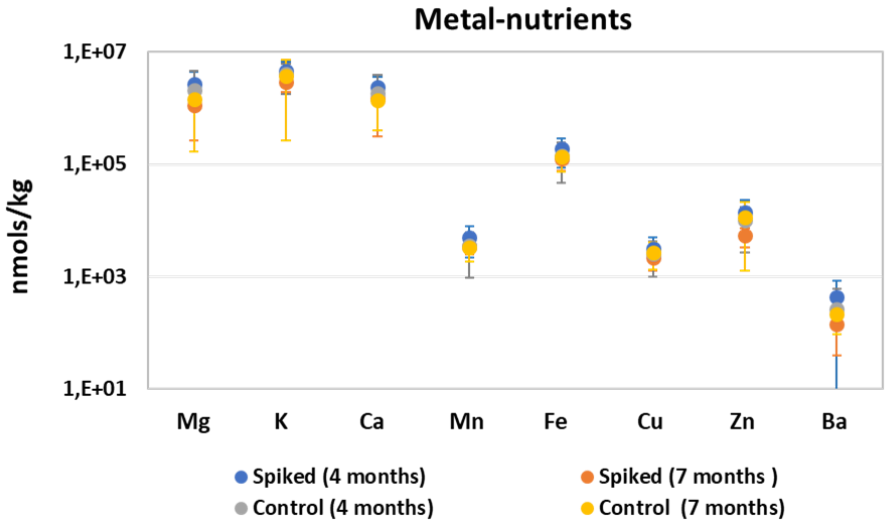

Figure 5.4. MAN and MIN concentrations in xylem-sap in control and spiked conditions at 4 and 7 months of growth

We found that the MAN components are more than one order of magnitude higher than the MIN components, However, the concentrations of both macro and micronutrients do not change between control and spiked conditions and for both sampling times. Since we have not found variations in the electrolyte and organic acids concentration, these resulst indicates that REE enrichments does not significantly impact plant physiology. It is necessary to highlight that this result is just an indication because in our conditions the REE amount added is very low.

As mentioned before, xylem-sap analysis represents an analytical challenge both for the low trace elements concentration and for the low amount of sap extracted that imply the impossibility to apply pre-concentration techniques and make replicas (each sample is related to the instant (t) in which the xylem-sap was collected). Validation studies showed that REE determination in xylem-sap is affected by high variability. Therefore, to verify the significance of the amount of the analytes determined (REE, MAN and MIN), we tested a data series that deviate from the global behaviour of the majority of data itself. To evaluate the eventual presence of multivariate outliers we used the Mahalanobis (T_2) and Euclidean distance (Q). Mahalanobis distance is a measure of distance between two random values that allows quantification of the closeness between two probability distributions whereas, the Euclidean metric is the geometrical distance between two points within the multidimensional space (Leardi et al 2020). Both parameters (T_2 and Q) were calculated from the data matrix composed of 22 variables (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb Dy, Y, Ho, Er, Tm, Yb, Lu, Fe, Mn, Cu, Zn, Mg, K, Ca) and 35 Samples (6 control and 29 spiked for both sampling time) (Fig. 5.5). Data

were autoscaled and centred. The elaborations and the plots were carried out through the software CAT (Chemometric Agile Tool) (Leardi et al 2020).

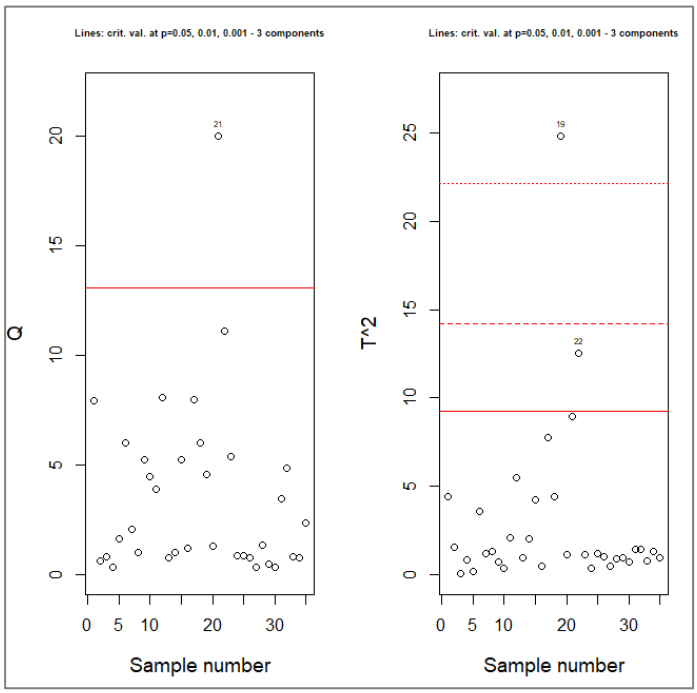

Figure 5.5. Mahalanobis (T^2) and Euclidean distance for MAN, MIN variables in xylem-sap for control and spiked samples

We found, at 95% of confidence, that only 3 spiked samples showed anomalous behaviour with respect to the others whereas, all other samples, both control and spiked, showed a homogeneous behaviour (Fig 5.17). Considering the high variability intrinsic of the system, we can consider these 3 points as outliers. The closeness between two probability distributions of REE, MAN and MIN in xylem-sap suggest that the *Vitis vinifera L.* behaviour is homogenous between the two different experimental conditions. Our results do not show evidence on the direct metabolism function of REE^{3+}, suggesting that contaminated REE substrate does not

influence the long-distance water transport transfer. Our study has provided indications on the methodological approach to perform trace analysis in xylem-sap, however, we must underline that in our experimentation the objective was to assess if *Vitis vinifera L.* was able to register even small variations of REE in the substrates. Therefore, to verify the real impact of REE^{3+} on the *Vitis vinifera L.* life new tests should be carried out increasing the concentration of REE in the substrate of growth.

Globally, we found that the REE enrichment of substrate influences neither the plant mass nor the total REE accumulation in aerial part. *Vitis vinifera L.* preferably accumulates REE in fine roots. However, both experimental investigations of the REE transfer dynamic in soil/plant system showed that *Vitis vinifera L.* accumulated REE proportionally to the amount of REE of substrates. This result means that *Vitis vinifera L.* uptake REE without selectivity. Also, we found that the REE* discriminates the environmental conditions of growth even when the substrate of growth is poorly enriched in REE, suggesting that REE* is a potential marker of the substrate of *Vitis vinifera L.* growth. The investigation of REE behaviour in xylem-sap allows, for the first time, to propose a conservative REE transfer mechanism. Our results, in particular, showed that, despite the very low concentration values of REE in xylem-sap, ranging between 1-20 ng/l, REE* spectra in xylem-sap trace soil spiked conditions. This result indicates the absence of fractionation during REE transfer. The ability of *Vitis vinifera L.* organs to keep unaltered the REE pattern during the REE transfer from soil to leaves, suggests that REE* can be used to differentiate plants coming from different soils.

Conclusions and perspectives

In this thesis, we have analysed the possible use of REE on tracing the geographical origin of *Vitis vinifera L.* An analytical methodology was developed to determine REE in soils and plant organs and to measure REE ultra-low amount in xylem-sap by ICP-MS. The method was validated through a metrological approach controlling of all sources of uncertainty. We have examined and discussed the REE accumulation and distribution in the plant organs, as well growth physiology variations during the stress potentially generated by the soil contamination. Although soil is a primary destination of most products containing REE, little was known on the potential impact of anthropogenic soil pollution from REE in agricultural and food products.

We found that, irrespectively of the REE amount present in the substrates of growth, leaves accumulate the same levels of REE in ‘polluted’ and ‘unpolluted’ conditions indicating that grapevine products obtained by *Vitis vinifera L*. cultivations should have a very low amount of REE. Since REE are considered emergent contaminants, our results are very important from the environmental point of view as, even if REE are non-essential trace elements, they may potentially enter into plant tissues and in the food-chains.

We showed that REE* trace the artificial enrichment of the substrate of growth and consequently REE spectra can be a useful tool assessing the quality and safety of ecosystem, discriminating the possible soil REE pollution. The REE* spectra, as well as tracing artificial substrate pollution, demonstrated the correspondence between the elemental profile of the *Vitis vinifera L.* organs with the substrates. This indicates that REE transfer is not selective but depends on the relative abundance of REE in the substrates of growth. Also, the similarity of the REE* shape between substrates and leaves suggest that the REE transfer in *Vitis vinifera L.* is not influenced by environmental factors (weathering, light exposure, substrate features), as atmospheric fallout.

The main point revealed in this work is that the REE distribution patterns discriminate the environmental conditions of growth, even when small differences in terms of relative abundance of REE are present in the substrates. This confirms that the REE* can be used to differentiate plants coming from different soil of growth.

These results are helpful in the establishment of geographical tracer strategies, howevere somemodifications are needed. In our investigation, we have used an equimolar solution of REE allowing verifying the reciprocal behaviour of each REE while such a proportion is not common in natural conditions. Considering that high differences between elements concentration can involve different reactivity of single elements, we believe that other experiments should be carried out using different kinds of soils as substrate of growth to verify the real and general discriminating power of REE*.

Overall, our data extend knowledge about the uptake of REE in *Vitis vinifera L*. and their behaviour in soil-plant relationships even from an environmental point of view, highlighting the potential of REE as biogeochemical tracers and as potential environmental indicators.

General References

Aide M.T., Aide C., (2012). Rare earth elements: their importance in understanding soil genesis. International Scholarly Research Notices, 2012.

Alonso E., Wallington,T., Sherman A., Everson M., Field F., Roth R., et al., (2012). An assessment of the rare earth element content of conventional and electric vehicles. SAE Int. J. Mater. Manuf. 5, 473-477.

Alves Dias P., Bobba S., Carrara S., Plazzotta B. (2020). The role of rare earth elements in wind energy and electric mobility, EUR 30488 EN, Publication Office of the European Union, Luxembourg.

Angelini, Renzo. *La vite e il vino.* Coltura & Cultura, 2007.

Araujo, P. (2009). Key aspects of analytical method validation and linearity evaluation. Journal of chromatography B, 877(23), 2224-2234.Belter M., Sajnóg A.,

Arbuzov S.I., Maslov S.G., Finkelman R.B., Mezhibor A.M., Ilenok, S.S., Blokhin, M.G., et al., (2018). Modes of occurrence of rare earth elements in peat from Western Siberia. Journal of Geochemical Exploration, 184, 40-48.

Arif N., Yadav V., Singh S., Singh S., Ahmad P., Mishra R.K. (2016). Influence of high and low levels of plant-beneficial heavy metal ions on plant growth and development. Frontiers in environmental science, 4, 69.

Aysha MK. (2017). Speciation and bioavailibility of rare earth elements (REEs) in ex-tin mining area: A case study from Lahat, Perak, Malaysia/Aysha Masood Khan. Doctoral dissertation, University of Malaya.

Balaram V. (2019). Rare earth elements: A review of applications, occurrence, exploration, analysis, recycling, and environmental impact. Geoscience Frontiers, 10(4) 1285-1303.

Byrne R.H., Li B., (1995). Comparative complexation behavior of the rare earths. Geochimica et Cosmochimica Acta, 59(22), 4575-4589.

Barceló J., Poschenrieder, C., Vázquez M.D., Gunsé B. (1993). Beneficial and toxic effects of chromium in plants: Solution culture, pot and field studies. In Studies in Environmental science (Vol. 55, pp. 147-171). Elsevier.

Bargagli R., (1998). Trace elements in terrestrial plants. Springer.

Bertoldi D., Larcher R., Bertamini M., Otto S., Concheri G., Nicolini G. (2011). Accumulation and distribution pattern of macro- and microelements and trace elements in Vitis vinifera L. cv. chardonnay berries. Journal of Agriculture and Food Chem. 59, 7224–7236.

Borrego J., Carro B., López-González N., De La Rosa J., Grande J.A., Gómez T., et al., (2012). Effect of acid mine drainage on dissolved rare earth elements geochemistry along a fluvial–estuarine system: the Tinto-Odiel Estuary (SW Spain). Hydrology Research, 43(3), 262-274.

Brioschi L., Steinmann M., Lucot E., Pierret M.C., Stille P., Prunier, J., (2013). Transfer of rare earth elements (REE) from natural soil to plant systems: implications for the environmental availability of anthropogenic REE. Plant Soil. 366(1-2), 143-163.

Buhtz A., Kolasa A., Arlt K., Walz C., Kehr J. (2004). Xylem sap protein composition is conserved among different plant species. Planta, 219(4), 610-618.

Calabrese S., Aiuppa A., Allard P., Bagnato E., Bellomo S., Brusca L., et al., (2011). Atmospheric sources and sinks of volcanogenic elements in a basaltic volcano (Etna, Italy). Geochim. Cosmochim. Acta 75, 7401-7425.

Cao M., (2001). Determination of stability constants for rare earth elements and fulvic acids extracted from different soils. Talanta 53, 1163–1170.

Censi P., Cibella F., Falcone E.E., Cuttitta G., Saiano F., Inguaggiato C. (2017). Rare earths and trace elements contents in leaves: A new indicator of the composition of atmospheric dust. Chemosphere. 169, 342-350.

Censi P., Saiano F., Pisciotta A., Tuzzolino N. (2014). Geochemical behaviour of rare earths in Vitis Vinifera grafted onto different rootstocks and growing on several soils. Sci. of Total. Environ. 473, 597-608.

Censi P., Tamburo E., Speziale S., Zuddas P., Randazzo L.A., Punturo R. (2011). Yttrium and lanthanides in human lung fluids, probing the exposure to atmospheric fallout. J. Hazard. Mater. 186, 1103–1110.

Ciacci, L., Reck, B.K., Nassar, N.T. Graedel, T.E. (2015). Lost by design. Environ. Sci. Technol. 49, 9443–9451.

Cochard, H., Herbette, S., Hernandez, E., Hölttä, T., Mencuccini, M. (2010). The effects of sap ionic composition on Xylem-sap vulnerability to cavitation. J. Exp. Bot. 61 (1).

De Boer J.L.M., Verweij W., Van Der Velde-Koerts T., Mennes W. (1996). Levels of rare earth elements in Dutch drinking water and its sources. Determination by inductively coupled plasma mass spectrometry and toxicological implications. A pilot study. Water Res. 30, 190-198.

De Souza S.V., Junqueira R.G., (2005). A procedure to assess linearity by ordinary least squares method. Analytica Chimica Acta, 552(1-2), 25-35.

Di Lorenzo R., Barbagallo M.G., Costanza P., Gugliotta E., Lino T. (2005). Pre dawn, stem and leaf water potential evolution in Vitis vinifera L. cv Nero d'Avola/1103 P. under different water regime. In Proceeding for the fourteenth international conference GESCO, Geisenheim, Germany pp. 493–498.

Diaz-Espejo A., Hernandez-Santana V. (2017). The phloem–Xylem-sap consortium: until death do them part. Tree Physiol. 37, 847–850.

Dinali G.S., Root R.A., Amistadi M.K., Chorover J., Lopes, G., Guilherme, L.R.G. (2019). Rare earth elements (REY) sorption on soils of contrasting mineralogy and texture. Environment international, 128, 279-291.

Ding S., Liang T., Yan J., Zhang Z., Huang, Z., Xie, Y. (2007). Fractionations of rare earth elements in plants and their conceptive model. Science in China Series C: Life Sciences, 50(1), 47-55.

Ellison S.L.R., Williams A., (2012). Quantifying Uncertainty in Analytical Measurement, Third edition. Eurachem/CITAC guide.

Goodenough K.M., Wall F., Merriman D. (2017). The rare earth elements: demand, global resources, and challenges for resourcing future generations, Natural Resources Research, n°27, pages 201 – 216.

Götzke L., Schaper G., März J., Kaden P., Huittinen N., Stumpf T. (2019). Coordination chemistry of f-block metal ions with ligands bearing bio-relevant functional groups. Coordination Chemistry Reviews, 386, 267-309.

Gupta N., Yadav K.K., Kumar V., Kumar S., Chadd R.P., Kumar A., (2018). Trace elements in soil-vegetables interface: translocation, bioaccumulation, toxicity and amelioration-a review. Science of The Total Environment.

Hossain M.A., Piyatida P., da Silva J.A.T., Fujita M., (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. Journal of Botany, 2012.

Jowitt S.M., Werner T.T., Weng Z., Mudd G.M. (2018). Recycling of the rare earth elements. Current Opinion in Green and Sustainable Chemistry, 13, 1-7

Kabata-Pendias A. (2011). Trace Elements in Soils and Plants. 4th Ed. New York: Taylor and Francis Group.

Kabata-Pendias, A., Pendias, H. (2001). Trace elements in soils and plants–CRC Press. Boca Raton.

Kant S., (2017). Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. Semin. Cell Dev. Biol. 74, 89–96.

Kegl T., Košak A., Lobnik A., Novak Z., Kralj A.K., Ban I. (2020). Adsorption of rare earth metals from wastewater by nanomaterials: a review. Journal of hazardous materials, 386, 121632.

Kovaríková M., Tomásková I., Soudek, P. (2019). Rare earth elements I plants. Biol. Plant., 63, 20–32.

Krzciuk K., Gałuszka, A. (2015). Prospecting for hyperaccumulators of trace elements: a review. Critical reviews in biotechnology, 35(4), 522-532.

Krzesłowska, M. (2011). The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. Acta physiologiae plantarum, 33(1), 35-51.

Laveuf C., Cornu S. (2009). A review on the potentiality of rare earth elements to trace pedogenetic processes. Geoderma. 154, 1–12.

Leardi R., Melzi C., Polotti, G. (2020). CAT (Chemometric Agile Software). Available online: http://gruppochemiometria.it/index.php/ software.

Li K., Li M., Xue D. (2012). Solution-phase electronegativity scale: Insight into the chemical behaviors of metal ions in solution. The Journal of Physical Chemistry A, 116(16), 4192-4198.

Liang T., Zhang S., Wang L., Kung H., Wang Y., Hu A., (2005). Environmental biogeochemical behaviors of rare earth elements in soil–plant systems. Environ Geochem Health 27:301–311.

Loell M., Albrecht C., Felix-Henningsen P. (2011). Rare earth elements and relation between their potential bioavailability and soil properties, Nidda catchment (Central Germany). Plant Soil 349:303–317.

Long, K. R., Van Gosen, B. S., Foley, N. K. Cordier, D. The principal rare earth elements deposits of the United States: A summary of domestic deposits and a global perspective. U.S. Geological Survey Scientific Investigations Report 2010–5220 (2010).

Magnusson B., and Örnemark U. (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0. Available from www.eurachem.org.

Marchionni S., Buccianti A., Bollati A., Braschi E., Cifelli F., Molin P., et al., (2016). Conservation of 87Sr/86Sr isotopic ratios during the winemaking processes of 'Red'wines to validate their use as geographic tracer. Food chemistry, 190, 777-785.

McCormick, J.M., (2016). Propagation of Uncertainty. CHEM 130 Chemical Principles I Laboratory Manual, 141 (p.p 141-146).

Miao L., Ma Y., Xu R., Yan W., (2011). Environmental biogeochemical characteristics of rare earth elements in soil and soil-grown plants of the Hetai goldfield, Guangdong Province, China. Environ. Earth Sci. 63, 501–511.

Migaszewski Z.M., Gałuszka A., (2015). The characteristics, occurrence, and geochemical behavior of rare earth elements in the environment: a review. Critical reviews in environmental science and technology, 45(5), 429-471.

Mihajlovic J., Rinklebe J., (2018). Rare earth elements in German soils-A review. Chemosphere, 205, 514-523. Nardini, A., Dimasi, F., Klepsch, M., Jansen, S., 2012. Ion-mediated enhancement of Xylem-sap hydraulic conductivity in four Acer species: Relationships with ecological and anatomical features. Tree Physiol. 32 (12), 1434–1441.

Nardini A., Salleo S., Jansen S. (2011). More than just a vulnerable pipeline: Xylem-sap physiology in the light of ion-mediated regulation of plant water transport. J. Exp. Bot. 62 14 4701–4718.

Netzer F., Schmid C., Herschbach C., Rennenberg H. (2017). Phosphorus-nutrition of European beech (Fagus sylvatica L.) during annual growth depends on tree age and P-availability in the soil. Environ Exp Bot 137:194–207.

Oddo E., Inzerillo S., Grisafi F., Sajeva M., Salleo S., Nardini, A. (2014). Does short-term potassium fertilization improve recovery from drought stress in laurel?. Tree physiology, 34(8), 906-913.

Pallardy S., (2008). Physiology of Woody Plants, third ed. Elsevier Inc.

Pisciotta A., Tutone L., Saiano F. (2017). Distribution of YLOID in soil-grapevine system (Vitis vinifera L.) as tool for geographical characterization of agro-food products. A two years case study on different grafting combinations. Food chemistry, 221, 1214-1220.

Pratt R.B., Jacobsen A.L. (2018). Identifying which conduits are moving water in woody plants: a new HRCT-based method. Tree Physiol. 38, 1200–1212.

Pryseley A., Mintiens K., Knapen K., Van der Stede Y., Molenberghs, G. (2010). Estimating precision, repeatability, and reproducibility from Gaussian and non-Gaussian data: a mixed models approach. Journal of applied statistics, 37(10), 1729-1747.

Punturo R., D'Antone C., Pepi S., Vaccaro C. (2018). Rare earth elements absorption patterns in grapevine "Vitis vinifera L." cultivated in carbonate terrains (south-eastern Sicily, Italy). Environmental earth sciences, 77(24), 801.

Pupyshev A.A., Semenova E.V. (2001). Formation of doubly charged atomic ions in the inductively coupled plasma. Spectrochim Acta Part B At Spectrosc;56:2397-418.

Redling K. (2006). Rare earth elements in agriculture with emphasis on animal husbandry. (Ludwig-Maximilians-Universität München (Doctoral dissertation, lmu).

Reimann C., De Caritat P. (2000). Intrinsic flaws of element enrichment factors (EFs) in environmental geochemistry. Environ. Sci. Technol. 34, 5084e5091.

Reimann C., De Caritat P. (2005). Distinguishing between natural and anthropogenic sources for elements in the environment: regional geochemical surveys versus enrichment factors. Sci. Total Environ. 337, 91-107.

Reimann C., Englmaier P., Fabian K., Gough L., Lamothe P., Smith D. (2015). Biogeochemical plant-soil interaction: variable element composition in leaves of four plant species collected along a south-north transect at the southern tip of Norway. Sci. Total Environ. 506-507, 480-495.

Richter B., Gurk S., Wagner D., Bockmayr M., Fischer, M. (2019). Food authentication: Multi-elemental analysis of white asparagus for provenance discrimination. Food Chem. 286, 475-482.

Sasmaz M., Obek, E., Sasmaz A. (2018). The accumulation of La, Ce and Y by Lemna minor and Lemna gibba in the Keban gallery water, E lazig T urkey. Water Environ. J. 32, 75-83.

Scalenghe R., Territo C., Petit S., Terribile F., Righi D. (2016). The role of pedogenic overprinting in the obliteration of parent material in some polygenetic landscapes of Sicily (Italy). Geoderma Reg. 7, 49-58.

Shan X., Wang H., Zhang S., Zhou H., Zheng Y., Yu H. (2003). Accumulation and uptake of light rare earth elements in a hyperaccumulator Dicropteris dichotoma. Plant Science, 165(6), 1343-1353.

Skoog D.A., West, D.M., Holler F.J. (1988). Fundamentals of Analytical Chemistry, 5th Ed.; Saunders College Publishing: New York, p. 39-42.

Sojka, M., Choiński, A., Ptak, M., Siepak, M. (2021). Causes of variations of trace and rare earth elements concentration in lakes bottom sediments in the Bory Tucholskie National Park, Poland. Scientific reports, 11(1), 1-18.

Sucharovà J., Suchara I., Hola M., Marikova S., Reimann C., Boyd R. (2012). Top-/bottom-soil ratios and enrichment factors: what do they really show?. Applied Geochemistry, 27(1), 138-145.

Sursyakova, V. V., Burmakina, G. V., Rubaylo, A.I. (2016). Influence of analyte concentration on stability constant values determined by capillary electrophoresis. Journal of chromatographic science, 54(7), 1253-1262.

Taylor S.R., McLennan S.M. (1988). The significance of the rare earths in geochemistry and cosmochemistry. Handbook on the physics and chemistry of rare earths, 11, 485-578.

Thomas P.J., Carpenter D., Boutin C., Allison J.E. (2014). Rare earth elements (REEs): effects on germination and growth of selected crop and native plant species. Chemosphere, 96, 57-66.

Tomašević M., Rajšić S., Đorđević D., Tasić M., Krstić J., Novaković V. (2004). Heavy metals accumulation in tree leaves from urban areas. Environmental Chemistry Letters, 2(3), 151-154.

Tommasi F., Thomas P.J., Pagano G., Perono G.A., Oral, R., Lyons, D.M. (2020). Review of rare earth elements as fertilizers and feed additives: a knowledge gap analysis. Archives of Environmental Contamination and Toxicology, 1-10.

Turra C. (2018). Sustainability of rare earth elements chain: from production to food–a review. Int. J. of Environ. Health Res. 28, 23-42.

Tyler G., (2004). Rare earth elements in soil and plant systems — a review. Plant and Soil. 267:191–206.

USGS, (2020). USGS Mineral Commodity Summaries (2020). Reston, Virginia. https:// doi.org/10.3133/mcs2020. Voncken, J.H.L., 2016. The Rare

Verma nee Juneja S., Prakash S., (2008). Electrophoretic study of chromium speciation in xylem sap of maize (Winter Crop). Chemical Speciation Bioavailability, 20(2), 55-63-

Vermeire M.L., Cornu S., Fekiacova Z., Detienne M., Delvaux B., Cornélis J.T., (2016). Rare earth elements dynamics along pedogenesis in a chronosequence of podzolic soils. Chemical Geology. 446, 163-174.

Wang L., Huang X., Zhou Q., (2008). Effects of rare earth elements on the distribution of mineral elements and heavy metals in horseradish. Chemosphere. 73(3), 314-319.

Wang L., He J., Xia A., Cheng M., Yang Q., Du C., et al., (2017). Toxic effects of environmental rare earth elements on delayed outward potassium channels and their mechanisms from a microscopic perspective. Chemosphere. 181, 690-698.

Zhenggui W., Ming Y., Xun Z., Fashui H., Bing L., Chunhua, Y. (2001). Rare earth elements in naturally grown fern Dicranopteris linearis in relation to their variation in soils in South-Jiangxi region (Southern China). Environmental Pollution, 114(3), 345-355.

Yan D., Ro S., Sunam O., Kim S., (2020). On the Global Rare Earth Elements Utilization and Its Supply-Demand in the Future. In IOP Conference Series: Earth and Environmental Science. (IOP Publishing) (Vol. 508, No. 1, p. 012084).

Yu K.F., Kamber B.S., Lawrence M.G., Greig A., Zhao J.X. (2007). High-precision analysis on annual variations of heavy metals, lead isotopes and rare earth elements in mangrove tree rings by inductively coupled plasma mass spectrometry. Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. Atoms 255, 399-408.

Ye X.X., Wang G.Z., Zhang, Y.X., Zhao, H.J. (2018). Hydroxyapatite nanoparticles in root cells: reducing the mobility and toxicity of Pb in rice. *Environmental Science: Nano*, *5*(2), 398-407.

Yuan M., Guo M.N., Liu W.S., Liu C., van der Ent A., Morel J.L. (2017). The accumulation and fractionation of Rare Earth Elements in hydroponically grown Phytolacca americana L. Plant Soil. 421, 67-82.

Zhao J., Du X., Cheng N., Chen L., Xue X., Zhao J., (2016). Identification of monofloral honeys using HPLC-ECD and chemometrics. Food Chemistry, 194, 167–174.

Zheng T., Haider M.S., Zhang K., Jia H, Fang, J. (2020). Biological and functional properties of Xylem-sap sap extracted from grapevine (cv. Rosario Bianco). Scientia Horticulturae, 272, 109563.

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List of Abbreviations

CRM	Certified Reference Material
DW	Dried Weight
ESI-	Negative-ion Electro-Spray Ionization
HPLC-MS/MS	High Performance Liquid Chromatography-Mass Spectrometry
HREE	Heavy Rare Earth Elements
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IDL	Instrumental Detection limit
IQL	Instrumental Quantification limit
ISTD	Internal Standard
KED	Kinetic Energy Discrimination
LREE	Light rare Earth Elements
MAN	Macronutrients
MDL	Method Detection Limit
MIN	Micronutrients
MQL	Method Quantification Limit
MRM	Multiple Reaction Monitoring
QC	Quality Control
REC	Recovery
REE*	Rare Earth Elements spectra
REE	Rare Earth Elements
RM	Reference Material
RSD	Relative Standard Deviation
SW	Spiked Water
TF	Translocation Factors
U	Uncertainty
Uc	Combined Uncertainty
UCC	Upper Continental Crust
U_{dil}	Dilution Uncertainty
U_{rec}	Recovery Uncertainty
U_{ref}	Reference Material Uncertainty
U_{rep}	Repeatability Uncertainty
$u_{stock\ sol}$	Stock standard solution Uncertainty
VIM	Vocabulaire International de Metrolgie
Xy	Xylem-sap matrix

Appendix A

1. Validation parameters

1.1. Mandel test: The linearity of the calibration process was investigated by means of Mandel's Fitting Test Value (Mandel, 1964). This is a statistical test to ascertain the goodness of fit of the calibration curve. Specifically, Mandel's test defines which is the better fit of the experimental data, comparing of residual standard error between linear and quadratic models. (Raposo, 2016; Rawski et al., 2016).

1.2. Instrumental Detection limit (IDL)" is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified, it is estimated by using the expression:

$$Y_{DL} = y + 3s$$

Where y is the average value of the standard solution signal and s its corresponding standard deviation, obtained by measuring at least 10 independent samples.

1.3. Instrumental Quantification limit (IQL): is defined as the lowest concentration of analyte that can be determined quantitatively with an acceptable level of precision. The procedure for evaluating LOQ is equivalent to that of LOD, by measuring at least 10 independent sample blanks and using the factor 10 instead of 3:

$$Y_{QL} = y + 10s$$

1.4. Method detection and quantification limit (MDL, MQL): MDL and MQL should always be distinct by the instrument detection and quantification limits (IDL and IQL). IDL and IQL are based on measurements on standard solution, whereas MDL and MQL are calculated on measurement on real samples, IDL is often far smaller than a DL because does not take into account the matrix effects (Thompson et al., 2002; Magnusson et al 2014; EPA guidelines 2016; Belter et al 2014)

1.5. Precision: The definition of precision in the Vocabulaire International de Metrolgie (VIM) is: "closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions". Precision can be described through two quantities: repeatability and reproducibility. Repeatability is obtained from replication with the same analytical procedure, instrument and reagents, in the same laboratory, by the same analyst, in a "short" period of time, this can be calculated through the Relative Standard Deviation (RSD) (Thompson et al., 2002). This parameter depends only on the distribution of random errors and does not relate to the true or specified value (ISO 21748:2017; Magnusson et al., 2014; Pryseley et al., 2010).

To evaluate the precision, differences observed between two or more test results are examined. For this purpose, a critical value is required, rather than a standard deviation. Repeatability limit (r) is enabled by comparing the data set of results determined under repeatability conditions (Pryseley et al., 2010). This parameter is calculated by:

$$r = |x_1 - x_2| = t_{(p,\nu)} \cdot s_r \cdot \sqrt{2}$$

Where t, is the critical value of the Student t distribution at the 95% confidence level (Pryseley et al., 2010).

1.6. Trueness (or accuracy) is an index of the agreement between the experimental mean value and an accepted reference value. Method accuracy can be quantified in different ways, as indicated in the EURACHEM guide (Magnusson et al 2014): participation in collaborative trials; use of certified reference materials (CRM); use of reference materials (RM), This last case is applied when CRM is not available. Examples of RM are materials characterized in the laboratory by adding a known mass of the analyte (spiked materials).

1.6.1 Recovery (bias) is the ratio of the found value to the assumed value true, can be calculated as the ratio between the difference of the mean of several determinations of a test sample, obtained under repeatability condition, and its "true" or accepted concentration, as follows:

$$\%Rec. = \frac{C_0 - C_R}{C_R}\ x\ 100$$

where C_0 is the mean value obtained from repeated measurements of a CRM using the method under validation and C_R is the assigned value for the CRM. Bias can be expressed as the ratio (Rec), or percentage (%Rec) between C_0 and C_R and can be greater or lower than 100%. (Magnusson et al., 2014).

When CRM is not available recovery can be calculated through spiked materials as follows:

$$\%Rec. = \frac{C_{spike+balk} - C_{blank}}{C_{spike}}\ x\ 100$$

where $C_{spike+blank}$ is the mean value obtained from repeated measurements of a spiked sample, C_{blank} is the mean value obtained from repeated measurements of a sample before the spike addition, and C_{spike} is the known mass of the analyte added to the test portion.

Recovery calculation is fundamental when the determination method needs extraction or pre-treatments of the samples. Indeed, this parameter allows quantifying if the analyte present in the sample is effectively extracted. Taking into account eventual recovery variation is possible to correct the result analytically or to evaluate if it falls within a given range of acceptability.

1.7. F-test: This statistical tool allows to compare the variance of two data sets through the following expression:

$$F_{calc} = \frac{S_1^2}{S_2^2} < F_{tab}$$

where the s^2 is the variance, that corresponds to the square of the standard deviation. Conventionally the larger s^2 must be the numerator (Araujo, 2009). The performances are evaluated by comparing calculated F (Fcalc) with tabled F (F^{th}). F^{th} depends on the degrees of freedom (ν) for s_1, and s_2 (ν= *n*-1 where *n* is the number of replicas). If $F_{cal} < F_{th}$ it is possible

to claim that the two standard deviations are not significantly different with 95% of confidence. Thus, there is still a 5% chance that we draw the wrong conclusion.

2. Combined Uncertainty

Combined Uncertainty can be calculated using a functional relationship involving other measured quantities and their measured uncertainties. If measurand y is determined from input variables x1, x2, …, xn through a functional relationship, then uncertainties in the x's will propagate through the calculation to uncertainty in *y*. The general relationship between the uncertainty Uc (y) of a value y and the uncertainty of the independent parameters U(xn), on which it depends is described by:

$$\dot{U}_C\left(y\left(x_1, x_2, x_N\right)\right) = \sqrt{\sum_{i=1,N} \dot{U}\left(x_i\right)^2}$$

Table A1 summarizes the formulas used to calculate the single uncertainty contributions.

(Guide JCGM 100:2008; Magnusson et al., 2014)

Repeatability Uncertainty (U_{rep})	$\boldsymbol{U_{rip} = \frac{u(\bar{x})}{\bar{x}} = \frac{r}{\bar{x}\sqrt{n}}}$	r = repatability limit $\bar{x}$ = experimental value mean n = number of replicas
Recovery Uncertainty (U_{rec})	$\boldsymbol{U_{rec} = \sqrt{\frac{S^2}{n} + u_{CRM}^2}}$	S^2 =variance n = number of replicas u_{CRM} = uncertainty of the CRM
Standard Material Uncertainty ($U_{st.material}$)	$\boldsymbol{U_{St.material} = \frac{a}{\sqrt{3}}}$	a= relative error of the material
Stock standard solution Uncertainty ($U_{stock\ sol.}$)	$\mathbf{U_{stock\ sol.} = \frac{\frac{5}{1000}}{\sqrt{3}}}$	1000=standard solution concentration 5= declared material uncertain
Reference Material Uncertainty ($U_{ref.}$)	$\boldsymbol{u_{ref} = \sqrt{u_{Stock\ sol.}^2 + u_{I\ dil.}^2 + u_{II\ dil.}^2 + u_{III\ dil}^2}}$	u_{dil}=Dilution Uncerainty $u_{stock\ sol}$ =Stock standard solution Uncertainty

Table A1 Formulas for calculating the uncertainty contributions

3. Quality control

Quality control (QC) was performed through X-chart and duplicate analysis. X-chart looks for changes in the average value of X-measurements as time goes on, whereas duplicate analysis looks for systematic differences between two consecutive results. Although several types of control charts can be applied, here will be described the Control Chart of the Mean (x-chart) that was used in this thesis. X-chart is constituted by five lines: one for the Mean, two Warning Limits and two Action Limits (see Fig 1).

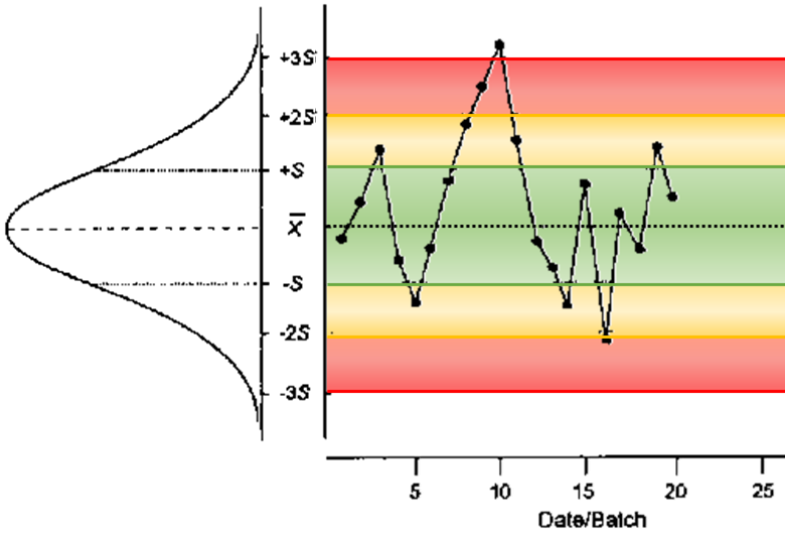

Figure A1. X-Chart

Points to draw the X-chart are collected each time a result for the control sample is obtained in a batch of test samples. The basic assumption is that when a control result falls within a distance of 2s from the mean, the system was under control and the results of the batch as a whole can be accepted. A control result beyond the distance of 2s from the mean (the "Warning Limit") signals that something may be wrong or tends to go wrong, while a control result beyond 3s (the "Control Limit" or "Action Limit") indicates that the system was

statistically out of control and that the results have to be rejected: the batch has to be repeated after sorting out what went wrong and after correcting the system.

Reference

Araujo, P. (2009). Key aspects of analytical method validation and linearity evaluation. Journal of chromatography B, 877(23), 2224-2234.Belter M., Sajnóg A.,

Barałkiewicz D., (2014). Over a century of detection and quantification capabilities in analytical chemistry–Historical overview and trends. Talanta, 129, 606-616.

EPA guidelins Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (pdf) (December 2016, 821-R-16-006).

ISO/IEC Guide 99:2007, International vocabulary of metrology - Basic and general concepts and associated terms (VIM). ISO, Geneva, (2007). Available from http://www.bipm.org as JGCM 200:2008.

Joint Committee for Guides in Metrology (JCGM) 100: 2008. Evaluation of measurement data — Guide to the expression of Uncertainty in Measurement, First Edition September 2008.

Magnusson B., U. Örnemark U., (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0. Available from www.eurachem.org.

Mandel J., (1964). The Statistical Analysis of Experimental Data, Interscience Publ. J. Wiley & Sons New York.

Pryseley A., Mintiens K., Knapen K., Van der Stede Y., Molenberghs, G., (2010). Estimating precision, repeatability, and reproducibility from Gaussian and non-Gaussian data: a mixed models approach. Journal of applied statistics, 37(10), 1729-1747.

Raposo F., (2016). Evaluation of analytical calibration based on least-squares linear regression for instrumental techniques: A tutorial review. TrAC Trends in Analytical Chemistry, 77, 167-185.

Rawski R.I., Sanecki P.T., Kijowska, K.M., Skitat, P.M., Saletnik, D.E., (2016). Regression analysis in analytical chemistry. Determination and validation of linear and quadratic regression dependencies. South African Journal of Chemistry, 69, 166-173.

Thompson, M., Ellison, S. L., Wood, R. (2002). Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure and Applied Chemistry, 74(5), 835-855.