Consiglio Nazionale delle Ricerche

UNIVERSITÀ DI SIENA
1240

UNIVERSITÀ DI PISA

GIOVANISÌ Regione Toscana

DEPARTMENT OF LIFE SCIENCES

Ph.D SCHOOL IN LIFE SCIENCES

XXXV Cycle

COORDINATOR Prof. Simona Maccherini

Geographical traceability of extra virgin olive oil from Valdichiana Senese (Tuscany, Italy)

Scientific-Disciplinary sector: BIO/03

TUTOR: Prof. Stefano Loppi

Candidate: Martina Grattacaso

Co-tutor: Prof. Maria João Cabrita
(University of Évora)

Co-tutor: Prof. Giuseppe Protano

A.A. 2021-2022

Index

ABSTRACT

Extra virgin olive oil (EVOO) is derived from the pressing of the drupes of the *Olea europaea* tree and is renowned for its health benefits due to the presence of several monounsaturated fats, antioxidants, and polyphenols. Indeed, EVOO is a typical food used in the Italian gastronomic tradition because the *terroir* of Italy allows the production of high-quality olive oil. For this reason, to ensure transparency, to prevent fraud and enhance consumer confidence, methods were developed to determine the geographical origin and authentication of EVOOs. Methods like elemental chemical analysis, compositional analysis, and isotopic analysis play central roles in determining the geographical origin. The area selected for the study is Valdichiana Senese, in Southern Tuscany, Italy, acclaimed for its EVOOs, renowned for its bitter and spicy attributes and its high-quality.

This thesis is divided in four parts:

- The concentrations of 12 potential toxic elements (As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, U, V, and Zn; PTEs) were determined in soils and EVOOs collected in 2019-20 with the aim of assessing the potential risk for human health related to PTE intake from the consumption of Valdichiana Senese EVOO as well as to identify any contamination by PTEs in olive grove soils and evaluate the associated ecological risk. The data, obtained by ICP-MS analyses, were used to calculate the Health Risk Index (HRI) for EVOOs and the Potential Ecological Risk Index (PERI) for soils. The results indicated that although EVOOs from Valdichiana Senese may present somewhat slightly high concentrations of Cu and Zn, the associated health risk is negligible. The concentration of PTEs in olive grove soils was comparable to their geochemical background in soils from Southern Tuscany. A low to

moderate Cu contamination emerged only in two cases, probably because of the diffuse use of Cu-based products in agriculture. Overall, the ecological risk was very low.

- In order to establish the geographical traceability of Valdichiana Senese EVOOs were considered physical and chemical properties of the olive grove soils and the concentration of 36 elements in the olive grove soils (total content and bioavailable fraction), in olive pulps and EVOOs collected in 2020-21. In the pursuit of relationships between soil, olive fruit and EVOO chemical composition, REEs were excluded due to their undetectability in olives and EVOOs. Pearson correlations revealed Sb as is the only element correlated between bioavailable soil fractions and olives among all farms. Instead, in soils with only marine sands and sandstones substratum, positive correlations were observed between total contents of Fe and V in soils and EVOOs and the content of U with olive pulps. Also, were found correlations of Pb and Sb in the soil bioavailable fraction and olives.
- Volatile organic compounds (VOC) and fatty acid (FA) profiles in EVOOs collected in 2021-22 and 2020-21 from Valdichiana Senese and Alentejo, Portugal, were compared to classify EVOOs according to their origin area. Utilising HS-SPME-GC/MS and GC-FID analysis were identified 107 compounds in Italian EVOOs, while 77 in those Portuguese, with only 43 in common. Volatiles were divided in classes (alcohols, aldehydes, alkanes, esters, and terpenoids) and 2-Hexenal is the compound most abundant in both EVOOs. For FAs, oleic, palmitic, and linoleic are the acids with the highest percentages. Discriminant analysis confirmed that volatile compounds and fatty acids are valid to distinguish EVOOs based on their year and area of production.
- Additionally, the study evaluated the quality of EVOOs from 2019 to 2021, focusing on free acidity, peroxide values, and total phenols content. Valdichiana Senese EVOOs showed an excellent ability to maintain unaltered characteristics. Weather conditions were observed to affect the quantity of phenols. In addition, the same parameters were used to

reveal insights into stability and factors influencing quality parameters in Valdichiana Senese EVOO from the 2021-22 harvest to 12 months later. The linear regression analysis allowed to see that the peroxide values and the phenols content were highly influenced by the time, but not acidity.

1. INTRODUCTION

1.1. EXTRA VIRGIN OLIVE OIL

Olive oil is the product obtained from the crushing of the drupes of olive, a fruit-bearing tree belonging to the Oleaceae family. The olive tree (*Olea europaea* L.; Fig.1) thrives in the Mediterranean region, known for its warm temperate climate characterised by long, arid summers (Loumou & Giourga, 2003). This evergreen tree, reaching heights of 3-20 m, exhibits growth characteristics influenced by environmental factors, such as soil and climate, as well as cultivar selection and cultural practices (Boskou, 2006). These factors impact the shape, density, and overall development of the tree's canopy, with some specimens growing as tall as 15 m with trunk diameters ranging from 1.5 to 2 m. Notable traits of this species include its remarkable ability to adapt to extreme environmental and growing conditions, as well as its longevity. Worldwide, there are approximately 2000 known cultivars of olive trees, alongside with many cases of synonyms, ecotypes, and local varieties, contributing to the immense genetic diversity of olive germplasm (Ilarioni & Proietti, 2014).

Figure 1. *Olea europaea* tree.

Olea europaea produces an oval-shaped drupe as fruit, whose weight ranges from 2 to 12 g, although in some varieties it may weigh up to 20 g (Boskou, 2006). The drupe is composed of pericarp, divided further in endocarp and mesocarp, and epicarp (Paiva-Martins & Kiritsakis, 2017). The endocarp, or fruit pit, is a rigid and woody structure that encloses the seed at the centre of the fruit. Externally there is the mesocarp, the fruit pulp, and then the epicarp, the external layer of cells (Rugini et al., 2016). In an olive fruit, the average composition consists of approximately 22% oil, 50% water, and the remaining 19% comprises carbohydrates (pectin, cellulose (6%), and hemicelluloses), proteins (1.6%), minerals (1.5%), volatile compounds (such as aldehydes, alcohols, esters, hydrocarbons, ketones, and furans), as well as lignin (Seçmeler & Galanakis, 2019; Conde et al., 2008).

Olive oil is obtained by physical extraction from the fruits of *O. europaea*. No treatments are applied to the olives besides washing, settling, centrifugation, and filtration. Olive oil is usually classified in categories mainly based on quality parameters, such as acidity and peroxide index, established by national and international regulations (Grossi et al., 2014). According to Regulation (EEC) No 2568/91, there are four categories used to classify olive oils:

- Extra Virgin Olive Oil (EVOO): the highest quality olive oil obtained through the pressing of olives; in order to prevent any alteration of the oil, the extraction is carried out at temperatures <27°C. The free acidity (expressed in % of oleic acid) must be <0.8%, even if optimal values are 0.1-0.3%. The Regulation provides that the limit on the number of peroxides in an EVOO is 20, above which the oil is considered of low quality. In addition, the oil is considered acceptable if this value is <12, excellent if <7.
- Virgin Olive Oil (VOO): is always obtained through the pressing of olives and its free acidity does not exceed 2%.
- Lampante Virgin Olive Oil (LVOO): is not suitable for consumption and requires a refining process to address its organoleptic defects. Lampante virgin olive oil is the classification given to all oils obtained through physical processes that have a free acidity exceeding 2%.
- Refined Olive Oil (ROO): has a free acidity greater than 2% and requires a refining process before it can be consumed.

EVOO is considered the highest quality olive oil and is one of the most important components of the Mediterranean diet. It has been shown that a habitual use of EVOO in the diet has positive effects on human health, such as maintaining a normal level of cholesterol, because the significant presence of oleic acid reduces the low-density lipoprotein (LDL) cholesterol and increases the high-density lipoprotein (HDL) cholesterol in the bloodstream (Mata et al., 1992). Indeed, it protects the cells from oxidation (Lioupi et al., 2020) and decreases the risk of cardiovascular disease (Servili et al., 2013; Visioli & Galli, 2002). Scientific research has consistently demonstrated the numerous benefits associated with the consumption of extra virgin olive oil (Foscolou et al., 2018). Rich in monounsaturated fats, antioxidants, and polyphenols, this oil is known to reduce the risk of heart disease, lower cholesterol levels, and contribute to overall well-being (Žanetić et al., 2021). Additionally, its anti-inflammatory properties and potential anticancer effects have further enhanced its reputation as a fundamental component of a healthy lifestyle.

Being a food highly consumed, at least in the Mediterranean area, several studies have focused on determining the concentration of potentially toxic elements (PTEs) in EVOO (Kabaran et al., 2020; Zaanouni et al., 2018; Angioni, 2010; Bakkali et al., 2009). Indeed, food consumption is the main pathway of PTE accumulation in the human body (Mendil et al., 2009). The intake of PTEs from olive oil consumption depends on their concentration in this food and the amount consumed. The US Environmental Protection Agency (US EPA), the International Olive Council, the FAO/WHO Codex and the European Commission (International Olive Council, 2019; World Health Organization, 2004; Commission of the European Communities, 2006; JECFA, 2003) provided guidelines about the intake of some PTEs through olive oil consumption. The concentration of PTEs in olive oils is mainly dependent upon the physical, geochemical, and biological features of soils of olive groves, combined with the characteristics of the olive tree cultivars. These features regulate the bioavailability of PTEs in the soil as well as root uptake and translocation from roots to fruits (Al-Habahbeh et al., 2021). Other factors influencing PTE content in olive oils are olive treatments to extract the oil (La Pera et al., 2002), and the manufacture of foodstuff (Benincasa et al., 2007). Consequently, it is very important to investigate the concentration of PTEs in the olive grove soils, as they may contribute to the abundance of these chemicals in olives and olive oils. On the other hand, PTEs can have high concentrations in soils due to natural geochemical anomalies or contamination caused by several human activities, such as the excessive use of agrochemicals, intense vehicular traffic, and industrial fallout (Zwolak et al., 2019; Micó et al., 2006).

In Italy, EVOO is a typical food used in the gastronomic tradition and each region produces EVOOs with different taste and biological properties, depending on the plant cultivar as well as on climate, landscape and geopedological features (Aprea et al., 2018). The large numbers of native olive tree varieties and the diverse cultivation landscapes stand as the strength of Italian olive cultivation. Each cultivar narrates the story of its area as the climate, soil, topography, and most importantly, the history, culture, and traditions: the *terroir.* The *terroir* is a combination of numerous factors that contribute to the uniqueness of a product and make it immediately recognizable. In the case of olive trees, the variety, soil composition, altitude and climate make high-quality oil an exclusive product in the world, impossible to replicate in other regions of the planet (Rellini et al., 2022). Indeed, olive oils from different territories often have distinct flavours and aroma.

The combination of numerous volatile compounds contributes to the formation of the oil's aroma. These volatile compounds are naturally occurring chemical substances that evaporate at room temperature and can be detected through the olfactory receptors. In high-quality products, the volatile compounds arise from natural biochemical reactions that occur within the drupe during the crushing and extraction of oil at the mill (Kalua et al., 2007). The volatile compounds responsible for most of the sensory perceptions experienced during the tasting of

EVOO are characterised by a low (<300 Da) molecular weight (Cecchi & Alfei, 2013). They belong to various chemical classes, including aldehydes, alcohols, esters, ethers, hydrocarbons, ketones, furans, terpenes (Martins et al., 2020; Kalua et al., 2007; Angerosa et al., 2004) and phenols (Vichi et al., 2011; Vichi et al., 2008). In addition, a well-balanced composition of fatty acids and the presence of minor components make olive oil unique among other oils (Sánchez & Harwood, 2002).

The global spread of food products has led to increased awareness of local traditional products, but at the same time has unfortunately brought challenges in controlling the proliferation of imitations, counterfeits, and adulterated items, posing health risks to consumers and harming economies worldwide. In this context, geographical traceability plays a decisive role to ensure the authenticity and the quality of food products.

1.2. GEOGRAPHICAL TRACEABILITY

The geographical traceability describes the journey of food from their origin to the arrival to consumers. The main aims of this practice are, not only to guarantee transparency and authenticity (Mahne Opatić et al., 2017), but also to prevent unfair practices and preserve food safety (Bora et al., 2018; Rashmi et al., 2017). This assurance of quality and protection, improves the confidence of consumers in foods they buy (Opara & Mazaud, 2001). In recent decades, a lot of research has focused on the development of some strategies to trace the geographical origin of food products. The main traceability methodologies currently in use are based on the recording of the information describing each stage of the supply chain. In Europe there are strict regulations governing the classification of EVOO and guaranteeing its authenticity and superior quality (Commission of the European Communities, 2012):

- Protected Designation of Origin (PDO): in order to meet the requirements for geographical indication, every step of production, processing, and preparation of EVOO must occur within the designated region.
- Protected Geographical Indication (PGI): at least one of the production, processing, or preparation stages occurs within the specific region of EVOO production.

However, the high economic value and distinctive attributes of EVOO make it susceptible to non-compliance and fraud. The risk is related to its premium value, which is driven by superior quality and varies depending on the geographical origin (Casadei et al., 2021). Indeed, despite the restrictions and the control activities, EVOO is one of the foods most subjected to fraudulent activities, as reported by the European Parliament (2014). The most common fraud is dilution, where EVOO is adulterated with lampante, hazelnut or soybean oil (Tibola et al., 2018; Bajoub et al., 2017). In some cases, seed oil is artificially coloured with beta-carotene or copper complex of chlorophyll and sold as VOO (Aceto et al., 2019; Fang et al., 2015). More difficult to detect is when an EVOO is sold as local, but, in fact, is produced in other areas of Italy or are used olives of foreign origin.

For this reason, methods have been developed based on scientific and innovative approaches aimed to determine the geographical origin and authentication of food. The most widely used are the followings.

- Elemental chemical analysis: this is one of the most widely used methods to authenticate or trace a food product using multi-elemental analytical techniques (Pucci et al., 2022; Aceto et al., 2019; Damak et al., 2019; Beltrán et al., 2015; Benincasa et al., 2007). This method consists of the determination of concentrations of many chemical elements including trace elements such as rare earth elements (REEs), which usually have concentrations lower than 0.1%, in different environmental matrices among which the soil. The importance of soil in studies focused on the geographical traceability is due to the significant role it plays in regulating the distribution of chemical elements across the hydrosphere and biosphere. In fact, several physical, chemical, and biological properties of the soil (i.e. texture, geochemical and mineralogical features, microorganisms activity; Janin et al., 2014) rule the mobility and bioavailability of chemical elements in the soil and their transfer to plants. Plants take up the bioavailable fraction of chemical elements in the soil through the roots and translocate them to the aerial parts including the edible. Consequently, the elemental composition of the olive oil reflects, in some way and proportion, that of the soil of origin. For this kind of analysis, inductively coupled plasma mass spectrometry (ICP-MS) is the preferred analytical technique due to its high sensitivity (Aceto et al., 2019; Lepri et al., 2011).

- Compositional analysis: literature extensively demonstrates that factors such as cultivar, climatic conditions of the cultivation region, agricultural practices, ripeness level, storage conditions, and fruit processing techniques all play a significant role in the development and presence of the compounds responsible for the aroma of EVOO (Tomé-Rodríguez et al., 2021; Lukić et al., 2019; Genovese et al., 2018; Peres et al., 2017; Angerosa et al., 2004). These factors influence both the type and quantity of volatile and phenolic compounds present in EVOO. Even olives of the same variety, cultivated in identical environmental conditions, produce oils with distinct volatile compounds, as is also observed for a single cultivar grown in different regions (Baccouri et al., 2008; Benincasa et al., 2003).
- Isotopic analysis: this technique is able to distinguish the isotopes of a chemical element and calculate their isotopic ratios. The most widely used isotopes for characterization

of food are light stable isotopes of chemical elements, such as carbon (C), oxygen (O) and hydrogen (H) and subordinately nitrogen (N), and sulfur (S) (Zhao & Zhao, 2020; Portarena et al., 2017; Schellenberg et al., 2010). Analysing the stable isotopic composition in plants can provide insights into the geographical and climatic conditions of their growth environment. Indeed, the $^{13}C/^{12}C$ ratios in plant compounds are affected by the photosynthetic processes (C3, C4 or CAM) and various environmental and physiological factors, including relative humidity, temperature, precipitation, water stress, plant age, and maturation (Portarena et al., 2014; Camin et al., 2009). The ratio of $^{16}O/^{18}O$ and $^{1}H/^{2}H$ is influenced by the hydrological cycle. In fact, the isotopic composition of rainfall is regulated by factors such as temperature, altitude, and distance from the ocean or specific location (Burgess et al., 2000). The isotopic ratios of these above-mentioned chemical elements are measured with the isotope-ratio mass spectrometry (IRMS) technique, where mass spectrometry is based on the ionisation of a molecule and its subsequent fragmentation into ions of different mass/charge ratio.

The geographical traceability of EVOO is crucial for its authenticity and provides insights on its quality, potential defects, and meeting consumer expectations. Moreover, traceability improves safety by identifying the geographic origin of the olives, which aids in tracking the oil back to its source and identifying any safety hazards. Promoting sustainability and traceability ensures the long-term viability of the olive oil industry and its communities by supporting local producers and sustainable farming practices.

1.3. VALDICHIANA SENESE

Valdichiana Senese is a territory of about 700 km^2 located at the easternmost sector of the Siena province in Southern Tuscany, Italy, and includes nine municipalities (Fig. 2).

Figure 2. Map of Valdichiana Senese.

Valdichiana Senese is part of a NW–SE oriented tectonic basin where sandy, silty-clayey, and clayey sediments of the Neogene-Quaternary succession (Tuscan Neoautochthonous) deposited in marine, lacustrine and continental settings from Miocene to Pleistocene (Bossio et al., 1998). At the borders of the tectonic basin formations of the Tuscan Nappe (non-metamorphic Tuscan Units), Subligurian Units, and Ligurian Units crop out (Carmignani et al., 2013). These formations consist mainly of sandstones, siltites, limestone, and shales.

The area is distinguished by gently-rolling hills characterised by vineyards, olive groves and cypress trees. The landscape and the sub-Mediterranean climate (average annual temperature of 14°C, annual rainfall of about 850 mm) enable an important agricultural activity characterised by the production of high-quality foodstuff such as DOCG (Denomination of Controlled and Guaranteed Origin) wines and elephant garlic. Moreover, Valdichiana Senese is well known also for the high-quality EVOO. The area has a long tradition of olive cultivation, dating back to Etruscan times (Cherubini, 2012). The unique microclimate of the region, along with the careful cultivation and harvesting practices, result in a distinctive and flavourful EVOO. The EVOO from Valdichiana Senese is largely consumed by the local population and also exported elsewhere and abroad.

The Valdichiana Senese region has a variety of olive cultivars, among which the most common are *Frantoio*, *Leccino*, *Moraiolo*, and *Pendolino*. However, it is possible to find cultivars at risk of genetic erosion, such as *Minuta di Chiusi* and *Maremmano* (Regione Toscana, 2023). Cultivars are frequently blended in different proportions to attain the desired flavour profile; but the specific characteristics of the resulting EVOO can also vary depending on factors such as soil and harvesting practices.

The EVOO from Valdichiana Senese is particularly renowned and characterised by two important organoleptic features: the bitter and the spicy (Valdichiana Living, 2021). These attributes are distinctive of high-quality olive oils with a high presence of polyphenols, compounds well known for their anti-cancer, anti-inflammatory and antioxidant properties (Serreli & Deiana, 2018; Parkinson & Cicerale, 2016; Cicerale et al., 2012).

2. AIMS OF THE STUDY

In this thesis, various features of the soil, olives, and especially of the EVOO from the Valdichiana Senese region were investigated and characterised.

Firstly, the total content of 12 selected potentially toxic elements (PTEs), such as As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, U, V, and Zn, was measured in EVOOs and olive grove soils from Valdichiana Senese collected in 2019-2020. These analytical data were used to achieve the following goals: i) identifying any contamination by PTEs in olive grove soils and assessing the associated environmental risk, ii) assessing the potential risk for human health related to PTE intake from the consumption of Valdichiana Senese EVOO.

Secondly, the total elemental content and bioavailable fraction of 36 chemical elements (As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Rb, S, Sb, Sr, Tl, U, V, Zn, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) were measured in olive grove soils, as well as their concentration in olive fruits and EVOOs collected in 2020-21. Additionally, the chemical-physical properties of soil samples, such as pH, electrical conductivity, cation exchange capacity (CEC), organic carbon content, and particle size distribution were measured. The data obtained were analysed to identify correlations among the olive grove soils and the related olive fruits and the EVOO. Indeed, the objective was to determine the geographical traceability of the EVOO from the Valdichiana Senese region.

Thirdly, the organic volatile compound and fatty acid profiles of EVOOs from Valdichiana Senese collected in 2020-21 and 2021-22, were determined. Furthermore, differences in volatile and fatty acid profiles were evaluated between EVOOs from Valdichiana Senese and monovarietal EVOOs collected in 2021-22 from the Alentejo region in Portugal. Given the influence of factors like cultivar and climatic conditions on volatiles and fatty acids, this study aimed to verify if these components could allow to classify EVOOs according to their origin area.

Lastly, this study involved an assessment of the quality of EVOOs provided in 2019, 2020, and 2021. To this purpose, key quality parameters like free acidity, peroxide values, and total phenol content were chosen. Also, to evaluate the stability of quality over a year, the current study examined Valdichiana Senese EVOO from the 2021-22 harvest to 12 months later. Even if stored at room temperature and in dark glass bottles, EVOO usually undergoes qualitative and quantitative modifications due to oxidative and hydrolytic reactions. Consequently, the quality parameters (free acidity, peroxide value, and total phenol content) were continuously monitored throughout the year and their relationships with storage time were determined by using a linear regression analysis.

3. MATERIALS AND METHODS

For this project 19 farms over the 9 municipalities within Valdichiana Senese were selected: Cetona (N=2), Chianciano Terme (N=1), Chiusi (N=2), Montepulciano (N=3), San Casciano dei Bagni (N=2), Sarteano (N=1), Sinalunga (N=4), Torrita di Siena (N=2) and Trequanda (N=2). In the thesis, the farms were anonymized and randomly assigned a letter.

3.1. SOIL GEOCHEMISTRY

3.1.1. Sampling

Between January and February 2020, soil samples were collected from only 18 olive groves. The parent rocks of sampled soils were marine sands and sandstones (MSS) and quartz-feldspathic-micaceous sandstones (QFS), and, to a lesser extent, the following lithologies: marine silty-marly clays (MSC), shales with subordinate limestone (SHL), alluvial deposits (ALL), limestone, dolomitic limestone, and dolomite (LMD), continental sands and conglomerates (CSC). Subordinate parent rocks of soil samples were shales and silty-marly shales (SSS), limestone with chert (CLM), and jaspers (JSP).

The 18 olive groves selected in Valdichiana Senese extend over both a single geolithological unit consisting of MSS (N=8), QFS (N=2), MSC (N=2), or SHL (N=1), and different types of rocks and sediments (up to four lithologies) represented by MSS, QFS, MSC, ALL, LMD, CSC, SSS, CLM, and JSP (N=5).

To identify any contamination by PTEs, in each olive grove 5 soil samples were collected at a depth of 20-30 cm using a shovel according to a stratified random design based on the geolithological and pedological features.

Furthermore, during March-May 2021, a second soil sampling campaign took place, as part of the traceability project. In this case, soil samples were collected at a depth of 20-60 cm, taking a portion of interest of 40 cm using an Edelman auger. This depth level of the soil was selected because it is considered the portion where the roots of the olive tree are more active in the uptake of micronutrients. After removing rock fragments, plant roots and residual surface vegetation, the soil samples were placed in plastic bags. In addition, the geographical coordinates of each sampling point were recorded. To acquire a soil sample representative of the entire olive grove, the calculation of the number of samples was determined based on the extent of the olive groves: for olive groves with an area <5000 m^2, two samples were collected, whereas for groves >5000 m^2, one sample was taken per every half hectare. For olive groves with 2 or more lithologies, the number of samples was determined based on the area covered by each lithology. In Table 1 the number of samples collected for each farm is shown.

Table 1. Area (m^2) and number of soil samples collected from each olive grove.

Farm	Area (m^2)	n. of samples
a	12168	3
b	2550	2
c	4654	2
d	4818	2
e	27303	5
f	25513	5
g	20526	4
h	13429	3
j	59805	12
k	29442	6
i	25041	5
l	25452	5
m	47438	8
n	41739	8
o	25776	5
p	50890	10
q	19931	4
r	14450	3
s	75050	14

3.1.2. Laboratory preparation

In the laboratory, the soil samples were placed in rectangular plastic basins and dried in a ventilated oven at 40°C. Afterwards, a composite soil sample representative of the entire pedological substratum of each olive grove was prepared, for comparing it with the composite EVOO sample from the same olive grove. The composite soil sample of the olive groves extending over a single geolithological unit was made by mixing equal amounts of the samples collected. For the olive groves extending over different types of rocks and sediments, the mixed amounts of the samples were weighted according to the areal extent of each lithology.

Once dried, the composite soil samples were sieved at 2 mm and the resulting particle size fraction was placed in a special plastic container and mixed manually, in order to obtain a first homogenization. Then samples were divided into 4 parts (quadrants) of equal size. The soil particles from 2 opposing quadrants (quadrants II and IV) were collected and stored, while the remaining 2 quadrants (I and III) were mixed once more. This procedure was repeated until the weight of a single quadrant was approximately 100 g. Finally, the soil particles of quadrant I were placed in an agate jar with marbles of different sizes. The jar was placed inside a mechanical pulveriser, which allows it to rotate on itself at a high speed. This movement allows further homogenization and pulverisation, increasing the free surface of the particles, so as to facilitate the solubilization process. Finally, the powder obtained was stored in polyethylene containers until the analysis.

During the preparation phase, only non-metallic materials were used (i.e. plastic, wood, rubber etc.), in order to avoid any possible contamination.

3.1.3. Determination of chemical-physical properties

Soil is crucial in studies on geographical traceability because it influences the availability of chemical elements for plants through various soil properties. Consequently, the following chemical-physical properties were measured for soil samples collected in 2021: pH, electrical conductivity, cation exchange capacity (CEC), organic carbon content and particle size distribution.

pH and electrical conductivity

The determination based on a soil pH was carried out using the procedure defined in the US EPA protocol 9045/D (2004), solid: liquid ratio 1:2.5. In detail, 50 mL of ultrapure water was added to 20 g of soil sample (particle size < 2 mm). The soil-water mixture was placed in a plastic tube and kept under agitation for 2 h. The mixture was then centrifuged for 15 min at 3,500 rpm (PK110, Alc). The supernatant was separated by filtration and pH was measured using a pH-meter (HD 8705, Delta OHM). For each soil sample, 3 measurements were taken, and the value provided is their mean after log-transformation.

After measuring the pH values, the same filtrate was used to determine the electrical conductivity with a conductivity-meter (HD 8706, Delta OHM); the results were expressed in mS/cm.

Cation exchange capacity

The method adopted to determine the cation exchange capacity (CEC) of soil samples is that proposed by Hendershot and Douquette (1986), which measures the effective CEC through the determination of the concentration of calcium, magnesium, potassium, and aluminium released in solution by ion-exchange with ammonium ion (NH^{4+}). Two g of the pulverised soil sample were added to 20 mL of 1 M solution of ammonium chloride (NH_4Cl), according to a solid:liquid ratio 1:10. The mixture was placed in a test tube, covered, and kept under agitation for 2 h. Afterwards, samples were centrifuged for 15 min at 3,500 rpm and the supernatant was filtered. The extract was analysed by inductively coupled plasma-optical emission spectrometry (ICP-OES) using an Optima 2000 DV spectrometer (Perkin Elmer) to determine the concentrations of exchangeable cations.

The value of the CEC was calculated with the following formula:

$$CEC = \frac{Ca}{200} + \frac{Mg}{120} + \frac{K}{390} + \frac{Al}{90}$$

Organic carbon content

The content of organic carbon in the soil samples was assessed following the procedure described by Walkley-Black (1934), which makes use of the determination of the amount of excess potassium dichromate ($K_2Cr_2O_7$). Ten mL of 1 N solution of potassium dichromate were added to 0.5 g of pulverised soil samples, and, subsequently, 20 mL of sulphuric acid. The solution was stirred for 1 min and allowed to stand for 30 minutes, before adding 200 mL of bidistilled water. Then, 10 mL of orthophosphoric acid and 0.5 mL of phenolphthalein were added as indicators. The excess potassium dichromate was determined by titration with a solution of iron (II) sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$). The same procedure was performed for a blank sample.

The content of organic carbon, expressed as percentage, was calculated using the following formula:

$$\%C\ org = (S - T)\left(\frac{10 * 0{,}003 * 100}{S * W}\right)$$

where:

S = mL of the sulphate solution and iron (II) heptahydrate needed to titrate the blank; T = mL of the sulphate solution and iron (II) heptahydrate needed to titrate the sample; 10 = volume of potassium dichromate solution (mL); 0.003 = meq of C; W = soil weight (g).

<u>Particle size distribution</u>

The soil particle size distribution as percent content of sandy, silty, and clayey fractions was determined by the hydrometer method.

In this method 125 mL of sodium hexametaphosphate were added to 40 g of soil sample sieved at 2 mm. The mixture was shaken for 15 minutes and then poured into a cylinder, and the volume was adjusted to reach a final value of 1 L by adding bidistilled water. Measurements were recorded at time intervals of 30 sec, 1, 2, 4, 8, 15, 30 min and 1, 2, 4, 8, 24 h. This test measures the falling speed of soil particles, i.e. the distance covered by each single soil particle in an established time interval. The readings of these distances were carried out using a hydrometer introduced into the cylinder containing the water/soil mixture. The density values read on the hydrometer allowed it to determine the distance travelled by soil particles and consequently their diameter.

The percentage of soil particles was determined using the Stokes law (Raviolo, 1998):

$$\% = R'S * \frac{100}{PS}\left(\frac{Y_S}{Y_S * Y_W}\right)$$

where:

R'S = correct density; PS = soil dry weight (g); Y_S = mean specific gravity of soil (2.7 g/cm^3); Y_W = water specific gravity (1g/ cm^3).

3.1.4. Bioavailable fraction of chemical elements

The specimen to determine the bioavailable fraction of the selected chemical elements in soil samples collected during 2021, was obtained by shaking for 16 h at room temperature 1 g of pulverised sample in a 40 mL of 0.11 M acetic acid solution. Afterwards, the mixture was centrifuged at 3,500 rpm for 15 min and the obtained supernatant was filtered and stored at 4°C.

3.1.5. Total content of chemical element

For the determination of the total content of the selected chemical elements, all soil samples collected in 2020 and 2021 were solubilized by alkaline fusion using the following procedure. In a platinum vessel, 0.1 g of lithium tetraborate ($Li_2B_4O_7$) and 0.4 g of lithium metaborate ($LiBO_2$) were added to 0.1 g of soil sample and mixed together using a glass rod. The mixture was melted in a muffle at 950°C for 90 min (LM 312, Linn Elektro Therm). Finally, the melt was dissolved by adding 40 mL of a 5% nitric acid (HNO_3) solution. Then, the solution obtained was filtered and made up to a final volume of 50 mL adding ultrapure water.

3.2. EVOO AND OLIVE CHARACTERIZATION

3.2.1. Extra virgin olive oil

Sampling

Towards the end of 2019, 2020 and 2021, the EVOO samples representative of the extra virgin olive oil produced in each of the selected olive groves, were provided by the producers involved in the present study. The EVOO bottles were always maintained in a climatic room at 17°C and in the dark until the analysis.

In 2019, unfortunately farm *c* was unable to provide its EVOO sample; consequently, the assessment of the health risk related to PTEs was conducted on 18 EVOOs.

In 2020, farm *d* supplied two samples of extra virgin olive oil from the same olive grove: one from a single variety (*Minuta di Chiusi*), the other one was a combination of 2 cultivars (*Correggiolo* and *Morellino*). In addition, because farms *n* and *o* have olive groves far away one from the other, they provided EVOOs from each of them, 4 and 2 samples, respectively.

Due to the prolonged summer drought and the damage caused by the olive fly, four farms were unable to produce EVOO in 2021; therefore, the extra virgin olive oil samples provided in 2020 were utilised for the analysis of volatiles and fatty acids.

Determination of the total elemental content

For the chemical analysis, EVOO samples were solubilized using the following procedure. About 250 mg of EVOO were put in a Teflon bomb and mineralized with 3 mL of nitric acid (HNO_3) and 1 mL of hydrogen peroxide (H_2O_2) in a microwave lab station (Milestone Ethos 900). Once cooled, samples were filtered and brought to a final volume of 50 mL by the addition of ultrapure water. For each run, a standard reference material and a *blank* sample were solubilized with the same procedure.

Quality of EVOO

Among the various parameters, it was decided to proceed with the determination of acidity, total phenolic content, and peroxide number.

In order to verify the change in EVOO quality within one year, five EVOOs produced in 2021-2022 were analysed at 0, 4, 8, and 12 months. All determinations were done in triplicate.

Free acidity

In a 250 mL glass flask 20 g of extra virgin olive oil were weighted. The substance was dissolved in 100 mL of diethyl ether and 95% ethanol (2:1). The solution was neutralised with 0.3 mL of phenolphthalein and titrated with a 0.1 M potassium hydroxide solution. The acidity was expressed as % oleic acid calculated using the following formula:

$$Acidity = \frac{V * c * M}{m * 10}$$

where:

V = mL of the potassium hydroxide solution consumed; c = exact concentration (mol/L) of the potassium hydroxide solution used; M = molecular mass, in g/mol, of the acid used for the expression of the result (oleic acid M = 282); m = weight of the sample (g).

Total polyphenol content

The total polyphenol content was determined by UV spectrophotometry according to the method proposed by Alessandri et al. (2014).

In a centrifuge tube 2.5 g of EVOO were weighted and diluted with 2.5 mL of *n*-hexane. Three extractions were carried out with 2.5 mL of a methanol-water mixture 80:20 (v/v), 2 min of vortex and then 5 min of centrifuge at 5000 g. At the same time, a blank was prepared by replacing the extract sample with 1 mL of methanol. In a tube containing 1 mL of extract 0.25 mL of Folin-Ciocâlteu reagent, 1.5 mL of 20% sodium carbonate (Na_2CO_3) and deionized water up to 10 mL were added; then the samples were kept in the dark for 45 min. Lastly, the spectrophotometric analysis was performed at λ = 725 nm with an Agilent 8453E. The final result, expressed in mg/kg of gallic acid, was obtained through a calibration curve with range from 25 to 75 µg/mL, using the following formula:

$$Total\ phenols = \frac{AU_{765}}{K * m}$$

where:

AU_{765} = absorbance of the sample compared to the blank; K = slope of the calibration curve of gallic acid (AU/µg); m = sample weight (g).

Peroxide index

After weighing between 2 and 5 g of EVOO, in a flask 25 mL of chloroform-glacial acetic acid (3:2) were added and the solution was stirred until dissolution. Quickly, 0.5 mL of potassium iodide (KI) were added, and the flask was closed, shaken for 1 min, and then left at room temperature in the dark for 5 min. Afterwards, the solution was immediately diluted with 75 mL of deionised water and titrated with a 0.002 N sodium thiosulphate solution using the starch solution as indicator. The number of peroxides, expressed as milliequivalent active oxygen per kilogram (meq O_2/kg), was given by the following formula:

$$Number\ of\ peroxides = \frac{V * N * 1000}{m}$$

where:

V = volume in mL of sodium thiosulphate solution used for the test; N = exact normality of the sodium thiosulphate solution; m = weight of the sample in grams.

Compositional characterization

In 15 EVOOs produced in 2021-2022 and 6 EVOOs of 2020-2021, the profile of volatile organic compounds and fatty acids was defined.

These analyses were performed at the Departamento de Fitotecnia, Escola de Ciências e Tecnologia of the University of Évora (Portugal). As a comparison with Tuscan samples, 11 EVOOs produced in Alentejo were also analysed. Alentejo is a region located in the south of Portugal with a landscape defined by rolling hills, plains, and cork oak forests and an area of approximately 31000 km^2. The region experiences a Mediterranean climate, with hot summers, with temperatures >40°C, mild winters, and an average annual rainfall of 500-600 mm.

Alentejo is an important agricultural region, with a strong focus on olive oil production. Indeed, it is the major producer of EVOO in Portugal, accounting for >70% of the country's EVOO production. The four main traditional olive varieties in the region are *Galega*, *Cordovil de Serpa*, *Verdeal Alentejana*, and *Cobrançosa*. Each variety offers unique characteristics and flavours used to produce high-quality EVOOs.

The analysed Portuguese EVOO samples are from several regions of the Alentejo: Alter do Chão (N=1), Pias (N=1), Redondo (N=1), Reguengos de Monsaraz (N=4), Sousel (N=1) and Vidigueira (N=3).

Extractions of volatile organic compounds

HS-SPME extraction was performed using a carboxen/divinylbenzene/polydimethylsiloxane fiber (CAR/DVB/ PDMS, 1 cm, 50/30 µm film thickness (df)) supplied from Supelco, (Bellefonte, PA, USA), after being conditioned following manufacturer recommendations.

0.6 g of sodium chloride were added to 3 g of EVOO, and the mixture was placed in a vial sealed with a PTFE/silicone septum. Each sample was analysed three times. After the vials were put in a SepSolve Analytical Sample Preparation Robot, the samples were stabilised at 40°C for 5 min then extracted for 30 min keeping the same temperature. The analytes were thermally desorbed by exposing the fiber in the GC injection port at 260°C for 3 min in splitless mode. To guarantee the absence of contaminants, fiber blanks were processed every 6 samples. Sample preparation and chromatographic analysis were based on the work of Martins et al. (2020). All samples were analysed in triplicate.

GC/TOF-MS analyses were performed by an Agilent 8890 GC system coupled with a Bench TOF-Select system (Markes International) featuring Tandem Ionization that provides variable-energy electron ionisation. Chromatographic separation was achieved by a DB-Wax capillary column (60 m x 0.25 mm i.d. x 0.25mm d_f) supplied by Agilent (USA). The oven temperature program began at 40ºC, held for 5 min, raised at 4ºC min^{-1} up to 240ºC and held for 10 min. Carrier gas was helium at a constant flow of 1.3 mL min^{-1}. The MS transfer line and source temperature were set at 250ºC.

The MS optimization option was set to operate with a mass range between 30–400 *m/z*; filament voltage was set at 1.60 V. Electron ionisation energies explored was 70 eV. TOF-DS software (Markes International) and NIST Spectral Library & Search Software (version 2020) were used for control, acquisition, and spectra analysis.

The volatile compounds were identified by matching their structure, mass spectra, and molecular weight with reference compounds in the NIST mass spectral library. In addition, the linear retention index (LRi) values, used for the identification of volatiles in each sample, were calculated using a hydrocarbon mixture (C7–C30) and were compared with LRIs described in the literature. The results for each compound are expressed as the percentage ratio of the peak area for that compound relative to the total peak area.

Analysis of fatty acids

Analysis of fatty acids (FAs) in extra virgin olive oil samples was performed through trans-esterification with a cold methanol solution of KOH, following the Official method (IOOC, 2001). Briefly, in a glass tube of 5 mL approximately 0.1 g of the EVOO sample was mixed with n-heptane (1:20, w:v) and later 0.2 mL of 2 N solution of KOH in methanol were added. The final solution was stirred in vortex for 30 seconds, and when the upper layer, containing the fatty acid methyl esters (FAME) became clear, 1 mL of this solution was directly transferred to the vial for the gas chromatography (GC) analysis. The analyses were performed by a HP 6890 Series GC System with flame ionisation detector (FID) and an automatic sampler injector Agilent 7683 Series injector.

Chromatographic separation was achieved by a SP^{TM}-2380 capillary column (60m×0.25mm i.d.,0.2 μm df) supplied by Supelco, Sigma-Aldrich, USA.

The oven temperature program began at 140 °C hold for 5 min, raised at 4 °C min^{-1} up to 240 °C and remained at 240°C for 10 min. Hydrogen was used as carrier gas at a flow rate of 1.2 mL/min and the temperature of the injector and detector was set at 250 and 260ºC respectively. 1 mL of each sample was injected with a 20:1 split ratio. Identification was carried out by comparison of retention times of each peak with retention times of a Supelco^{TM} 37 Component FAME Mix (Supelco, Bellefonte, PA) injected using the same conditions. The percentage of individual FAs was calculated based on the sum of peak areas for each FAME identified, for semi-quantification purposes. Both data acquisition and processing were accomplished by software Clarity (DataApex, Prague).

3.2.2. Olive fruits

Sampling

Olive fruits were sampled in the period September-November 2020. The sampling included a random collection throughout the olive grove, and the drupes were randomly taken at different heights and cardinal exposures, in order to obtain a representative sample. Approximately 500 g of olives were harvested for each farm.

Preparation

After the harvest, the olives were washed with deionized water, dried and kept in the oven at 40°C for 2 hours, to facilitate the separation of the pit from the pulp. The drupes were cut into pieces one by one with a plastic knife to avoid any possible contamination by metals. Later the olives were subjected to cryogenic grinding.

Specifically, liquid nitrogen was used to press the olives that, placed inside a ceramic mortar, come into contact with and become hard and rigid, allowing easy pulverisation through the use of a pestle. Once pulverised, the olives were freeze-dried using a freeze-dryer in order to eliminate the residual water content and obtain a dry weight, necessary for the analysis.

Determination of the total elemental concentration

As far EVOOs (§ 3.2.1.), olive samples were solubilized with a combination of ultra-pure acids. Solubilisation of olives was achieved by adding to 0.25 g of sample and using a microwave apparatus. Subsequently, each solution was filtered and adjusted to a final volume of 50 mL by the addition of ultrapure water.

3.3. REAGENTS AND STANDARDS

Reagents of VWR Chemicals and Carlo Erba were used for sample preparation and analysis. As certified standard materials were used NIST 2709 (*San Joaquin soil*), GBW 07311 (*Stream sediment*) and GBW 07411 (*Chinese soil*) for soil analysis, and GBW 07604 (*Poplar leaves*), STD 1.09469.0100 (*Multi-element standard II dissolved in oil*), GBW 07603 (*Branches leaves*) for EVOO and olive analysis.

3.4. CHEMICAL ANALYSIS

For the chemical characterization of soil, olive and EVOO samples the concentration of macro, micro and trace elements (As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Rb, S, Sb, Sr, Tl, U, V, Zn, and REEs such as La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) was determined by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) using a NexION 350 spectrometer (Perkin Elmer).

Recoveries ranged from 97% (K) to 102% (Ni) for NIST 2709, 93% (Mg) to 109% (Ni) for GBW 07311, 94% (V) to 108% (Ni) for GBW 07411 in soils, 93% (Cu) to 101% (Sm) for GBW 07604, 96% (Tb) to 101% (Co) for GBW 07603 in olives, and 95 % (Fe) to 107% (Co) for GBW 07604, 99% (Mg) to 101% (Zn) for STD 1.09469.0100, 95% (Cr) to 105% (Zn) for GBW 07603 in EVOOs.

For the risk indices, only the concentration of 12 PTEs (As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, U, V, and Zn) in EVOO and soil samples was measured. Recoveries ranged from 97% (As) to 105% (Cd) for NIST 2709, 91% (Cd) to 107% (Cr) for GBW 07411, 95% (As) to 108% (U) for GBW 07604, 98% (Cu) to 102% (Pb) for STD 1.09469.0100, and 99% (As) to 108% (Ni) for GBW 07603.

3.5. STATISTICAL ANALYSIS

Since the concentrations of the investigated PTEs in both EVOO and soil samples approached a normal distribution (Shapiro–Wilk test, $p<0.05$), their descriptive statistics were given as min, max, mean, and standard deviation. For the statistical calculations, the concentrations of PTEs that were below the limit of quantification (LOQ), were taken as the LOQ value.

The data for the determination of the geographical correlation were reported as mean values ± standard deviation. To find the correlation between the results, correlation analysis was run and visualised using the *ggcorplot* package of R (Wickham, 2016). Correlations with a p value <0.05 were taken into consideration.

The reported results of volatile and fatty acids in EVOO samples were expressed as the percentage of minimum and maximum values, and as mean ± standard deviation. A linear discriminant analysis (LDA) was run using the *MASS* package of R (Venables & Ripley, 2002). The use of LDA is common for identifying patterns between two or more classes. For this project it was used to classify the EVOOs correctly according to their geographical origin (Italy, Portugal) and the harvest year (2020-2021, 2021-2022). The *Ggplot* package of R (Wickham, 2016) was used for data visualisation and graphical representation.

For the quality assessment, the data were reported as mean ± standard deviation. Also, to evaluate the stability of quality, the regression analysis was performed by package *lattice* (Sarkar, 2008) and package *grid* (R Core Team, 2023) for graphics. The statistical significance level was set at $p <0.05$ and the final choice was selected by F-tests among marginal models.

3.6. HEALTH AND ECOLOGICAL RISK

The assessment of soil contamination, ecological risk, and health risk was based on the following specific indices: Geoaccumulation Index (I_{geo}), Potential Ecological Risk Index (PERI), and Health Risk Index (HRI). These indices were used to assess: i) the PTE contamination levels in the olive grove soils (I_{geo}); ii) the potential risk for the environment related to the PTE contamination level in the olive grove soils (PERI); iii) the risk associated with the intake of PTEs through the consumption of the Valdichiana Senese EVOO (HRI). The calculations were performed on the total content of PTEs found in soils and EVOOs collected in 2019-2020.

3.6.1. Geoaccumulation Index

To identify contamination by PTEs in olive grove soils of Valdichiana Senese, the Geoaccumulation Index (I_{geo}) was considered and calculated as follows:

$$Igeo = log_2(C_{element}/(1.5 * C_{background}))$$

where: $C_{element}$ is the concentration of the chemical element (PTE) in the soil sample; $C_{background}$ is the geochemical background of the chemical element (PTE) corrected by a 1.5 factor (Odat, 2015; Zhiyuan et al., 2011). In this study, the geochemical background of PTEs was assessed by means of their concentrations in uncontaminated surface soils of Southern Tuscany originated from the main lithologies by which the olive grove soils of Valdichiana Senese derived (Protano, 2021): marine sands and sandstones (MSS), quartz-feldspathic-micaceous sandstones (QFS), marine silty-marly clays (MSC), shales with subordinate limestone (SHL), limestone, dolomitic limestone and dolomite (LMD), shales and silty-marly shales (SSS).

Table 2 lists the I_{geo} categories used to evaluate the PTE contamination level in the olive grove soils of Valdichiana Senese (Zhiyuan et al., 2011).

Table 2. Soil contamination levels based on the value of the Geoaccumulation Index (I_{geo}).

Geoaccumulation Index (I_{geo})	
$0 < I_{geo}$	Uncontaminated
$0 < I_{geo} < 1$	Low to moderately contaminated
$1 < I_{geo} < 2$	Moderately contaminated
$2 < I_{geo} < 3$	Moderately to strongly contaminated
$3 < I_{geo} < 4$	Strongly contaminated
$4 < I_{geo} < 5$	Strongly to extremely contaminated
$I_{geo} > 5$	Extremely contaminated

3.6.2.Potential Ecological Risk Index

The Potential Ecological Risk Index (PERI) determines whether a certain level of soil contamination corresponds to a potential risk for the environment. According to Kars and Dengiz (2020), the PERI was calculated through chemical and ecotoxicological data as follows:

$$PERI = \sum ERF = \sum T * CF$$

where: ERF is the Ecological Risk Factor obtained by multiplying the toxic-response factor (T) of each PTE by its contamination factor (CF). The toxic-response factors (T) of the analysed PTEs were: As = 10, Cd = 30, Co = 5, Cr = 2, Cu = 5, Ni = 5, Pb = 5, Sb = 7, Tl = 10, U = 5, V = 2, and Zn = 1 (Rahman et al., 2019; Hakanson, 1980). The contamination factor (CF) was calculated for each PTE as the ratio between the element concentration in the olive grove soil ($C_{element}$) and the highest concentration of its geochemical background ($C_{background}$) (Pandey et al., 2015).

As shown in Table 3, ERF and PERI values were classified into 5 and 4 categories, respectively (Vannini et al., 2021; Luo et al., 2007).

Table 3. Ecological risk levels based on the values of the Ecological Risk Factor (ERF) and the Potential Ecological Risk Index (PERI).

Ecological Risk Factor (ERF)		**Potential Ecological Risk Index (PERI)**	
ERF < 40	Low ecological risk	PERI < 65	Low risk
40 < ERF < 80	Moderate ecological risk	65 < PERI < 130	Moderate risk
80 < ERF < 160	Considerable ecological risk	130 < PERI < 260	Considerable risk
160 < ERF < 320	High considerable ecological risk	PERI > 460	Very high risk
ERF > 320	Significant high ecological risk		

3.6.3. Health risk index

The Health Risk Index (HRI) evaluates the risk associated with the consumption of food. It can be calculated using the following equation:

$$HRI = \frac{DIPTE}{R_{fd}}$$

In this study the value of DIPTE (Daily Intake of PTE) was calculated using the formula proposed by Khan et al. (2017):

$$DIPTE = \frac{C_{EVOO} * D_{food\ intake}}{B_{av.\ weight}}$$

where: C_{EVOO} is the average concentration (mg/kg) of PTE in EVOO samples; D_{food} intake is the daily dose of EVOO (40 g) considering its maximum daily consumption; $B_{av.\ weight}$ is the average body weight (70 kg).

The values of R_{fd} (reference dose in mg/kg/day) for the analysed PTEs were: As = 0.0003, Cd = 0.005, Co = 0.0055, Cr = 1.5, Cu = 0.040, Ni = 0.020, Pb = 0.0035, Sb = 0.004, Tl = 0.0003, U = 0.003, V = 0.009, Zn = 0.3. These R_{fd} values were taken from the IRIS (Integrated Risk Information System) of the US EPA (Integrated Risk Information System US EPA, 2013).

A value of HRI >1 for a given PTE is indicative of a potential risk for human health (Kumar et al., 2016).

4. RESULTS and DISCUSSION

4.1. HEALTH AND ECOLOGICAL RISK FROM POTENTIALLY TOXIC ELEMENTS IN SOIL AND EVOOs

In soils of olive groves from Valdichiana Senese sampled in 2019, the highest concentrations were found for Cr (68.7-190 mg/kg), V (58.2-180 mg/kg) and Ni (36.2-127 mg/kg; Tab. 4). Lower contents were measured for Zn, Cu, Pb, Co, and As as their levels in soil samples were in the ranges 56.9-115, 25.5-67.8, 17.5-42.3, 7.2-22.5, and 5.2-14.8 mg/kg, respectively (Tab. 4). In order of abundance, in soils of olive groves from Valdichiana Senese the above-mentioned PTEs are followed by U, Sb, Tl, and Cd with concentrations of 1.8-3.3, 0.76-1.45, 0.19-0.50, and 0.14-0.56 mg/kg, respectively.

Table 4. Concentrations (mg/kg) of potentially toxic elements (PTEs), expressed as mean ± standard deviation of 3 measures on each sample of olive grove soil from Valdichiana Senese.

Farm	As	Cd	Co	Cr	Cu	Ni	Pb	Sb	Tl	U	V	Zn
a	6.4 ± 0.2	0.2 ± 0.00	14.1 ± 0.3	111 ± 3.09	44.4 ± 0.4	68.1 ± 1.1	17.8 ± 0.2	0.9 ± 0.02	0.3 ± 0.02	2.3 ± 0.0	89.4 ± 0.3	72.6 ± 0.7
b	8.6 ± 0.1	0.2 ± 0.01	16.6 ± 0.2	137 ± 0.98	48 ± 0.4	88.4 ± 0.9	23.6 ± 0.7	1 ± 0.01	0.4 ± 0.01	3.3 ± 0.1	108 ± 0.7	96.2 ± 1.7
c	8.9 ± 0.8	0.2 ± 0.02	13.6 ± 0.5	124 ± 2.62	42.2 ± 0.2	73.8 ± 1.1	19.9 ± 0.9	1 ± 0.01	0.3 ± 0.03	2.4 ± 0.1	95 ± 2.2	88.5 ± 0.3
d	6.9 ± 0.2	0.2 ± 0.03	12.6 ± 0.8	127 ± 6.92	27.8 ± 0.5	65.3 ± 4	17.5 ± 0.6	0.9 ± 0.01	0.3 ± 0.02	2.7 ± 0.2	87 ± 2.0	66.8 ± 0.6
e	9.3 ± 0.5	0.1 ± 0.02	16.4 ± 0.2	140 ± 5.03	25.5 ± 0.5	85.1 ± 1.6	20.6 ± 0.8	1 ± 0.04	0.4 ± 0.02	2.7 ± 0.1	110 ± 3.0	80.9 ± 0.4
f	8.8 ± 0.2	0.2 ± 0.01	12.3 ± 0.5	111 ± 2.9	52.6 ± 0.4	67.1 ± 1.4	24.8 ± 1	0.9 ± 0.02	0.2 ± 0.01	2.8 ± 0.1	82.8 ± 3.2	70.6 ± 0.5
g	7.3 ± 0.6	0.2 ± 0.03	11.9 ± 0.3	87.2 ± 1.03	32.7 ± 0.2	60.2 ± 2.5	17.5 ± 0.2	0.8 ± 0.02	0.2 ± 0.03	1.8 ± 0.1	75.2 ± 2.8	62.4 ± 0.3
h	9.1 ± 0.8	0.2 ± 0.02	9.46 ± 0.3	92.6 ± 6.06	31.4 ± 0.2	58.3 ± 2	17.7 ± 0.6	1 ± 0.05	0.3 ± 0.02	2.2 ± 0.1	69.7 ± 3.6	56.9 ± 0.4
j	14.8 ± 0.2	0.4 ± 0.03	17.7 ± 0.3	149 ± 2.44	45.7 ± 0.3	88.7 ± 1.1	25 ± 0.2	1.5 ± 0.08	0.4 ± 0.01	3.1 ± 0.0	132 ± 2.1	84.5 ± 0.6
k	5.4 ± 0.4	0.3 ± 0.03	18.9 ± 1.2	186 ± 10.1	67.8 ± 0.5	62.6 ± 3.5	22.3 ± 0.6	1 ± 0.05	0.2 ± 0.01	2.9 ± 0.2	180 ± 8.5	115 ± 0.7
i	9.3 ± 0.3	0.3 ± 0.01	13.4 ± 0.3	114 ± 1.9	40.9 ± 0.3	63.6 ± 1.3	31.3 ± 0.7	1 ± 0.05	0.5 ± 0.01	2.5 ± 0.1	89.8 ± 2.0	83.5 ± 0.4
l	6.5 ± 0.5	0.2 ± 0.02	15.2 ± 0.2	199 ± 6.56	42.3 ± 0.2	127 ± 4.2	42.3 ± 0.6	1 ± 0.03	0.3 ± 0.01	2.5 ± 0.1	87.3 ± 3.9	66.1 ± 0.5
m	7.1 ± 0.1	0.2 ± 0.01	13.8 ± 0.4	175 ± 4.51	58 ± 0.6	99.8 ± 0.7	28.7 ± 0.7	1.1 ± 0.06	0.3 ± 0.01	2.3 ± 0.1	86.5 ± 2.0	74.5 ± 0.3
n	7.9 ± 0.5	0.2 ± 0.07	12.3 ± 0.2	113 ± 0.66	34.7 ± 0.3	60.7 ± 0.6	18.3 ± 0.2	1 ± 0.02	0.4 ± 0.03	2.1 ± 0.0	91.9 ± 5.4	62.9 ± 0.7
o	9.34 ± 0.7	0.2 ± 0.01	18.7 ± 1	159 ± 5.82	35.7 ± 1.1	98.5 ± 6.2	27.2 ± 0.5	1 ± 0.03	0.4 ± 0.00	3 ± 0.1	99.9 ± 1.0	81.4 ± 0.8
p	5.2 ± 0.1	0.2 ± 0.03	22.5 ± 0.4	190 ± 4.54	60 ± 0.3	99.9 ± 2.7	21.8 ± 0.1	0.9 ± 0.02	0.3 ± 0.03	2.4 ± 0.1	152 ± 2.1	109 ± 0.7
q	5.6 ± 0.4	0.2 ± 0.02	16.9 ± 0.7	133 ± 3.91	31.9 ± 0.3	81.1 ± 2.4	20.6 ± 0.4	0.9 ± 0.03	0.2 ± 0.02	2.2 ± 0.1	97.6 ± 5.1	75.8 ± 1
r	8.8 ± 0.5	0.2 ± 0.01	10.9 ± 0.2	87.4 ± 1.11	29.1 ± 0.6	53.5 ± 0.9	18.4 ± 0.9	0.8 ± 0.01	0.2 ± 0.01	1.9 ± 0.1	72.3 ± 1.1	75.1 ± 1.1
s	8.7 ± 0.7	0.6 ± 0.06	7.21 ± 0.1	68.7 ± 0.83	30 ± 0.1	36.2 ± 0.4	32.8 ± 0.2	0.9 ± 0.03	0.2 ± 0.02	1.8 ± 0.0	58.2 ± 1.4	95.4 ± 0.5

In Table 5 are reported the concentrations of PTEs in soils of olive groves from Valdichiana Senese formed by a single geolithological unit (MSS, MSC, QFS, and SHL soils) and different types of rocks and sediments (DRS soils), as well as the respective geochemical background expressed as interval of PTE concentrations in uncontaminated surface soils of Southern Tuscany originated by the same parent rocks of olive grove soils (Protano, 2021). The analytical data shows that the concentrations of PTEs in MSS, MSC, QFS, SHL, and DRS soils of olive groves from Valdichiana Senese were comparable to the respective geochemical background in soils of Southern Tuscany. The only exceptions were the higher concentrations of Cr, Cu, Ni, Pb and V in some olive grove soils formed by marine sands and sandstones (MSS), quartz-feldspathic-micaceous sandstones (QFS), and shales with subordinate limestone (SHL). Moreover, the highest concentrations of Cr and V measured in the latter soils exceeded the respective contamination threshold in agricultural soils: 150 mg/kg for Cr and 90 mg/kg for V as established by the Italian Ministerial Decree 46/2019 (Ministero dell'Ambiente della Tutela del Territorio e del Mare, 2019).

Table 5. Concentrations (mg/kg) of PTEs in soils of olive groves from Valdichiana Senese (vs) grouped by geolithological substratum, and the respective geochemical background concentrations (bg) in soils of Southern Tuscany originated by the same parent rocks of olive grove soils. For QFS and SHL soils the background values for V are not available.

PTE		MSS		MSC		QFS		SHL		DRS	
		min	max	min	max	min	max	min	max	min	max
As	*vs*	6.42	9.32	5.56	9.29	6.51	7.06	5.43		5.16	14.8
	bg	6.99	10.1	3.35	11.3	8.10	24.4	4.64	5.40	1.47	24.4
Cd	*vs*	0.14	0.22	0.21	0.28	0.15	0.16	0.28		0.19	0.56
	bg	0.14	0.54	0.10	0.95	0.74	0.90	0.13	0.27	0.10	1.35
Co	*vs*	9.50	16.6	13.4	16.9	13.8	15.2	18.9		7.21	22.5
	bg	10.3	14.0	10.8	22.0	10.0	13.5	21.7	34.4	10.0	34.4
Cr	*vs*	87.2	139.6	114.4	132.6	175.2	198.9	185.6		68.7	190.0
	bg	59.2	99.1	69.8	201.1	150.9	162.2	114.1	140.0	59.2	201.1
Cu	*vs*	25.5	52.6	31.9	40.9	42.3	58.0	67.8		30.0	60.0
	bg	13.9	34.3	21.9	37.6	13.3	16.2	38.5	52.2	13.3	86.8
Ni	*vs*	53.5	88.4	63.6	81.1	99.8	126.6	62.6		36.2	99.9
	bg	37.8	60.5	36.6	94.2	73.9	77.9	59.3	80.8	36.6	101.7
Pb	*vs*	17.5	24.8	20.6	31.3	28.7	42.3	22.3		18.3	32.8
	bg	19.9	27.7	14.1	37.5	18.0	23.2	16.16	23.18	12.8	37.5
Sb	*vs*	0.76	0.99	0.87	1.03	0.99	1.07	1.02		0.93	1.45
	bg	0.81	1.25	0.32	1.27	1.02	1.24	0.11	1.01	0.11	1.33
Tl	*vs*	0.19	0.39	0.23	0.50	0.27	0.31	0.20		0.24	0.44
	bg	0.20	0.35	0.11	0.56	0.29	0.32	0.35	0.37	0.09	0.56
U	*vs*	1.80	3.28	2.24	2.52	2.32	2.47	2.88		1.78	3.09
	bg	1.69	3.05	1.49	2.99	1.74	2.97	2.23	2.65	1.45	3.05
V	*vs*	69.7	110.1	89.8	97.6	86.5	87.3	179.9		58.2	152.4
	bg	59.5	87.1	84.5	120.0	-		-		59.5	120.0
Zn	*vs*	56.9	96.2	75.8	83.5	66.1	74.5	114.9		62.9	109.2
	bg	41.9	97.8	72.0	127.5	72.1	109.8	113.1	135.6	41.9	135.6

The high Cu concentrations in some olive grove soils from Valdichiana Senese are likely related to the intensive use of Cu-based products in agriculture (Vázquez-Blanco et al., 2020; Komárek et al., 2010). The concentration peaks of Cr, Ni, and V in the investigated soils have probably a natural (geogenic) origin due to local enrichments of these PTEs in MSS, QFS, and SHL parent rocks enhanced by pedogenetic processes. In fact, Cr, Ni, and V can reach concentrations higher than 100 mg/kg in: i) shales and schists such as SHL in this study (Reimann & de Caritat, 1998); ii) rocks and sediments with important contents of femic minerals, micas, magnetite, and Fe-Mn oxyhydroxides (QFS and MSS in this study) in which Cr, Ni and V can replace Fe, Mg and Al (Gonnelli & Renella, 2012). Likewise, high natural levels of Pb can be found in lithologies rich in feldspars and micas (QFS in this study) due to isomorphic substitutions of Pb for K and Ca in these minerals (Steinnes, 2013). Lastly, it should be noted that the worldwide mean concentration of V in soil (90 mg/kg; Reimann & de Caritat, 1998) corresponds to its contamination threshold in agricultural soils (Ministero dell'Ambiente della Tutela del Territorio e del Mare, 2019).

The concentrations of PTEs measured in MSS, MSC, QFS, SHL and DRS soils and the upper limit of the respective geochemical background (Tab. 5) were used to calculate the Igeo of these PTEs in the olive grove soils from Valdichiana Senese. Based on the Igeo classification shown in Table 2, except two cases, all the analysed PTEs had values of Igeo<0, suggesting the absence of contamination in the sampled soils (Fig. 3). Igeo values >0 were found only for Cu (1.26, 0.80) in two farms, indicating a low to moderate contamination by this PTE, probably due to agriculture practices based on the intensive use of Cu-based products (Vázquez-Blanco et al., 2020; Komárek et al., 2010). High levels of Cu in agricultural soils of Southern Tuscany have been already found in several other studies (Vannini et al., 2021; Ballabio et al., 2018; Protano & Rossi, 2014).

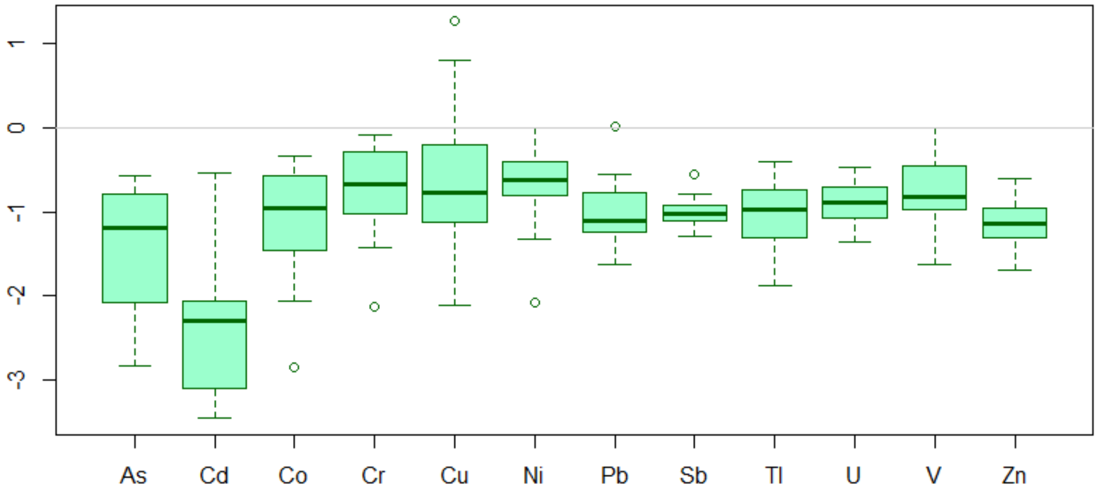

Figure 3. Boxplot of I_{geo} values of PTEs in olive grove soils from Valdichiana Senese.

As a further assessment of the contamination degree by PTEs in the investigated soils, the Ecological Risk Factor (ERF) was used to depict the potential environmental risk from individual PTEs based on their concentration in the olive grove soils from Valdichiana Senese. As shown in Figure 4, the ERF values for all the analysed PTEs were <40, suggesting a low ecological risk. The highest ERF values were found for Cd (30) and Cu (18) in two farms with SHL and QFS soils, respectively. The relatively high ERF value for Cd is determined by the high toxic-response factor of this chemical element (T=30) that significantly enhances the low value of the contamination factor (CF) resulting by a very slight difference between Cd concentration in SHL soil (0.28 mg/kg) and the upper limit of its geochemical background (0.27 mg/kg). Instead, the ERF value for Cu has been obtained by the same olive grove soil with a moderate level of contamination by this PTE.

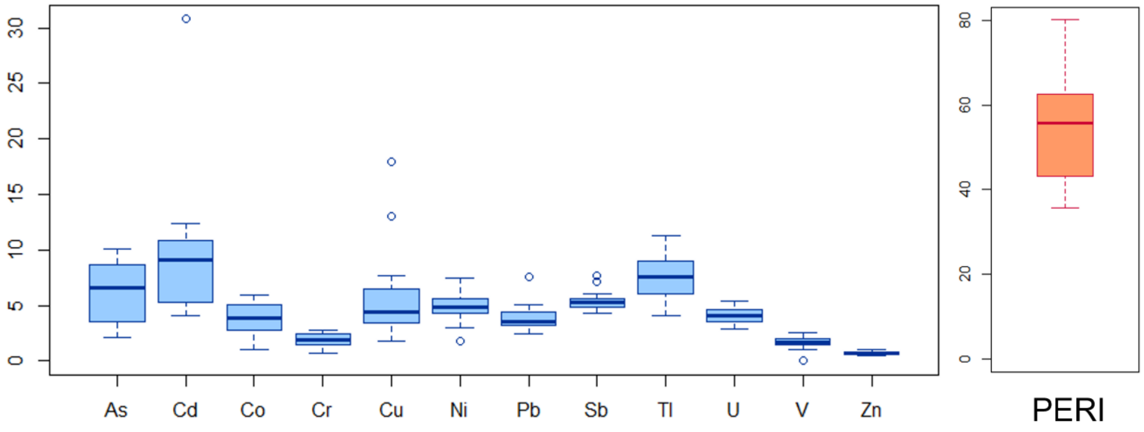

Figure 4. Boxplot of ERF and PERI values calculated with the concentration of PTEs in each olive grove soil from Valdichiana Senese.

The Potential Ecological Risk Index (PERI) obtained by the mean of ERF values of the analysed PTEs for each soil sample (Fig. 4) is <65 indicative of a low ecological risk (the lowest grade of the scale), except for the farm with the high ERF for Cd that has a PERI value of 80.

Among the analysed PTEs in the EVOO samples from Valdichiana Senese (year 2019), Zn, Cu, and Ni had the highest concentrations (1530-6050, 760-5000, and 260-1678 µg/kg, respectively), followed by Cr and Pb (140-236, and 64-147 µg/kg, respectively; Tab. 6). The concentrations were <100 µg/kg for V, Cd, Sb, and <10 µg/kg for Co, U, Tl; the levels of As were always below the limit of quantification (LOQ) of 1 µg/kg (Tab. 6).

Furthermore, it should be noted that: i) the concentrations of Cd and Tl were below their detection limits (20 and 1 µg/kg, respectively) except in two EVOO samples; ii) the concentrations of Co, Sb and V were below their LOQ (3, 6 and 4 µg/kg, respectively) in a variable number of samples, and the remaining samples were in the ranges 3-9.6 µg/kg for Co, 7-18.2 µg/kg for Sb and 8.7-84.5 µg/kg for V. As reported in Table 6, most PTEs showed a high variability of their concentrations in EVOO samples from Valdichiana Senese that could depend on several factors such as the olive tree cultivar, the physical, geochemical, and biological features of olive grove soils, as well as on the agricultural practices and olive manufacturing (Ghane et al., 2021).

Table 6. Concentrations (µg/kg) of potentially toxic elements (PTEs), expressed as mean ± standard deviation of 3 measures on each sample of extra virgin olive oils (EVOOs) from Valdichiana Senese.

Farm	As	Cd	Co	Cr	Cu	Ni	Pb	Sb	Tl	U	V	Zn
a	<1.0	<20.0	4.6 ± 2	213 ± 18	1373 ± 10	687 ± 22	93.4 ± 20.6	<6.0	<1.0	4.7 ± 0.6	43.3 ± 6.2	4358 ± 726
b	<1.0	<20.0	3.2 ± 1	213 ± 48	1446 ± 33	492 ± 43	104.8 ± 12.1	18 ± 8.4	<1.0	3.8 ± 1.2	84.5 ± 11	3247 ± 277
d	<1.0	<20.0	<3.0	229 ± 24	1282 ± 127	571 ± 63	118.8 ± 11.2	14 ± 9.2	<1.0	3.3 ± 0.7	8.7 ± 4.6	2182 ± 436
e	<1.0	<20.0	<3.0	186 ± 15	937 ± 65	709 ± 37	85 ± 9.2	7 ± 4	<1.0	4.5 ± 0.7	<4.0	5686 ± 694
f	<1.0	<20.0	6 ± 1	176 ± 38	1120 ± 50	1087 ± 15	93.4 ± 5.5	<6.0	<1.0	4.2 ± 2.1	<4.0	3735 ± 470
g	<1.0	<20.0	9.6 ± 5	205 ± 27	1436 ± 68	537 ± 19	130.3 ± 6.3	<6.0	<1.0	4.9 ± 1.2	<4.0	1530 ± 93
h	<1.0	<20.0	4.5 ± 2	140 ± 42	780 ± 35	260 ± 12	66.1 ± 3.4	9 ± 4.3	<1.0	3.1 ± 0.2	<4.0	2679 ± 170
j	<1.0	<20.0	<3.0	189 ± 49	1211 ± 32	1445 ± 96	66.3 ± 9	<6.0	<1.0	5.3 ± 2.4	<4.0	4439 ± 680
k	<1.0	<20.0	3 ± 1	193 ± 5	1300 ± 19	593 ± 38	103.8 ± 9.5	<6.0	<1.0	4.8 ± 1.1	<4.0	1596 ± 494
i	<1.0	<20.0	<3.0	170 ± 41	760 ± 42	370 ± 18	63.5 ± 3.3	15 ± 3.9	<1.0	4.3 ± 1.3	<4.0	2291 ± 494
l	<1.0	<20.0	<3.0	217 ± 22	1037 ± 62	1678 ± 93	82.5 ± 8.7	8 ± 1.4	<1.0	4.9 ± 0.5	10.5 ± 4.2	6050 ± 152
m	<1.0	<20.0	<3.0	218 ± 36	1664 ± 117	911 ± 38	140.4 ± 6.5	<6.0	<1.0	5.2 ± 1.2	<4.0	2363 ± 155
n	<1.0	<20.0	9.4 ± 5	232 ± 25	5000 ± 220	1612 ± 98	119.1 ± 10.1	11 ± 7.3	<1.0	4.8 ± 0.4	30.4 ± 13	4606 ± 530
o	<1.0	64.7 ± 0.01	3 ± 1	166 ± 7	1035 ± 45	425 ± 27	81.3 ± 5.2	9 ± 4.7	<1.0	4.1 ± 0.8	<4.0	5188 ± 499
p	<1.0	<20.0	<3.0 ±	182 ± 4	1261 ± 74	405 ± 24	100.5 ± 16.7	7 ± 3.1	<1.0	4.1 ± 0.3	16.1 ± 11	2781 ± 72
q	<1.0	<20.0	7.3 ± 3	204 ± 12	1758 ± 53	560 ± 39	147 ± 8.5	<6.0	<1.0	4.4 ± 0.6	10.9 ± 5.5	2930 ± 53
r	<1.0	<20.0	<3.0	170 ± 15	949 ± 59	321 ± 14	68 ± 4.8	<6.0	<1.0	3.2 ± 1.1	<4.0	3841 ± 505
s	<1.0	<20.0	<3.0	236 ± 22	1321 ± 25	514 ± 22	102.6 ± 7.1	12 ± 7.5	2.3 ± 1	5.6 ± 0.9	<4.0	4041 ± 295

The comparison with other olive oils from Italy, Europe, and non-European countries (Tab. 7) shows that the EVOOs from Valdichiana Senese have: i) As concentrations constantly lower; ii) comparable concentrations of Cd, Co, Cr, Cu, Ni, Pb, Sb, V, and Zn; iii) Cu contents in some EVOO samples exceeding the concentration peak in Italian, European and non-European olive oils. Lastly, Tl and U are potentially toxic elements not commonly analysed in olive oils and therefore very few data are available for comparison (Castillo et al., 1999).

Table 7. Concentrations (min and max values in µg/kg) of potentially toxic elements (PTEs) in extra virgin olive oils (EVOOs) from Valdichiana Senese. For comparison, the min and max concentrations of the investigated PTEs measured in olive oils from Italy (Telloli et al., 2023; Ziarati et al., 2019; Angioni, 2010; Benincasa et al., 2007; La Pera et al., 2002), Europe (Llorent-Martínez et al., 2014; Bakkali et al., 2009; Pehlivan et al., 2008; Zeiner et al., 2005; Roca et al., 2000; Castillo et al., 1999), and non-European countries (Telloli et al., 2023; Haj Heidary et al., 2022; Ziarati et al., 2019; Zaanouni et al., 2018; Savio et al., 2014; Bakkali et al., 2012; Zhu et al., 2011; Bakkali et al., 2009) are also reported.

PTEs	Valdichiana Senese		Italy		Europe		Non-European countries	
	min	max	min	max	min	max	min	max
As	<1.0	-	0.1	26.6	1.2	50	0.05	36
Cd	<20.0	64.7	0.002	396	1.1	150	0.02	4180
Co	<3.0	9.6	0.02	0.41	<1.0	5450	4.3	11
Cr	140	236	116	437	0.8	2000	6.6	30
Cu	760	5000	0.12	58.5	7.7	4510	0.28	1110
Ni	260	1678	0.30	14180	3	2260	1.4	14440
Pb	63.5	147	0.44	10110	<1.0	50	0.06	18780
Sb	<6.0	18.2	0.19	0.41	2.9	100	7.1	2030
Tl	<1.0	2.3	-	-	<0.5	-	-	-
U	3.1	5.6	-	-	<0.25	-	-	-
V	<4.0	84.5	-	-	100	460	-	120
Zn	1530	6050	0.7	385	8.8	4030	1240	13900

The highest PTE concentrations in EVOO samples from Valdichiana Senese were used for determining the values of the Health Risk Index (HRI), in order to assess the risk associated with PTE intake through the EVOO consumption. The results of calculation show that the HRI values were well below the safety limit (HRI=1) for all the analysed PTEs (Tab. 8).

Table 8. Values of the Daily Intake (DIPTE, mg/kg/day) and Health Risk Index (HRI) of the PTEs analysed in the EVOO samples from Valdichiana Senese.

PTE	DIPTE	HRI
As	5.71×10^{-7}	1.91×10^{-3}
Cd	3.71×10^{-5}	7.43×10^{-3}
Co	5.71×10^{-6}	1.04×10^{-3}
Cr	1.31×10^{-4}	8.76×10^{-5}
Cu	2.86×10^{-3}	7.14×10^{-2}
Ni	9.59×10^{-4}	4.79×10^{-2}
Pb	8.40×10^{-5}	2.40×10^{-3}
Sb	1.03×10^{-5}	2.57×10^{-3}
Tl	1.14×10^{-6}	3.81×10^{-3}
U	3.20×10^{-6}	1.07×10^{-3}
V	4.57×10^{-5}	5.08×10^{-4}
Zn	3.46×10^{-3}	1.15×10^{-2}

Same results on the absence of risk consuming olive oils are also reported by Kabaran (2020) and Haj Heidary (2022). Therefore, the Valdichiana Senese EVOOs can be used without any potential health risk for consumers.

4.2. GEOGRAPHICAL TRACEABILITY

To establish the geographical traceability of EVOOs from Valdichiana Senese, this study considered: i) physical and chemical properties of the olive grove soils; ii) concentrations of major, minor and trace elements in the olive grove soils, including for each chemical elements both the total content and the bioavailable fraction in soil samples; iii) concentrations of macro, micro, and trace elements in olive pulps and EVOOs. The above-mentioned analytical determinations were carried out on samples of olive grove soils, olive pulps and EVOOs collected in 2020-21.

4.2.1. Soil properties

In Table 9 the values of pH, electrical conductivity (EC), cation exchange capacity (CEC), organic carbon content, and particle size distribution of olive grove soil samples from Valdichiana Senese are reported. Below, the classification proposed by Costantini et al. (2006) was used to categorise the values of the above-mentioned soil properties.

The pH has an essential role in controlling mobility of chemical elements in the soil and their availability for plants, as this chemical property controls several reactions and processes in the soil such as precipitation, coprecipitation and adsorption. Negative charges are led by high pH conditions, while low pH conditions facilitate positive charges (Neina, 2019).

Most olive grove soils from Valdichiana Senese are slightly to moderately alkaline soils as pH levels ranged from 7.7 to 8.1, in agreement with the features of soil parent rocks consisting of lithologies in which carbonates, mainly calcite, represent primary minerals [i.e., marine sands and sandstones (MSS), marine silty-marly clays (MSC), shales with subordinate limestone (SHL)]. To confirm this, soils from farms *l* and *m*, formed by a siliciclastic lithology [quartz-feldspathic-micaceous sandstones (QFS)], showed a neutral pH (6.7 and 7.2).

Electrical conductivity in soil samples from Valdichiana Senese results as negligible, because it falls < 500 µS/cm.

Soils from farms *a*, *b*, *h*, *m*, and *o* had a low organic carbon content (<0.9 %); on the opposite, soils from farms *j*, *k*, *i*, *l*, and *s* showed a very high quantity of organic carbon (Corg from 1.88 to 2.57%). The remaining olive grove soils from Valdichiana Senese had a medium organic carbon content within the range 0.92-1.35 %.

The cation exchange capacity (CEC) reflects the soil ability to hold and provide nutrients to plants based on the availability of negative charges on the surface of the solid constituents in the soil (i.e. clay minerals and organic matter). The cation exchange capacity is influenced by soil pH, mineralogy, and texture (Jones & Jacobsen, 2005). Olive grove soils collected in 2020-21 from Valdichiana Senese can be divided into 2 categories based on the values of cation exchange capacity (Costantini et al., 2006): 8 soil samples had a moderate CEC (20.4-24.6 cmol/kg), while the remaining 11 showed a high CEC (25.1-34.5 cmol/kg). It is worth noting that the lowest values of CEC (20.4-20-8 cmol/kg) characterised the soils formed by siliciclastic lithologies represented by the quartz-feldspathic-micaceous sandstones (QFS).

Sand, silt, and clay are the three classes of particle sizes necessary to classify soils. By their respective percentages, the soil texture can be determined using a textural triangle. The texture of Valdichiana Senese soil samples exhibit a minor variability (Tab. 9). In fact, the majority of these soil samples are loam soils according to classification of the United States Department of Agriculture (USDA), while the other soils are distributed between the clay-loam, silt-loam and sandy-loam classes.

Table 9. Values of pH, electrical conductivity (EC in µS/cm), organic carbon content (%), cation exchange capacity (cmol/kg), as well as percentage of sand, silt and clay and texture class of olive grove soils collected in 2020-21 from Valdichiana Senese. P.R. = parent rock of soil sample.

Sample	pH	EC	Corg	CEC	Sand	Silt	Clay	Texture	P.R.
a	7.8	323	0.59	27.5	23.5	50.6	25.9	Silty-loam	MMS
b	7.9	333	0.59	28.9	25.6	50.5	23.9	Silty-loam	MMS
c	7.8	318	1.22	25.1	46.4	39.9	13.7	Loam	MMS
d	7.7	313	1.28	26.8	26.2	44.8	29	Clay-loam	MMS
e	8.0	358	1.02	29.7	22	44.8	33.1	Clay-loam	MMS
f	7.9	293	0.92	24.6	38.4	49	12.6	Loam	MMS
g	7.9	300	1.33	23.6	51.9	34.3	13.6	Loam	MMS
h	7.8	305	0.84	24.3	40.7	44.7	14.6	Loam	MMS
j	7.7	460	1.88	34.5	39.9	38.4	21.7	Loam	DRS
k	7.9	348	2.57	33.9	37.8	40.0	22.2	Loam	SHL
i	7.7	460	1.88	28.6	31.4	45.5	23.1	Loam	MSC
l	6.7	308	1.91	20.4	46.7	37.6	15.7	Loam	QFS
m	7.2	143	0.79	20.4	51.3	35.4	13.3	Loam	QFS
n	7.8	308	1.35	27.1	55	33.3	11.6	Sandy-loam	MMS/QFS
o	7.8	380	0.83	20.8	35.2	43.8	21	Loam	QFS
p	7.8	313	1.00	29.5	41.4	36.3	22.1	Loam	DRS
q	7.7	370	1.33	26.3	37.8	45.3	16.9	Loam	MSC
r	8.0	293	0.92	21.1	64.5	23.2	12.1	Sandy-loam	MMS
s	7.9	413	2.31	21.7	60.9	26	13	Sandy-loam	DRS

MSS, marine sands, and sandstones; QFS, quartz-feldspathic-micaceous sandstones; MSC, marine silty-marly clays; SHL, shales with subordinate limestone; DRS, different types of rocks and sediments.

4.2.2. Major, minor and trace elements in olive grove soils

Table 10, 11, 12, and 13 report the total contents of major, minor and trace elements, including Rare Earth Elements (REEs), in olive grove soil samples collected during 2020-21 in the Valdichiana Senese area.

The major chemical elements such as Fe, Ca, Mg, Na, and K, exhibited variable total contents in soil samples in the range 17122-42857, 4891-221944, 2603-21505, 2905-17396, and 8778-22523 mg/kg, respectively (Tab. 10). In agreement with the lithological and mineralogical features of parent rock, the soils formed by the quartz-feldspathic-micaceous sandstones (QFS) showed the highest total contents of Mg, Na, and K, and the lowest ones of Ca.

The minor chemical elements such as S, P, and Mn, had similar total contents in soil samples in the range 422-1422, 390-1724, 290-1291 mg/kg, respectively (Tab. 11).

Using the mean of total contents as reference value (Tabb. 11 and 12), the analysed trace elements (except REEs) follow this order of abundance in olive grove soils from Valdichiana Senese (data in mg/kg):

Ba (330) > Sr (182) > Cr (124) > V (98) > Rb (89) > Zn (73) > Ni (71) > Cu (36) > Pb (22) > Co (14) > As (8.1) > U (2.3) > Sb (1.1) > Tl (0.4) > Cd (0.3).

The trace elements listed above had total contents in soil samples from Valdichiana Senese distributed within rather narrow ranges of values (Tabb. 11 and 12). However, it is to be noted the following for the levels of Ba, Cr, Ni, Pb, Rb, Sr, and Tl in the investigated olive grove soils. The highest total contents of Ba (378-491 mg/kg), Cr (163-225 mg/kg), Ni (77.9-150), Pb (24.7-30.7 mg/kg), Rb (101-128 mg/kg), and Tl (0.42-0.63 mg/kg) characterised the soils formed by the quartz-feldspathic-micaceous sandstones (QFS). These geochemical features agree with the siliciclastic nature of QFS enriched in feldspars, micas, and other detrital minerals (i.e., magnetite), that are able to host trace elements such as Ba, Cr, Ni, Pb, Rb, and Tl. Moreover, Pb reached the peak of concentration (46.2 mg/kg) in soil sample from farm *h*, whose parent rock is represented by the marine sands and sandstones (MSS). Among the trace elements analysed in soil samples, Sr showed the widest range of total contents (71.2-689 mg/kg) mainly ruled by the amount of carbonates (calcite) in the soil. In fact, the lowest levels of Sr were measured in soils derived by the siliciclastic quartz-feldspathic-micaceous sandstones.

About the total contents of Rare Earth Elements (REEs) in soil samples from Valdichiana Senese (Tabb. 13 a, b), analytical data indicated that the sum of 14 REEs (ΣREE), 7 Light REEs (La, Ce, Pr, Nd, Sm, Eu, and GD; ΣLREE) and 7 Heavy REEs (Tb, Dy, Ho, Er, Tm, Yb, and Lu; ΣHREE) are normally lower in soils formed by the marine sands and sandstones (MSS) and silty-marly clays (MSC) than in soils formed by the other parent rocks considered in this study.

Lastly, the total contents of As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, U, V, and Zn in soil samples collected in 2019-20 and 2020-21 are comparable.

Table 10. Total contents (mg/kg) of major chemical elements in olive grove soils collected in 2020-21 from Valdichiana Senese, expressed as mean ± standard deviation of 3 measures on each soil sample.

Sample	Ca	Mg	Na	K	Fe
a	36336 ± 300	10639 ± 59	11726 ± 138	16382 ± 66	33097 ± 336
b	47744 ± 464	12043 ± 143	12455 ± 28	16513 ± 78	34650 ± 755
c	35218 ± 262	12760 ± 162	13516 ± 125	19382 ± 145	30942 ± 766
d	44819 ± 594	10455 ± 78	13262 ± 35	15124 ± 112	27831 ± 351
e	60258 ± 288	14767 ± 224	8757 ± 30	19017 ± 153	39651 ± 646
f	42973 ± 544	8644 ± 75	13743 ± 111	14331 ± 160	29150 ± 562
g	91027 ± 875	3803 ± 18	6274 ± 29	11184 ± 54	25750 ± 547
h	71275 ± 246	8413 ± 113	11739 ± 10	14883 ± 68	30519 ± 391
j	57604 ± 486	12460 ± 87	6172 ± 54	16346 ± 140	42211 ± 390
k	67341 ± 1246	8724 ± 56	4178 ± 23	12042 ± 68	42857 ± 854
i	221944 ± 2472	5901 ± 4	4121 ± 13	8778 ± 73	23559 ± 1199
l	6242 ± 8	16911 ± 227	17396 ± 73	22162 ± 231	36959 ± 317
m	5389 ± 43	15764 ± 272	16537 ± 38	22523 ± 167	35902 ± 741
n_1	64039 ± 430	9517 ± 92	2905 ± 3	15192 ± 42	39141 ± 1370
n_2	6968 ± 22	21505 ± 194	13801 ± 65	21964 ± 181	42593 ± 832
n_3	20536 ± 185	4938 ± 58	15237 ± 48	13838 ± 48	18691 ± 152
n_4	8183 ± 26	5147 ± 94	15586 ± 88	15334 ± 74	20569 ± 213
o_1	7783 ± 76	13228 ± 224	15044 ± 143	20474 ± 56	31952 ± 1432
o_2	4891 ± 35	11139 ± 172	14959 ± 9	16896 ± 52	30771 ± 701
p	23630 ± 123	13423 ± 173	9991 ± 39	20365 ± 80	42397 ± 757
q	81023 ± 1617	4231 ± 29	9521 ± 45	12339 ± 92	38716 ± 852
r	30748 ± 430	9776 ± 120	10479 ± 59	16780 ± 180	22952 ± 99
s	95386 ± 290	2603 ± 31	6467 ± 17	11015 ± 52	17122 ± 118

Table 11. Total contents (mg/kg) of minor and trace elements in olive grove soils collected in 2020-21 from Valdichiana Senese, expressed as mean ± standard deviation of 3 measures on each soil sample.

Sample	S	P	Mn	Sb	Cd	Tl	As	Co	Pb	Cu
a	722 ± 4	726 ± 19	781 ± 8	0.95 ± 0.06	0.16 ± 0.06	0.34 ± 0.03	6.788 ± 0.36	13.9 ± 0.5	18.8 ± 0.7	59.4 ± 2.2
b	825 ± 11	817 ± 34	656 ± 3	1.15 ± 0.06	0.25 ± 0.07	0.34 ± 0.03	8.579 ± 1.05	12.4 ± 0.7	22.3 ± 0.7	29.9 ± 1.5
c	669 ± 12	840 ± 27	600 ± 10	1.22 ± 0.02	0.57 ± 0.06	0.32 ± 0.01	9.11 ± 0.37	12.6 ± 0.5	23 ± 0.9	34.5 ± 1.7
d	638 ± 23	847 ± 25	560 ± 9	0.95 ± 0.04	0.32 ± 0.14	0.31 ± 0.03	6.962 ± 0.43	10.8 ± 0.1	17.1 ± 0.3	23.8 ± 0.4
e	663 ± 12	663 ± 20	653 ± 14	1.19 ± 0.11	0.17 ± 0.11	0.39 ± 0.03	10.39 ± 0.31	15.8 ± 0.7	20.8 ± 1.0	30.4 ± 1.3
f	533 ± 1	651 ± 33	503 ± 7	1.07 ± 0.08	0.29 ± 0.12	0.31 ± 0.01	7.698 ± 0.36	10.5 ± 0.3	19.5 ± 0.7	44.4 ± 2.1
g	422 ± 19	525 ± 13	713 ± 3	0.97 ± 0.05	0.21 ± 0.06	0.26 ± 0.03	6.406 ± 0.03	10.8 ± 0.5	19.3 ± 1.3	40.5 ± 0.2
h	767 ± 24	866 ± 60	530 ± 7	1.01 ± 0.08	0.17 ± 0.13	0.27 ± 0.03	7.252 ± 0.37	10.8 ± 0.5	46.2 ± 1.3	31.3 ± 1.4
j	802 ± 16	990 ± 26	761 ± 6	1.17 ± 0.06	0.26 ± 0.12	0.31 ± 0.03	8.295 ± 0.51	17.3 ± 0.3	20.5 ± 0.9	49.8 ± 1.4
k	857 ± 21	1724 ± 53	800 ± 3	0.96 ± 0.08	0.35 ± 0.1	0.16 ± 0.02	5.272 ± 0.37	16.9 ± 0.6	21.4 ± 0.3	51.9 ± 1.8
i	1422 ± 10	554 ± 50	394 ± 18	1.01 ± 0.04	0.21 ± 0.07	0.37 ± 0.02	8.284 ± 0.29	8.94 ± 0.5	18.8 ± 1.5	28.5 ± 2.0
l	731 ± 15	713 ± 16	758 ± 9	1.17 ± 0.05	0.20 ± 0.08	0.44 ± 0.01	7.454 ± 0.17	17.2 ± 0.2	28.3 ± 1.4	40.4 ± 1.2
m	638 ± 28	675 ± 24	605 ± 10	1.13 ± 0.03	0.27 ± 0.12	0.44 ± 0.01	7.894 ± 0.46	13.3 ± 0.4	24.9 ± 1.0	39.8 ± 0.9
n_1	542 ± 8	1448 ± 24	401 ± 6	1.0 ± 0.12	0.21 ± 0.04	0.31 ± 0.04	11.72 ± 0.36	14.8 ± 1.0	16.6 ± 0.9	35.3 ± 2.0
n_2	481 ± 12	679 ± 29	717 ± 2	1.35 ± 0.02	0.34 ± 0.05	0.47 ± 0.04	7.432 ± 0.34	19.6 ± 0.5	30.7 ± 1.7	45 ± 1.3
n_3	512 ± 21	416 ± 12	405 ± 3	0.86 ± 0.04	0.19 ± 0.06	0.3 ± 0.02	7.935 ± 0.26	8.89 ± 0.3	11.7 ± 0.4	20.3 ± 1.0
n_4	520 ± 15	480 ± 30	538 ± 1	0.95 ± 0.04	0.22 ± 0.08	0.37 ± 0.02	11.65 ± 0.67	14.2 ± 0.5	16.4 ± 1.1	27.3 ± 1.8
o_1	459 ± 9	597 ± 22	525 ± 16	1.11 ± 0.06	0.17 ± 0.1	0.42 ± 0.03	7.292 ± 0.18	15.5 ± 0.9	25.3 ± 1.7	37.6 ± 2.2
o_2	558 ± 14	390 ± 26	290 ± 9	1.05 ± 0.06	0.19 ± 0.03	0.63 ± 0.03	6.459 ± 0.18	12.8 ± 0.6	24.7 ± 1.4	25.1 ± 0.8
p	763 ± 10	826 ± 66	1270 ± 7	1.11 ± 0.06	0.12 ± 0.09	0.45 ± 0.02	4.864 ± 0.49	19.4 ± 0.3	21 ± 0.8	51.2 ± 3.1
q	445 ± 9	643 ± 29	1291 ± 8	1.02 ± 0.04	0.27 ± 0.05	0.38 ± 0.01	5.245 ± 0.17	16.1 ± 0.2	21.9 ± 0.4	33.7 ± 0.5
r	689 ± 15	509 ± 48	676 ± 4	1.1 ± 0.06	0.15 ± 0.07	0.21 ± 0.01	13.29 ± 0.21	10.3 ± 0.3	13.6 ± 0.4	26.4 ± 1.2
s	630 ± 4	648 ± 12	678 ± 5	1.11 ± 0.05	0.48 ± 0.14	0.28 ± 0.02	10.85 ± 0.74	7.92 ± 0.4	24.4 ± 0.8	22.7 ± 0.7

Table 12. Total contents (mg/kg) of trace elements in olive grove soils collected in 2020-21 from Valdichiana Senese, expressed as mean ± standard deviation of 3 measures on each soil sample.

Sample	U	Zn	Ni	V	Cr	Rb	Sr	Ba
a	2.2 ± 0.1	70.55 ± 1.5	62.8 ± 2.7	99.51 ± 2.6	117.5 ± 2.6	89.82 ± 1.1	151.7 ± 8.97	347.1 ± 7.81
b	2.4 ± 0.1	75.61 ± 2.4	62.89 ± 2.4	92.75 ± 0.8	114 ± 1	90.13 ± 0.5	171.5 ± 12.2	356.5 ± 7.88
c	2 ± 0.1	71.38 ± 1.8	76.08 ± 1.3	87.45 ± 2.5	117.8 ± 1.6	99.30 ± 3	151.8 ± 16.9	370.6 ± 13.2
d	2.5 ± 0.1	56.42 ± 1.2	57.53 ± 0.6	80.68 ± 0.7	101.9 ± 1.3	78.13 ± 1.4	149.3 ± 8.3	320 ± 8.25
e	2.6 ± 0.1	76.37 ± 2	77.61 ± 3.5	124.1 ± 3	136.4 ± 2.2	115.18 ± 3.3	239.1 ± 6.72	318.5 ± 6.59
f	3.1 ± 0.1	57.94 ± 1.1	63.53 ± 2.3	77.71 ± 0.7	99.06 ± 1	72.39 ± 2	154.6 ± 5.64	300.6 ± 7.14
g	1.7 ± 0.1	64.29 ± 1.2	67.37 ± 3	78.82 ± 1.1	91.92 ± 1.1	61.87 ± 1.3	180.8 ± 0.67	246.1 ± 3.72
h	2.4 ± 0.1	61.08 ± 1	50.46 ± 2	73.93 ± 1.5	92.31 ± 2.7	79.74 ± 2.4	225.5 ± 6.28	260.7 ± 7.37
j	2.4 ± 0.1	85.16 ± 2	64.44 ± 2.4	157 ± 3.3	144.1 ± 3.2	94.02 ± 2	205.1 ± 6.62	289.8 ± 5.2
k	2.4 ± 0.2	111.8 ± 2.6	56.09 ± 3.5	176.1 ± 2.8	164.9 ± 1	72.82 ± 2.7	264.7 ± 4.9	218.1 ± 3.06
i	1.5 ± 0.1	72.51 ± 1.1	47.09 ± 1	61.85 ± 2.8	70.99 ± 2.4	71.34 ± 3.6	689.2 ± 9.97	211.6 ± 8.83
l	2.9 ± 0.1	85.14 ± 2.1	129.1 ± 3	98.77 ± 2.9	187.8 ± 4.1	121.24 ± 2.7	100.7 ± 8.99	490.9 ± 10.3
m	2.3 ± 0.1	67.04 ± 2.4	101.3 ± 3.3	94.14 ± 2.6	164.6 ± 1.3	117.16 ± 3.3	91.41 ± 5.94	461.1 ± 11.1
n_1	2.3 ± 0.4	88.1 ± 4.1	55.17 ± 6.2	186.4 ± 1.1	157.2 ± 3.9	84.47 ± 1	141.6 ± 6.55	220.4 ± 3.9
n_2	2.8 ± 0.1	83.67 ± 2.6	149.9 ± 4.1	120 ± 1	224.9 ± 2.6	128.03 ± 0.8	71.21 ± 12.8	461.4 ± 7.93
n_3	1.5 ± 0.1	43.95 ± 1	47.54 ± 0.5	55.04 ± 0.6	84.13 ± 1.9	69.75 ± 2.6	110.8 ± 6.1	295.2 ± 4.61
n_4	1.6 ± 0.1	59.15 ± 1.9	58.05 ± 1	62.73 ± 0.4	82.35 ± 0.9	76.84 ± 1.1	94.15 ± 1.87	325.7 ± 5.44
o_1	2.5 ± 0.2	87.84 ± 3.6	85.13 ± 4.6	79.5 ± 1.9	122.7 ± 4	100.59 ± 2.7	85.48 ± 16.5	402.4 ± 17.9
o_2	2.8 ± 0.1	60.49 ± 1.4	77.92 ± 1.9	83.61 ± 0.5	120.6 ± 1.8	88.00 ± 1.7	76.54 ± 13.1	378.2 ± 13.4
p	2.1 ± 0.1	83.16 ± 3.2	84 ± 3	139.7 ± 2.4	163.3 ± 2.8	115.93 ± 1.2	119.1 ± 7.62	397.7 ± 10.5
q	2.2 ± 0.1	71.62 ± 2.6	82.46 ± 2.2	102.3 ± 1.4	128.5 ± 0.9	95.62 ± 1.1	147.9 ± 2.73	365.5 ± 13.2
r	1.7 ± 0.1	56.68 ± 1.7	42.92 ± 1.8	65.68 ± 1.1	78.41 ± 0.4	59.59 ± 0.9	299.5 ± 6.46	248.8 ± 6.34
s	2.5 ± 0.2	79.86 ± 0.2	32.24 ± 1.3	64.31 ± 0.3	77.6 ± 0.8	58.05 ± 0.7	274.7 ± 6.75	300.5 ± 7.17

Table 13a. Total contents (mg/kg) of Rare Earth Elements (REE) in olive grove soils collected in 2020-21 from Valdichiana Senese, expressed as mean ± standard deviation of 3 measures on each soil sample.

Sample	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy
a	28.33 ± 2.40	55.28 ± 3.98	6.85 ± 0.56	26.90 ± 2.58	5.19 ± 0.36	1.06 ± 0.06	4.43 ± 0.31	0.71 ± 0.07	4.09 ± 0.26
b	28.56 ± 2.21	54.57 ± 3.13	7.00 ± 0.51	26.88 ± 1.94	5.46 ± 0.33	1.07 ± 0.07	4.76 ± 0.30	0.74 ± 0.07	4.34 ± 0.40
c	26.62 ± 2.15	52.99 ± 3.77	6.43 ± 0.45	24.62 ± 1.82	4.99 ± 0.34	0.99 ± 0.07	4.26 ± 0.27	0.66 ± 0.04	3.79 ± 0.32
d	26.71 ± 1.55	53.76 ± 2.94	6.44 ± 0.34	24.65 ± 0.74	4.91 ± 0.28	0.92 ± 0.04	4.36 ± 0.32	0.66 ± 0.03	3.99 ± 0.18
e	31.58 ± 2.08	59.35 ± 3.81	7.45 ± 0.50	28.63 ± 2.33	5.42 ± 0.32	1.09 ± 0.05	4.63 ± 0.30	0.69 ± 0.04	4.06 ± 0.29
f	25.08 ± 1.68	49.26 ± 3.05	6.16 ± 0.37	23.79 ± 1.50	4.85 ± 0.27	0.88 ± 0.04	4.28 ± 0.25	0.68 ± 0.04	3.83 ± 0.27
g	22.27 ± 1.66	44.29 ± 3.11	5.45 ± 0.32	21.41 ± 1.64	4.30 ± 0.35	0.90 ± 0.07	3.79 ± 0.33	0.57 ± 0.02	3.26 ± 0.29
h	24.29 ± 2.04	47.43 ± 2.24	5.90 ± 0.51	23.94 ± 2.14	4.54 ± 0.36	0.89 ± 0.09	3.92 ± 0.36	0.60 ± 0.07	3.45 ± 0.45
j	34.58 ± 2.54	63.97 ± 3.13	8.13 ± 0.52	32.21 ± 2.00	5.93 ± 0.31	1.28 ± 0.09	5.18 ± 0.36	0.78 ± 0.06	4.25 ± 0.35
k	34.92 ± 2.38	61.87 ± 2.02	8.13 ± 0.51	33.34 ± 1.81	5.97 ± 0.40	1.32 ± 0.08	5.27 ± 0.27	0.78 ± 0.04	4.48 ± 0.34
i	22.58 ± 1.49	42.44 ± 3.18	5.31 ± 0.36	20.86 ± 1.61	3.90 ± 0.11	0.78 ± 0.08	3.37 ± 0.21	0.51 ± 0.04	2.80 ± 0.23
l	34.13 ± 2.30	69.65 ± 3.12	8.23 ± 0.61	32.52 ± 1.42	6.43 ± 0.30	1.23 ± 0.06	5.44 ± 0.43	0.84 ± 0.06	4.76 ± 0.32
m	29.90 ± 1.73	59.42 ± 3.44	7.26 ± 0.53	28.24 ± 1.75	5.58 ± 0.32	1.16 ± 0.04	4.82 ± 0.21	0.76 ± 0.04	4.52 ± 0.31
n_1	36.81 ± 2.51	65.79 ± 3.15	8.50 ± 0.52	32.06 ± 2.13	5.89 ± 0.37	1.27 ± 0.10	5.06 ± 0.37	0.75 ± 0.05	4.17 ± 0.26
n_2	34.52 ± 2.94	65.85 ± 3.19	8.38 ± 0.69	32.94 ± 2.44	6.65 ± 0.34	1.36 ± 0.14	5.79 ± 0.48	0.91 ± 0.08	5.19 ± 0.45
n_3	18.33 ± 1.29	37.96 ± 2.70	4.43 ± 0.27	17.32 ± 0.61	3.52 ± 0.26	0.69 ± 0.03	2.93 ± 0.19	0.45 ± 0.04	2.56 ± 0.19
n_4	22.52 ± 1.84	44.09 ± 3.34	5.38 ± 0.47	20.98 ± 1.65	4.02 ± 0.34	0.82 ± 0.05	3.50 ± 0.37	0.53 ± 0.05	3.09 ± 0.34
o_1	26.30 ± 1.59	53.26 ± 2.81	6.25 ± 0.35	24.01 ± 0.87	4.62 ± 0.16	0.94 ± 0.04	4.15 ± 0.25	0.65 ± 0.05	3.76 ± 0.13
o_2	29.64 ± 2.32	61.33 ± 3.38	6.93 ± 0.67	26.45 ± 2.00	5.19 ± 0.31	1.00 ± 0.09	4.61 ± 0.34	0.71 ± 0.07	4.10 ± 0.34
p	36.55 ± 3.23	72.04 ± 3.98	8.83 ± 0.77	33.93 ± 2.25	6.42 ± 0.54	1.26 ± 0.11	5.35 ± 0.45	0.82 ± 0.08	4.56 ± 0.49
q	30.00 ± 2.54	57.75 ± 2.30	7.24 ± 0.52	28.08 ± 1.75	5.51 ± 0.35	1.09 ± 0.09	4.75 ± 0.29	0.73 ± 0.04	4.18 ± 0.33
r	21.15 ± 1.30	45.22 ± 2.58	5.01 ± 0.28	19.22 ± 0.72	3.82 ± 0.24	0.79 ± 0.03	3.25 ± 0.15	0.50 ± 0.03	2.81 ± 0.24
s	19.79 ± 1.42	38.46 ± 2.63	4.73 ± 0.29	18.39 ± 1.32	3.75 ± 0.18	0.84 ± 0.04	3.30 ± 0.16	0.53 ± 0.04	3.00 ± 0.27

Table 13b. Total contents (mg/kg) of Rare Earth Elements (REE) in olive grove soils collected in 2020-21 from Valdichiana Senese, expressed as mean ± standard deviation of 3 measures on each soil sample. Table also reports the sum of 14 REEs (ΣREE), 7 Light REEs (ΣLREE) and 7 Heavy REEs (ΣHREE).

Sample	Ho	Er	Tm	Yb	Lu	Σ_{HREE}	Σ_{LREE}	Σ_{REE}
a	0.74 ± 0.06	2.25 ± 0.21	0.32 ± 0.02	2.21 ± 0.25	0.34 ± 0.02	10.66	128.04	138.70
b	0.81 ± 0.07	2.43 ± 0.16	0.36 ± 0.03	2.34 ± 0.23	0.36 ± 0.03	11.38	128.30	139.68
c	0.68 ± 0.07	2.16 ± 0.15	0.32 ± 0.02	2.07 ± 0.24	0.32 ± 0.04	10.00	120.90	130.90
d	0.75 ± 0.05	2.31 ± 0.17	0.35 ± 0.03	2.22 ± 0.25	0.35 ± 0.04	10.62	121.75	132.37
e	0.74 ± 0.06	2.36 ± 0.20	0.33 ± 0.03	2.21 ± 0.22	0.35 ± 0.04	10.74	138.15	148.89
f	0.73 ± 0.05	2.24 ± 0.26	0.32 ± 0.03	2.15 ± 0.10	0.34 ± 0.02	10.29	114.30	124.59
g	0.59 ± 0.04	1.77 ± 0.11	0.25 ± 0.02	1.69 ± 0.17	0.27 ± 0.03	8.40	102.41	110.81
h	0.62 ± 0.06	1.85 ± 0.19	0.27 ± 0.03	1.78 ± 0.25	0.29 ± 0.04	8.86	110.91	119.77
j	0.80 ± 0.08	2.44 ± 0.19	0.35 ± 0.03	2.29 ± 0.21	0.35 ± 0.04	11.26	151.28	162.54
k	0.79 ± 0.04	2.44 ± 0.16	0.34 ± 0.03	2.21 ± 0.23	0.34 ± 0.03	11.38	150.82	162.20
i	0.51 ± 0.05	1.61 ± 0.16	0.23 ± 0.03	1.51 ± 0.15	0.23 ± 0.03	7.39	99.24	106.63
l	0.87 ± 0.07	2.69 ± 0.25	0.39 ± 0.04	2.65 ± 0.23	0.39 ± 0.05	12.59	157.63	170.22
m	0.81 ± 0.07	2.49 ± 0.21	0.37 ± 0.04	2.42 ± 0.21	0.37 ± 0.02	11.73	136.38	148.11
n_1	0.76 ± 0.06	2.29 ± 0.20	0.33 ± 0.03	2.12 ± 0.19	0.33 ± 0.04	10.75	155.38	166.13
n_2	0.95 ± 0.09	2.88 ± 0.33	0.42 ± 0.03	2.77 ± 0.33	0.42 ± 0.05	13.54	155.49	169.03
n_3	0.46 ± 0.03	1.44 ± 0.08	0.22 ± 0.02	1.45 ± 0.10	0.22 ± 0.01	6.80	85.18	91.98
n_4	0.55 ± 0.05	1.70 ± 0.20	0.25 ± 0.03	1.58 ± 0.16	0.25 ± 0.03	7.95	101.31	109.26
o_1	0.70 ± 0.04	2.13 ± 0.11	0.31 ± 0.02	2.08 ± 0.21	0.32 ± 0.03	9.95	119.53	129.48
o_2	0.73 ± 0.08	2.28 ± 0.21	0.36 ± 0.04	2.28 ± 0.23	0.35 ± 0.04	10.81	135.15	145.96
p	0.82 ± 0.09	2.58 ± 0.30	0.38 ± 0.04	2.52 ± 0.25	0.37 ± 0.05	12.04	164.38	176.42
q	0.78 ± 0.08	2.36 ± 0.22	0.33 ± 0.03	2.23 ± 0.23	0.34 ± 0.03	10.95	134.42	145.37
r	0.50 ± 0.04	1.55 ± 0.05	0.21 ± 0.01	1.50 ± 0.14	0.22 ± 0.02	7.29	98.46	105.75
s	0.55 ± 0.05	1.63 ± 0.16	0.24 ± 0.02	1.54 ± 0.16	0.23 ± 0.03	7.71	89.26	96.97

For the 36 major, minor and trace elements above-mentioned, the concentrations in the bioavailable fraction of the olive grove soils collected in 2020-21 were also measured.

As shown in Table 14, the bioavailable concentrations of major chemical elements (Fe, Ca, Mg, Na, and K) in soil samples were variable similarly to their total contents. The bioavailable concentrations of Ca were significantly lower in soil samples derived by the siliciclastic quartz-feldspathic-micaceous sandstones (< 5000 mg/kg) and increased up to 91486 mg/kg as the Ca total contents increased likely related to the abundance of carbonates, mainly calcite, in the soil. The same distribution is exhibited by Fe whose lowest levels in the bioavailable fraction (≤ 20 mg/kg) characterised soils formed by the siliciclastic QFS sandstones. Within the range of the bioavailable concentrations of the analysed major chemical elements, anomalous high levels were measured for Fe in soil sample *r* and for Mg in soil samples *e* and *j* (Tab. 14).

Also, the bioavailable concentrations of minor chemical elements in soil samples were variable in the range 18-484 mg/kg for S, 1.7-136 mg/kg for P, and 17-545 mg/kg for Mn (Tab. 14). The highest levels of P in the bioavailable fraction (48-136 mg/kg) were found in soils whose parent rock consists of the siliciclastic quartz-feldspathic-micaceous sandstones, while those of Mn in soils formed by the marine sands and sandstones (MMS). Among the analysed minor chemical elements, anomalous high bioavailable concentrations of S and P were detected in soils samples *i* and *l*, respectively (Tab. 14).

Table 14. Bioavailable concentrations (mg/kg) of major and minor chemical elements in olive grove soils collected in 2020-21 from Valdichiana Senese, expressed as mean ± standard deviation of 3 measures on each soil sample.

Sample	Ca	Mg	Na	K	Fe	S	P	Mn
a	29678 ± 757	615 ± 31	51.7 ± 2.1	192 ± 8.1	29 ± 2.5	27 ± 7.3	6.5 ± 0.3	267 ± 5.1
b	37521 ± 355	742 ± 18	57.7 ± 1.1	263 ± 6.2	38 ± 0.5	50 ± 2.0	7.4 ± 0.1	290 ± 4.2
c	28261 ± 474	589 ± 22	45.1 ± 1.4	443 ± 6.7	27 ± 1.1	26 ± 4.6	31 ± 1.2	246 ± 4.3
d	37938 ± 51	628 ± 5	55.4 ± 1.4	185 ± 5.2	38 ± 1.9	59 ± 2.6	18 ± 0.3	246 ± 5.9
e	49760 ± 818	1273 ± 33	79.9 ± 1.6	212 ± 6.7	47 ± 0.2	49 ± 1.9	2.7 ± 0.1	251 ± 12
f	36811 ± 439	388 ± 16	51.2 ± 1.6	135 ± 4.7	48 ± 1.8	39 ± 2.0	4.6 ± 0.2	244 ± 7.1
g	84677 ± 2576	603 ± 24	48.9 ± 1.4	141 ± 2.4	76 ± 0.9	57 ± 2.4	2.3 ± 0.3	329 ± 5.8
h	64869 ± 1007	714 ± 29	121 ± 3.6	247 ± 5.1	58 ± 2.1	77 ± 3.1	9.7 ± 0.3	298 ± 9.8
j	47891 ± 890	1228 ± 54	77.3 ± 2.7	210 ± 2.8	46 ± 0.9	134 ± 3.4	5.1 ± 0.3	351 ± 5.9
k	55798 ± 2624	473 ± 8	140 ± 3.4	158 ± 5.3	51 ± 2.6	45 ± 2.5	4 ± 0.2	351 ± 1.9
i	91486 ± 2986	456 ± 20	61.6 ± 2.5	192 ± 1.5	84 ± 6.1	484 ± 2.6	5.1 ± 0.1	17 ± 0.4
l	1851 ± 17	587 ± 22	41 ± 0.8	105 ± 2.5	8.2 ± 0.9	76 ± 2.4	136 ± 3.3	59 ± 2.9
m	1691 ± 16	721 ± 27	49.3 ± 2.0	199 ± 4.4	15 ± 0.5	118 ± 5.5	78 ± 1.4	112 ± 0.4
n_1	51712 ± 370	349 ± 11	93 ± 4.1	179 ± 4.6	8.5 ± 0.7	24 ± 5.3	1.9 ± 0.3	70 ± 2.7
n_2	2943 ± 47	313 ± 10	37.6 ± 1.6	116 ± 2.2	7.4 ± 1.0	18 ± 2.3	86 ± 2.2	155 ± 0.6
n_3	16118 ± 507	200 ± 8	57.7 ± 8.6	91 ± 6.7	55 ± 1.5	20 ± 2.7	8.6 ± 0.6	249 ± 2.0
n_4	5415 ± 19	172 ± 8	37.1 ± 1.5	145 ± 4.7	30 ± 2.3	19 ± 7.0	18 ± 0.2	87 ± 1.9
o_1	4136 ± 55	335 ± 12	50.5 ± 1.8	200 ± 4.6	20 ± 0.7	84 ± 3.3	7.2 ± 0.2	183 ± 2.2
o_2	2189 ± 82	451 ± 21	61.8 ± 3.3	135 ± 3.8	9.7 ± 0.7	83 ± 2.0	48 ± 2.3	43 ± 0.6
p	16985 ± 84	438 ± 9	57.7 ± 1.0	175 ± 3.5	20 ± 0.9	83 ± 11	7.5 ± 0.1	230 ± 2.7
q	25678 ± 513	668 ± 27	80.9 ± 3.8	226 ± 3.9	35 ± 2.8	60 ± 3.7	5.2 ± 0.3	200 ± 7.0
r	75080 ± 885	854 ± 13	55.9 ± 0.8	165 ± 3.9	252 ± 5.0	119 ± 6.2	1.7 ± 0.1	545 ± 9.7
s	89000 ± 1941	443 ± 12	42.4 ± 1.3	183 ± 9.2	75 ± 3.5	45 ± 2.3	7.8 ± 1.4	400 ± 15

As for the total contents, mean of the bioavailable concentrations of trace elements (except REEs) in olive grove soils from Valdichiana Senese (Tabb. 15 and 16) was used as reference value to determine their order of abundance in the availability fraction of the investigated soils. Based on these values, the order was as follows (data in µg/kg):

Sr (85427) > Ba (17761) > Ni (2727) > Zn (2505) > Co (1491) > Cu (638) > Cr (276) > Pb (240) > Rb (81) > Cd (53) > As (40) > V (31) > U (26) > Sb (6).

Table 15. Bioavailable concentrations (µg/kg) of trace elements in olive grove soils collected in 2020-21 from Valdichiana Senese, expressed as mean ± standard deviation of 3 measures on each soil sample.

Sample	Sb	Cd	As	Co	Pb	Cu	U
a	3.5 ± 0.1	60.9 ± 8.6	16.9 ± 3.3	874 ± 22.1	176 ± 3	1838 ± 48	38 ± 0.4
b	8.9 ± 0.8	61 ± 8.2	24.2 ± 2.6	1161 ± 44.2	262 ± 25	282 ± 11	23 ± 1.2
c	6.1 ± 0.5	43 ± 6.2	132 ± 5.8	1274 ± 39	155 ± 18	280 ± 5.2	20 ± 1.6
d	8.4 ± 0.5	53 ± 1.4	40.9 ± 5.4	985 ± 42	128 ± 11	272 ± 5.3	30 ± 0.3
e	3.3 ± 1.1	59.8 ± 9.3	16.4 ± 1.3	1190 ± 23.1	206 ± 17	176 ± 1.2	54 ± 7.2
f	9.4 ± 1.2	53.4 ± 3.3	33.5 ± 12	1720 ± 45.8	260 ± 3.1	1516 ± 81	55 ± 1.2
g	4.9 ± 0.4	71.9 ± 7.0	1.8 ± 5.3	1114 ± 47.4	26 ± 2.7	181 ± 7	6.9 ± 2.0
h	12 ± 0.8	69.4 ± 1.9	22.5 ± 6.5	2185 ± 52.2	2422 ± 41	369 ± 14	38 ± 2.2
j	5.5 ± 0.8	85.6 ± 2.3	18.3 ± 3.3	2061 ± 24.4	139 ± 5.4	443 ± 21	26 ± 2.6
k	4.9 ± 0.4	90.3 ± 8.8	4.1 ± 2	1772 ± 52.2	130 ± 1.3	379 ± 21	16 ± 1.0
i	6.6 ± 0.4	8.6 ± 2.0	23.5 ± 12	36 ± 3.0	2.3 ± 0.2	249 ± 4.1	35 ± 6.3
l	3.4 ± 0.5	27.8 ± 4.0	114 ± 9.6	555 ± 20.7	108 ± 6.4	1443 ± 50	7.6 ± 0.8
m	3.2 ± 0.6	17.2 ± 4.9	92.5 ± 6.1	1405 ± 3.9	143 ± 6.4	1916 ± 38	8.8 ± 0.5
n_1	4.2 ± 0.1	30.7 ± 7.8	76.3 ± 8.1	250 ± 12.6	76.7 ± 0.4	561 ± 34	9.2 ± 1.0
n_2	4.6 ± 0.3	40.2 ± 5.2	89.8 ± 3.3	2764 ± 113	186 ± 3.4	809 ± 9.6	7.5 ± 0.9
n_3	4.9 ± 1.7	77.7 ± 8.7	0.5 ± 1.6	3297 ± 69.2	172 ± 5.5	243 ± 3.3	18 ± 1.3
n_4	3.2 ± 0.7	25.1 ± 6.8	65.2 ± 5.9	248 ± 18	66.4 ± 5.2	546 ± 7	10 ± 1.1
o_1	5.2 ± 0.2	38.2 ± 7.1	26.3 ± 0.8	3179 ± 127	296 ± 7.1	1251 ± 30	35 ± 1.6
o_2	4.1 ± 1.1	17.2 ± 2.3	58.9 ± 4.4	1833 ± 83.8	285 ± 12	444 ± 17	10 ± 0.2
p	3.6 ± 1.2	41.8 ± 4.5	14.7 ± 7.2	1081 ± 7.6	69.6 ± 12	620 ± 19	11 ± 1.4
q	3.2 ± 0.4	63.3 ± 5.5	14.8 ± 8.5	218 ± 10.8	55.7 ± 4.8	281 ± 12	33 ± 1.5
r	6.5 ± 1.1	64.2 ± 6.6	21.9 ± 4.7	3923 ± 5.8	156 ± 7.4	421 ± 21	60 ± 5.1
s	13 ± 0.5	130 ± 6.1	11.7 ± 3.1	1162 ± 35	4.2 ± 2.7	144 ± 2.3	42 ± 9.7

Table 16. Bioavailable concentrations (µg/kg) of trace elements in olive grove soils collected in 2020-21 from Valdichiana Senese, expressed as mean ± standard deviation of 3 measures on each soil sample.

Sample	Zn	Ni	V	Cr	Rb	Sr	Ba
a	2185 ± 148	2506 ± 25.3	18 ± 3.7	265 ± 52	56 ± 3.4	75961 ± 1068	22451 ± 513
b	2750 ± 20	1978 ± 50.9	20 ± 5.2	268 ± 31	85 ± 5.7	96148 ± 1398	32159 ± 758
c	2371 ± 29	2323 ± 80.5	21 ± 2.9	312 ± 40	130 ± 14	70374 ± 1020	9066 ± 188
d	2125 ± 178	1975 ± 138	23 ± 3.8	280 ± 12	88 ± 10	82116 ± 2888	19903 ± 330
e	2085 ± 89	1862 ± 19.9	13 ± 6.4	318 ± 14	80 ± 17	151297 ± 3131	12311 ± 119
f	3672 ± 161	2342 ± 40.6	55 ± 15	377 ± 15	97 ± 9.9	85077 ± 748	14882 ± 525
g	1867 ± 123	2692 ± 54.8	26 ± 7.6	98 ± 15	71 ± 20	115167 ± 913	13496 ± 221
h	2530 ± 135	2519 ± 129	25 ± 9.3	230 ± 2.7	91 ± 18	148127 ± 2211	12095 ± 59
j	1789 ± 52	2211 ± 141	15 ± 7.7	235 ± 14	61 ± 4.1	119928 ± 1617	13859 ± 141
k	2475 ± 62	1535 ± 28.5	21 ± 6.9	187 ± 24	34 ± 9.9	162358 ± 1869	10358 ± 194
i	1488 ± 120	2076 ± 77	40 ± 6.1	70 ± 23	130 ± 28	229044 ± 4553	8213 ± 223
l	3173 ± 148	4405 ± 38.9	44 ± 14	235 ± 17	29 ± 4.3	6782 ± 83	25389 ± 757
m	2534 ± 91	5263 ± 98.8	60 ± 7.2	328 ± 27	92 ± 3.9	7524 ± 89	23418 ± 423
n_1	4311 ± 239	2661 ± 50	16 ± 4.6	330 ± 16	71 ± 16	15353 ± 317	17308 ± 579
n_2	3163 ± 175	4458 ± 209	23 ± 3.2	231 ± 36	73 ± 8.9	10617 ± 142	28491 ± 312
n_3	1832 ± 114	1650 ± 70.8	45 ± 17	312 ± 19	36 ± 4	67834 ± 1861	10862 ± 233
n_4	2943 ± 50	2124 ± 193	41 ± 1.5	344 ± 20	66 ± 15	32411 ± 661	10214 ± 79
o_1	2904 ± 130	4229 ± 67	43 ± 6.3	307 ± 17	119 ± 12	12405 ± 403	16745 ± 158
o_2	2309 ± 123	2577 ± 101	28 ± 5.0	217 ± 26	71 ± 16	6728 ± 108	20042 ± 174
p	2456 ± 35	2241 ± 12.8	28 ± 8.8	507 ± 29	41 ± 8.3	38152 ± 380	30018 ± 408
q	1937 ± 77	2625 ± 6.2	31 ± 4.1	474 ± 18	137 ± 14	52318 ± 404	23218 ± 383
r	2768 ± 275	5330 ± 72.6	40 ± 10	357 ± 18	124 ± 0.9	203556 ± 2185	12649 ± 432
s	1939 ± 178	1138 ± 10.2	32 ± 9.4	60 ± 21	73 ± 7.0	175534 ± 2928	21349 ± 209

Analytical data of trace elements reported in Tables 15 and 16 suggested that:

i) for most of the analysed trace elements concentrations in the bioavailable fraction of soil samples showed a variability wider than the respective total contents;

ii) soils formed by the marine sands and sandstone (MSS) had preferentially the highest bioavailable concentrations of Cd (43-130 µg/kg) and Sr (32411-203556 µg/kg);

iii) soils formed by the quartz-feldspathic-micaceous sandstones (QFS) had preferentially the highest bioavailable concentrations of Cu (809-1916 µg/kg) and Ni (2577-5263 µg/kg);

iv) anomalous low bioavailable concentrations were measured in soil sample *i* for Cd, Co, Cr, and Pb, in soil sample *s* for Cr and Pb, in soil sample *g* for As and Cr, and in soil samples *n3* and *k* for As;

v) anomalous high bioavailable concentrations were detected in soil sample *h* for Pb.

Differently to their total contents, the lowest bioavailable concentrations of Rare Earth Elements (REEs) in soil samples from Valdichiana Senese (Tabb. 17a, b), were mostly determined in soils formed by the quartz-feldspathic-micaceous sandstones (QFS). This feature is evident considering both the sum of 14 REEs (ΣREE) and that of 7 Light REEs (ΣLREE) or 7 Heavy REEs (ΣHREE).

It can be noticed that sample *i* has REE concentrations much lower compared to the other farms. Indeed, the sum of REEs in sample *i* measures 50.2 µg/kg, while the other samples range from 365.2 to 7197.6 µg/kg.

Table 17a. Concentrations (µg/kg) of Rare Earth Elements (REEs) in the bioavailable fraction of olive grove soils collected from Valdichiana Senese in 2020-21, expressed as mean ± standard deviation of 3 measures on each sample.

Sample	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy
a	550 ± 18	1039 ± 28	158 ± 6.2	798 ± 52	209 ± 17	54 ± 4.0	207 ± 8.9	26 ± 1.2	134 ± 4.0
b	421 ± 15	673 ± 20	109 ± 1.4	551 ± 11	136 ± 3.5	37 ± 0.5	136 ± 2.9	16 ± 0.0	83 ± 1.8
c	517 ± 17	948 ± 28	137 ± 3.4	649 ± 34	151 ± 8.1	38 ± 2.0	157 ± 10	21 ± 0.2	106 ± 9.7
d	395 ± 14	710 ± 22	106 ± 1.6	530 ± 44	135 ± 7.3	35 ± 1.9	130 ± 7.3	17 ± 0.7	82 ± 6.3
e	1113 ± 22	2158 ± 44	321 ± 14	1602 ± 60	392 ± 19.	95 ± 3.7	404 ± 14	51 ± 1.2	250 ± 5.5
f	668 ± 20	1279 ± 64	205 ± 4.9	981 ± 21	247 ± 4.8	61 ± 3.1	252 ± 6.7	33 ± 0.8	168 ± 4.4
g	501 ± 14	742 ± 21	129 ± 7.0	658 ± 28	150 ± 5.2	37 ± 1.6	147 ± 5.3	17 ± 0.5	82 ± 7.3
h	517 ± 9.3	975 ± 40	140 ± 6.5	718 ± 30	169 ± 9.0	41 ± 1.8	175 ± 4.2	22 ± 0.9	101 ± 7.0
j	605 ± 12	986 ± 33	150 ± 6.2	747 ± 13	186 ± 2.4	45 ± 1.6	181 ± 5.7	22 ± 1.2	109 ± 3.4
k	439 ± 17	785 ± 29	122 ± 3.5	651 ± 29	146 ± 2.9	36 ± 1.7	151 ± 3.5	17 ± 0.2	80 ± 1.9
i	11 ± 0.6	14 ± 2.8	2.0 ± 0.5	9.0 ± 0.4	5.0 ± 0.9	1.8 ± 0.6	3.0 ± 0.4	0.2 ± 0.1	2.0 ± 0.7
l	87 ± 5.9	87 ± 15	21 ± 0.5	86 ± 6.9	31 ± 3.1	10 ± 1.4	24 ± 2.3	4.1 ± 0.3	22 ± 3.9
m	105 ± 3.9	135 ± 9.7	26 ± 1.0	114 ± 0.6	35 ± 3.7	11 ± 1.0	32 ± 1.1	5.5 ± 0.5	28 ± 2.1
n_1	529 ± 20	1059 ± 33	168 ± 7.0	855 ± 36	195 ± 9.9	45 ± 2.5	193 ± 17	25 ± 1.1	122 ± 5.0
n_2	80 ± 2	98 ± 1.8	22 ± 0.6	100 ± 4.5	34 ± 3.3	12 ± 1.1	30 ± 2.2	4.3 ± 0.4	27 ± 4.4
n_3	246 ± 7.8	391 ± 9.9	73 ± 4.6	393 ± 34	94 ± 6.9	25 ± 1.8	90 ± 3.5	12 ± 1.1	66 ± 8.7
n_4	155 ± 7.8	207 ± 5	43 ± 1.8	218 ± 12	59 ± 1.4	16 ± 2.6	58 ± 2.1	8 ± 0.3	42 ± 0.2
o_1	93 ± 4.7	219 ± 0.9	29 ± 1.6	153 ± 23	41 ± 2.3	12 ± 0.6	42 ± 1.8	6 ± 0.4	33 ± 5.5
o_2	55 ± 6.4	132 ± 14	22 ± 1.7	103 ± 14	40 ± 5.8	12 ± 0.7	37 ± 4	5.1 ± 0.7	31 ± 4.7
p	206 ± 1.2	358 ± 7.4	58 ± 1.7	283 ± 15	81 ± 5.7	25 ± 1.0	80 ± 2.6	10 ± 0.9	57 ± 2.0
q	430 ± 19	677 ± 27	132 ± 2.0	677 ± 17	184 ± 7.0	46 ± 4.2	181 ± 15	23 ± 1.2	119 ± 4.5
r	1052 ± 16	2164 ± 34	324 ± 7.6	1678 ± 26	464 ± 7.3	123 ± 2.4	501 ± 16	73 ± 3.2	381 ± 11
s	82 ± 2.4	114 ± 5.2	15 ± 0.2	82 ± 7.9	24 ± 1.1	8.8 ± 0.5	20 ± 1.2	1.9 ± 0.6	9.0 ± 0.8

Table 17b. Concentrations (µg/kg) of Rare Earth Elements (REEs) in the bioavailable fraction of olive grove soils collected from Valdichiana Senese in 2020-21, expressed as mean ± standard deviation of 3 measures on each sample. Table also reports the sum of 14 REEs (ΣREE), 7 Light REEs (ΣLREE) and 7 Heavy REEs (ΣHREE).

Sample	**Ho**	**Er**	**Tm**	**Yb**	**Lu**	**ΣHREE**	**ΣLREE**	**ΣREE**
a	24 ± 1.0	63 ± 3.2	7.4 ± 0.5	41 ± 2.4	6.0 ± 2.4	301.70	3014.70	3316.6
b	15 ± 0.8	39 ± 1.8	4.5 ± 0.3	29 ± 1.8	3.9 ± 1.8	191.10	2063.40	2254.5
c	20 ± 2.1	56 ± 3.5	6.2 ± 1.1	38 ± 3.0	6.3 ± 3.0	123.20	2597.20	2850.4
d	15 ± 1.1	41 ± 4.5	4.3 ± 0.5	26 ± 0.5	4.2 ± 0.5	188.80	2041.00	2229.8
e	42 ± 2.2	110 ± 5.9	11 ± 0.4	68 ± 3.3	11 ± 3.3	543.10	6084.70	6627.8
f	31 ± 2.4	82 ± 5.7	9.8 ± 0.9	53 ± 2.3	7.8 ± 2.3	384.40	3693.40	4077.8
g	14 ± 1.3	37 ± 1.0	3.6 ± 0.2	22 ± 0.4	3.1 ± 0.4	178.40	2364.40	2542.8
h	20 ± 1.3	49 ± 3.1	5.3 ± 0.7	33 ± 1.4	4.5 ± 1.4	233.90	2735.40	2969.3
j	19 ± 1.0	49 ± 3.4	5.0 ± 0.7	32 ± 2.5	4.8 ± 2.5	240.50	2900.00	3140.5
k	14 ± 0.6	36 ± 1.4	3.7 ± 0.3	20 ± 1.7	3.2 ± 1.7	173.90	2329.90	2503.8
i	0.2 ± 0.2	1 ± 0.5	0.1 ± 0.0	0.9 ± 0.4	<0.1	4.55	45.80	50.2
l	4.6 ± 0.4	12 ± 1.7	1.5 ± 0.3	9.9 ± 2.3	1.4 ± 2.3	55.50	346.10	401.6
m	5.4 ± 0.5	15 ± 0.5	1.8 ± 0.1	13 ± 1.0	1.6 ± 1.0	70.00	457.80	527.8
n_1	22 ± 1.8	58 ± 5.1	5.9 ± 0.5	36 ± 4.3	5.2 ± 4.3	272.80	3044.30	3317.1
n_2	4.5 ± 1.0	14 ± 2.1	1.5 ± 0.4	12 ± 0.2	1.4 ± 0.2	64.60	375.50	440.1
n_3	12 ± 0.5	30 ± 2.3	3.5 ± 0.7	20 ± 1.5	3.0 ± 1.5	146.00	1311.50	1457.5
n_4	7.8 ± 0.5	24 ± 1.5	2.3 ± 0.1	15 ± 1.1	2.1 ± 1.1	101.80	755.50	857.3
o_1	5.7 ± 0.0	15 ± 1.7	1.9 ± 0.3	15 ± 6.0	1.5 ± 6.0	77.50	589.20	666.7
o_2	6 ± 0.5	17 ± 0.9	1.9 ± 0.2	14 ± 1.5	1.9 ± 1.5	76.70	400.80	477.5
p	9.6 ± 0.6	26 ± 1.5	3.1 ± 0.2	20 ± 1.3	2.5 ± 1.3	128.80	1090.80	1219.6
q	21 ± 1.1	53 ± 3.0	6 ± 0.6	35 ± 3.2	4.8 ± 3.2	262.10	2327.10	2589.2
r	74 ± 3.7	187 ± 9.1	22 ± 0.9	135 ± 7.1	20 ± 7.1	891.60	6306.00	7197.6
s	1.5 ± 0.1	3.9 ± 1.4	0.4 ± 0.2	2.4 ± 0.4	0.3 ± 0.4	19.40	345.80	365.2

In order to assess the level of mobility of the analysed chemical elements in olive grove soil samples from Valdichiana Senese, that is their tendency to be released and mobilised into the soil solution, the mobility factor index (MFI) was calculated as the ratio between the bioavailable concentration and total content (x 100) of a chemical element in soil sample (Kabala & Singh, 2001).

Using the mean of MFI as reference value, the order of mobility of major and minor chemical elements in the investigated soil samples was as follows (data in %): Ca (76) > Mn (35) > S (11) > Mg (7) > P (3) > K (1) > Na (0.8) > (0.2). It should be noted that the high MFI values of Ca and, to a lesser extent, of Mn are due to the fact that the bioavailable (extractable) fraction accounts for the carbonate-bound fraction together with the water-soluble and exchangeable fractions.

Among the trace elements, the most mobile ones resulted in Sr, Cd and Co on the basis of an average MFI value of 42, 23 and 12%, respectively. The comment made for Ca is valid also to explain the high Sr concentrations in the bioavailable fraction of soils samples, because Sr is a vicariant of Ca in several minerals including calcite (carbonate). In the order of mobility of trace elements follow Ba, Ni, Zn, and Cu with MFI values normally in the range 10-1%. Trace elements such as U, Pb, Sb, As, Cr, Rb, and V have MFI values generally lower than 1%.

4.2.3. Macro, micro and trace elements in olives

The concentrations of macro, micro, and trace elements in olive pulp samples collected in 2020-21 are reported in Tables 18 and 19.

The macro and micro elements exhibited rather homogeneous concentrations in olive pulp samples in the range: 19922-30457 mg/kg for K, 908-1844 mg/kg for P, 749-1410 for Ca, 649-1039 mg/kg for S, 351-555 mg/kg for Mg, 47-296 mg/kg for Na, 12-41 mg/kg for Fe, and 4-5 mg/kg for Mn (Tab. 18). While the soil samples formed by the quartz-feldspathic-micaceous sandstones (QFS) presented the highest concentrations of Mg, Na and K, and the lowest of Ca, these geochemical features were not reflected in the chemical composition of olives from olive trees grown on these soils.

Table 18. Concentrations (mg/kg) of macro and micro elements in olive pulp samples collected from Valdichiana Senese in 2020-21, expressed as mean ± standard deviation of 3 measures on each sample.

Sample	Ca	Mg	Fe	Mn	Na	K	P	S
a	824 ± 58	357 ± 27	19 ± 1.5	4.9 ± 0.1	62.9 ± 5	24629 ± 1614	1597 ± 127	828 ± 6
b	1098 ± 65	555 ± 46	26 ± 2.8	5.1 ± 0.1	63.4 ± 5	27340 ± 1842	1770 ± 160	1039 ± 5
c	749 ± 11	382 ± 15	18 ± 1.3	3.6 ± 0.2	125 ± 6	28842 ± 286	1785 ± 36	889 ± 10
d_1	1077 ± 76	433 ± 52	15 ± 0.8	4.1 ± 0.1	161 ± 12	20764 ± 626	1042 ± 71	659 ± 11
d_2	1202 ± 71	365 ± 26	20 ± 2.3	4.5 ± 0.1	296 ± 37	19922 ± 917	908 ± 57	649 ± 7
e	920 ± 34	410 ± 21	15 ± 0.4	4.2 ± 0.2	52.7 ± 3	26644 ± 880	1673 ± 53	821 ± 4
f	909 ± 36	375 ± 14	12 ± 0.3	4.5 ± 0.1	146 ± 8	27247 ± 588	1816 ± 72	772 ± 5
g	1046 ± 55	386 ± 20	20 ± 0.3	4.5 ± 0.1	117 ± 7	25599 ± 720	1572 ± 56	818 ± 5
h	866 ± 46	391 ± 25	30 ± 1.7	4.7 ± 0.3	102 ± 8	30457 ± 1493	1785 ± 110	962 ± 5
j	943 ± 25	393 ± 19	16 ± 2.8	4.1 ± 0.2	92.4 ± 5	26693 ± 225	1714 ± 31	948 ± 4
k	1410 ± 56	377 ± 19	18 ± 0.6	4.6 ± 0.2	49.3 ± 2	20910 ± 814	1844 ± 92	840 ± 9
i	839 ± 9	351 ± 12	13 ± 1.2	3.7 ± 0.1	62.3 ± 2	25867 ± 395	1827 ± 45	906 ± 5
l	1106 ± 42	390 ± 20	17 ± 0.7	4.5 ± 0.1	61.5 ± 3	22752 ± 532	1406 ± 79	735 ± 4
m	1245 ± 71	375 ± 32	14 ± 0.9	4.4 ± 0.0	85.2 ± 4	23577 ± 484	1825 ± 33	884 ± 7
n	980 ± 18	457 ± 37	41 ± 3.8	5.3 ± 0.0	61.7 ± 5	23694 ± 1046	1622 ± 144	800 ± 12
o	1049 ± 74	385 ± 33	16 ± 1.4	3.9 ± 0.1	62.5 ± 6	23866 ± 1466	1670 ± 144	828 ± 8
p	1288 ± 55	423 ± 41	13 ± 0.3	3.9 ± 0.1	60.5 ± 3	26239 ± 1242	1539 ± 89	864 ± 10
q	817 ± 23	447 ± 32	17 ± 0.3	5 ± 0.2	71.9 ± 4	24951 ± 334	1611 ± 73	906 ± 4
r	953 ± 58	362 ± 26	18 ± 1.5	3.5 ± 0.1	46.9 ± 4	21885 ± 1212	1274 ± 87	931 ± 3
s	988 ± 58	368 ± 26	18 ± 1.7	4.3 ± 0.2	64 ± 4	20247 ± 1137	1441 ± 114	844 ± 2

Based on the average value of their concentrations, trace elements (except REEs) in olive pulp samples from Valdichiana Senese had the following order of abundance (data in µg/kg):

Rb (13289) > Zn (12499) > Cu (8929) > Sr (3646) > Ni (843) > Ba (804) > Cr (324) > Pb (257) > V (87.5) > Co (30) > As (15) > Cd (12) > Sb (11) > U (5).

No concentrations higher than the LOQ (1 µg/kg) were identified for Tl in the olive pulp samples. In addition, numerous samples had concentrations of As and Cd lower than the respective LOQ (7 and 10 µg/kg, respectively; Tab. 19).

Taking into account soil geochemistry, concentrations of Ba, Cr, Ni, Pb, Rb, Sr, and Tl in olive pulp samples from olive groves extending on soils derived by the quartz-feldspathic-micaceous sandstones (QFS), were expected to be higher than in the other samples. Instead, Ba, Cr, Pb, Rb, and Sr showed a rather uniform distribution across all olive pulp samples, and only Ni had higher concentrations in olives from olive trees grown on soils formed by QFS.

Table 19. Concentrations (expressed in mg/kg or in µg/kg) of trace elements in olive pulp samples collected from Valdichiana Senese in 2020-21, expressed as mean ± standard deviation of 3 measures on each sample.

Sample	**Cu** mg/kg	**Zn** mg/kg	**Rb** mg/kg	**Sr** mg/kg	**Ba** mg/kg	**V** µg/kg	**Cr** µg/kg	**Co** µg/kg	**Ni** µg/kg	**As** µg/kg	**Cd** µg/kg	**Pb** µg/kg	**U** µg/kg	**Sb** µg/kg
a	10.7 ± 0.3	11.6 ± 0.2	12.7 ± 0.4	2.8 ± 0.12	0.6 ± 0.02	70.1 ± 2.6	317 ± 61	28 ± 2.6	679 ± 31.7	22.7 ± 4.7	15 ± 4.0	316 ± 13	4.8 ± 0.8	4.7 ± 0.0
b	10.2 ± 0.2	18.3 ± 0.1	20.8 ± 0.5	3.2 ± 0.13	0.9 ± 0.04	69.6 ± 7.7	356 ± 51	52 ± 2.8	628 ± 22.9	27.6 ± 2.8	13 ± 4.9	360 ± 19	4.9 ± 0.2	10 ± 5.5
c	8.15 ± 0.1	11 ± 0.4	3.49 ± 0.1	3.7 ± 0.17	0.7 ± 0.04	124 ± 12	544 ± 75	36 ± 2.9	683 ± 39.3	19.8 ± 4.7	11 ± 6.3	308 ± 19	5.4 ± 1.0	34 ± 4.0
d_1	7.36 ± 0.2	12.1 ± 0.1	9.72 ± 0.3	4.3 ± 0.23	1 ± 0.04	46.4 ± 2.3	245 ± 65	14 ± 4.3	869 ± 75.3	8.6 ± 8.1	15 ± 4.9	246 ± 15	6.6 ± 1.6	7.3 ± 1.0
d_2	6.99 ± 0.3	14.4 ± 0.6	12.2 ± 0.4	4.7 ± 0.15	1.1 ± 0.05	66.3 ± 4.5	269 ± 81	11 ± 2.7	728 ± 66	11.8 ± 8.7	14 ± 7.5	392 ± 28	6.6 ± 1.2	11 ± 1.8
e	10.8 ± 0.1	16.5 ± 0.3	10.2 ± 0.3	5.3 ± 0.10	0.6 ± 0.01	89 ± 5.6	332 ± 64	77 ± 5.0	448 ± 43.5	<7.0	12 ± 5.5	225 ± 4.8	5.2 ± 0.7	7.3 ± 1.7
f	7.53 ± 0.3	8.38 ± 0.4	12.8 ± 0.4	2.5 ± 0.13	0.4 ± 0.03	106 ± 5.2	325 ± 37	20 ± 3.0	681 ± 66	<7.0	<10	238 ± 17	4.5 ± 0.9	13 ± 2.4
g	7.77 ± 0.2	9.9 ± 0.3	16.3 ± 0.5	2.8 ± 0.14	0.6 ± 0.05	95.8 ± 9.7	166 ± 56	19 ± 4.9	514 ± 15.3	25.5 ± 17	<10	112 ± 7.6	4.3 ± 0.3	8.2 ± 3.3
h	10.2 ± 0.5	18.8 ± 0.9	8.74 ± 0.4	3 ± 0.16	1.2 ± 0.04	90.3 ± 6.3	453 ± 72	31 ± 1.7	614 ± 68.2	37.4 ± 3.0	12 ± 3.9	560 ± 41	5.4 ± 1.3	48 ± 4.6
j	12.1 ± 0.1	11.3 ± 0.5	15.2 ± 0.5	3.7 ± 0.16	0.5 ± 0.02	108 ± 4	235 ± 55	60 ± 4.6	681 ± 64.7	16.6 ± 6.0	<10	118 ± 8.2	3.6 ± 0.7	2.0 ± 0.0
k	10.6 ± 0.2	11.6 ± 0.3	8.88 ± 0.2	4 ± 0.11	0.4 ± 0.02	64.9 ± 1.4	360 ± 37	23 ± 2.4	401 ± 36.7	<7.0	13 ± 7.7	265 ± 11	3.7 ± 0.5	2.1 ± 0.0
i	8.85 ± 0.4	13.4 ± 0.4	21.1 ± 0.8	5.8 ± 0.16	0.3 ± 0.02	91.8 ± 5.6	370 ± 39	35 ± 6.4	594 ± 52.4	<7.0	12 ± 1.2	184 ± 11	5.1 ± 0.7	4.7 ± 1.2
l	8.47 ± 0.3	8.35 ± 0.6	14.5 ± 0.5	3.9 ± 0.15	2.2 ± 0.13	103 ± 9.6	353 ± 36	40 ± 3.7	1167 ± 45	14.4 ± 10	14 ± 3.8	284 ± 11	5.3 ± 0.1	6.0 ± 1.2
m	9.87 ± 0.2	15.1 ± 0.2	11.9 ± 0.2	2.8 ± 0.10	0.6 ± 0.00	80 ± 6.9	269 ± 57	17 ± 3.3	3456 ± 179	18.3 ± 9.8	12 ± 6.4	254 ± 6.9	4.5 ± 1.3	4.1 ± 1.0
n	6.89 ± 0.1	9.23 ± 0.1	17.3 ± 0.5	3.2 ± 0.13	0.9 ± 0.05	99.4 ± 4.6	282 ± 15	11 ± 1.5	692 ± 44.9	<7.0	<10	137 ± 8.7	3.1 ± 0.4	3.9 ± 0.0
o	9.15 ± 0.3	11.7 ± 0.5	10.7 ± 0.4	3.5 ± 0.11	0.8 ± 0.06	101 ± 8.6	290 ± 37	25 ± 2.9	812 ± 114	<7.0	<10	229 ± 15	5.0 ± 0.7	4.3 ± 2.0
p	7.39 ± 0.3	9.39 ± 0.6	13.4 ± 0.7	3.8 ± 0.17	1.3 ± 0.09	104 ± 4.8	230 ± 48	14 ± 3.6	782 ± 26.7	15 ± 7.1	<10	241 ± 26	3.6 ± 1.4	6.2 ± 3.8
q	10.7 ± 0.2	12.1 ± 0.4	9.72 ± 0.1	2.9 ± 0.00	0.7 ± 0.02	83.5 ± 8.1	491 ± 24	28 ± 5.1	973 ± 85.6	<7.0	<10	145 ± 3.1	3.6 ± 0.9	7.3 ± 2.8
r	6.68 ± 0.3	14.3 ± 1	25.2 ± 1.2	4.7 ± 0.22	0.5 ± 0.05	77 ± 7.3	230 ± 45	31 ± 6.7	524 ± 15.7	26.5 ± 20	15 ± 3.0	281 ± 27	3.9 ± 0.4	2.8 ± 0.0
s	8.32 ± 0.3	12.5 ± 0.4	10.7 ± 0.3	2.5 ± 0.15	0.8 ± 0.06	82 ± 1.8	371 ± 58	25 ± 7.5	935 ± 30.9	<7.0	<10	238 ± 0.5	3.6 ± 0.7	37 ± 4.0

About the REEs (Tab. 20), only La, Ce, Pr, and Nd had concentrations in olive pulp samples mostly higher than their limit of quantification (LOQ; Tab. 20). On the contrary, the levels of the other REEs in olive samples were frequently (Sm, Gd, and Dy) or constantly (Eu, Tb, Ho, Er, Tm, Yb, and Lu) lower than the respective LOQs (1.0 µg/kg for Sm, Gd, Dy, Tb, and Tm, 0.4 µg/kg for Eu, 0.2 for Ho, 2.0 µg/kg for Er, 0.3 µg/kg for Yb and Lu).

Table 20. Concentrations (µg/kg) of Rare Earth Elements (REEs) in olive pulp samples collected from Valdichiana Senese in 2020-21, expressed as mean ± standard deviation of 3 measures on each sample.

Sample	La	Ce	Pr	Nd	Sm	Gd	Dy
a	7.39 ± 0.7	15.1 ± 2.2	1.5 ± 0.3	7.44 ± 5.3	1.9 ± 1.5	1.1 ± 0.5	<1.0
b	25.9 ± 2.8	17 ± 3.3	1.7 ± 0.5	8.78 ± 1.3	<1.0	<1.0	<1.0
c	7.17 ± 2.1	10.7 ± 2.7	1.8 ± 0.8	8.34 ± 2	<1.0	<1.0	<1.0
d_1	6.63 ± 0.6	9.36 ± 0.2	1.3 ± 0.7	7.77 ± 1.4	<1.0	<1.0	<1.0
d_2	12.4 ± 1.1	17.6 ± 2.5	2 ± 0.5	10.9 ± 1.1	1.9 ± 1.6	<1.0	<1.0
e	6.62 ± 1	8.19 ± 1	1.3 ± 0.2	<2.0	<1.0	<1.0	<1.0
f	10.4 ± 1.2	16 ± 3.2	2.2 ± 0.5	9.46 ± ±	<1.0	<1.0	1.2 ± 0.4
g	5.12 ± 1.4	8.08 ± 1.3	1 ± 0.2	2.05 ± ±	<1.0	<1.0	<1.0
h	<1.0	12.6 ± 1.5	2.1 ± 0.5	8.86 ± 0.6	<1.0	<1.0	<1.0
j	14.2 ± 1.8	20.2 ± 0.5	2.3 ± 0.9	9.04 ± 2.8	1.4 ± 1.3	<1.0	<1.0
k	6.82 ± 0.9	14.4 ± 0.9	1.8 ± 0.8	4.7 ± 1.1	1.7 ± 1.1	<1.0	<1.0
i	12.9 ± 0.7	19.2 ± 1.8	1.5 ± 0.7	<2.0	<1.0	<1.0	<1.0
l	10.8 ± 2.9	12.7 ± 0.8	1.4 ± 0.7	6.48 ± 1.8	<1.0	<1.0	<1.0 ± ±
m	26.4 ± 0.5	55.9 ± 3.4	6.3 ± 0.4	24.8 ± 1.2	4.3 ± 1	2.6 ± 2.2	3.1 ± 0.9
n	8.13 ± 0.3	13.9 ± 2.9	1.7 ± 0.6	5.24 ± 1.6	<1.0	<1.0	<1.0
o	8.37 ± 1.1	10.9 ± 0.4	1.8 ± 0.7	7.84 ± 6.9	<1.0	<1.0	<1.0
p	6.39 ± 0.5	10.8 ± 3.6	1.8 ± 0.8	7.56 ± 1.5	<1.0	<1.0	<1.0
q	7.15 ± 2.1	9.39 ± 0.8	1.6 ± 0.5	7.38 ± 0.6	1.6 ± 1.5	<1.0	<1.0
r	7.76 ± 0.6	11.3 ± 0.9	2 ± 0.5	8.21 ± 0.7	1.5 ± 1.1	<1.0	<1.0
s	6.39 ± 0.3	11.2 ± 2.2	1.8 ± 0.4	4.9 ± 1	<1.0 ±	<1.0	<1.0

4.2.4. Macro, micro and trace elements in EVOOs

In the extra virgin olive oils (EVOOs) from Valdichiana Senese collected in 2020-21 were determined the concentrations of the 36 chemical elements analysed in soil and olive samples.

The macro and micro elements are listed below with their minimum and maximum concentrations (Tab. 21): S (28334-49386 µg/kg), Ca (21750-49645 µg/kg), Na (3398-12106 µg/kg), K (1201-7500 µg/kg), Mg (2203-6494 µg/kg), P (2581-5715 µg/kg), Fe (2480-4586 µg/kg), and Mn (69-161 µg/kg).

EVOO samples from olive groves extending on soils derived by QFS, do not show higher concentrations of Mg, Na, and K and lower ones of Ca and Fe, as observed in the respective olive samples.

Analysing trace element concentrations detected in EVOOs from Valdichiana Senese (Table 22), the following observations can be noted:

i) in all EVOO samples As, Tl and Cd had concentrations lower than their LOQ (17, 17, and 1 µg/kg, respectively) and therefore they were not reported in Table 22;

ii) Zn, Cu, Ni, Sr, Cr, Pb, Ba, Rb, and U exhibited the following range: 1076-6961, 1052-3235, 209-1707, 328-525, 199-320, 68-1169, 55-4283, 7-67, and 4-9 µg/kg, respectively;

iii) EVOO sample *a* had anomalous high concentration of Ba and Co, as well as sample *r* of Pb;

iv) Rb, V, Co, and Sb exhibited in 3, 6, 13, and 10 EVOO samples, respectively, concentrations below their LOQ (3, 4, 2.5, 5.5 µg/kg, respectively);

v) the concentrations of PTEs (As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Tl, U, V, and Zn) measured in EVOO samples collected in 2020-21 were comparable with those of EVOO samples produced in 2019-20 in these farms, with few exceptions;

vii) while the bioavailable fraction of soils formed by the marine sands and sandstones (MMS) had high concentrations of Cd and Sr, and soils derived by the quartz-feldspathic-micaceous sandstones (QFS) showed high levels of Cu and Ni, these features were not found in EVOOs from olive groves extending of these types of soils.

Table 21. Concentrations (µg/kg) of macro and micro elements in EVOOs collected from Valdichiana Senese in 2020-21, expressed as mean ± standard deviation of 3 measures on each sample.

Sample	Ca	Mg	Fe	Mn	Na	K	P	S
a	33400 ± 635	3156 ± 14	3596 ± 21	70 ± 1.2	7921 ± 53	5436 ± 126	5715 ± 760	38223 ± 2307
b	38896 ± 456	3904 ± 32	3518 ± 53	95.6 ± 8.8	5569 ± 158	3281 ± 512	5026 ± 1038	49386 ± 1354
c	36485 ± 163	3068 ± 11	4586 ± 45	161 ± 6.6	11863 ± 24.7	6944 ± 53.8	4259 ± 246	41056 ± 3461
d_1	21750 ± 244	2707 ± 66	2713 ± 16	84.6 ± 9.1	4599 ± 114	1201 ± 154	3997 ± 486	38210 ± 503
d_2	38262 ± 330	2508 ± 14	2904 ± 6.5	104 ± 7.6	9798 ± 135	4408 ± 65.6	3510 ± 490	40706 ± 1263
e	28581 ± 374	3536 ± 17	2971 ± 19	98.9 ± 7.6	10061 ± 34	1572 ± 23	4575 ± 1050	39478 ± 4022
f	27712 ± 502	3060 ± 3.8	2798 ± 37	126 ± 3.1	10225 ± 91.4	6047 ± 109	4306 ± 853	38265 ± 2837
g	30856 ± 372	3562 ± 21	3349 ± 18	106 ± 9.4	6107 ± 123	3331 ± 502	3878 ± 348	38154 ± 2696
h	23666 ± 706	2842 ± 28	2695 ± 16	88 ± 2.1	4223 ± 101	2085 ± 68.7	2998 ± 463	28727 ± 4254
j	31447 ± 640	3606 ± 27	3164 ± 25	114 ± 7.1	8208 ± 127	3662 ± 37.9	3786 ± 252	32681 ± 3084
k	39052 ± 132	2203 ± 49	2779 ± 33	106 ± 13	9709 ± 152	3734 ± 123	4236 ± 587	37023 ± 2067
i	22418 ± 246	2537 ± 68	3302 ± 28	84.9 ± 13	4972 ± 78.9	1993 ± 75.4	2821 ± 732	33751 ± 2417
l	29057 ± 295	2644 ± 24	3046 ± 9	89.9 ± 11	6733 ± 127	3895 ± 516	4328 ± 1326	38467 ± 2764
m	49345 ± 244	4926 ± 26	3846 ± 37	137 ± 22	8445 ± 154	4358 ± 91.1	4541 ± 410	28334 ± 756
n1	35136 ± 218	3799 ± 25	2823 ± 17	105 ± 12	10843 ± 160	1327 ± 21	4740 ± 333	46695 ± 1160
n2	37706 ± 140	4202 ± 22	2664 ± 22	107 ± 10	6610 ± 110	2008 ± 59.8	4661 ± 756	45529 ± 2657
n3	31578 ± 284	2698 ± 56	2480 ± 43	110 ± 5	5517 ± 125	1795 ± 410	3419 ± 201	40858 ± 2720
n4	40251 ± 89	4230 ± 33	2563 ± 13	114 ± 0.7	12106 ± 201	2292 ± 339	3895 ± 1417	46416 ± 1961
o1	30770 ± 235	2682 ± 27	2999 ± 19	94 ± 18	7753 ± 74.8	4155 ± 47.3	2581 ± 344	35553 ± 4185
o2	34360 ± 234	3058 ± 19	2574 ± 23	89.4 ± 7.4	3398 ± 27.8	1659 ± 35	3574 ± 987	35764 ± 789
p	27362 ± 171	2538 ± 17	3721 ± 12	68.7 ± 2.1	12105 ± 120	7500 ± 148	4679 ± 1109	32219 ± 3587
q	28905 ± 658	2566 ± 11	3156 ± 38	95.4 ± 3.1	4517 ± 67.1	2297 ± 30.4	4120 ± 747	37483 ± 1281
r	24947 ± 460	6494 ± 52	2658 ± 33	104 ± 6.1	8371 ± 41.1	2430 ± 315	3271 ± 422	37779 ± 3324
s	24718 ± 197	2531 ± 9.8	2957 ± 16	111 ± 16	9835 ± 108	3767 ± 156	3841 ± 784	36517 ± 2669

Table 22. Concentrations (µg/kg) of trace elements in EVOOs collected from Valdichiana Senese in 2020-21, expressed as mean ± standard deviation of 3 measures on each sample.

Sample	Cu	Zn	Rb	Sr	Ba	V	Cr	Co	Ni	Pb	U	Sb
a	3235 ± 43	3766 ± 102	66 ± 5.8	516 ± 24	4283 ± 107	23 ± 17	260 ± 22	23 ± 8.7	775 ± 39	189 ± 19	9.2 ± 1.1	35 ± 11
b	1235 ± 22	1755 ± 475	24 ± 0.5	493 ± 12	108 ± 8.9	26 ± 5.6	270 ± 59	<2.0 ± ±	593 ± 11	121 ± 11	6.4 ± 1.8	<5.0
c	1605 ± 108	5147 ± 618	22 ± 1.2	387 ± 6	117 ± 6.5	13 ± 7.1	266 ± 53	6.8 ± 2.4	1006 ± 66	235 ± 12	6.3 ± 1.5	17 ± 4.2
d_1	1243 ± 103	2003 ± 142	20 ± 3.4	426 ± 22	72 ± 8.5	9 ± 6.0	240 ± 13	4.3 ± 0.6	1161 ± 99	169 ± 3	4.4 ± 1.7	<5.0
d_2	1684 ± 94	1536 ± 363	15 ± 2.6	328 ± 26	99 ± 6.6	<4.0	267 ± 29	<2.0	209 ± 24	103 ± 7.3	5.2 ± 0.4	<5.0
e	1407 ± 33	3717 ± 579	28 ± 3.5	407 ± 12	117 ± 11	56 ± 7.6	233 ± 24	<2.0	1099 ± 54	141 ± 11	5.7 ± 0.2	10 ± 3.3
f	1211 ± 30	1076 ± 90	67 ± 5.0	389 ± 32	98 ± 9.7	4.2 ± 2.3	199 ± 16	<2.0	402 ± 16	68 ± 7.2	4.3 ± 0.8	9 ± 5.5
g	1333 ± 40	3645 ± 245	35 ± 1.1	426 ± 13	182 ± 22.4	<4.0 ±	320 ± 20	8.2 ± 4.6	900 ± 30	202 ± 23	6.0 ± 1.4	8 ± 5.3
h	1109 ± 83	1178 ± 560	24 ± 0.3	373 ± 9	153 ± 3.8	<4.0 ±	233 ± 32	<2.0	854 ± 15	145 ± 14	4.3 ± 0.6	29 ± 17
j	1329 ± 96	2360 ± 329	27 ± 8.5	432 ± 15	147 ± 14.9	12 ± 5.1	237 ± 49	<2.0	497 ± 16	105 ± 3	4.3 ± 0.3	19 ± 6.4
k	1339 ± 45	1103 ± 392	44 ± 3.1	386 ± 13	99 ± 14.6	12 ± 7.5	228 ± 10	<2.0	343 ± 15	345 ± 9.3	5.9 ± 0.9	6 ± 3.1
i	1123 ± 62	2558 ± 475	15 ± 1.8	336 ± 20	71 ± 14.1	9 ± 5.0	213 ± 6	9.7 ± 1.6	536 ± 43	281 ± 13	5.1 ± 0.4	<5.0
l	1327 ± 95	2511 ± 466	25 ± 4.3	426 ± 3	111 ± 7	17 ± 8.4	233 ± 48	<2.0	464 ± 29	251 ± 3.9	5.9 ± 1.6	15 ± 11
m	1253 ± 88	6961 ± 661	25 ± 2.6	525 ± 41	112 ± 12.3	<4.0	242 ± 55	7.5 ± 0.7	1309 ± 23	149 ± 8.7	5.9 ± 2.7	<5.0
n_1	1443 ± 16	1299 ± 110	7 ± 3.0	436 ± 57	78 ± 16.3	14 ± 3.2	212 ± 41	<2.0	981 ± 93	118 ± 13	4.5 ± 2.0	13 ± 6.7
n_2	1257 ± 69	1754 ± 84	<3.0	444 ± 39	73 ± 8.7	<4.0	237 ± 15	6.2 ± 3.4	691 ± 56	143 ± 16	4.4 ± 0.2	<5.0
n_3	1407 ± 45	2423 ± 293	<3.0	422 ± 16	55 ± 1.2	16 ± 5.6	199 ± 41	7.3 ± 4.4	878 ± 33	177 ± 9.5	4.5 ± 1.2	<5.0
n_4	1740 ± 22	1501 ± 284	<3.0	447 ± 29	95 ± 1.4	<4.0	232 ± 43	<2.0	922 ± 44	157 ± 6	5.2 ± 0.4	8 ± 4.4
o_1	1524 ± 125	3026 ± 137	29 ± 4.2	446 ± 18	135 ± 13.8	29 ± 9.5	210 ± 39	<2.0	844 ± 27	140 ± 6.7	7.3 ± 1.2	<5.0
o_2	1052 ± 79	2454 ± 419	16 ± 3.5	459 ± 22	85 ± 14.3	39 ± 8.4	246 ± 18	2.9 ± 0.6	568 ± 49	116 ± 5.3	4.5 ± 1.2	<5.0
p	1438 ± 65	3185 ± 17	49 ± 1.5	379 ± 22	109 ± 5.1	35 ± 2.2	266 ± 21	4.7 ± 1.9	1555 ± 52	139 ± 5.4	5.3 ± 1.4	<5.0
q	1445 ± 39	2624 ± 281	14 ± 2.1	388 ± 7	110 ± 16.4	76 ± 11	229 ± 23	<2.0	808 ± 30	158 ± 4	4.9 ± 0.8	10 ± 3.4
r	1622 ± 121	n.a.	17 ± 2.7	359 ± 9	121 ± 11	14 ± 7.8	207 ± 40	5.6 ± 3.5	1707 ± 24	1169 ± 55	5.5 ± 0.5	11 ± 0.6
s	1591 ± 89	1400 ± 263	25 ± 1.6	359 ± 16	200 ± 2.2	11 ± 6.8	274 ± 38	<2.0	484 ± 20	107 ± 10	5.7 ± 1.3	14 ± 5.5

n.a., data not available

Regarding the concentrations of Rare Earth Elements (Tab. 23) in EVOOs 2020-21 from Valdichiana Senese, only La, Ce, and Pr were detected in all samples. Ho and Er levels higher than their LOQ (<0.1 and <1 µg/kg, respectively) were measured only in one EVOO sample each. No concentrations of Nd, Sm, Eu, Gd, Tb, Dy, Tm, Yb, and Lu exceeded the respective LOQ (3.7, 1.7, 0.9, 1.8, 3, 2.2, 4.8, 1.3, and 2.3 µg/kg, respectively).

Similarly to their corresponding contents in olive samples, EVOOs from olive grove soils formed by QFS did not show any significant differences compared to other EVOOs, as it was evident in the bioavailable fractions of QFS soils.

Table 23. Concentrations (µg/kg) of Rare Earth Elements (REEs) in EVOOs collected from Valdichiana Senese in 2020-21, expressed as mean ± standard deviation of 3 measures on each sample.

Sample	**La**	**Ce**	**Pr**	**Ho**	**Er**
a	4.92 ± 1.0	5.97 ± 1.0	<0.5	<0.1	<1.0
b	9.63 ± 1.11	16.8 ± 0.5	1.8 ± 0.4	<0.1	<1.0
c	6.01 ± 1.11	9.48 ± 1.1	1.6 ± 0.5	<0.1	<1.0
d_1	7.44 ± 0.71	11.3 ± 1.3	1.0 ± 0.3	<0.1	<1.0
d_2	5.41 ± 1.49	7.84 ± 1.6	1.6 ± 0.7	0.5 ± 0.2	<1.0
e	11.6 ± 1.4	22.5 ± 2.2	2.8 ± 0.5	<0.1	2.3 ± 0.8
f	5.39 ± 0.51	9.08 ± 2.5	1.1 ± 0.5	<0.1	<1.0
g	11.0 ± 0.91	23.8 ± 1.5	2.6 ± 1	<0.1	<1.0
h	9.28 ± 2.11	15.6 ± 0.2	1.5 ± 0.4	<0.1	<1.0
j	6.93 ± 0.75	12.2 ± 2.4	1.2 ± 0.3	<0.1	<1.0
k	6.65 ± 2.0	10.1 ± 1.5	0.9 ± 0.4	<0.1	<1.0
i	4.38 ± 1.41	6.91 ± 2.0	1.6 ± 0.6	<0.1	<1.0
l	6.16 ± 0.57	8.16 ± 1.9	1.2 ± 0.2	<0.1	<1.0
m	7.43 ± 1.0	10.6 ± 0.6	0.8 ± 0.2	<0.1	<1.0
n_1	6.29 ± 2.0	13.7 ± 1.5	0.6 ± 0.3	<0.1	<1.0
n_2	3.06 ± 2.35	5.44 ± 1.4	0.8 ± 0.4	<0.1	<1.0
n_3	5.48 ± 1.05	8.65 ± 2.0	<0.5	<0.1	<1.0
n_4	4.34 ± 1.18	6.95 ± 1.2	0.8 ± 0.3	<0.1	<1.0
o_1	7.17 ± 2.09	12.8 ± 1.7	1.3 ± 0.6	<0.1	<1.0
o_2	6.05 ± 1.13	12.5 ± 1.6	<0.5	<0.1	<1.0
p	7.38 ± 1.41	13.3 ± 2.3	1.0 ± 0.4	<0.1	<1.0
q	9.9 ± 1.72	25.5 ± 0.8	2.1 ± 0.5	<0.1	<1.0
r	7.01 ± 1.2	8.47 ± 1.1	1.4 ± 0.4	<0.1	<1.0
s	7.05 ± 1.51	12.6 ± 2.9	1.1 ± 0.6	<0.1	<1.0

Considering that elemental composition of olives and EVOO could reflect some geochemical features of the soil of origin, all analytical data presented above were used to identify relationships between the concentrations of the analysed chemical elements in the olive grove soil samples and in the related olive fruit and EVOO samples collected in 2020-21 in the Valdichiana Senese area.

In this frame, REEs are often used in studies of geographical traceability (Barbera et al., 2022; Aceto et al., 2019; Zhao & Yang, 2019; Aceto et al., 2018; Catarino et al., 2014), because their concentrations in foods reflect the geochemical characteristics of the cultivation area. In particular, the studies on EVOO traceability usually compare REE concentrations in soils and olives and/or EVOOs in a way to identify relevant correlations between the two matrices. Unfortunately, in this study, concentrations of almost all Rare Earth Elements in both olives and EVOOs were lower than the respective LOQ, preventing finding relationships between soil, olive fruit and EVOO chemical composition.

To individuate the relationships between the (geo)chemistry of olive grove soils, olive fruits, and EVOOs and determine the geographical traceability of Valdichiana Senese EVOO, a Pearson correlation analysis was performed (Fig. 5).

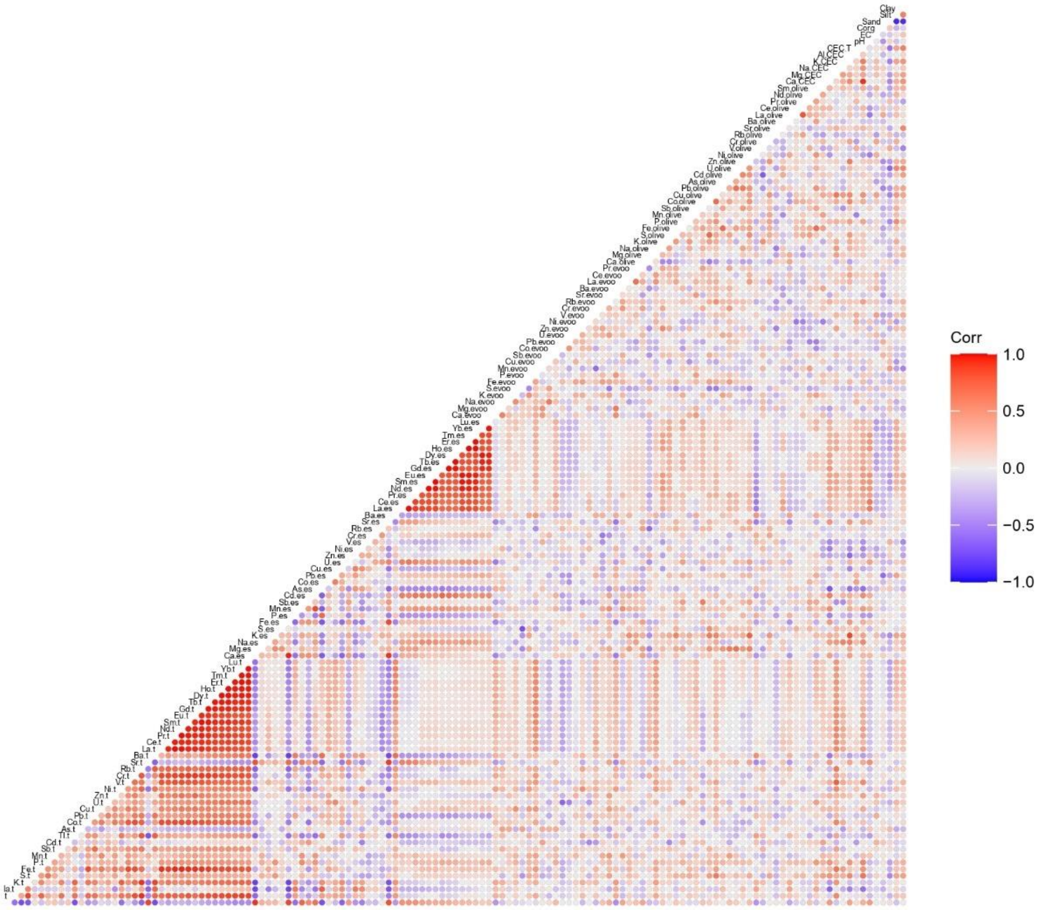

Figure 5. Correlation matrix between chemical and physical properties of soils, concentrations of 36 chemical elements in the olive grove soils, including both the total elemental content and the bioavailable fraction, olive pulps and EVOOs from Valdichiana Senese collected in 2020-21. Pearson's correlation ranged from +1 (red) to -1 (blue).

Considering correlations with p-value <0.05 and r >0.5, Pearson analysis determined 813 correlations (144 negatives and 669 positives) among soil properties, element total contents and bioavailable concentrations in soils, and element concentrations in olives and EVOOs. Most of the positive correlations were found between the total content of several chemical elements in the olive grove soils (52.5%; i.e. Ni with Tl or Ba and Na), as well as between the concentration in the soil bioavailable fraction (20.6%; i.e., Fe and Sr, or Ca and Sb, and especially between the REEs; red spots in Fig. 5). In addition, a smaller percentage of correlations (7.3%) was identified between the bioavailable concentration and its total content in soil samples (i.e., As and Na or Mn and P).

Considering the soil-plant relationship, only 18 positive correlations were observed between olive grove soil geochemistry (both total element content and bioavailable element concentration) and olive elemental composition, and 20 correlations between soil geochemistry and EVOO composition. For example, it emerges that the phosphorus concentration in EVOO samples is correlated with the total content of Cu and REE (La, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) in the olive grove soil samples. However, despite a significant relationship, in reality there is no evidence suggesting that Cu and REEs in the soil influence the amount of P in EVOO. Indeed, almost all the identified correlations are just coincidental and without significance in the context of the relationships in the soil-plant system.

Anyway, antimony (Sb) is the only chemical element whose concentrations in the bioavailable fraction of olive grove soils are positively correlated to its concentrations in olives (p-value <0.001 and r =0.64; Fig. 6).

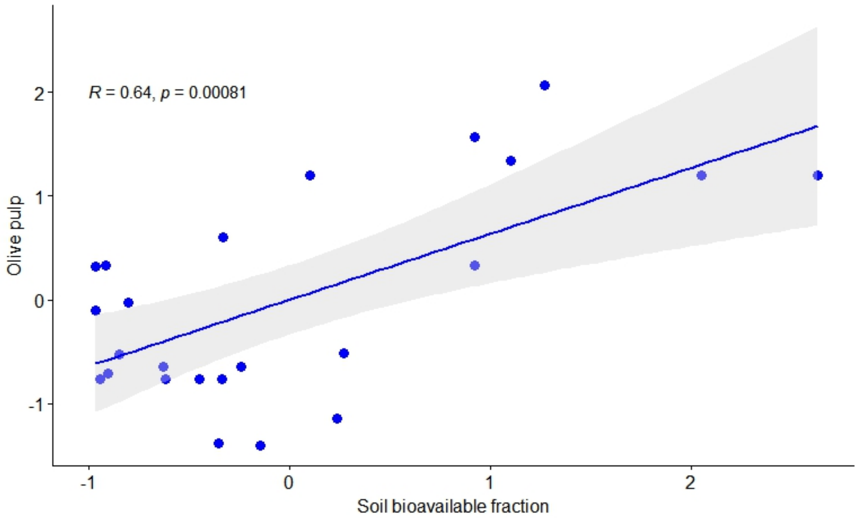

Figure 6. Linear correlation between the concentration of Sb in soil bioavailable fraction (mg/kg) and olive pulp (µg/kg) from Valdichiana Senese.

As the previous results did not show relevant correlations between the geochemistry of olive grove soils and the elemental composition of olives and/or EVOOs from Valdichiana Senese, a Pearson correlation analysis was applied only to soil, olive and EVOO samples from olive groves with the same geolithological background. This approach included olive grove soil, olive and EVOO samples from 12 olive groves (*a*, *b*, *c*, d_1, d_2, *e*, *f*, *g*, *h*, n_3, n_4, and *r*), whose geolithological substratum consists of the marine sands and sandstones (MSS).

Examining Figure 7 it results that the number of correlations between the elemental composition of soil, olive and EVOO samples from olive groves extending on MSS, is greater compared to those reported in Figure 5 for all samples. Indeed, a total of 1292 correlations with $p <0.05$ and $r >0.5$ was identified, 218 negatives and 1074 positives. As seen for the analysis including all the samples, most of the positive correlations (72%) were found between the total contents of several chemical elements as well as the bioavailable concentrations in soils. A smaller percentage of correlations were detected between soil geochemistry and elemental composition of olives or EVOOs (18%).

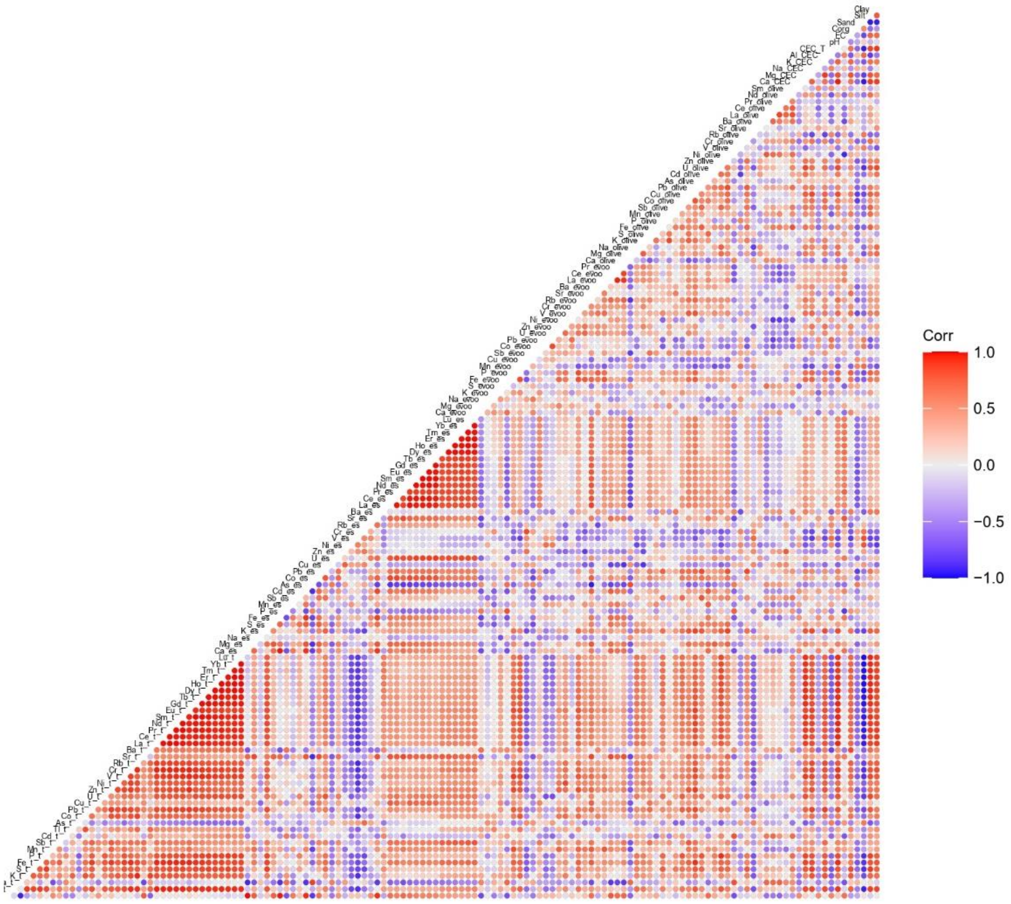

Figure 7. Correlation matrix between chemical and physical parameters of soils, concentrations of 36 chemical elements in the olive grove soils, including both the total elemental content and the bioavailable fraction, olive pulps and EVOOs from Valdichiana Senese collected in 2020-21 from olive groves with a geolithological substratum consisting of marine sands and sandstones (MSS). Pearson's correlation ranged from +1 (red) to -1 (blue).

Unfortunately, once again, most of the identified correlations seem to be coincidental. Anyway, some relationship was found between the element abundance in olive grove soils and olives or EVOOs. Total contents of Fe and V in soil samples resulted positively correlated with their concentrations in EVOOs (Fig. 8a), as well as soil total contents of U with its concentrations in olive pulps (Fig. 8b). Furthermore, the positive correlations between the concentrations of Pb and Sb in the soil bioavailable fraction and in olives are noteworthy (Fig. 8c).

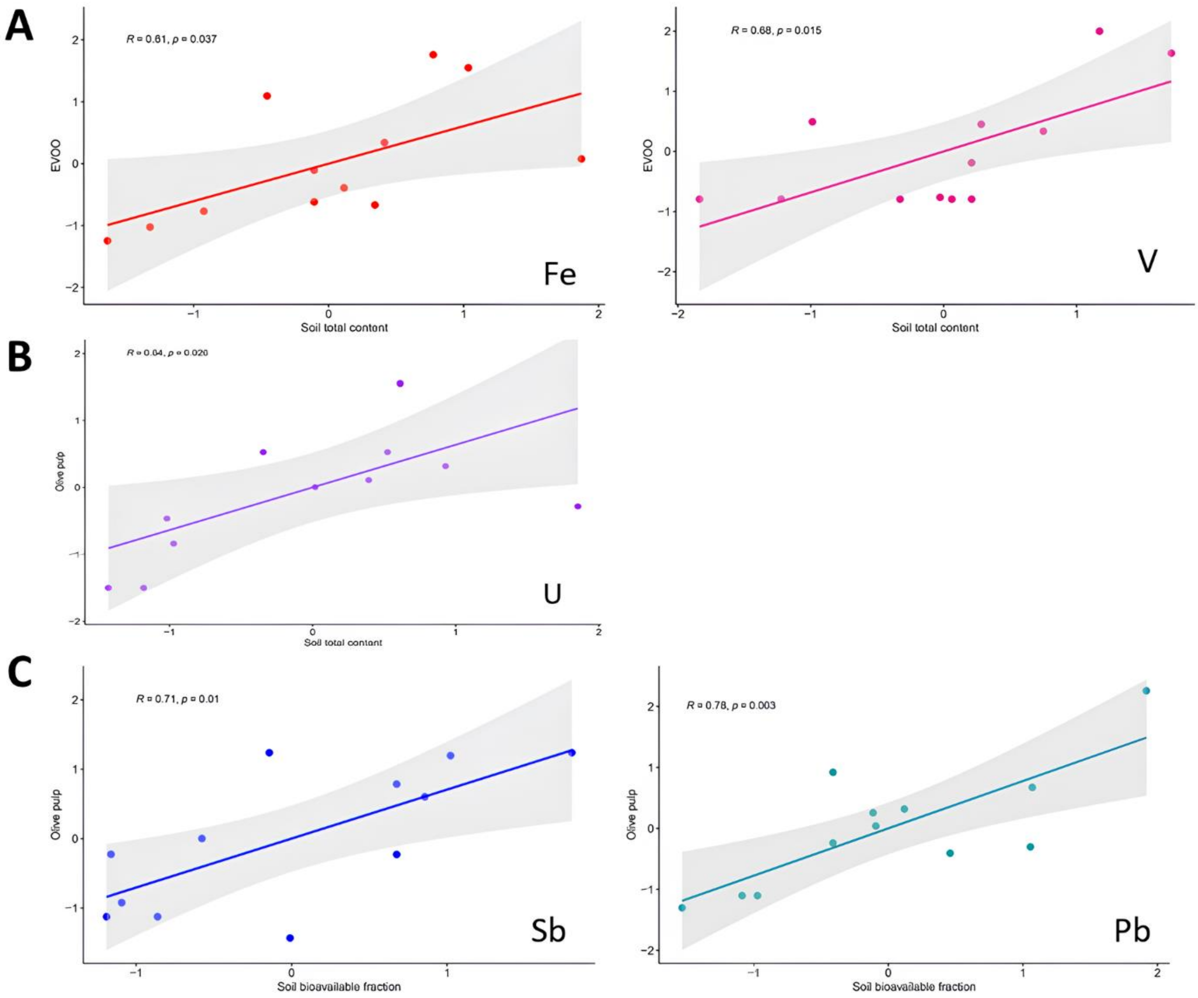

Figure 8. A) Linear correlation between A) the concentrations of Fe and V in the soil total content (mg/kg) and in EVOO (µg/kg); B) the concentrations of U in the soil total content (mg/kg) and in olive pulp (µg/kg) the from MMS olive groves of Valdichiana Senese; C) the concentrations of Sb and Pb in the soil bioavailable fraction (mg/kg) and olive pulp (µg/kg) from MMS olive groves of Valdichiana Senese.

In previous studies, Damak et al. (2021) and Lučić et al. (2023) have already identified Fe as a chemical element of interest in geographical traceability of olive oil, because its total content in soils resulted positively correlated to concentration in EVOOs.

Regarding Pb, Sb, U, and V, even if analysed in other works (Lučić et al., 2023; Beltrán et al., 2015), they have never exhibited statistically significant correlations between soil contents and olive/EVOO concentrations.

Lastly, the limited number of soil-olives/EVOO chemical correlations identified in the present study could be due to the pedological and geolithological uniformity of the selected olive groves in Valdichiana Senese. In fact, most of the investigated olive grove soils formed by the same parent rock consisting of marine sands and sandstone (MSS). This suggests that a more comprehensive sampling design extended to soils and related parent rocks characterised by a wider pedological, geological and geochemical variability, could provide better results to geographical traceability.

4.3 CHARACTERIZATION OF FATTY ACIDS AND ORGANIC VOLATILE COMPOUND PROFILES

This study investigated the profile of organic volatile compounds and fatty acids in 15 EVOOs produced in 2021-2022, 6 EVOOs of 2020-2021 from Valdichiana Senese and 11 EVOOs produced in 2021-22 from Alentejo, through HS-SPME-GC/MS and GC-FID analysis, respectively. The percentage ratio of each peak area compared to the total peak area was used to calculate the relative amount of each compound and expressed as a percentage (%).

In Table 24 are reported the volatiles found in at least one of EVOO from Valdichiana Senese, showing also other relevant parameters for their characterization, such as retention time, chemical family and chemical formula and characteristic fragmentation pattern (ions m/z). In addition, the percentages corresponding to the minimum, maximum, mean, and standard deviation (S.D.) were reported for each volatile.

A chromatogram of a Valdichiana Senese EVOO is shown in Figure 9.

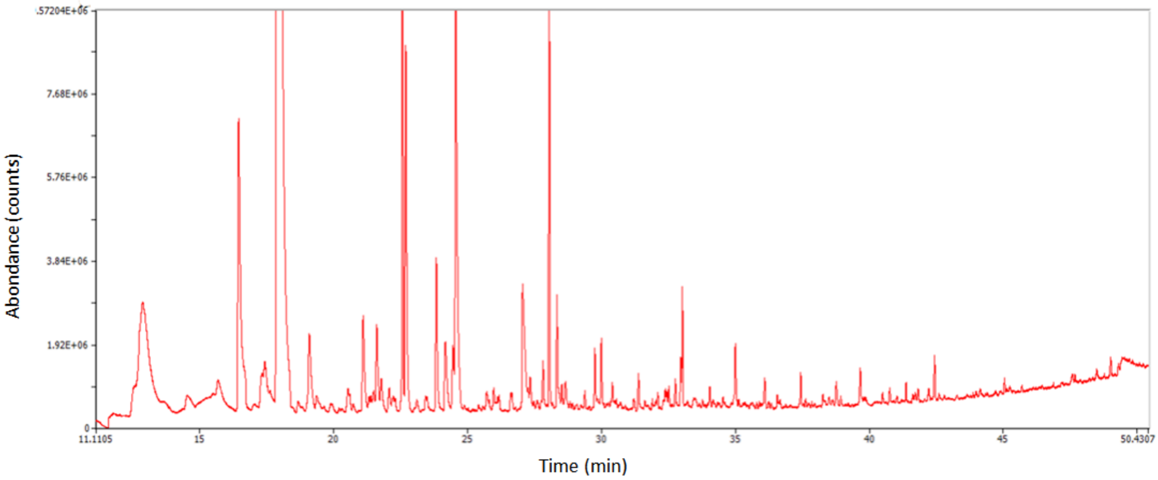

Figure 9. HS-SPME-GC/MS chromatogram of an EVOO sample from Valdichiana Senese.

A total of 107 compounds belonging essentially to the chemical classes of the alcohols, aldehydes, alkanes, esters, and terpenoids were identified. The respective mean amounts for these classes are 32.3%, 54.5%, 0.24%, 3.75%, and 4.2%. The few compounds described as other belong to classes of acids, ketones, or ethers.

The most abundant compounds include one aldehyde (2-Hexenal) and 3 alcohols (2-Hexen-1-ol, 1-Hexenol, and 3-Hexen-1-ol), that constitute 70% of the total amount. The first two compounds are well known to be the major volatiles of olive oils (Kiritsakis, 1998). These compounds are created during the olive oil production process. In detail, lipoxygenases enzymes, during the pressing of olives, produce 9- and 13-hydroperoxides of linolenic and linoleic acid (Olias et al., 1993). Among these, the 13-hydroperoxide of linoleic acid is divided by hydroperoxide lyases (HPL) producing cis-3-hexanal. Subsequently, cis-3-hexenal can undergo enzymatic reduction in 3-hexen-1-ol or isomerization to mutate into 2-hexenal. Lastly, 2-hexenal, after an enzymatic reduction, forms the 2-hexen-ol (Angerosa et al., 1999).

The high presence of 2-Hexenal in EVOO samples is reported in several studies (Mariotti et al., 2022; Aprea et al., 2018; Garcia et al., 2012), particularly in those involving the cultivar *Frantoio* and *Leccino* (Blasi et al., 2019; Veneziani et al., 2018), that are the most abundant cultivars in Valdichiana samples. In addition, the C6 aldehydes and alcohols are well known for their influence on the olive oil aroma, especially contributing to the sweetness and the green odour notes (Mariotti et al., 2022; García-Vico et al., 2017).

The exclusive compounds detected in Valdichiana Senese EVOO of 2021-22 were 1-dodecene, 2(3H)- furanone,5-ethyl dihydro-, 2,5 furandicarboxaldehyde, diphenyl ether, and isopropyl palmitate. Furthermore, o-xylene and dodecane-1-ethoxy were only found in one and two EVOO of 2021-22, respectively. In particular o-xylene, a monocyclic aromatic hydrocarbon, is an important source of contamination by volatiles in gasoline vapours. Anyway, its amount

(0.009%) is too low to be dangerous. Usually, the presence of o-xylene is due to olives kept in small rooms with vehicles (Biedermann et al., 1996).

Table 24. VOCs identified in at least one the analysed samples of Valdichiana Senese EVOO. The percentages of the minimum (min) and maximum (max) values, along with the mean and standard deviation (S.D.), are provided for each volatile compound.

Peak no.	RT[a]	Name	Family[b]	Ions *m/z*	Chemical Formula	Min	Max	Mean	S.D.
1	12.71	Hexanal	*Ald*	44/56/41	$C_6H_{12}O$	0.00	0.23	0.03	0.05
2	12.82	3-Ethyl-1,5-octadiene	*Alk*	41/69	$C_{10}H_{18}$	0.00	0.09	0.02	0.02
3	14.33	o-Xylene	*Alk*	91/106/105	C_8H_{10}	0.00	0.01	0.00	0.00
4	15.66	1-Hexen-3-ol	*Alc*	57/29/72	$C_6H_{12}O$	0.00	1.11	0.28	0.26
5	17.26	2-Hexenal*	*Ald*	41/55/83/69	$C_6H_{10}O$	0.00	0.11	0.03	0.03
6	17.89	2-Hexenal*	*Ald*	41/55/69	$C_6H_{10}O$	16.60	74.56	45.39	17.69
7	18.67	1-Dodecene	*Alk*	55/56/69	$C_{12}H_{24}$	0.00	0.98	0.12	0.31
8	19.04	β-Ocimene	*Terp*	93/91/41	$C_{10}H_{16}$	0.01	3.98	1.89	1.32
9	19.83	Acetic acid, hexyl ester	*Est*	43/56/84	$C_8H_{16}O_2$	0.00	0.40	0.07	0.10
10	20.16	1,2,4-trimethylbenzene	*Other*	105/120/119	C_9H_{12}	0.00	0.33	0.12	0.12
11	20.19	1,2,3-trimethylbenzene	*Other*	105/120/71	C_9H_{12}	0.00	0.20	0.07	0.06
12	20.28	2,7-Octanedione	*Other*	43/84/71	$C_8H_{14}O_2$	0.00	0.17	0.06	0.05
13	20.46	Octanal	*Ald*	43/44/57	$C_8H_{16}O$	0.03	0.49	0.14	0.15
14	21.04	Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene	*Other*	69/41	$C_{11}H_{18}$	0.00	9.77	3.64	2.15
15	21.32	Cyclopentanol	*Alc*	57/44/58	$C_5H_{10}O$	0.00	0.46	0.15	0.12
16	21.47	3-Hexen-1-ol, acetate	*Est*	43/67/82	$C_8H_{14}O_2$	0.00	4.49	0.84	1.33
17	21.61	2-Penten-1-ol*	*Alc*	57/41/44	$C_5H_{10}O$	0.65	2.68	1.51	0.59
18	21.75	2-Heptanal*	*Ald*	41/83/55/70	$C_7H_{12}O$	0.05	1.09	0.41	0.25
19	22.21	5-Hepten-2-one, 6-methyl-	*Other*	43/41/69	$C_8H_{14}O$	0.02	0.58	0.32	0.14
20	22.68	1-Hexanol	*Alc*	56/43/41/69	$C_6H_{14}O$	1.93	25.59	7.56	6.46
21	23.1	3-Hexen-1-ol*	*Alc*	41/67/82/55	$C_6H_{12}O$	0.09	0.90	0.33	0.22
22	23.25	Benzene, 1-ethyl-2,3-dimethyl	*Other*	119/134	$C_{10}H_{14}$	0.00	0.20	0.04	0.05
23	23.38	1,3-Cyclohexadiene, 1,3,5,5-tetramethyl-	*Other*	121/105/136	$C_{10}H_{16}$	0.00	0.72	0.30	0.20
24	23.82	3-Hexen-1-ol*	*Alc*	41/67/82/55	$C_6H_{12}O$	1.58	9.85	4.20	2.49
25	24.12	Nonanal	*Ald*	57/41/98	$C_9H_{18}O$	0.00	3.17	1.59	0.84
26	24.43	2,4 Hexadienal*	*Ald*	81/96/39	C_6H_8O	0.00	1.62	0.54	0.32
27	24.55	2-Hexen-1-ol	*Alc*	57/41	C6H12O	0.64	40.78	13.26	11.72
28	24.57	2-penten-1-ol, 4-methyl-	*Alc*	41/67/57	$C_6H_{12}O$	0.00	1.68	0.77	0.75
29	24.58	2,4-Hexadienal	*Ald*	81/96/53	C_6H_8O	0.00	5.24	2.29	1.10
30	24.88	2 Hexen-1-ol*	*Alc*	57/67/41/82	$C_6H_{12}O$	0.00	0.70	0.15	0.17
31	25.37	2-Octenal*	*Ald*	41/55/70	$C_8H_{14}O$	0.00	0.30	0.10	0.08
32	25.38	Dodecane,1-ethoxy	*Est*	58/72/43	$C_{13}H_{28}O$	0.00	0.06	0.06	0.00
33	25.69	Benzene, 1-methyl-4-(1-methylethenyl)	*Other*	117/132/91	$C_{10}H_{12}$	0.00	0.58	0.22	0.18

34	25.92	Cosmene*	*Terp*	119/91/134	$C_{10}H_{14}$	0.00	0.40	0.20	0.14
35	25.96	1-Octen-3-ol	*Alc*	57/43/72	$C_8H_{16}O$	0.00	0.43	0.21	0.13
36	26.02	Benzene, 1-ethenyl-4-ethyl-	*Other*	117/132	$C_{10}H_{12}$	0.00	0.16	0.09	0.05
37	26.12	1-Heptanol	*Alc*	56/70/41	$C_7H_{16}O$	0.00	0.62	0.20	0.13
38	26.45	trans-Sabinene hydrate	*Terp*	71/43/81/93	$C_{10}H_{18}O$	0.00	0.11	0.06	0.03
39	26.62	2,4-Heptadienal*	*Ald*	81/110/41	$C_7H_{10}O$	0.00	0.96	0.47	0.26
40	26.76	Furfural	*Ald*	95/39/67	$C_5H_4O_2$	0.00	17.97	1.06	4.22
41	26.95	Cyclosativene	*Terp*	161/204/105/119	$C_{15}H_{24}$	0.00	1.21	0.22	0.29
42	27.05	Acetic acid	*Other*	43/60	$C_2H_4O_2$	0.05	5.83	1.04	1.36
43	27.24	1-hexanol,2-ethyl	*Alc*	57/41/70	$C_8H_{18}O$	0.00	0.56	0.17	0.11
44	27.28	Copaene	*Terp*	161/109/105/93	$C_{15}H_{24}$	0.00	3.40	0.78	0.86
45	27.33	Ylangene	*Terp*	161/119/43	$C_{15}H_{24}$	0.00	0.32	0.10	0.15
46	27.55	2,4-Heptadienal*	*Ald*	81/110/41	$C_7H_{10}O$	0.00	0.32	0.18	0.08
47	27.57	Decanal	*Ald*	57/43/70	$C_{10}H_{20}O$	0.00	0.18	0.08	0.05
48	27.96	2-Hepten-1-ol	*Alc*	57/41/54	$C_7H_{14}O$	0.00	0.34	0.09	0.09
49	28.64	Benzaldehyde	*Ald*	106/67/51	C_7H_6O	0.02	1.34	0.72	0.29
50	28.78	2-Nonenal*	*Ald*	41/55/70	$C_9H_{16}O$	0.00	0.22	0.09	0.06
51	28.8	2-Nonenal*	*Ald*	41/55/70	$C_9H_{16}O$	0.00	0.24	0.07	0.05
52	29.07	Linalool	*Terp*	71/41/93	$C_{10}H_{18}O$	0.00	0.16	0.07	0.05
53	29.36	1-Octanol	*Alc*	56/41/69	$C_8H_{18}O$	0.04	0.98	0.36	0.20
54	29.84	Benzene, 1,3-diethenyl-	*Other*	130/115	$C_{10}H_{10}$	0.00	0.12	0.05	0.03
55	30.07	Dimethyl Sulfoxide	*Other*	62/78/45	C_2H_6OS	0.01	0.46	0.24	0.16
56	30.18	5- Methyl Furfural	*Ald*	109/53	$C_6H_6O_2$	0.00	0.26	0.06	0.06
57	30.21	Trans-α-bergamotene	*Terp*	119/93/69	C15H24	0.00	0.18	0.10	0.04
58	30.48	Hexadecane	*Alk*	57/43/71	$C_{16}H_{34}$	0.00	0.25	0.10	0.05
59	30.67	4-Cyclopentene-1,3-dione	*Other*	96/42/68	$C_5H_4O_2$	0.00	0.14	0.05	0.04
60	30.85	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-*	*Alc*	71/111/43	$C_{10}H_{18}O$	0.00	0.06	0.03	0.01
61	31.18	2-Octen-1-ol*	*Alc*	57/41/54	$C_8H_{16}O$	0.00	0.19	0.09	0.05
62	31.39	Ethanol, 2-(2-ethoxyethoxy)-	*Alc*	45/59	$C_6H_{14}O_3$	0.01	1.28	0.53	0.26
63	31.6	Benzoic acid, methyl ester	*Est*	105/77/136	$C_8H_8O_2$	0.00	0.65	0.20	0.18
64	31.69	3-methylbenzaldehyde	*Ald*	119/91	$C_8H_8O_2$	0.00	0.12	0.06	0.03
65	32.03	Butyrolactone	*Other*	42/86	$C_4H_6O_2$	0.00	0.23	0.09	0.07
66	32.06	2-Decenal*	*Ald*	70/55/41	$C_{10}H_{18}O$	0.02	0.53	0.23	0.15
67	32.3	Benzeneacetaldehyde	*Ald*	91/120	C_8H_8O	0.00	0.55	0.13	0.15
68	32.42	1-Nonanol	*Alc*	56/41/70	$C_9H_{20}O$	0.02	0.41	0.20	0.10
69	32.87	Benzoic acid, ethyl ester	*Est*	105/77/122/150	$C_9H_{10}O_2$	0.00	0.86	0.23	0.32
70	33.19	3-Nonen-1-ol*	*Alc*	68/55/41	$C_9H_{18}O$	0.00	0.12	0.07	0.03
71	33.39	Butanoic acid, 3-methyl-	*Other*	60/74/97	$C_5H_{10}O_2$	0.00	0.35	0.16	0.10
72	33.53	1-Butanol, 4-butoxy-	*Alc*	57/71/41	$C_8H_{18}O_2$	0.00	0.29	0.08	0.06
73	33.93	Dodecanal	*Ald*	57/68/82	$C_{12}H_{24}O$	0.00	0.03	0.02	0.01

74	33.99	Valencene	*Terp*	91/161/79/41/55	$C_{15}H_{24}$	0.00	0.55	0.22	0.14
75	34.07	2(3H)- Furanone,5-ethyldihydro-	*Other*	85/56/42/70	$C_6H_{10}O_2$	0.00	0.09	0.04	0.03
76	34.14	Benzaldehyde, 4-ethyl-	*Ald*	134/119/105	$C_9H_{10}O$	0.00	0.38	0.11	0.07
77	34.34	α-Muurolene	*Terp*	105/161/204/93	$C_{15}H_{24}$	0.00	0.48	0.10	0.14
78	34.43	1-Imidazole, 1,2-dimethyl*	*Other*	96/54/42	$C_5H_8N_2$	0.00	0.36	0.09	0.12
79	34.67	Acetic acid, phenylmethyl	*Est*	69/108/44	$C_9H_{10}O_2$	0.00	0.02	0.01	0.01
80	34.95	α-Farnesene	*Terp*	93/41/69	$C_{15}H_{24}$	0.00	1.28	0.38	0.32
81	35.01	Benzaldehyde, 4-ethyl	*Ald*	134/133/105/91	$C_9H_{10}O$	0.00	0.23	0.11	0.06
82	35.33	Pentanoic acid	*Other*	60/73	$C_5H_{10}O_2$	0.00	0.31	0.10	0.09
83	36.07	Methyl salicylate	*Est*	120/92/152	$C_8H_8O_3$	0.05	1.46	0.51	0.31
84	36.34	Ethanol, 2-(2-butoxyethoxy)-	*Alc*	57/41/75	$C_8H_{18}O_3$	0.02	0.28	0.07	0.06
85	36.82	2-Butenoic acid,2-methyl-	*Other*	100/55/39/82	$C_5H_8O_2$	0.00	0.31	0.05	0.09
86	37.51	Ethanone, 1-(2,4-dimethylphenyl)-	*Other*	133/105/148	$C_{10}H_{12}O$	0.00	0.23	0.04	0.05
87	37.9	5,9-Undecadien-2-one, 6,10-dimethyl-*	*Other*	69/43/136/107	$C_{13}H_{22}O$	0.00	0.66	0.07	0.15
88	38.21	Hexanoic acid	*Other*	60/73	$C_6H_{12}O_2$	0.00	12.18	0.81	2.61
89	38.5	Benzoic acid, 2-ethyl-, methyl ester	*Est*	133/103/77	$C_{10}H_{12}O_2$	0.00	0.20	0.07	0.07
90	38.77	Benzyl alcohol	*Alc*	79/108/51	C_7H_8O	0.02	0.90	0.38	0.27
91	39.66	Phenylethyl alcohol	*Alc*	91/92/122/65	$C_8H_{10}O$	0.01	2.00	0.80	0.49
92	39.85	1,4 Butanediol	*Other*	42/71/57	$C_4H_{10}O_2$	0.00	0.36	0.12	0.10
93	40.73	1-Dodecanol	*Alc*	56/69/83/97/41	$C_{12}H_{26}O$	0.00	0.44	0.18	0.10
94	40.98	Heptanoic acid	*Other*	60/73/87	$C_7H_{14}O_2$	0.00	0.64	0.09	0.16
95	41.59	2,5 Furandicarboxaldehyde	*Ald*	124/95/39	$C_6H_4O_3$	0.00	0.51	0.15	0.21
96	42.2	Diphenyl ether	*Est*	170/141/77/51	$C_{12}H_{10}O$	0.00	0.64	0.23	0.18
97	42.23	Phenol	*Other*	94/66/40	C_6H_6O	0.00	0.41	0.15	0.10
98	42.38	Isopropyl myristate	*Est*	102/228/60/43/73	$C_{17}H_{34}O_2$	0.02	20.35	1.47	4.46
99	42.56	Peruviol	*Terp*	69/93/107/81	$C_{15}H_{26}0$	0.00	0.13	0.04	0.04
100	43.53	Octanoic acid	*Other*	43/60/145/85	$C_8H_{16}O_2$	0.00	0.20	0.04	0.06
101	44.81	Hexadecanal	*Ald*	83/57/71/96/41	$C_{16}H_{32}O$	0.00	0.13	0.04	0.04
102	45.39	2-Phenoxyethanol	*Est*	94/138/77	$C_8H_{10}O_2$	0.01	0.10	0.04	0.03
103	45.67	1-Tetradecanol	*Alc*	83/69/56/97	$C_{14}H_{30}O$	0.00	0.09	0.04	0.02
104	46.06	Nonanoic acid	*Other*	43/60/73/86	$C_9H_{18}O_2$	0.00	0.14	0.04	0.04
105	47.12	Isopropyl palmitate	*Est*	102/256/60/73	$C_{19}H_{38}O_2$	0.00	0.04	0.02	0.01
106	49.42	Glycerol	*Alc*	61/43	$C_3H_8O_3$	0.00	1.52	0.56	0.46
107	53.32	5-Hydroxymethylfurfural	*Ald*	97/41/126	$C_6H_6O_3$	0.00	1.53	0.39	0.76

[a] Retention time (minutes); * Isomer compounds. [b] Alc-alcohol, Ald-aldehyde, Alk-alkanes, Est-ester, and Terp-terpenoids. Value of 0.00 in the minimum value column indicates that the volatile compound was not detected, but for quantification purposes, it was considered as 0.

For the volatiles in Alentejo EVOOs, described in Table 25, 77 compounds were identified, only 43 in common with the Italian EVOOs. As for Valdichiana samples, Alentejo EVOOs are divided into classes of the alcohols, aldehydes, alkanes, esters, and terpenoids. Comparing the composition of the two EVOOs, Alentejo EVOOs have higher mean values for aldehydes (59.5%) and alkanes (1.4%) than Valdichiana Senese samples, and lower for alcohols (22%), esters (2.7%) and terpenoids (1.8%). Furthermore, the compounds classified as other include mainly ketones and acids.

A chromatogram of an Alentejo EVOO is shown in Figure 10.

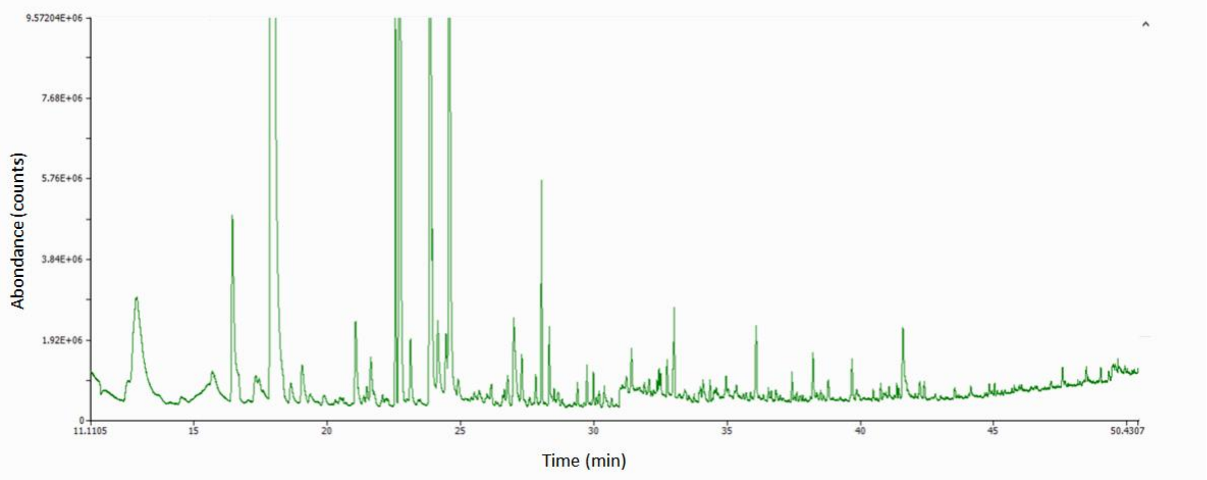

Figure 10. HS-SPME-GC/MS chromatogram of an EVOO sample from Alentejo.

In the 11 Portuguese samples the isolated and identified compounds with the highest percentages are 2-Hexenal (50.5%) and its isomer (3.4%), 3-Hexen-1-ol (13.2%), 1-Hexanol (5.6%), and 3-Hexenal (3.6%), similar composition of Italian EVOOs. As mentioned before, these alcohols and aldehydes are responsible for the distinctive green sensory perception in olive oils. For example, 1-Hexanol is associated with the fragrance of freshly cut grass (Iraqi et al., 2005).

Among all the volatiles identified, compounds as Benzoic acid-ethyl ester, 1-Pentene-3-ethyl-, 2H-Pyran-2-one-tetrahydro-6-propyl, 1,3,7-Octatriene, 3,7-dimethyl were detected only once. Regarding 1,3,7-Octatriene, 3,7-dimethyl, its presence was already reported by Iraqi (2005) in a study on Moroccan green olives. Instead, Benzoic acid-ethyl ester is already mentioned in an olive oil study (Sales et al., 2019), and it was also identified in vanilla extract (Adahchour et al., 2004).

The volatile composition of Alentejo EVOO was already studied by Martins et al. (2020), always using a head-space solid phase microextraction coupled with HS-SPME-GC/MS analysis. It can be noted that, even if in Alentejo profile of 2017 were found 107 compounds, they have in common 50 compounds and 2-hexenal is always the volatiles more abundant.

Table 25. VOCs identified in at least one the analysed samples of Alentejo EVOO. The percentages of the minimum (min) and maximum (max) values, along with the mean and standard deviation (S.D.), are provided for each volatile compound.

Peak no.	RT^a (min)	Name	Family^b	Ions *m/z*	Chemical Formula	Min	Max	Mean	S.D.
1	11.05	3-Ethyl-1,5-octadiene*°	*Alk*	41/69	$C_{10}H_{18}$	0.31	1.66	0.84	0.43
2	11.26	1-Penten-3-one	*Other*	55/84	C_5H_8O	0.06	1.56	0.41	0.41
3	13.25	3-Ethyl-1,5-octadiene*	*Alk*	41/69	$C_{10}H_{18}$	0.06	0.89	0.54	0.26
4	13.75	2-Pentenal, (E)-	*Ald*	55/83/84	C_5H_8O	0.11	0.78	0.27	0.19
5	14.82	1-Pentene, 3-ethyl-	*Alk*	41/69/55	C_7H_{14}	0.00	0.01	0.00	0.00
6	14.9	3-Hexenal*	*Ald*	41/55/69	$C_6H_{10}O$	0.02	7.85	3.59	2.64
7	15.34	1-Penten-3-ol	*Alc*	57/41	$C_5H_{10}O$	0.48	2.70	1.05	0.64
8	16.4	Heptanal	*Ald*	70/55/44	$C_7H_{14}O$	0.00	0.17	0.06	0.06
9	16.96	2-Hexenal*°	*Ald*	41/55/69	$C_6H_{10}O$	1.41	5.08	3.35	1.31
10	17.53	2-Hexenal*°	*Ald*	41/55/69	$C_6H_{10}O$	23.43	68.14	50.48	13.80
11	17.96	1,3,7-Octatriene, 3,7-dimethyl-	*Terp*	93/79/105	$C_8H_{16}O$	0.00	0.02	0.00	0.00
12	18.41	1-Pentanol	*Alc*	42/55/70	$C_5H_{12}O$	0.02	0.26	0.09	0.08
13	18.58	β-Ocimene°	*Terp*	93/91/41	$C_{10}H_{16}$	0.05	5.44	1.01	1.59
14	18.81	Styrene	*Other*	104/78/103	C_8H_8	0.00	0.11	0.01	0.03
15	19.29	Acetic acid, hexyl ester°	*Est*	43/56/84	$C_8H_{16}O_2$	0.00	3.54	0.46	1.09
16	19.39	4,8-Dimethylnona-1,3,7-triene*	*Terp*	41/69/107	$C_{11}H_{18}$	0.00	0.21	0.10	0.08
17	19.9	Octanal°	*Ald*	43/44/57	$C_8H_{16}O$	0.03	0.13	0.07	0.03
18	20.44	4,8-Dimethylnona-1,3,7-triene*	*Terp*	41/69/107	$C_{11}H_{18}$	0.00	0.21	0.10	0.08
19	20.73	2-Penten-1-ol°	*Alc*	57/41/44	$C_5H_{10}O$	0.66	5.67	1.82	1.45
20	20.74	3-Hexen-1-ol, acetate°	*Est*	43/67/82	$C_8H_{14}O_2$	0.18	11.17	1.86	3.35
21	21.13	2E-Heptenal°	*Ald*	41/83/55/70	$C_7H_{12}O$	0.03	0.20	0.11	0.06
22	21.27	2-Hexen-1-ol, acetate	*Est*	43/67/82	$C_8H_{14}O_2$	0.00	0.27	0.04	0.09
23	21.48	5-Hepten-2-one, 6-methyl°	*Other*	43/41/69	$C_8H_{14}O$	0.02	0.16	0.06	0.05
24	21.87	1-Hexanol°	*Alc*	56/43/41/69	$C_6H_{14}O$	2.31	9.38	5.57	2.25
25	22.22	3-Hexen-1-ol*°	*Alc*	41/67/82/55	$C_6H_{12}O$	5.07	24.49	13.19	6.55
26	22.39	2-Pentanone, 4-hydroxy-4-methyl	*Other*	43/59	$C_6H_{12}O$	0.01	0.04	0.03	0.01
27	22.65	2,4,6-Octatriene, 2,6-dimethyl-	*Terp*	121/105/136	$C_{10}H_{16}$	0.00	0.14	0.03	0.04
28	22.75	2-Cyclopenten-1-one, 2-methyl-	*Other*	67/96/53	C_6H_8O	0.00	0.02	0.01	0.00
29	22.93	3-Hexen-1-ol*°	*Alc*	41/67/82/55	$C_6H_{12}O$	0.07	0.38	0.19	0.09
30	23.39	Nonanal°	*Ald*	57/41/98	$C_9H_{18}O$	0.00	0.92	0.18	0.30
31	23.44	2,4-Hexadienal°	*Ald*	81/96/39	C_6H_8O	0.00	1.73	0.64	0.50
32	23.6	2-Hexen-1-ol*°	*Alc*	57/67/41/82	$C_6H_{12}O$	0.00	0.27	0.04	0.09
33	23.9	2-Hexen-1-ol*	*Alc*	57/67/41/82	$C_6H_{12}O$	0.01	0.04	0.02	0.01
34	24.07	Butanoic acid, hexyl ester	*Est*	71/43/89	$C_{10}H_{20}O_2$	0.00	0.04	0.01	0.01
35	24.63	2-Octenal (E)°	*Ald*	41/55/70	$C_8H_{14}O$	0.00	0.06	0.02	0.02
36	24.68	Octanoic acid, ethyl ester	*Est*	88/101/127	$C_{10}H_{20}O_2$	0	0.11	0.06	0.03
37	25.06	Cosmene*°	*Terp*	119/91/134	$C_{10}H_{14}$	0.00	0.11	0.02	0.03
38	25.16	Acetic acid°	*Other*	43/60	$C_2H_4O_2$	0.05	1.72	0.54	0.50
39	25.5	Butanoic acid, 3-hexenyl ester*	*Est*	67/71/82	$C_{10}H_{18}O_2$	0.00	0.11	0.02	0.03
40	25.97	2-Propanol, 1-(2-methoxy-1-methylethoxy)	*Alc*	59/45/103	$C_7H_{13}O_3$	0.00	0.03	0.01	0.01
41	26.3	1-Hexanol, 2-ethyl-	*Alc*	57/41/70	$C_8H_{18}O$	0.00	0.02	0.01	0.01

Peak no.	RT[a] (min)	Name	Family[b]	Ions *m/z*	Chemical Formula	Min	Max	Mean	S.D.
42	26.62	2,4- Heptadienal°	*Ald*	81/110/41	$C_7H_{12}O$	0.00	0.02	0.01	0.01
43	26.7	Cyclosativene°	*Terp*	161/204/105/119	$C_{15}H_{24}$	0.00	0.13	0.02	0.04
44	26.93	Copaene°	*Terp*	161/109/105/93	$C_{15}H_{24}$	0.00	1.21	0.23	0.37
45	27.37	7-Oxabicyclo[4.1.0]heptan-2-one	*Other*	55/57	$C_6H_8O_2$	0.01	0.12	0.06	0.04
46	27.61	Benzaldehyde°	*Ald*	106/67/51	C_7H_6O	0.01	0.07	0.04	0.02
47	28.43	1-Octanol°	*Alc*	56/41/69	C_7H_6O	0.03	0.19	0.08	0.05
48	29.33	Dimethyl sulfoxide°	*Other*	62/78/45	C_2H_6OS	0.01	0.19	0.08	0.05
49	29.52	4-Oxohex-2-enal	*Ald*	83/55	$C_6H_8O_2$	0.05	1.20	0.61	0.43
50	30.23	Caryophyllene	*Terp*	93/105/133	$C_{15}H_{24}$	0.00	0.07	0.01	0.02
51	30.42	Ethanol, 2-(2-ethoxyethoxy)°	*Alc*	45/59/72	$C_6H_{14}O_3$	0.01	0.23	0.08	0.07
52	30.61	Benzoic acid, methyl ester°	*Est*	105/77/136	$C_8H_8O_2$	0.00	0.10	0.03	0.03
53	30.83	Butyrolactone°	*Other*	42/86	$C_4H_6O_2$	0.00	0.01	0.00	0.00
54	31.24	2-Decenal, E°	*Ald*	70/55/41	$C_{10}H_{18}O_2$	0.01	0.08	0.03	0.02
55	31.48	1-Nonanol°	*Alc*	56/41/70	$C_9H_{20}O$	0.02	0.17	0.06	0.05
56	31.92	Benzoic acid, ethyl ester°	*Est*	105/77/122/150	$C_9H_{10}O_2$	0.00	0.07	0.01	0.02
57	32.21	3-Nonen-1-ol°	*Alc*	68/55/41	$C_9H_{18}O$	0.00	0.02	0.01	0.01
58	32.78	α-Muurolene*°	*Terp*	105/161/204/93	$C_{15}H_{24}$	0.00	0.17	0.03	0.06
59	33.48	Valencene°	*Terp*	91/161/79/41/55	$C_{15}H_{24}$	0.00	0.06	0.01	0.02
60	34.06	α-Farnesene°	*Terp*	93/41/69	$C_{15}H_{24}$	0.01	1.74	0.23	0.51
61	34.54	2(5H)-Furanone, 5-ethyl-	*Other*	83/55	$C_6H_8O_2$	0.03	0.45	0.24	0.14
62	35.1	Methyl salicylate°	*Est*	120/92/152	$C_8H_8O_3$	0.02	0.39	0.17	0.11
63	35.42	Ethanol, 2-(2-butoxyethoxy)°	*Alc*	57/41/75	$C_8H_8O_3$	0.03	0.18	0.08	0.05
64	36.23	2-Propanol, 1,1'-oxybis-	*Alc*	45/59/89	$C_9H_{20}O$	0.00	0.03	0.01	0.01
65	37.01	5,9-Undecadien-2-one, 6,10-dimethyl°	*Other*	69/43/136/107	$C_{13}H_{22}O$	0.00	0.07	0.02	0.02
66	37.5	Benzyl alcohol°	*Alc*	79/108/51	C_7H_8O	0.04	0.15	0.09	0.04
67	37.94	Tetradecane, 1-chloro-	*Alk*	57/91/71	$C_{14}H_{29}Cl$	0.00	0.08	0.02	0.02
68	38.11	Dimethyl sulfone	*Other*	79/94	$C_2H_6O_2S$	0.00	0.19	0.04	0.05
69	38.5	Phenylethyl alcohol°	*Alc*	91/92/122/65	$C_8H_{10}O$	0.07	0.59	0.24	0.15
70	39.78	1-Dodecanol°	*Alc*	56/69/83/97/41	$C_{12}H_{26}O$	0.00	0.05	0.02	0.01
71	40.25	2H-Pyran-2-one, tetrahydro-6-propyl-	*Other*	58/102	$C_9H_{10}O$	0.00	0.02	0.00	0.01
72	40.6	Phenol°	*Other*	94/66/40	C_6H_6O	0.01	0.02	0.01	0.00
73	41.57	Isopropyl myristate°	*Est*	102/228/60/43/73	$C_{17}H_{34}O_2$	0.01	0.11	0.04	0.03
74	42.21	Triacetin	*Other*	43/103	$C_9H_{14}O_6$	0.00	0.04	0.01	0.01
75	42.33	Benzoic acid, 2-methoxy-, methyl ester	*Est*	135/77/166	$C_9H_{10}O$	0.00	0.02	0.01	0.01
76	44.12	2-Phenoxyethanol°	*Other*	94/138/77	$C_8H_{10}O_2$	0.00	0.03	0.02	0.01
77	49.4	Benzofuran, 2,3-dihydro-	*Other*	119/120	C_8H_8O	0.00	0.02	0.01	0.01

[a] Retention time (minutes); * Isomer compounds. [b] Alc-alcohol, Ald-aldehyde, Alk-alkanes, Est-ester, and Terp-terpenoids. Value of 0.00 in the minimum value column indicates that the volatile compound was not detected, but for quantification purposes, it was considered as zero.

In the GC-FID analysis, 21 fatty acids were investigated in the EVOO samples (Table 26): myristoleic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), cis-10-heptadecenoic (C17:1), stearic (C18:0), oleic (C18:1n9c), linolelaidic (C18:2n6t), linoleic (C18:2n6c), y-linolenic (C18:3n6), cis-11-eicosenoic (C20:2), behenic (C22:0), erucic (C22:1n9), arachidonic (C20:4n6), lignoceric (C24:0), cis-5,8,11,14,17-eicosapentaenoic (C20:5n3), and nervonic (C24:1).

The main fatty acids in Valdichiana Senese EVOOs are oleic, palmitic, linoleic, and stearic acids, ranging between 72.8-77.6%, 12.1-13.9%, 5.2-8.5%, and 1.9-2.8%, respectively. Alentejo EVOOs exhibit similar range values for oleic (65.6-77.6%), palmitic (14.1-16.2%), and stearic acids (1.6-3.3%). A wider range for linoleic acid was found in Portuguese samples compared to the Italian EVOOs, varying from 0.01 to 12.5%. The percentage of oleic acid aligns with other studies on FAs in EVOO samples (Cerretani et al., 2011; Chiavaro et al., 2010; Romero et al., 2003; Sánchez-Muniz et al., 1998).

The levels of minor FAs, as linolenic and behenic acids, is in accordance with the limit established by the Commission Regulation for this olive oil category (Commission Regulation, 2011). Instead, eicosenoic acid exceeds the 0.4% limit, with a mean of 0.67% for Valdichiana Senese EVOOs, 0.82% in Alentejo EVOOs, and reaching 1% in one Portuguese EVOO. The reason for this variation warrants further investigation but one should be aware that even an honest error in quantification could be the reason, since we are dealing with an FID detector. In a previous work with similar olive oils from Alentejo, the values found for this fatty acid were within the limits (Milinovic et al., 2019).

In addition, calculating the ratio between the amounts of oleic and linoleic acid (O/L), it was possible to determine the stability and the rancidity of EVOO samples. In all the samples, except one, the O/L ratio was >7, indicating stability and low rancidity.

Table 26. GC-FID quantified FA-components in Valdichiana Senese and Alentejo EVOOs. The percentages of the minimum (min) and maximum (max) values, along with the mean and standard deviation (S.D.), are provided for each fatty acid.

Peak no.	Name	Abbreviation	Valdichiana Senese				Alentejo			
			Min	Max	Mean	S.D.	Min	Max	Mean	S.D.
1	Myristoleic acid	C14:0	0.007	0.010	0.009	0.001	0.009	0.016	0.011	0.003
2	Pentadecanoic acid	C15:0	0.002	0.006	0.003	0.001	0.005	0.013	0.009	0.002
3	Palmitic acid	C16:0	12.084	13.901	13.189	0.486	14.094	16.223	15.214	0.777
4	Palmitoleic acid	C16:1	0.735	1.132	0.918	0.121	1.047	2.743	1.698	0.603
5	Heptadecanoic acid	C17:0	0.037	0.091	0.048	0.012	0.093	0.161	0.133	0.020
6	cis-10-Heptadecenoic acid	C17:1	0.066	0.160	0.085	0.020	0.194	0.345	0.279	0.048
7	Stearic acid	C18:0	1.946	2.826	2.308	0.207	1.620	3.302	2.453	0.723
8	Oleic acid	C18:1n9c	72.804	77.575	74.672	1.321	65.636	77.607	71.830	3.262
9	Linolelaidic acid	C18:2n6t	0.002	0.005	0.004	0.001	0.005	0.009	0.007	0.001
10	Linoleic acid	C18:2n6c	5.209	8.469	7.224	0.852	0.010	12.457	6.630	3.453
11	y-Linolenic acid	C18:3n6	0.340	0.470	0.375	0.031	0.334	0.512	0.407	0.061
12	cis-11-Eicosenoic acid	C20:1	0.554	0.743	0.672	0.049	0.607	1.056	0.824	0.165
13	Linolenic acid	C18:3n3	0.241	0.341	0.278	0.032	0.197	0.319	0.265	0.036
14	Heneicosanoic acid	C21:0	0.011	0.015	0.014	0.001	0.009	0.021	0.015	0.003
15	cis-11,14-Eicosadienoic acid	C20:2	0.000	0.002	0.002	0.000	0.001	0.004	0.002	0.001
16	Behenic acid	C22:0	0.096	0.129	0.112	0.011	0.099	0.145	0.121	0.016
17	Erucic acid	C22:1n9	0.001	0.003	0.002	0.000	0.002	0.004	0.002	0.001
18	Arachidonic acid	C20:4n6	0.010	0.024	0.019	0.005	0.002	0.032	0.022	0.009
19	Lignoceric acid	C24:0	0.036	0.065	0.050	0.008	0.049	0.091	0.067	0.013
20	cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5n3	0.003	0.064	0.013	0.018	0.002	0.041	0.010	0.011
21	Nervonic acid	C24:1	0.001	0.006	0.004	0.001	0.001	0.006	0.003	0.001

In this study, a multivariate parametric Linear Discriminant Analysis (LDA) was applied to differentiate EVOO samples from different areas (Valdichiana Senese and Alentejo) and produced in different years (2020 and 2021). Usually, LDA is a commonly used method for underlining differences between groups and simplifying their classification.

The data obtained for volatiles and fatty acids from HS-SPME-GC/MS and GC-FID analysis were processed using LDA, in order to evaluate their impact on EVOO classification. Statistical analyses were performed considering the mean percentage of volatile compounds and fatty acids in EVOO samples from Valdichiana Senese (2021 and 2020), and Alentejo (2021), as reported in Table 24, 25, and 26.

For the volatiles, 1-Hexanol, 2-ethyl- had the most considerable impact on discrimination for both function 1 and function 2. As shown in Figure 11, the first discriminant function explains 89.3% of the variance. In the two-dimensional space defined by the two functions, EVOOs from different areas are well discriminated. Regarding the production year, the EVOOs of Valdichiana Senese exhibit a nearly complete separation, with the exception of 2 samples.

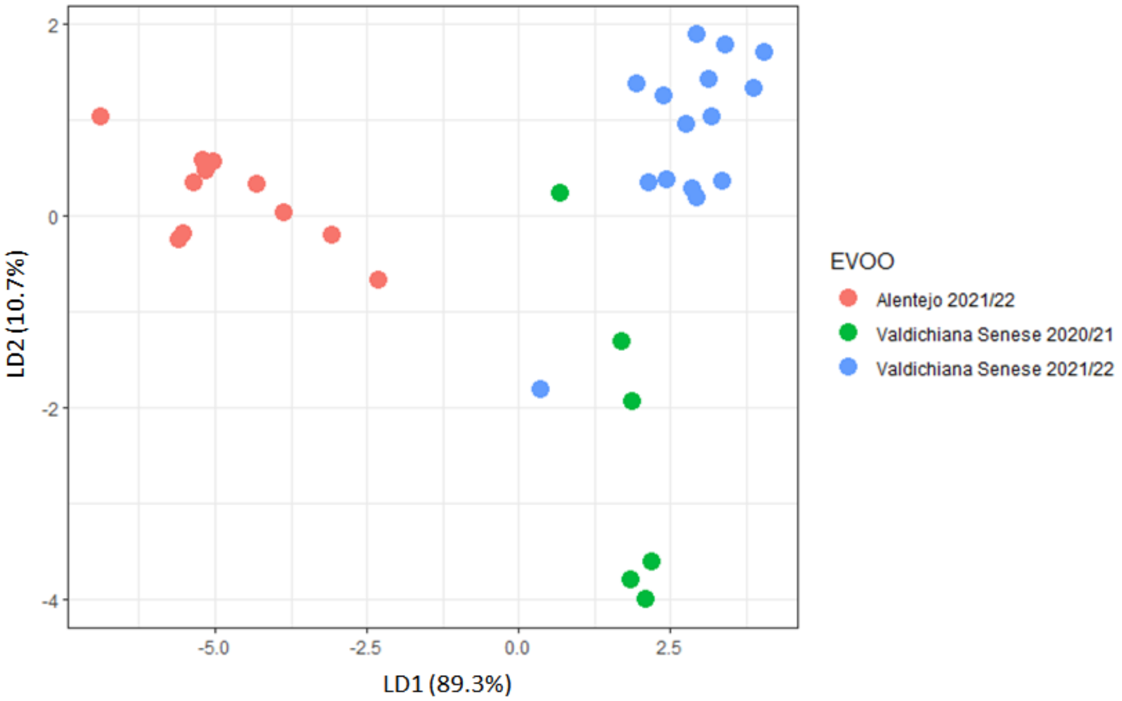

Figure 11. Linear discriminant analysis of VOCs in EVOO samples categorised into 3 groups according to region and year.

Instead, for the FAs composition, function 1 explains 96.8% of the variance and is positively correlated with myristoleic acid, while function 2 is associated with erucic acid. Figure 12 shows a complete separation based on FAs of EVOO according to their origin, but also on year of production for EVOO from Valdichiana Senese.

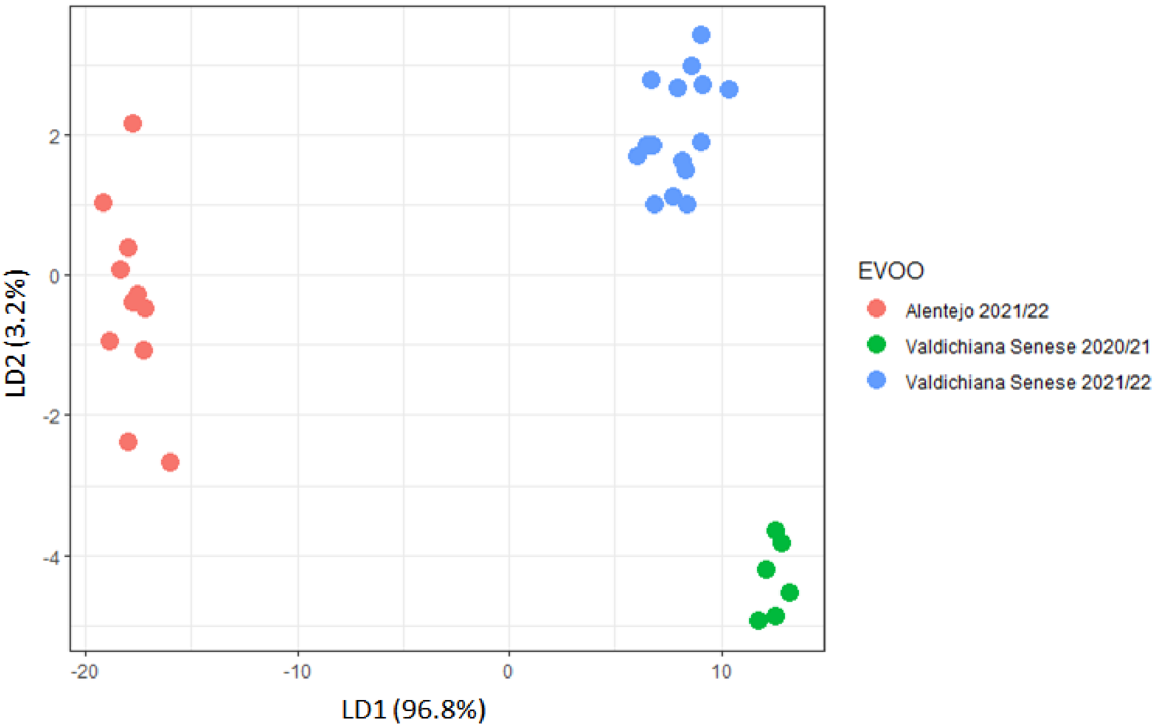

Figure 12. Linear discriminant analysis of FAs in EVOO samples categorised into 3 groups according to region and year.

In conclusion, the results obtained confirm that VOC and FA composition can be used for the geographical classification of EVOOs, in line with previous studies where volatiles and fatty acids were used as discriminants for geographical origin (Cecchi et al., 2020; Kosma et al., 2017; Cajka et al., 2010). Consequently, these markers are considered valid tools for quality control and authentication of the EVOO. Furthermore, the study suggests that fatty acids are valid in distinguishing EVOOs based on their year of production.

4.4 QUALITY AND STABILITY

In the last part of this thesis a depth analysis was conducted on the quality of EVOOs provided in 2019, 2020, and 2021. Since the EVOO quality is related to its oxidative stability, the ripeness of the olives and chemical composition, the evaluation was based on key parameters, specifically, the determination of free acidity, peroxide values, and total phenol content.

The first idea was to assess how the quality of EVOO changed each year and compare the different results. Unfortunately, due to the impact of the coronavirus pandemic and delays in the delivery of reagents, EVOOs from 2019 and 2020 were analysed 18 and 6 months, respectively, after pressing.

In addition, for farm *c* was impossible to provide its EVOO in 2019. In 2020, farm *d* supplied two EVOOs, one made from a single variety (*Minuta di Chiusi*), the other was a mixture of 2 cultivars (*Correggiolo* and *Morellino*). Instead, in 2021 farms *d*, *g*, *l*, and *o* could not produce EVOO as a consequence of a long summer drought and the olive fly damages.

In relation to the investigation of the quality of Valdichiana Senese EVOO the first parameter examined is free acidity, which indicates the percentage of oleic acid. The increase of free acidity value over time is due to the hydrolysis of triglycerides, which leads to an increase in the fatty acid levels and, consequently, a reduction in the quality of the EVOO (Alonso-Salces et al., 2011).

Examining the free acidity values reported in Table 27, it can be noticed that all the samples, even if they were analysed 18 months after the pressing, have values below the limit (0.8% of oleic acid) required by Regulation (EEC) No 2568/91. Moreover, the majority of those still have values considered optimal (<0.3%). These data highlight the stability of the quality of EVOOs from Valdichiana Senese and, consequently, their ability to maintain the status of extra virgin.

Table 27. Mean ± standard deviation of free acidity (% oleic acid) of EVOOs from Valdichiana Senese of 2019-20, 2020-21, and 2121-22; n.a - data not available.

Farm	2019-20	2020-21	2021-22
a	0.494 ± 0.01	0.220 ± 0.01	0.218 ± 0.01
b	0.324 ± 0.01	0.224 ± 0.01	0.212 ± 0.00
c	n.a.	0.226 ± 0.01	0.222 ± 0.02
d_1	0.511 ± 0.00	0.240 ± 0.01	n.a
d_2		0.195 ± 0.02	n.a
e	0.282 ± 0.01	0.212 ± 0.00	0.197 ± 0.00
f	0.310 ± 0.00	0.197 ± 0.00	0.201 ± 0.01
g	0.320 ± 0.01	0.169 ± 0.01	n.a.
h	0.592 ± 0.01	0.170 ± 0.00	0.194 ± 0.06
j	0.169 ± 0.00	0.190 ± 0.02	0.229 ± 0.01
k	0.437 ± 0.01	0.254 ± 0.01	0.260 ± 0.01
i	0.409 ± 0.01	0.183 ± 0.01	0.240 ± 0.00
l	0.479 ± 0.01	0.240 ± 0.01	n.a.
m	0.353 ± 0.02	0.320 ± 0.00	0.257 ± 0.05
n	0.470 ± 0.00	0.324 ± 0.00	0.282 ± 0.01
o	0.381 ± 0.01	0.195 ± 0.02	n.a
p	0.240 ± 0.01	0.240 ± 0.00	0.222 ± 0.01
q	0.437 ± 0.00	0.245 ± 0.01	0.229 ± 0.01
r	0.170 ± 0.00	0.212 ± 0.00	0.173 ± 0.02
s	0.155 ± 0.02	0.197 ± 0.01	0.200 ± 0.00

The second parameter chosen to evaluate the quality of the EVOO from Valdichiana Senese is the number of peroxides. In Table 28 are shown the results of three years of analysis (2019, 2020, and 2021). As decided by Regulation (EEC) No 2568/91, the peroxide limit for EVOO is 20 meq O_2/kg. Furthermore, EVOO is considered acceptable if the value is <12 meqO_2/kg and excellent if it is <7 meq O_2/kg.

The 2019 EVOOs, despite being analysed 18 months after the pressing, still have half of them with the peroxide number below the limit. This demonstrates, aligning with the results of acidity, a good tendency of EVOO from Valdichiana Senese in quality maintenance. In confirmation of this, also the 2020 EVOOs show uniformity, having good values and with only one oil exceeding the limit six months after the pressing. Instead, the majority of EVOOs from 2021 have excellent values, indicators of freshness, as expected from newly pressed oils.

Table 28. Mean ± standard deviation of peroxide number (meq O_2/kg) of EVOOs from Valdichiana Senese of 2019-20, 2020-21, and 2121-22; n.a - data not available.

Farm	2019-20	2020-21	2021-22
a	16.59 ± 0.57	7.12 ± 0.04	6.57 ± 0.53
b	26.21 ± 1.18	12.85 ± 0.25	8.41 ± 0.07
c	n.a.	21.65 ± 0.30	7.46 ± 0.38
d_1	22.02 ± 1.17	12.10 ± 0.18	n.a
d_2		12.54 ± 0.08	n.a
e	24.95 ± 2.40	7.39 ± 0.00	5.82 ± 0.03
f	26.12 ± 1.69	12.78 ± 0.38	8.09 ± 0.90
g	16.65 ± 1.18	9.34 ± 0.50	n.a.
h	13.92 ± 0.23	13.50 ± 1.80	9.01 ± 0.53
j	12.63 ± 1.49	5.24 ± 0.08	5.83 ± 0.02
k	16.43 ± 0.32	7.51 ± 0.66	6.42 ± 0.06
i	21.42 ± 0.26	7.84 ± 0.00	6.18 ± 0.87
l	12.48 ± 0.01	8.00 ± 0.37	n.a.
m	11.01 ± 0.89	8.80 ± 0.15	7.63 ± 0.17
n	20.50 ± 0.35	8.38 ± 0.14	7.51 ± 0.58
o	22.03 ± 0.13	10.44 ± 0.56	n.a
p	22.42 ± 0.03	8.02 ± 0.35	7.16 ± 0.17
q	17.19 ± 0.28	11.82 ± 0.31	7.51 ± 0.05
r	13.10 ± 1.46	8.36 ± 0.33	6.72 ± 0.15
s	20.95 ± 0.24	8.85 ± 0.22	6.50 ± 0.52

The final parameter considered is the total number of polyphenols. This parameter exhibits great variability, ranging from 50 to 1000 mg/kg (typically 100-300 mg/kg) in olive oils, divided in these three categories: low (50–200 mg GAE/kg), medium (200–500 mg GAE/kg), and high (500–1000 mg GAE/kg) (Nowak et al., 2021; Kalogeropoulos & Tsimidou, 2014). The Valdichiana EVOOs show a polyphenol content of <200 mg/kg for all the 2019 samples, reflecting a decline over time. Comparing the polyphenol content of EVOO 2020 and 2021, it can be noted that those of 2020 exhibit values more elevated than those of the following year.

As reported by Ben Hmida et al. (2022), high temperatures, as it was the summer of 2021, greatly reduce the amount of polyphenols in olive oil.

Samples *b* (2020) and *a* (2021) fall into the lowest category, despite EVOO of farm *a* being freshly pressed. This low polyphenol content is probably attributed to the fact that these olives were harvested at the end of November, unlike other farms which harvested in October. This is coherent with the phenomenon where polyphenol levels decrease with the advancement of olive maturation (El Qarnifa et al., 2019; Machado et al., 2013).

Table 29. Mean ± standard deviation of total number of polyphenols (mg/kg) of EVOOs from Valdichiana Senese of 2019-20, 2020-21, and 2121-22; n.a - data not available.

Farm	2019/20	2020/21	2021/22
a	86.8 ± 4.3	381.4 ± 26.4	61.7 ± 0.68
b	143.4 ± 10.6	184.6 ± 24.0	173.3 ± 16.4
c	n.a.	44.2 ± 0.7	158.6 ± 0.95
d_1	110.2 ± 3.5	165.1 ± 15.7	n.a
d_2		170.5 ± 9.3	n.a
e	59.9 ± 0.7	393.0 ± 19.9	275.8 ± 9.89
f	70.3 ± 5.4	220.2 ± 15.1	240.6 ± 9.87
g	150.4 ± 16.3	184.2 ± 10.1	n.a.
h	116.5 ± 8.7	144.6 ± 34.8	166.2 ± 3.58
j	180.1 ± 2.0	392.4 ± 32.6	314.0 ± 20.2
k	118.1 ± 7.2	334.7 ± 3.6	268.6 ± 12.7
i	112.1 ± 2.4	371.9 ± 21.8	244.1 ± 17.3
l	90.1 ± 2.2	219.6 ± 20.9	n.a.
m	143.6 ± 17.9	247.3 ± 26.8	274.5 ± 8.31
n	136.5 ± 8.4	269.3 ± 9.7	258.4 ± 2.67
o	100.9 ± 5.5	386.2 ± 35.5	n.a
p	134.7 ± 9.5	445.5 ± 28.8	232.0 ± 1.27
q	134.9 ± 3.7	375.7 ± 12.1	237.8 ± 10.4
r	139.8 ± 12.0	259.9 ± 23.9	176.3 ± 8.73
s	148.0 ± 6.9	340.1 ± 18.7	210.4 ± 3.14

In addition, to estimate the stability of the quality, this work examined Valdichiana Senese EVOO 2021 from the harvest to 12 months later. The quality parameters reported above were monitored at 0, 4, 8, and 12 months in 5 EVOO samples.

Table 30 presents the acidity values of all analysed samples and their evolution in 12 months of storage. It is evident that, after one year, the free acidity in the EVOO samples remained <0.8% of oleic acid. This ensures that they can continue to be classified as extra virgin olive oil, as defined by Regulation (EEC) No 2568/91. The samples *f* and *n* are characterised by a minimal increase, 2% and 6% respectively, from the initial analysis. Instead, the EVOOs *a* and *i* show a growth of 34% and 27% only from the fourth month. Sample *m* exhibits a rise in free acidity throughout the entire year, and the highest oleic acid content (0.33%) at the conclusion of the experiment.

Table 30. Mean of free acidity (% oleic acid) ± standard deviation of EVOOs from Valdichiana Senese at 0, 4, 8 and 12 months of storage.

Farm	T0	T4	T8	T12
a	0.22 ± 0.01	0.22 ± 0.01	0.25 ± 0.01	0.29 ± 0.01
f	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.00	0.21 ± 0.01
i	0.20 ± 0.00	0.20 ± 0.01	0.23 ± 0.01	0.26 ± 0.04
m	0.26 ± 0.05	0.29 ± 0.02	0.32 ± 0.01	0.33 ± 0.01
n	0.28 ± 0.01	0.28 ± 0.02	0.28 ± 0.01	0.29 ± 0.02

In Figure 13 were shown the correlations of free acidity in each sample with the months of storage. Being a parameter that increases during the time, the intercept values are always positive with a range of 0.006-0.001, indicating a minimal growth of the oleic acid in EVOOs. Also, for farms *a*, *f*, *i*, and *n* the p values reported are >0.05, evidencing that the free acidity does not depend on the time. The only exception is the farm *m*, where the interaction between the two variables is statistically significant (p=0.034).

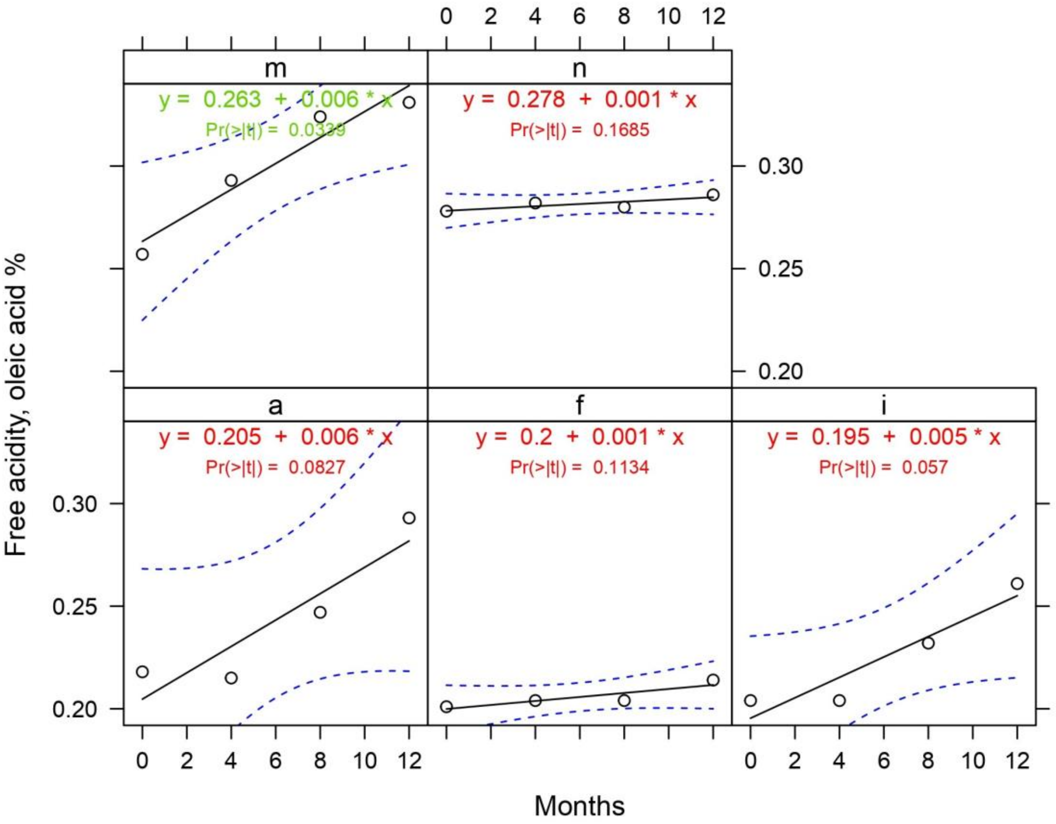

Figure 13. Evolution of EVOO free acidity within a year is described by a linear regression for each farm. The dot lines represent 95% of confidence intervals. The equation of linear regression and the value of Pr(>|t|) are written in green if there is statistical significance or in red if it is non-significance.

The primary oxidation of EVOO is also indicated by the number of peroxides. The presence of peroxides can significantly compromise the nutritional value and overall sensory quality of EVOO. While the initial peroxide numbers of Valdichiana Senese EVOOs were observed to be low at the start of the experiment (Tab. 31), unfortunately, these samples did not respect the regulatory limit (<20 meq O_2/kg) after one year, except one. Indeed, between the eighth and twelfth months, 3 of the EVOOs exceed the limit of 20 meq O_2/kg. Similarly, the *n* farm's EVOO exhibited the same trend between the fourth and eighth months. These specific samples showed a threefold increase (*m* and *n*) or a fourfold increase (*a* and *i*) in peroxide levels. Comparing these results with those reported in Table 28, it can be noted the differences of speed of oxidation respecting EVOOs from 2019 and 2020. This phenomenon may be attributed to the elevated aridity and intense olive fly activity during the summer of 2021, where the olives could have incurred damage before the crushing process. Damaged olives tend to generate higher levels of peroxides (Rojnić et al., 2014).

Finally, the *f* sample exhibited a gradual growth throughout the year and concluded the experiment with a value of 18.85 meq O_2/kg, being within the limits stipulated by Regulation (EEC) No 2568/91.

Table 31. Mean of number of peroxides (meq O_2/kg) ± standard deviation of EVOOs from Valdichiana Senese at 0, 4, 8 and 12 months of storage.

Farm	T0	T4	T8	T12
a	6.57 ± 0.53	9.05 ± 0.70	17.27 ± 0.26	25.09 ± 2.49
f	8.09 ± 0.90	9.67 ± 0.13	14.71 ± 0.33	18.85 ± 5.04
i	6.18 ± 0.87	10.20 ± 0.16	17.26 ± 0.11	26.17 ± 1.02
m	7.63 ± 0.17	11.53 ± 0.37	19.80 ± 0.10	21.62 ± 2.86
n	7.51 ± 0.58	10.58 ± 0.04	22.55 ± 0.61	23.28 ± 1.81

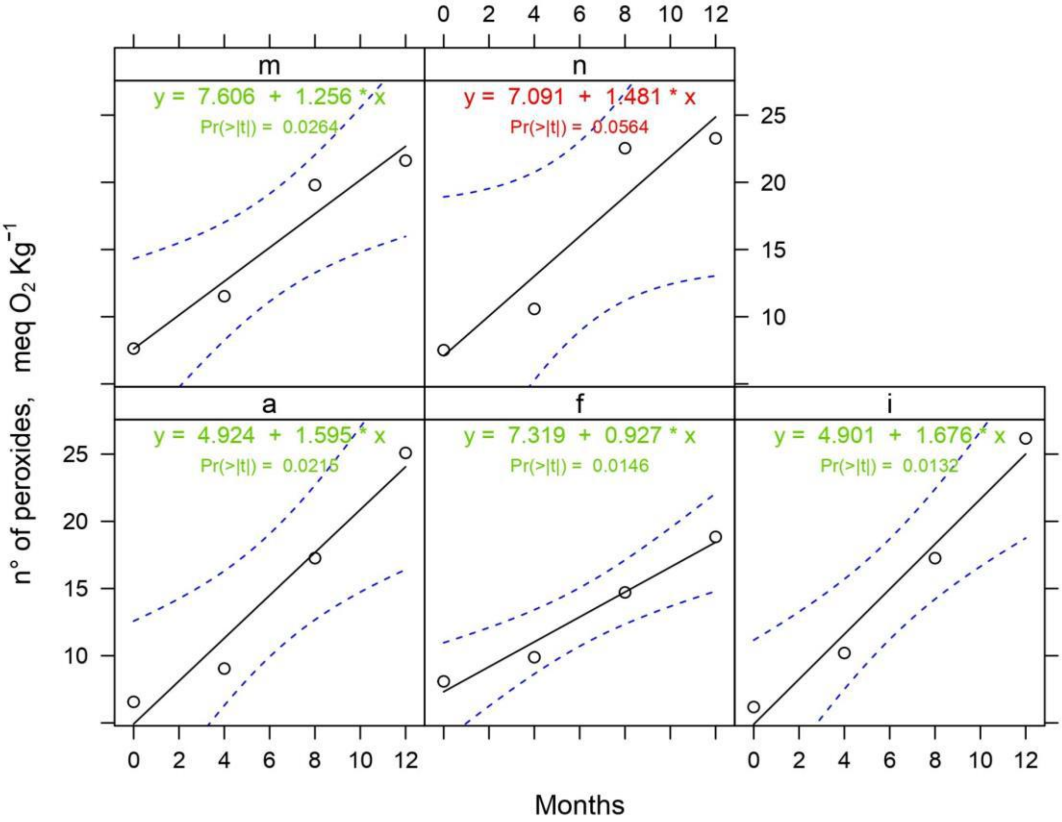

Figure 14. Evolution of EVOO peroxide number within a year is described by a linear regression for each farm. The dot lines represent 95% of confidence intervals. The equation of linear regression and the value of Pr(>|t|) are written in green if there is statistical significance or in red if it is non-significance.

Examining the linear regression shown in Figure 14 reveals a strong correlation between the increment of the peroxide level and the passing time for the most of EVOOs analysed. Even if farm *n* is the only sample with a p value>0.05 (0.056), it can be described as marginally significant, and this value is probably influenced by the breadth of its confidence interval.

Lastly, in the case of total polyphenols in 4 EVOO samples, as shown in Table 32, were classified within the level medium-low, ranging between 275 and 241 mg/kg a. Throughout the year, the EVOOs from Valdichiana Senese maintained an intermediate level (>150 mg/kg), undergoing only a modest decrease of around 30% in their total polyphenol content, with farm *n* showing a 14% reduction. However, an exception is observed in the EVOO from farm *a*, which suffered a more considerable loss of 58%, resulting in an extremely low content of 28 mg/kg. Indeed, as reported above, sample *a* falls into the lowest category probably due to a late olive harvest.

Table 32. Mean of total number of polyphenols (mg/kg) ± standard deviation of EVOOs from Valdichiana Senese at 0, 4, 8 and 12 months of storage

Farm	T0	T4	T8	T12
a	61.7 ± 0.7	46.2 ± 1.3	34.5 ± 1.2	25.3 ± 2.54
f	240.6 ± 9.9	235.9 ± 4.7	212.4 ± 0.6	205.1 ± 22.8
i	251.9 ± 17.8	215.4 ± 3.2	185.7 ± 4.3	158.8 ± 11.8
m	274.5 ± 8.3	246.3 ± 3.2	210.1 ± 1.2	195.1 ± 12.7
n	258.4 ± 2.7	230.0 ± 13.8	190.6 ± 1.4	180.3 ± 16.4

In contrast to the other parameters, as seen in Figure 15, the interactions between the two variables (total phenol content and time) are statistically significant for all the EVOOs investigated (p<0.03). Indeed, it can be observed two divisions based on the value of intercept, representing the rate at which the phenols decrease. For farms *f*, *i*, and *m* the intercepts ranged between -6.8 and -7.7, indicating a greater rate of phenol decrease than farms *a* and *n*, whose intercepts are around -3.

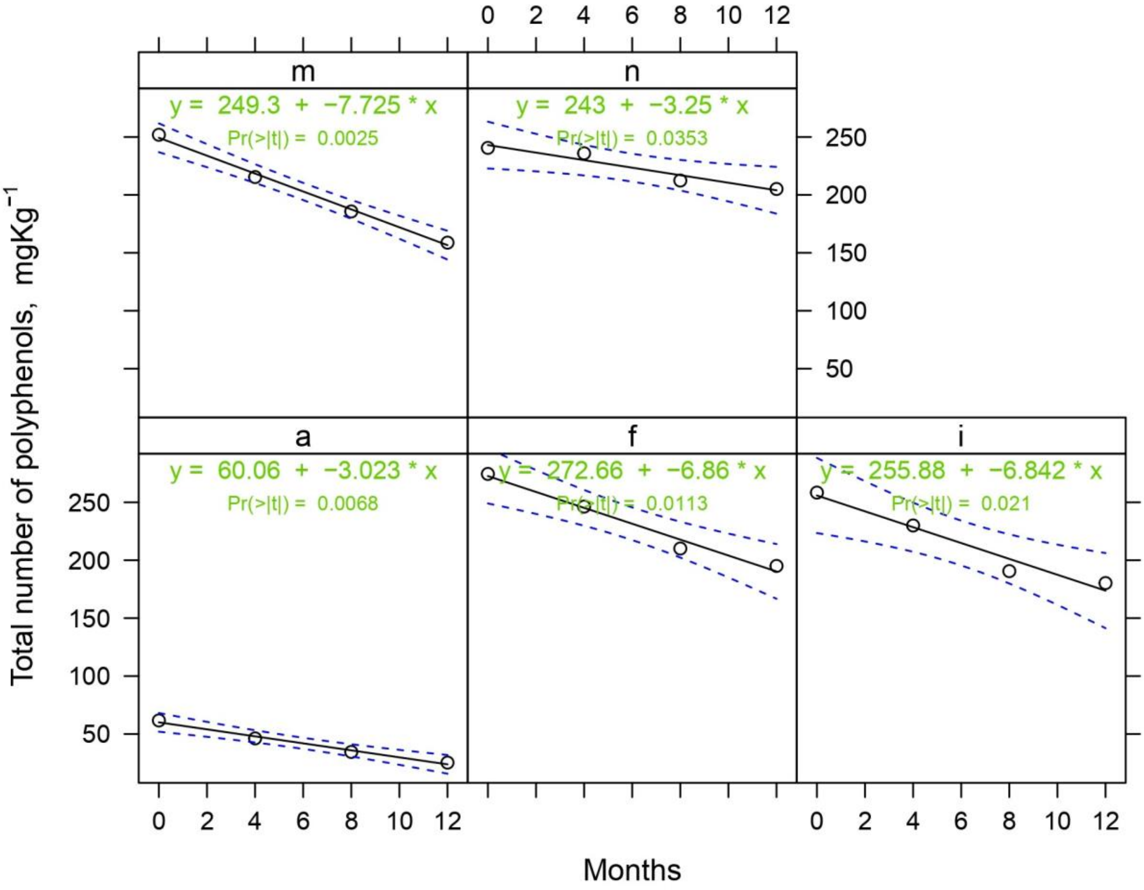

Figure 15. Evolution of EVOO total polyphenols within a year is described by a linear regression for each farm. The dot lines represent 95% of confidence intervals. The equation of linear regression and the value of Pr(>|t|) are written in green if there is statistical significance or in red if it is non-significance.

The EVOOs from Valdichiana Senese demonstrated overall stability in free acidity, with concerns arising in peroxide levels and notable variations in polyphenol content. Furthermore, it highlights the importance of monitoring and management in EVOO production and evidences the impact of factors such as olive harvest timing and environmental conditions on these parameters.

5. CONCLUSION

The results of this study indicated that although EVOOs from Valdichiana Senese may present somewhat slightly high concentrations of Cu and Zn, the associated health risk, based on the daily intake and the highest concentrations measured, is definitely negligible. The concentrations of PTEs in olive grove soils are comparable to their geochemical background in soils from Southern Tuscany formed by the same parent rocks of Valdichiana Senese soils. Only a low to moderate Cu contamination emerged in two cases, probably as a consequence of the diffuse use of Cu-based products in agriculture. Overall, the ecological risk is low.

To determine the geographical traceability of EVOOs from Valdichiana Senese various factors were considered, including soil properties, elemental concentrations in soils (total content and bioavailable fraction), olive pulps, and EVOOs. Soil formed by quartz-feldspathic-micaceous sandstones (QFS) exhibited high concentrations of Mg, Na, K, Ba, Cr, Ni, Pb, Rb, and Tl with low values of Ca and Fe. Instead, in the bioavailable fraction, soils formed by the marine sands and sandstone (MSS) have higher values of Cd and Sr, along with Cu with Ni for soils formed by QFS. Unfortunately, olive pulp and EVOO analyses indicated that soil geochemistry did not reflect in their composition. Most of REEs have levels lower than their respective LOQ, impeding relevant correlations. To individuate the relationships between the olive grove soils, olive fruits, and EVOOs were performed Pearson correlations. The statistical analysis identified 813 correlations, with most positive correlations within soil elements. Few correlations were observed between soil-plant systems, indicating coincidental relationships. Sb showed a positive correlation between its concentrations in soil bioavailable fraction and olive pulps. A more focused analysis on olive groves with marine sand and sandstone (MSS) backgrounds revealed 1292 correlations, predominantly between soil elements, especially between REEs. Some significant correlations emerged, such as positive relationships between soil total contents and their concentrations in EVOOs for Fe and V, between soil total content and olive pulps in U, and soil bioavailable fraction and olive pulps for Pb and Sb. These findings, coherent with previous studies on Fe, highlight the potential of certain elements for traceability. However, the limited correlations may be attributed to sampling methodologies that did not permit to obtain the expected results.

For classifying Valdichiana Senese EVOOs according to their origin area, its organic volatile compound and fatty acid profiles were evaluated and then compared with those of EVOOs from Alentejo, Portugal. In the HS-SPME-GC/MS analysis 107 volatiles were identified in Valdichiana Senese EVOOs, 77 compounds in Alentejo, with 43 compounds in common. In both EVOOs 2-Hexenal was the volatile most abundant, but the major alcohols were different in terms of amount and as distinct compounds. The GC-FID analysis revealed a predominance of oleic, palmitic, linoleic, and stearic acids with similar ranges in both EVOOs. By the Linear Discriminant Analysis (LDA), it was demonstrated that the volatiles, but especially fatty acids, allow a clear separation of EVOOs based on geographical origin, and on the year of production.

Lastly, in the comprehensive analysis about the quality of Valdichiana Senese EVOOs, free acidity and peroxide values remained below the regulatory limit, exhibiting the stability of EVOOs and indicating freshness. Total phenol content showed variability during the 3 years, where were highlighted the impact of harvest timing on its quantity. Additionally, year-long analysis of Valdichiana Senese EVOO highlights its stability and quality attributes. Free acidity stays within limits, but some samples exceed peroxide level regulations, potentially due to environmental factors. In conclusion, the study underscores the intricate interplay of factors characterising the quality of Valdichiana Senese EVOOs.

ACKNOWLEDGEMENTS

This thesis is part of a research project funded by the Strada del Vino Nobile di Montepulciano and Sapori della Valdichiana Senese; therefore, my first thanks go to them for providing me with the opportunity to undergo this experience, as well as to all the farms that participated in the project for their willingness, always ready to assist me.

A sincere thanks goes to my supervisor, Prof. Stefano Loppi, for offering me the opportunity to conduct this research, for his guidance, encouragement, and the esteem he has shown towards me during the years of my doctorate.

I also need to express my gratitude to my co-supervisor, Prof. Giuseppe Protano, along with Dr. Francesco Nannoni and Dr. Luigi Di Lella, for guiding me in my research journey with important advice, contributing to my professional growth, and always making me feel part of their group.

Special thanks also go to Prof. Maria Cabrita and her research group for the contribution to achieving this goal, providing me with the opportunity to develop a part of my project in her laboratory, and instilling in me her great passion for research.

I would also like to thank all my colleagues at work, whose friendship and time spent together have made these years joyful and serene.

Finally, I would like to express my gratitude to my parents, my friends and Enrico, who have always encouraged and lovingly supported me in all moments, both good and bad.

6. REFERENCES

Aceto, M., Bonello, F., Musso, D., Tsolakis, C., Cassino, C., & Osella, D. (2018). Wine Traceability with Rare Earth Elements. *Beverages*, *1*, 23. https://doi.org/10.3390/beverages4010023

Aceto, M., Calà, E., Musso, D., Regalli, N., & Oddone, M. (2019). A preliminary study on the authentication and traceability of extra virgin olive oil made from Taggiasca olives by means of trace and ultra-trace elements distribution. *Food Chemistry*, 125047. https://doi.org/10.1016/j.foodchem.2019.125047

Adahchour, M., Beens, J., Vreuls, R., Batenburg, A., & Brinkman, U. (2004). Comprehensive two-dimensional gas chromatography of complex samples by using a 'reversed-type' column combination: application to food analysis. *Journal of Chromatography A*, *1–2*, 47–55. https://doi.org/10.1016/s0021-9673(04)01288-9

Alessandri, S., Ieri, F., & Romani, A. (2014). Minor Polar Compounds in Extra Virgin Olive Oil: Correlation between HPLC-DAD-MS and the Folin-Ciocalteu Spectrophotometric Method. *Journal of Agricultural and Food Chemistry*, *4*, 826–835. https://doi.org/10.1021/jf403104a

Al-Habahbeh, K. A., Al-Nawaiseh, M. B., Al-Sayaydeh, R. S., Al-Hawadi, J. S., Albdaiwi, R. N., Al-Debei, H. S., & Ayad, J. Y. (2021). Long-Term Irrigation with Treated Municipal Wastewater from the Wadi-Musa Region: Soil Heavy Metal Accumulation, Uptake and Partitioning in Olive Trees. *Horticulturae*, *6*, 152. https://doi.org/10.3390/horticulturae7060152

Alonso-Salces, R. M., Holland, M. V., & Guillou, C. (2011). 1H-NMR fingerprinting to evaluate the stability of olive oil. *Food Control*, *12*, 2041–2046. https://doi.org/10.1016/j.foodcont.2011.05.026

Angerosa, F., Basti, C., & Vito, R. (1999). Virgin Olive Oil Volatile Compounds from Lipoxygenase Pathway and Characterization of Some Italian Cultivars. *Journal of Agricultural and Food Chemistry*, *3*, 836–839. https://doi.org/10.1021/jf980911g

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., & Montedoro, G. (2004). Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. *Journal of Chromatography A*, *1–2*, 17–31. https://doi.org/10.1016/s0021-9673(04)01298-1

Angioni, A. (2010). Olive Cultivar, Period of Harvest, and Environmental Pollution on the Contents of Cu, Cd, Pb, and Zn. In *Olives and Olive Oil in Health and Disease Prevention* (pp. 307–311). Elsevier. http://dx.doi.org/10.1016/B978-0-12-374420-3.00033-4

Aprea, E., Gasperi, F., Betta, E., Sani, G., & Cantini, C. (2018). Variability in volatile compounds from lipoxygenase pathway in extra virgin olive oils from Tuscan olive germoplasm by quantitative SPME/GC-MS. *Journal of Mass Spectrometry*, *9*, 824–832. https://doi.org/10.1002/jms.4274

Baccouri, O., Bendini, A., Cerretani, L., Guerfel, M., Baccouri, B., Lercker, G., Zarrouk, M., & Daoud Ben Miled, D. (2008). Comparative study on volatile compounds from Tunisian and Sicilian monovarietal virgin olive oils. *Food Chemistry*, *2*, 322–328. https://doi.org/10.1016/j.foodchem.2008.03.066

Bajoub, A., Bendini, A., Fernández-Gutiérrez, A., & Carrasco-Pancorbo, A. (2017). Olive oil authentication: A comparative analysis of regulatory frameworks with especial emphasis on quality and authenticity indices, and recent analytical techniques developed for their assessment. A review. *Critical Reviews in Food Science and Nutrition*, *5*, 832–857. https://doi.org/10.1080/10408398.2016.1225666

Bakkali, K., Ballesteros, E., Souhail, B., & Ramos Martos, N. (2009). Determinación de trazas metálicas en aceites vegetales de España y Marruecos mediante espectroscopía de absorción con cámara de grafito después de la digestión en horno de microondas. *Grasas y Aceites*, *5*, 492–499. https://doi.org/10.3989/gya.031909

Bakkali, K., Martos, N. R., Souhail, B., & Ballesteros, E. (2012). Determination of Heavy Metal Content in Vegetables and Oils From Spain and Morocco by Inductively Coupled Plasma Mass Spectrometry. *Analytical Letters*, *8*, 907–919. https://doi.org/10.1080/00032719.2012.655658

Ballabio, C., Panagos, P., Lugato, E., Huang, J.-H., Orgiazzi, A., Jones, A., Fernández-Ugalde, O., Borrelli, P., & Montanarella, L. (2018). Copper distribution in European topsoils: An assessment based on LUCAS soil survey. *Science of The Total Environment*, 282–298. https://doi.org/10.1016/j.scitotenv.2018.04.268

Barbera, M., Saiano, F., Tutone, L., Massenti, R., & Pisciotta, A. (2022). The Pattern of Rare Earth Elements Like a Possible Helpful Tool in Traceability and Geographical Characterization of the Soil-Olive System (*Olea europaea* L.). *Plants*, *19*, 2579. https://doi.org/10.3390/plants11192579

Beltrán, M., Sánchez-Astudillo, M., Aparicio, R., & García-González, D. L. (2015). Geographical traceability of virgin olive oils from south-western Spain by their multi-elemental composition. *Food Chemistry*, 350–357. https://doi.org/10.1016/j.foodchem.2014.07.104

Ben Hmida, R., Gargouri, B., Chtourou, F., Abichou, M., Sevim, D., & Bouaziz, M. (2022). Study on the Effect of Climate Changes on the Composition and Quality Parameters of Virgin Olive Oil “Zalmati” Harvested at Three Consecutive Crop Seasons: Chemometric Discrimination. *ACS Omega*, *44*, 40078–40090. https://doi.org/10.1021/acsomega.2c04813

Benincasa, C., De Nino, A., Lombardo, N., Perri, E., Sindona, G., & Tagarelli, A. (2003). Assay of Aroma Active Components of Virgin Olive Oils from Southern Italian Regions by SPME-GC/Ion Trap Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, *3*, 733–741. https://doi.org/10.1021/jf0258095

Benincasa, C., Lewis, J., Perri, E., Sindona, G., & Tagarelli, A. (2007). Determination of trace elements in Italian virgin olive oils and their characterization according to geographical origin by statistical analysis. *Analytica Chimica Acta*, *2*, 366–370. https://doi.org/10.1016/j.aca.2006.12.040

Biedermann, M., Grob, K., & Morchio, G. (1996). On the origin of benzene, toluene, ethylbenzene, and the xylenes in virgin olive oil-further results. *Zeitschrift Fur Lebensmittel-Untersuchung Und -Forschung*, *3*, 224–229. https://doi.org/10.1007/bf01192868

Blasi, F., Pollini, L., & Cossignani, L. (2019). Varietal Authentication of Extra Virgin Olive Oils by Triacylglycerols and Volatiles Analysis. *Foods*, *2*, 58. https://doi.org/10.3390/foods8020058

Bora, F. D., Donici, A., Rusu, T., Bunea, A., Popescu, D., & Bunea, C. I. (2018). Elemental Profile and ^{207}Pb/^{206}Pb, ^{208}Pb/^{206}Pb, ^{204}Pb/^{206}Pb, ^{87}Sr/^{86}Sr Isotope Ratio as Fingerprints for Geographical Traceability of Romanian Wines. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *1*, 223–239. https://doi.org/10.15835/nbha46110853

Boskou, D. (2006). *Olive Oil: Chemistry And Technology* (2nd ed., pp. 13–19). Academic Press and AOCS Press. https://doi.org/10.1016/B978-1-893997-88-2.50006-7

Bossio, A., Costantini, A., Foresi, L. M., Lazzarotto, A., Mazzantu, R., Mazzei, R., Pascucci, V., Salvatorini, G., Sandrelli, F., & Terzuoli, A. (1998). Neogene-Quaternary sedimentary evolution in the western side of the Northern Apennines (Italy). *Memorie Della Società Geologica Italiana*, *52*, 513–525.

Burgess, S. S. O., Adams, M. A., Turner, N. C., & Ward, B. (2000). Characterisation of hydrogen isotope profiles in an agroforestry system: implications for tracing water sources of trees. *Agricultural Water Management*, *3*, 229–241. https://doi.org/10.1016/s0378-3774(00)00105-0

Cajka, T., Riddellova, K., Klimankova, E., Cerna, M., Pudil, F., & Hajslova, J. (2010). Traceability of olive oil based on volatiles pattern and multivariate analysis. *Food Chemistry*, *1*, 282–289. https://doi.org/10.1016/j.foodchem.2009.12.011

Camin, F., Larcher, R., Bontempo, L., Perini, M., Bertoldi, D., & Nicolini, G. (2009). Geographical traceability of Italian and European extra-virgin olive oils. *Annual Report IASMA Research Centre.*

Carmignani, L., Conti, P., Cornamusini, G., & Pirro, A. (2013). Geological map of Tuscany (Italy). *Journal of Maps*, *4*, 487–497. https://doi.org/10.1080/17445647.2013.820154

Casadei, E., Valli, E., Panni, F., Donarski, J., Farrús Gubern, J., Lucci, P., Conte, L., Lacoste, F., Maquet, A., Brereton, P., Bendini, A., & Gallina Toschi, T. (2021). Emerging trends in olive oil fraud and possible countermeasures. *Food Control*, 107902. https://doi.org/10.1016/j.foodcont.2021.107902

Castillo, J. R., Jiménez, M. S., & Ebdon, L. (1999). Semiquantitative simultaneous determination of metals in olive oil using direct emulsion nebulization. *J. Anal. At. Spectrom.*, *9*, 1515–1518. https://doi.org/10.1039/a900754g

Catarino, S., Madeira, M., Monteiro, F. M. G., Caldeira, I., Rosa, T., Martins, P., Sousa, R. B., & Curvelo-Garcia, A. S. (2014). Multi-elemental analysis throughout soil-wine system as a generator of information on geographic origin. *Conference: 37th World Congress of Vine and Wine, At Mendoza, Argentina.*

Cecchi, L., Migliorini, M., Giambanelli, E., Rossetti, A., Cane, A., Mulinacci, N., & Melani, F. (2020). Authentication of the geographical origin of virgin olive oils from the main worldwide producing countries: A new combination of HS-SPME-GC-MS analysis of volatile compounds and chemometrics applied to 1217 samples. *Food Control*, 107156. https://doi.org/10.1016/j.foodcont.2020.107156

Cecchi, T., & Alfei, B. (2013). Volatile profiles of Italian monovarietal extra virgin olive oils via HS-SPME–GC–MS: Newly identified compounds, flavors molecular markers, and terpenic profile. *Food Chemistry*, *3*, 2025–2035. https://doi.org/10.1016/j.foodchem.2013.05.090

Cerretani, L., Maggio, R. M., Barnaba, C., Toschi, T. G., & Chiavaro, E. (2011). Application of partial least square regression to differential scanning calorimetry data for fatty acid quantitation in olive oil. *Food Chemistry*, *4*, 1899–1904. https://doi.org/10.1016/j.foodchem.2011.02.041

Cherubini, G. (2012). *La lunga storia degli oliveti= The Long History of Olive Groves*. 11–34.

Chiavaro, E., Estrada, M. T. R., Bendini, A., & Cerretani, L. (2010). Correlation between thermal properties and chemical composition of Italian virgin olive oils. *European Journal of Lipid Science and Technology*, *5*, 580–592. https://doi.org/10.1002/ejlt.200900254

Cicerale, S., Lucas, L. J., & Keast, R. S. J. (2012). Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. *Current Opinion in Biotechnology*, *2*, 129–135. https://doi.org/10.1016/j.copbio.2011.09.006

Commission of the European Communities. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006. Setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*.

Commission of the European Communities. (2012). Regulation (EU) No 1151/2012 of the European Parliament and the Council of 21 November 2012. Quality schemes for agricultural products and foodstuffs. *Official Journal of the European Union*.

Commission Regulation (EEC). (2011). Commission Regulation of 24 January 2011 amending Regulation No 2568/91/EEC on the characteristics of olive oil and olive pomace oil and on the relevant methods of analysis. *Official Journal of the European Union, 23*, 1–13.

Conde, C., Delrot, S., & Gerós, H. (2008). Physiological, biochemical and molecular changes occurring during olive development and ripening. *Journal of Plant Physiology*, *15*, 1545–1562. https://doi.org/10.1016/j.jplph.2008.04.018

Costantini, E. A. C. (2006). *Metodi di valutazione dei suoli e delle terre* (Vol. 7). Firenze: Cantagalli.

Damak, F., Asano, M., Baba, K., Ksibi, M., & Tamura, K. (2019). Comparison of Sample Preparation Methods for Multielements Analysis of Olive Oil by ICP-MS. *Methods and Protocols*, *3*, 72. https://doi.org/10.3390/mps2030072

Damak, F., Bougi, M. S. M., Araoka, D., Baba, K., Furuya, M., Ksibi, M., & Tamura, K. (2021). Soil geochemistry, edaphic and climatic characteristics as components of Tunisian olive terroirs: Relationship with the multielemental composition of olive oils for their geographical traceability. *Euro-Mediterranean Journal for Environmental Integration*, *1*. https://doi.org/10.1007/s41207-021-00241-y

EC. (1991). Commission regulation EC No. 2568/91 July 11, 1991. *Official EC Journal L*.

El Qarnifa, S., El Antari, A., & Hafidi, A. (2019). Effect of Maturity and Environmental Conditions on Chemical Composition of Olive Oils of Introduced Cultivars in Morocco. *Journal of Food Quality*, 1–14. https://doi.org/10.1155/2019/1854539

European Parliament. (2014). European Parliament resolution of 14 January 2014 on the food crisis, fraud in the food chain and the control thereof (2013/2091(INI)).

Fang, M., Tsai, C.-F., Wu, G.-Y., Tseng, S.-H., Cheng, H.-F., Kuo, C.-H., Hsu, C.-L., Kao, Y.-M., Shih, D. Y.-C., & Chiang, Y.-M. (2015). Identification and quantification of Cu-chlorophyll adulteration of edible oils. *Food Additives & Contaminants: Part B*, *3*, 157–162. https://doi.org/10.1080/19393210.2015.1025861

Foscolou, A., Critselis, E., & Panagiotakos, D. (2018). Olive oil consumption and human health: A narrative review. *Maturitas*, 60–66. https://doi.org/10.1016/j.maturitas.2018.10.013

Garcia, B., Magalhães, J., Fregapane, G., Salvador, M. D., & Paiva-Martins, F. (2012). Potential of selected Portuguese cultivars for the production of high quality monovarietal virgin olive oil. *European Journal of Lipid Science and Technology*, *9*, 1070–1082. https://doi.org/10.1002/ejlt.201200046

García-Vico, L., Belaj, A., Sánchez-Ortiz, A., Martínez-Rivas, J., Pérez, A., & Sanz, C. (2017). Volatile Compound Profiling by HS-SPME/GC-MS-FID of a Core Olive Cultivar

Collection as a Tool for Aroma Improvement of Virgin Olive Oil. *Molecules*, *1*, 141. https://doi.org/10.3390/molecules22010141

Genovese, A., Caporaso, N., Leone, T., Paduano, A., Mena, C., Perez-Jimenez, M. A., & Sacchi, R. (2018). Use of odorant series for extra virgin olive oil aroma characterisation. *Journal of the Science of Food and Agriculture*, *3*, 1215–1224. https://doi.org/10.1002/jsfa.9293

Ghane, E. T., Poormohammadi, A., Khazaei, S., & Mehri, F. (2021). Concentration of Potentially Toxic Elements in Vegetable Oils and Health Risk Assessment: a Systematic Review and Meta-analysis. *Biological Trace Element Research*, *1*, 437–446. https://doi.org/10.1007/s12011-021-02645-x

Gonnelli, C., & Renella, G. (2012). Chromium and Nickel. In *Environmental Pollution* (pp. 313–333). Springer Netherlands. http://dx.doi.org/10.1007/978-94-007-4470-7_11

Grossi, M., Di Lecce, G., Gallina Toschi, T., & Riccò, B. (2014). A novel electrochemical method for olive oil acidity determination. *Microelectronics Journal*, *12*, 1701–1707. https://doi.org/10.1016/j.mejo.2014.07.006

Haj Heidary, R., Golzan, S. A., Mirza Alizadeh, A., Hamedi, H., & Ataee, M. (2022). Probabilistic health risk assessment of potentially toxic elements in the traditional and industrial olive products. *Environmental Science and Pollution Research*, *4*, 10213–10225. https://doi.org/10.1007/s11356-022-22864-6

Hakanson, L. (1980). An ecological risk index for aquatic pollution control.a sedimentological approach. *Water Research*, *8*, 975–1001. https://doi.org/10.1016/0043-1354(80)90143-8

Hendershot, W. H., & Duquette, M. (1986). A Simple Barium Chloride Method for Determining Cation Exchange Capacity and Exchangeable Cations. *Soil Science Society of America Journal*, *3*, 605–608. https://doi.org/10.2136/sssaj1986.03615995005000030013x

Ilarioni, L., & Proietti, P. (2014). Olive tree cultivars. In *The Extra-Virgin Olive Oil Handbook* (pp. 59–67). John Wiley & Sons, Ltd. http://dx.doi.org/10.1002/9781118460412.ch5

International Olive Council. (2019). *Trade Standard applying to olive oils and olive pomace oils.*

Integrated Risk Information System, *US EPA*. (2013, March 15). US EPA. https://www.epa.gov/iris

Iraqi, R., Vermeulen, C., Benzekri, A., Bouseta, A., & Collin, S. (2005). Screening for Key Odorants in Moroccan Green Olives by Gas Chromatography−Olfactometry/Aroma Extract Dilution Analysis. *Journal of Agricultural and Food Chemistry*, *4*, 1179–1184. https://doi.org/10.1021/jf040349w

Janin, M., Medini, S., & Técher, I. (2014). Methods for PDO olive oils traceability: state of art and discussion about the possible contribution of strontium isotopic tool. *European Food Research and Technology*, *5*, 745–754. https://doi.org/10.1007/s00217-014-2279-8

JECFA. (2003). *Summary and conclusions of the 61st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Rome, Italy.*

Jones, C., & Jacobsen, J. (2005). Plant nutrition and soil fertility. *Nutrient Management Module*, *2(11)*, 1–11.

Kabala, C., & Singh, B. R. (2001). Fractionation and Mobility of Copper, Lead, and Zinc in Soil Profiles in the Vicinity of a Copper Smelter. *Journal of Environmental Quality*, *2*, 485–492. https://doi.org/10.2134/jeq2001.302485x

Kabaran, S., Güleç, A., & Besler, T. H. (2020). Are There Any Potential Health Risk of Heavy Metals Through Dietary Intake of Olive Oil that Produced In Morphou, Cyprus. *Progress in Nutrition*, *3*, e2020018. https://doi.org/10.23751/pn.v22i3.8098

Kalogeropoulos, N., & Tsimidou, M. (2014). Antioxidants in Greek Virgin Olive Oils. *Antioxidants*, *2*, 387–413. https://doi.org/10.3390/antiox3020387

Kalua, C. M., Allen, M. S., Bedgood, D. R., Bishop, A. G., Prenzler, P. D., & Robards, K. (2007). Olive oil volatile compounds, flavour development and quality: A critical review. *Food Chemistry*, *1*, 273–286. https://doi.org/10.1016/j.foodchem.2005.09.059

Kars, N., & Dengiz, O. (2020). Assessment of potential ecological risk index based on heavy metal elements for organic farming in micro catchments under humid ecological condition. *Eurasian Journal of Soil Science (EJSS)*, *3*, 194–201. https://doi.org/10.18393/ejss.719167

Khan, Z. I., Ahmad, K., Akram, N. A., Mehmood, N., & Yasmeen, S. (2017). Heavy metal contamination in water, soil and a potential vegetable garlic (*Allium sativum* L.) in Punjab, Pakistan. *Pakistan Journal of Botany*, *49(2)*, 547–552.

Kiritsakis, A. K. (1998). Flavor components of olive oil-A review. *Journal of the American Oil Chemists' Society*, *6*, 673–681. https://doi.org/10.1007/s11746-998-0205-6

Komárek, M., Čadková, E., Chrastný, V., Bordas, F., & Bollinger, J.-C. (2010). Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. *Environment International*, *1*, 138–151. https://doi.org/10.1016/j.envint.2009.10.005

Kosma, I., Vatavali, K., Kontakos, S., Kontominas, M., Kiritsakis, A., & Badeka, A. (2017). Geographical Differentiation of Greek Extra Virgin Olive Oil from Late-Harvested

Koroneiki Cultivar Fruits. *Journal of the American Oil Chemists' Society*, *11*, 1373–1384. https://doi.org/10.1007/s11746-017-3036-5

Kumar, M., Rahman, M. M., Ramanathan, A. L., & Naidu, R. (2016). Arsenic and other elements in drinking water and dietary components from the middle Gangetic plain of Bihar, India: Health risk index. *Science of The Total Environment*, 125–134. https://doi.org/10.1016/j.scitotenv.2015.08.039

La Pera, L., Lo Curto, S., Visco, A., La Torre, L., & Dugo, G. (2002). Derivative Potentiometric Stripping Analysis (dPSA) Used for the Determination of Cadmium, Copper, Lead, and Zinc in Sicilian Olive Oils. *Journal of Agricultural and Food Chemistry*, *11*, 3090–3093. https://doi.org/10.1021/jf0113124

Lepri, F. G., Chaves, E. S., Vieira, M. A., Ribeiro, A. S., Curtius, A. J., DeOliveira, L. C. C., & DeCampos, R. C. (2011). Determination of Trace Elements in Vegetable Oils and Biodiesel by Atomic Spectrometric Techniques—A Review. *Applied Spectroscopy Reviews*, *3*, 175–206. https://doi.org/10.1080/05704928.2010.529628

Lioupi, A., Nenadis, N., & Theodoridis, G. (2020). Virgin olive oil metabolomics: A review. *Journal of Chromatography B*, 122161. https://doi.org/10.1016/j.jchromb.2020.122161

Llorent-Martínez, E. J., Fernández-de Córdova, M. L., Ortega-Barrales, P., & Ruiz-Medina, A. (2014). Quantitation of Metals During the Extraction of Virgin Olive Oil from Olives Using ICP-MS after Microwave-assisted Acid Digestion. *Journal of the American Oil Chemists' Society*, *10*, 1823–1830. https://doi.org/10.1007/s11746-014-2511-5

L'olio extra vergine di oliva. Toscano, buono e sano. (2021). ValdichianaLiving.It. https://www.valdichianaliving.it/esplora/olio-extravergine

Loumou, A., & Giourga, C. (2003). *Agriculture and Human Values*, *1*, 87–95. https://doi.org/10.1023/a:1022444005336

Lučić, M., Špika, M. J., Mikac, N., Pošćić, F., Rengel, Z., Romić, M., Begić, H. B., Fiket, Ž., Turk, M. F., Bačić, N., Leder, R., Petric, I. V., Urlić, B., Žanetić, M., Runjić, M., Selak, G. V., Vitanović, E., Klepo, T., Rošin, J., & Perica, S. (2023). Traceability of Croatian extra virgin olive oils to the provenance soils by multielement and carbon isotope composition and chemometrics. *Food Chemistry*, 136401. https://doi.org/10.1016/j.foodchem.2023.136401

Lukić, I., Carlin, S., Horvat, I., & Vrhovsek, U. (2019). Combined targeted and untargeted profiling of volatile aroma compounds with comprehensive two-dimensional gas chromatography for differentiation of virgin olive oils according to variety and geographical origin. *Food Chemistry*, 403–414. https://doi.org/10.1016/j.foodchem.2018.07.133

Luo, W., Lu, Y., Giesy, J. P., Wang, T., Shi, Y., Wang, G., & Xing, Y. (2007). Effects of land use on concentrations of metals in surface soils and ecological risk around Guanting Reservoir, China. *Environmental Geochemistry and Health*, *6*, 459–471. https://doi.org/10.1007/s10653-007-9115-z

Machado, M., Felizardo, C., Fernandes-Silva, A. A., Nunes, F. M., & Barros, A. (2013). Polyphenolic compounds, antioxidant activity and l-phenylalanine ammonia-lyase activity during ripening of olive cv. “Cobrançosa” under different irrigation regimes. *Food Research International*, *1*, 412–421. https://doi.org/10.1016/j.foodres.2012.12.056

Mahne Opatić, A., Nečemer, M., Lojen, S., & Vidrih, R. (2017). Stable isotope ratio and elemental composition parameters in combination with discriminant analysis classification model to assign country of origin to commercial vegetables – A preliminary study. *Food Control*, 252–258. https://doi.org/10.1016/j.foodcont.2017.05.010

Mariotti, R., Núñez-Carmona, E., Genzardi, D., Pandolfi, S., Sberveglieri, V., & Mousavi, S. (2022). Volatile Olfactory Profiles of Umbrian Extra Virgin Olive Oils and Their Discrimination through MOX Chemical Sensors. *Sensors*, *19*, 7164. https://doi.org/10.3390/s22197164

Martins, N., Jiménez-Morillo, N. T., Freitas, F., Garcia, R., Gomes da Silva, M., & Cabrita, M. J. (2020). Revisiting 3D van Krevelen diagrams as a tool for the visualization of volatile profile of varietal olive oils from Alentejo region, Portugal. *Talanta*, 120276. https://doi.org/10.1016/j.talanta.2019.120276

Mata, P., Alvarez-Sala, L. A., Rubio, M. J., Nuño, J., & De Oya, M. (1992). Effects of long-term monounsaturated- vs polyunsaturated-enriched diets on lipoproteins in healthy men and women. *The American Journal of Clinical Nutrition*, *4*, 846–850. https://doi.org/10.1093/ajcn/55.4.846

Mendil, D., Uluözlü, Ö. D., Tüzen, M., & Soylak, M. (2009). Investigation of the levels of some element in edible oil samples produced in Turkey by atomic absorption spectrometry. *Journal of Hazardous Materials*, *1–3*, 724–728. https://doi.org/10.1016/j.jhazmat.2008.10.046

Micó, C., Recatalá, L., Peris, M., & Sánchez, J. (2006). Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. *Chemosphere*, *5*, 863–872. https://doi.org/10.1016/j.chemosphere.2006.03.016

Milinovic, J., Garcia, R., Rato, A. E., & Cabrita, M. J. (2019). Rapid Assessment of Monovarietal Portuguese Extra Virgin Olive Oil's (EVOO's) Fatty Acids by Fourier-Transform Near-

Infrared Spectroscopy (FT-NIRS). *European Journal of Lipid Science and Technology*, *3*. https://doi.org/10.1002/ejlt.201800392

Ministero dell’Ambiente della Tutela del Territorio e del Mare. (2019). «Dm Ambiente, 1 Marzo 2019, n. 46. Regolamento relativo agli interventi di bonifica, di ripristino ambientale e di messa in sicurezza, d’emergenza, operativa e permanente, delle aree destinare alla produzione agricola e all’allevamento. Attuazione Articolo 241, Dlgs 152/2006».

Neina, D. (2019). The Role of Soil pH in Plant Nutrition and Soil Remediation. *Applied and Environmental Soil Science*, 1–9. https://doi.org/10.1155/2019/5794869

Nowak, D., Gośliński, M., & Popławski, C. (2021). Antioxidant Properties and Fatty Acid Profile of Cretan Extra Virgin Bioolive Oils: A Pilot Study. *International Journal of Food Science*, 1–6. https://doi.org/10.1155/2021/5554002

Odat, S. (2015). Application of Geoaccumulation Index and Enrichment Factors on the Assessment of Heavy Metal Pollution along Irbid/zarqa Highway-Jordan. *Journal of Applied Sciences*, *11*, 1318–1321. https://doi.org/10.3923/jas.2015.1318.1321

Olias, J. M., Perez, A. G., Rios, J. J., & Sanz, L. C. (1993). Aroma of virgin olive oil: Biogenesis of the “green” odor notes. *Journal of Agricultural and Food Chemistry*, *12*, 2368–2373. https://doi.org/10.1021/jf00036a029

Opara, L. U., & Mazaud, F. (2001). Food Traceability from Field to Plate. *Outlook on Agriculture*, *4*, 239–247. https://doi.org/10.5367/000000001101293724

Paiva-Martins, F., & Kiritsakis, A. (2017). Olive fruit and olive oil composition and their functional compounds. In *Olives and Olive Oil as Functional Foods* (pp. 81–115). John Wiley & Sons, Ltd. http://dx.doi.org/10.1002/9781119135340.ch5

Pandey, B., Agrawal, M., & Singh, S. (2015). Ecological risk assessment of soil contamination by trace elements around coal mining area. *Journal of Soils and Sediments*, *1*, 159–168. https://doi.org/10.1007/s11368-015-1173-8

Parkinson, L., & Cicerale, S. (2016). The Health Benefiting Mechanisms of Virgin Olive Oil Phenolic Compounds. *Molecules*, *12*, 1734. https://doi.org/10.3390/molecules21121734

Pehlivan, E., Arslan, G., Gode, F., Altun, T., & Musa Özcan, M. (2008). Determination of some inorganic metals in edible vegetable oils by inductively coupled plasma atomic emission spectroscopy (ICP-AES). *Grasas y Aceites*, *3*. https://doi.org/10.3989/gya.2008.v59.i3.514

Peres, F., Martins, L. L., & Ferreira-Dias, S. (2017). Influence of enzymes and technology on virgin olive oil composition. *Critical Reviews in Food Science and Nutrition*, *14*, 3104–3126. https://doi.org/10.1080/10408398.2015.1092107

Portarena, S., Baldacchini, C., & Brugnoli, E. (2017). Geographical discrimination of extra-virgin olive oils from the Italian coasts by combining stable isotope data and carotenoid content within a multivariate analysis. *Food Chemistry*, 1–6. https://doi.org/10.1016/j.foodchem.2016.07.135

Portarena, S., Gavrichkova, O., Lauteri, M., & Brugnoli, E. (2014). Authentication and traceability of Italian extra-virgin olive oils by means of stable isotopes techniques. *Food Chemistry*, 12–16. https://doi.org/10.1016/j.foodchem.2014.04.115

Protano, G. (2021). *«Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy. Personal communication».*

Protano, G., & Rossi, S. (2014). Relationship between soil geochemistry and grape composition in Tuscany (Italy). *Journal of Plant Nutrition and Soil Science*, *4*, 500–508. https://doi.org/10.1002/jpln.201300055

Pucci, E., Palumbo, D., Puiu, A., Lai, A., Fiorani, L., & Zoani, C. (2022). Characterization and Discrimination of Italian Olive (*Olea europaea sativa*) Cultivars by Production Area Using Different Analytical Methods Combined with Chemometric Analysis. *Foods*, *8*, 1085. https://doi.org/10.3390/foods11081085

R Core Team (2023). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Rahman, M. S., Hossain, M. B., Babu, S. M. O. F., Rahman, M., Ahmed, A. S. S., Jolly, Y. N., Choudhury, T. R., Begum, B. A., Kabir, J., & Akter, S. (2019). Source of metal contamination in sediment, their ecological risk, and phytoremediation ability of the studied mangrove plants in ship breaking area, Bangladesh. *Marine Pollution Bulletin*, 137–146. https://doi.org/10.1016/j.marpolbul.2019.02.032

Rashmi, D., Shree, P., & Singh, D. K. (2017). Stable isotope ratio analysis in determining the geographical traceability of Indian wheat. *Food Control*, 169–176. https://doi.org/10.1016/j.foodcont.2017.03.025

Regione Toscana. (2023). *Consultazione Razze e Varietà Locali*. http://germoplasma.arsia.toscana.it/index.php?option=com_content&view=article&id=4&Itemid=109&act2=L&EROSGEN=-1&ShowCrit=0&ATTRIBUTO=3

Reimann, C., & Caritat, P. (1998). *Chemical Elements in the Environment | SpringerLink*. SpringerLink; Springer Berlin Heidelberg. https://link.springer.com/book/10.1007/978-3-642-72016-1

Rellini, I., Demasi, M., Scopesi, C., Ghislandi, S., Salvidio, S., Pini, S., & Stagno, A. (2022). Evaluation of the environmental components of the Taggiasca "*Terroir*" olive (Imperia, Italy). *BELS - Bulletin of Enviromental and Life Sciences*, V. 4 N. 1 (2022): Bulletin of Environmental and Life Sciences. https://doi.org/10.15167/2612-2960/BELS2022.4.1.2107

Roca, A., Cabrera, C., Lorenzo, M. a L., & López, M. a C. (2000). Levels of calcium, magnesium, manganese, zinc, selenium and chromium in olive oils produced in Andalusi. *Grasas y Aceites*, *6*. https://doi.org/10.3989/gya.2000.v51.i6.455

Rojnić, I. D., Bažok, R., & Barčić, J. I. (2014). Reduction of olive fruit fly damage by early harvesting and impact on oil quality parameters. *European Journal of Lipid Science and Technology*, *1*, 103–111. https://doi.org/10.1002/ejlt.201400150

Romero, A., Cuesta, C., & Sánchez-Muniz, F. J. (2003). Cyclic FA monomers in high-oleic acid sunflower oil and extra virgin olive oil used in repeated frying of fresh potatoes. *Journal of the American Oil Chemists' Society*, *5*, 437–442. https://doi.org/10.1007/s11746-003-0717-x

Rugini, E., Baldoni, L., Muleo, R., & Sebastiani, L. (2016). *The Olive Tree Genome* (pp. 13–25). Springer.

Sales, C., Portolés, T., Johnsen, L. G., Danielsen, M., & Beltran, J. (2019). Olive oil quality classification and measurement of its organoleptic attributes by untargeted GC–MS and multivariate statistical-based approach. *Food Chemistry*, 488–496. https://doi.org/10.1016/j.foodchem.2018.07.200

Sánchez, J., & Harwood, J. L. (2002). *European Journal of Lipid Science and Technology*, *9–10*, 564–573. https://doi.org/10.1002/1438-9312(200210)104:9/10<564::aid-ejlt564>3.0.co;2-5

Sánchez-Muniz, F. J., Oubiña, P., Benedí, J., Ródenas, S., & Cuesta, C. (1998). A preliminary study on platelet aggregation in postmenopausal women consuming extra-virgin olive oil and high-oleic acid sunflower oil. *Journal of the American Oil Chemists' Society*, *2*, 217–223. https://doi.org/10.1007/s11746-998-0034-7

Sarkar, D., (2008). Lattice: Multivariate Data Visualization with R_. Springer, New York. ISBN 978-0-387-75968-5, <http://lmdvr.r-forge.r-project.org>.

Savio, M., Ortiz, M. S., Almeida, C. A., Olsina, R. A., Martinez, L. D., & Gil, R. A. (2014). Multielemental analysis in vegetable edible oils by inductively coupled plasma mass spectrometry after solubilisation with tetramethylammonium hydroxide. *Food Chemistry*, 433–438. https://doi.org/10.1016/j.foodchem.2014.03.041

Schellenberg, A., Chmielus, S., Schlicht, C., Camin, F., Perini, M., Bontempo, L., Heinrich, K., Kelly, S. D., Rossmann, A., Thomas, F., Jamin, E., & Horacek, M. (2010). Multielement stable isotope ratios (H, C, N, S) of honey from different European regions. *Food Chemistry*, *3*, 770–777. https://doi.org/10.1016/j.foodchem.2009.12.082

Seçmeler, Ö., & Galanakis, C. M. (2019). Olive Fruit and Olive Oil. In *Innovations in Traditional Foods* (pp. 193–220). Elsevier. http://dx.doi.org/10.1016/B978-0-12-814887-7.00008-3

Serreli, G., & Deiana, M. (2018). Biological Relevance of Extra Virgin Olive Oil Polyphenols Metabolites. *Antioxidants*, *12*, 170. https://doi.org/10.3390/antiox7120170

Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Di Maio, I., Selvaggini, R., & Taticchi, A. (2013). Biological Activities of Phenolic Compounds of Extra Virgin Olive Oil. *Antioxidants*, *1*, 1–23. https://doi.org/10.3390/antiox3010001

Steinnes, E. (2013). *Lead.* SpringerLink; Springer Netherlands. https://link.springer.com/chapter/10.1007/978-94-007-4470-7_14

Telloli, C., Tagliavini, S., Passarini, F., Salvi, S., & Rizzo, A. (2023). ICP-MS triple quadrupole as analytical technique to define trace and ultra-trace fingerprint of extra virgin olive oil. *Food Chemistry*, 134247. https://doi.org/10.1016/j.foodchem.2022.134247

Tibola, C.S., da Silva, S.A., Dossa, A.A., & Patrício, D.I. (2018). Economically Motivated Food Fraud and Adulteration in Brazil: Incidents and Alternatives to Minimize Occurrence. *Journal of Food Science*, *8*, 2028–2038. https://doi.org/10.1111/1750-3841.14279

Tomé-Rodríguez, S., Ledesma-Escobar, C. A., Penco-Valenzuela, J. M., & Priego-Capote, F. (2021). Cultivar influence on the volatile components of olive oil formed in the lipoxygenase pathway. *LWT*, 111485. https://doi.org/10.1016/j.lwt.2021.111485

US EPA. (2004). Method 9045D: Soil and Waste pH, in Test Methods for Evaluating Solid Waste, SW846. *USA: EPA*.

Vannini, A., Grattacaso, M., Canali, G., Nannoni, F., Di Lella, L. A., Protano, G., Biagiotti, S., & Loppi, S. (2021). Potentially Toxic Elements (PTEs) in Soils and Bulbs of Elephant Garlic (*Allium ampeloprasum* L.) Grown in Valdichiana, a Traditional Cultivation Area of Tuscany, Italy. *Applied Sciences*, *15*, 7023. https://doi.org/10.3390/app11157023

Vázquez-Blanco, R., Arias-Estévez, M., Bååth, E., & Fernández-Calviño, D. (2020). Comparison of Cu salts and commercial Cu based fungicides on toxicity towards microorganisms in soil. *Environmental Pollution*, 113585. https://doi.org/10.1016/j.envpol.2019.113585

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition.

Springer, New York. ISBN 0-387-95457-0

Veneziani, G., Esposto, S., Taticchi, A., Urbani, S., Selvaggini, R., Sordini, B., & Servili, M. (2018). Characterization of phenolic and volatile composition of extra virgin olive oil extracted from six Italian cultivars using a cooling treatment of olive paste. *LWT*, 523–528. https://doi.org/10.1016/j.lwt.2017.09.034

Vichi, S., Romero, A., Tous, J., & Caixach, J. (2011). The Activity of Healthy Olive Microbiota during Virgin Olive Oil Extraction Influences Oil Chemical Composition. *Journal of Agricultural and Food Chemistry*, *9*, 4705–4714. https://doi.org/10.1021/jf200642s

Vichi, S., Romero, A., Tous, J., Tamames, E. L., & Buxaderas, S. (2008). Determination of volatile phenols in virgin olive oils and their sensory significance. *Journal of Chromatography A*, *1–2*, 1–7. https://doi.org/10.1016/j.chroma.2008.09.067

Visioli, F., & Galli, C. (2002). Biological Properties of Olive Oil Phytochemicals. *Critical Reviews in Food Science and Nutrition*, *3*, 209–221. https://doi.org/10.1080/10408690290825529

Walkey, A., & Black, I. A. (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, *37(1)*, 29–38.

Wickham, H., (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

World Health Organization. (2004). *Evaluation of Certain Food Additives and Contaminants: Sixty-first Report of the Joint FAO/WHO Expert Committee on Food Additives (WHO Technical Report Series).*

Zaanouni, N., Gharssallaoui, M., Eloussaief, M., & Gabsi, S. (2018). Heavy metals transfer in the olive tree and assessment of food contamination risk. *Environmental Science and Pollution Research*, *19*, 18320–18331. https://doi.org/10.1007/s11356-018-1474-8

Žanetić, M., Jukić Špika, M., Ožić, M. M., & Brkić Bubola, K. (2021). Comparative Study of Volatile Compounds and Sensory Characteristics of Dalmatian Monovarietal Virgin Olive Oils. *Plants*, *10*, 1995. https://doi.org/10.3390/plants10101995

Zeiner, M., Steffan, I., & Cindric, I. J. (2005). Determination of trace elements in olive oil by ICP-AES and ETA-AAS: A pilot study on the geographical characterization. *Microchemical Journal*, *2*, 171–176. https://doi.org/10.1016/j.microc.2004.12.002

Zhao, H., & Yang, Q. (2019). The suitability of rare earth elements for geographical traceability of tea leaves. *Journal of the Science of Food and Agriculture*, *14*, 6509–6514. https://doi.org/10.1002/jsfa.9930

Zhao, S., & Zhao, Y. (2020). Application and Preparation Progress of Stable Isotope Reference Materials in Traceability of Agricultural Products. *Critical Reviews in Analytical Chemistry*, 1–12. https://doi.org/10.1080/10408347.2020.1768359

Zhiyuan, W., Dengfeng, W., Huiping, Z., & Zhiping, Q. (2011). Assessment of Soil Heavy Metal Pollution with Principal Component Analysis and Geoaccumulation Index. *Procedia Environmental Sciences*, 1946–1952. https://doi.org/10.1016/j.proenv.2011.09.305

Zhu, F., Fan, W., Wang, X., Qu, L., & Yao, S. (2011). Health risk assessment of eight heavy metals in nine varieties of edible vegetable oils consumed in China. *Food and Chemical Toxicology*, *12*, 3081–3085. https://doi.org/10.1016/j.fct.2011.09.019

Ziarati, P., Mirmohammad Makki, F., Vambol, S., & Vambol, V. (2019). Determination of Toxic Metals Content in Iranian and Italian Flavoured Olive Oil. *Acta Technologica Agriculturae*, *2*, 64–69. https://doi.org/10.2478/ata-2019-0012

Zwolak, A., Sarzyńska, M., Szpyrka, E., & Stawarczyk, K. (2019). Sources of Soil Pollution by Heavy Metals and Their Accumulation in Vegetables: a Review. *Water, Air, & Soil Pollution*, *7*. https://doi.org/10.1007/s11270-019-4221-y