Macro- and microelements occurrence in soil and plant (leaves) samples of a representative and potentially sustainable Mediterranean vineyard

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Abstract

The study reported here concerns the geochemical distributions of macro- and trace elements (including potentially toxic elements, PTEs) in the vineyard soils of Alcubillas, which is one of the oldest, albeit not world-renowned, wine-growing areas in La Mancha (Central Spain). Soil and leaf samples were analyzed by X-ray fluorescence spectrometry to ascertain the levels of various elements in the soil and the plant. The potential toxicity of the elements was assessed with regard to the development of the vineyard. Despite the fact that fertilizers and pesticides are employed in the vineyards in this area, the results showed that the levels of trace elements in the soil samples did not exceed the reference values according to the pedochemical values for the region and Spain. This finding suggests that the study area is not polluted. The Biological Absorption Coefficient (BAC) was calculated to assess the assimilation of various elements from the soil to the leaves, and differences were found in the element absorption capacity of the vines. Some elements were not taken up by Vitis vinifera despite elements like Zr and Rb being present in relatively high concentrations in the soil. The production in these soils of grapes and wine does not represent a threat to human health or the ecosystem, because the farmers in this area are extremely careful to preserve the environment and they only farm to achieve moderate yields of grapes per hectare.

Introduction

Soil is an important part of the natural environment and its effect on wine quality and grape composition is highly complex because it has an influence on mineral nutrition in the grapevine, the uptake of water, root depth and rhizosphere temperature. In this respect, analysis of elements could be used (Greenough et al. 2005) since it is believed that these chemical elements present in the soil can be transferred to the plant and subsequently to the harvested grapes (Protano and Rossi 2014). The topic, however, is now being discussed further because it has not been demonstrated how these elements act on the sensory attributes of wines (Maltman 2013).

In viticulture, micronutrient deficiencies are corrected by the addition of trace elements that are essential for plant growth, e.g., Cu, Zn, Fe, Mn and B. Treatment of plants with fungicides, pesticides and herbicides also inputs chemicals into the vineyard soil. However, the presence of trace elements of natural origin in grapes and wine is mainly dependent on the mineralogical content of the soils, which are developed from various types of rocks.

Trace elements, including potentially toxic elements (PTEs, Hooda 2010), are found naturally in soils and
they can also be introduced and accumulated due to anthropogenic influences in agricultural, urban and industrial areas. The extensive use of fertilizers and fungicides (Kment et al. 2005; Komárek et al. 2010; Geana et al. 2013; Wightwick et al. 2013) leads to the accumulation of trace elements in vineyard soils and the bioavailability of such elements may be increased for plants. Other sources of trace elements include industrial pollution of sites (Moreno et al. 2007; Pohl 2007; Geana et al. 2013). It is not surprising, therefore, that numerous studies on viticultural areas have focused on the identification of the main macro- and microelements in the soil, grapes and wine (Huzum et al. 2012; Bora et al. 2013, 2015; Geana et al. 2013; Ungureanu et al. 2017).

The probability of anthropogenic contamination and the toxicological health risks associated with soil pollution can be evaluated by considering various indices (Mehr et al. 2017). Indeed, trace element data can be used, through predictive indices (such as the enrichment factor EF or Geocumulation Index, I-geo), to evaluate risks based on their total contents and toxicity (Gu et al. 2016; Müller 1969). On the other hand, the bioaccumulation coefficient (BAC) provides information about the capacity of a plant to uptake nutrients and this parameter can be measured in any plant tissue (root, leaf or fruit) (Kabata-Pendias 2001).

Although terroir studies have generally focused on heavily populated regions, a few studies have been conducted in smaller rural locations. In this respect, the objectives of this study were to provide an overview of the contents of some major and trace elements (including PTEs) in vineyard soils in a small and traditional rural location, namely Alcubillas (a small municipality of La Mancha, Central Spain), with special attention paid to those elements that under particular environmental circumstances may become toxic. The specific goals were (i) to provide a database for the studied macro- and microelements, (ii) to establish an ecological risk assessment for some PTEs in order to contribute to sustainable management of this agricultural area and (iii) to determine if certain elements are present in significant quantities in grapevine leaves in relation to soil composition.

Material and methods

Study area

The study area of Alcubillas is in the Castilla-La Mancha Community in Central Spain (Figure 1). The coordinates of this site are 38º 45’7” N and 3º 8’5” W and the area is 4,768 ha. The main crops that are cultivated in Alcubillas are vines, olive trees and cereals. There are also some small plots dedicated to orchards.

One of the main physiographic features is a virtually flat and karstified surface that is interrupted by residual reliefs in the form of inselberg and an alluvial plain. The major parent materials are calcareous sediments, limestones (with signs of karstification, as evidenced by the appearance of numerous ‘dolinas’), clay sediments of fluvial character, and quartzite sandstones (in some cases slates) in residual surfaces of the Paleozoic era. At this site the majority of the soils are Alfisols, Inceptisols and Entisols according to Soil Taxonomy (Soil Survey Staff 2014) and Luvisols, Calcisolos, Cambisolos, Regosolos and Leptosolos according to FAO-UNESCO-ISSS (2006). The dominant formation is constituted by Red soils (Alfisols) with calcic or petrocalcic horizons (Jiménez-Ballesta et al. 2020).

Figure 1

Sampling and laboratory methods

Soil samples from 10 profiles and 12 superficial horizons were collected. The sampling locations were selected from different geomorphological sites and vineyard types. Given that the majority of the active vine roots are located at a depth of 20–80 cm, the samples were taken from the Ap and Bt or Bw horizons. Leaf samples were collected along with the soil on which the plants were growing. The samples were air-dried naturally and sieved through a 2-mm sieve to remove the coarse fraction. The remaining fine-earth fraction was homogenized before chemical analysis was carried out. The contents of major and trace elements (Na, Mg, Al, Si, P, S, K, Ca, Fe, Mn, Ti, Ba, Sr, V, Cr, Ce, Rb, Ni, Zn, Nd, Pb, Nb, Co, Cs, Ga, La, Y, Zr, Sc, Th, U, Sn, Cu, As, Mo, Ta, Hf and W) in the soil and leaf samples were determined by X-ray fluorescence.
spectroscopy (XRF) using a Philips PW 2404 spectrophotometer with a maximum power of 4 kW (set of crystal analysers for LiF220, LiF200, Ge, PET and PX1, flow detector and twinkle detector). This analytical technique has been validated for solid matrices such as sediment (Stosnach and Mages 2009; Pessanha et al. 2010). Quality control was ensured by duplicate analysis of certified reference samples (BCR 62, SMR 1573A, SMR 1515).

**Statistical analysis**

The data were analyzed using Microsoft Office Excel 2018 and the software Statistical Package for Social Science (SPSS 24.0 for Windows, SPSS Inc., IL, U.S.A.), both under institutional licenses for the University of Castilla-La Mancha (Spain).

**Pollution indices: Geocumulation Index (I-geo)**

The level of contamination in sediments is commonly assessed and quantified using the so-called geo-accumulation (Igeo) parameter. This was originally defined by Müllner (1969) and is obtained using the formula:

$$I_{geo} = \frac{C_n}{B_n} \times 100$$  \[1\]

where $C_n$ denotes the measured value and $B_n$ is the geochemical background of the site. The factor 1.5 is applied to control variations in $B_n$ values caused by anthropogenic influences. The geo-accumulation index (Igeo) was defined into seven classes (Table 1) by Buccolieri et al. (2006).

**Table 1**

**Biological Absorption Coefficient (BAC)**

The BAC is the ratio between the plant and soil concentrations of each element. In this study the concentration in the leaf was used.

$$BAC = \frac{[\text{leaf}]}{[\text{soil}]}$$ \[2\]

**Results and Discussion**

**Macroelement contents in soils**

The results of macroelement measurements are summarized as mean, standard deviation (SD), minimum, and maximum values (Table 2).

**Table 2**

The vineyard soils from the Alcubillas site showed a heterogeneous concentration for the elemental composition (Table 2), with Si and Ca being the most abundant major elements followed by Al, influenced by the parent rock. The average soil contents in Ca were 134.4 g*kg\(^{-1}\) in topsoil (Ap horizons) and 161.6 g*kg\(^{-1}\) in subsoil (B horizons). These values (that highlight the calcareous character of the studied soils) are higher than the global average (15 g*kg\(^{-1}\), Kabata Pendias 2011 and other authors); in some case the Ca content even reached values up to 412.7 g*kg\(^{-1}\). It is worth pointing out that there are wine areas in the world with similar contents in Ca and many of these are highly appreciated for their wines. This is the case, for example, in the Champagne and Burgundy regions in France, Tuscany in Italy, and La Rioja in Spain.

Iron (Fe) and potassium (K) are present at lower levels than Ca. Iron – the average content of which in both horizons was 32.3 g*kg\(^{-1}\) – is necessary for the formation of chlorophyll, the respiration process of plants and the formation of certain proteins. The value for sulfur (S) obtained in the study area is 0.6 g*kg\(^{-1}\), a value below the average world background level (0.70 g*kg\(^{-1}\), Kabata-Pendias 2011).

The variances in the Si and Ca values are very large, while Na, Mg, S, P and Mn showed low variance. These variations can be explained as being the result of soil evolution and, to a lesser extent, ploughing and agricultural practices, as reported by Mirlean et al. (2007). Manganese (Mn) concentrations varied very little in the soils in the study area (between 0.7 and 0.6 g*kg\(^{-1}\)).
Trace element contents in soils

The results of microelemental measurements are summarized as mean, standard deviation (SD), minimum, and maximum values (Table 3).

Table 3

The data reported in Table 3 allow some general observations to be made. These data and the behaviour of some elements is discussed below.

Copper (Cu) and zinc (Zn)

Copper is also one of the most widely studied elements in wine-growing regions because the treatment and prevention of vine downy mildew is commonly carried out using the Bordeaux mixture \( \text{Ca(OH)}_2 + \text{CuSO}_4 \). However, copper becomes toxic to plants and some micro-organisms when it is present at high levels in soils as it disrupts nutrient-cycling and inhibits the mineralisation of essential nutrients.

In the soils under discussion here, copper was present in an acceptable medium level (Table 3), i.e., between 25.0 mg*kg\(^{-1}\) (topsoil) and 23.7 mg*kg\(^{-1}\) (subsoil). The world average level in soil is 30 mg*kg\(^{-1}\) (from 2 to 250 mg*kg\(^{-1}\), Adriano 2001) and the normal variation in soils is between 5 mg*kg\(^{-1}\) and 50 mg*kg\(^{-1}\) (Bloemen et al. 1995). A mean value of 35.4 mg*kg\(^{-1}\) has been determined in Spain (Peris et al. 2007), 24 mg*kg\(^{-1}\) (from 1 to 111 mg*kg\(^{-1}\)) in Portugal (Reis et al. 2007), 227 mg*kg\(^{-1}\) in France for the deep horizon (Chopin et al. 2008), while in Brazil the content is in the range from 50.1 mg*kg\(^{-1}\) (20 years) to 2197 mg*kg\(^{-1}\) (100 years) (Mirlean et al. 2007). The pedogeochemical baseline level for Castilla-La Mancha is 10.3 mg*kg\(^{-1}\) (Jimenez Ballesta et al. 2010).

The average contents in Zn (Table 3) are 47.4 mg*kg\(^{-1}\) (topsoil) and 43.6 mg*kg\(^{-1}\) (subsoil). According to other authors the values for Zn range between 10 mg*kg\(^{-1}\) and 300 mg*kg\(^{-1}\) (Adriano 2001), with a world soil average of 50 mg*kg\(^{-1}\), so contamination is not evident for this element. In Castellon (Spain) the average value is 76.8 mg*kg\(^{-1}\) (Peris et al. 2007); in the Champagne region of France (Chopin et al. 2008) the values range from 318 mg*kg\(^{-1}\) in the topsoil to 208 mg*kg\(^{-1}\) in the deep horizon, with 75 mg*kg\(^{-1}\) as median; and in Portugal (Reis et al. 2007) a minimum value of 14 mg*kg\(^{-1}\) and a maximum value of 344 mg*kg\(^{-1}\) were determined for vineyard areas. Finally, the pedogeochemical baseline level for Castilla-La Mancha (Central Spain) is 35.7 mg*kg\(^{-1}\) (Jimenez Ballesta et al. 2010). Increased concentrations of Zn and Cu in soils under the long-term production of grapevine have been recorded in numerous studies (Romič and Romič 2003; Fishel 2014; Tóth et al. 2016).

Lead (Pb), cobalt (Co) and chromium (Cr)

In the vineyard soils studied, lead (Pb) has average values (Table 3) of 21.9 mg*kg\(^{-1}\) in topsoil and 20.2 mg*kg\(^{-1}\) in subsoil. In some cases moderately high Pb levels (31.6 mg*kg\(^{-1}\)) are detected in some soils when compared to the pedogeochemical baseline levels for Castilla-La Mancha (19.3 mg*kg\(^{-1}\), Jiménez Ballesta et al. 2010). This level is probably caused by the mechanized equipment used for works carried out in the vineyard. However, these values are still far below the total mean contents of other regions. For example, the lead content in Castellon, Spain, is 56.1 mg*kg\(^{-1}\) (Peris et al. 2007), in the Champagne region of France Pb ranges from 76 (deep horizon) to 141 mg*kg\(^{-1}\) (topsoil) (Chopin et al. 2008) and, in the case of the soils from Brestnik village, (Bulgaria), values range from 72.6 mg*kg\(^{-1}\) for a depth of 0–10 cm to 61.4 mg*kg\(^{-1}\) (Huzum et al. 2012).

In the present study, the chromium (Cr) content is in the range between 71.0 and 29.9 mg*kg\(^{-1}\) (Table 3), with an average of 51.4 mg*kg\(^{-1}\), in top horizons, whereas in the subsoil the values range between 70.9 and 25.5 mg*kg\(^{-1}\), with an average of 50.0 mg*kg\(^{-1}\). The chromium contents in soils worldwide differ greatly, i.e., in the range from 7 to 2221 mg*kg\(^{-1}\) (McBride 1994), and this depends mostly on the parent material and soil mineralogy. Mirlean et al. (2007) suggested a variation between 8.2 (young plantation) and 77.9 mg*kg\(^{-1}\). In Portugal the Cr content has an average of 31 mg*kg\(^{-1}\) (from a minimum of 3 to a maximum value of 243 mg*kg\(^{-1}\)) (Reis et al. 2007), while the values for some areas in Spain are quite similar, with a total mean
content of 32.2 mg·kg⁻¹ (Peris et al. 2007). With some exceptions, the chromium concentrations did not exceed the pedogeochanical baseline value for soils of Castilla-La Mancha (Jiménez Ballesta et al. 2010).

The average cobalt (Co) content is near to 10.5 mg·kg⁻¹ (Table 3) for both topsoil and subsoil, with values slightly higher than for the soils of Castilla-La Mancha (5.8 mg·kg⁻¹, Jiménez Ballesta et al. 2010). Cobalt is an element that is essential to human health but excess amounts can cause detrimental effects (ATSDR 2004). The transfer potential from soil to the edible parts of plants is rather low (Luo et al. 2010).

The average nickel (Ni) contents (Table 3) in the topsoil and subsoil of Alcubillas are similar (34.7 vs 33.9 mg·kg⁻¹, respectively). In terms of Ni values, a total mean content of 19.9 mg·kg⁻¹ was reported for the Castellon area of Spain (Peris et al. 2007) and a mean value of 28 mg·kg⁻¹ (with a minimum of 2 mg·kg⁻¹ and a maximum of 539 mg·kg⁻¹) was found in the Douro basin, Portugal (Reis et al. 2007). The levels of Ni in Alcubillas are slightly higher than the current Castilla-La Mancha pedogeochanical reference value (16.9 mg·kg⁻¹).

Strontium (Sr) and Molybdenum (Mo)

The contents of strontium in the surface and subsurface horizons are 125.9 mg·kg⁻¹ (Table 3). The mean worldwide background concentration for Sr in soil is 147.9 mg·kg⁻¹ (Kabata-Pendias 2011) and in Castilla-La Mancha it is 380.0 mg·kg⁻¹, although in this Community values of up to 3384.9 mg·kg⁻¹ have been found in soils on gypsum or gypsum marls (Conde et al. 2008). Alcubillas is an active agricultural area and therefore the levels determined cannot be attributed to the use of soil amendments (e.g., phosphorus fertilizers). Various anthropogenic activities, including the application of fertilizers, the generation of nuclear power, and the burning of coal to generate power, have led to increased levels of strontium (Sr) in soil (Burger and Lichtscheidl 2019). The great potential for the accumulation of Sr in plants and animals can pose serious environmental and human health hazards, which include cancers and disorders of the nervous system (Burger and Lichtscheidl 2019). Sar is able to affect the health of plants and animals because in high concentrations it is a potentially toxic metal (Evans and Barabash 2010). The average Mo content in the surface soils of Alcubillas was 0.9 mg·kg⁻¹ and in the subsurface soils it was 1.0 mg·kg⁻¹ (Table 3), with a maximum level of 1.3 mg·kg⁻¹. In comparison to the pedogeochanical references for Castilla-La Mancha these values are lower or similar and they are also lower than the mean background content of Mo in surface soils as a worldwide average (1.1 mg·kg⁻¹, Kabata-Pendias 2011).

Ecological risk assessment

The pollution index (I-geo) provides a useful tool to evaluate potential environmental contamination. On considering the I-geo (Table 3) values it can be observed that all values fall in the 0 class, which indicates the absence of contamination, i.e. almost all of the elements are of natural origin or from natural weathering processes. Only Cu, Ni and Co fall into the class 1 category and these elements are therefore influenced slightly by human activity.

Trace element contents in leaves. Soil/leaf ratios

For primary production through photosynthesis, in addition to water and carbon dioxide, plants require some inorganic mineral nutrients that are taken up through the root system. In this process elements are transferred from soil to plant and the leaf mineral composition should reflect the complex interaction between Vitis vinifera and the local soil composition, since it is one of main factors that limits plant productivity and quality. The Biological Absorption Coefficient (BAC) is the bioaccumulation of a given element and this is
the ratio between the leaf/soil concentrations (Kabatha-Pendias 2003). The BAC values are listed in Table 4.

The modified Kabata-Pendias BAC classification (2001) was used here to classify the major elements accumulated in the vine leaf. The elements Ca, K, Mg and S had high BAC values (greater than 1), whereas Sr and Zn had medium BAC values (between 0.4 and 0.7) and Ba, Pb, Cu had low values (between 0.4 and 0.10). Finally, V and Cr had very low values (<0.1). The BAC value obtained for Sr in this study is similar to those reported in the scientific literature (Kabata-Pendias and Sadurski 2004). If we compare the results obtained in other studies carried out in Castilla-La Mancha (Amorós et al. 2012b; Bravo et al. 2017) it can be observed that the values for Sr and Zn are higher (0.56 vs. 0.28 and 0.7 vs. 0.37, respectively); the values for Ba, V, Cr and Cu are lower (0.14 vs. 0.26; 0.06 vs. 0.16; 0.07 vs. 0.17 and 0.19 vs 1.86, respectively); while the value of Pb is similar (0.12 vs. 0.15).

Table 4

The relationship between the concentrations of major and trace elements studied in soils and leaves from grapevine cultivars of Alcubillas are represented in Figure 2. It can be seen that the higher the soil contents in Sr, Pb and Cr, the higher the contents of the same elements incorporated in the leaves. Amorós et al. (2012b) found a positive correlation between the amount of Sr in the soil and that in the leaf and grape. A similar trend was observed for Cu, Zn and, to a lesser extent, Ba, but less markedly than for the previous elements. Finally, the content of V in the leaf decreases as the content in the soil increases.

All of these data provide relevant information that could help to ascertain the nutritional status of the plant. Nevertheless, significant correlations were not found in this survey; the uptake of elements in grapevine can be influenced by soil, climate, geographic origin, and rootstock type.

Figure 2

The elementary compositions of the soils of Alcubillas allowed us to establish the pedogeochemical baseline levels and these are relevant because they allow the distinction of contaminated areas from uncontaminated ones. The absence of potentially polluting industrial activities in the area of study means that most of the potentially polluting chemical elements are at concentrations close to the pedogeochemical baseline levels of the region (Jimenez Ballesta et al. 2010), i.e., they are directly associated with the existing lithological units or weathering processes.

It should be noted that the foliar application of fungicides or the addition (direct or irrigated) of phosphate fertilizers (with impurities that contain heavy metals such as Cd, Hg and Pb, Ramalho et al. 1999; Nagajyoti et al. 2010) contribute to increased contents in soils, so it was expected that the vineyard soils of Alcubillas would have higher than normal contents in some PTEs. It is worth noting that Cu values have been reported for the soils of traditional wine regions throughout the world. For example, these values range from 20 to 500 mg·kg⁻¹ Cu in France (Flores-Vélez et al. 1996; Brun et al. 1998), 35 to 600 mg·kg⁻¹ in Spain (Arias et al. 2004), 2 to 375 mg·kg⁻¹ in Italy (Toselli et al. 2009), 100 to 210 mg·kg⁻¹ in Greece (Vavloidou et al. 2005) and 40 to 250 mg·kg⁻¹ in Australia (Pietrzak and Mcphail 2004). This is not the case for Alcubillas, where the common feature is the addition of organic residues that are mainly of plant origin (pruning residues) to which goat and sheep excrement is added.

In Alcubillas farmers are aware of this situation and they fertilize soils with only moderate doses with a low frequency of application. The farmers also control the changes that occur during the development and growth of the crop. In fact, even if the farmers are not aware of the origins, they observe structural, biochemical and physiological changes through visible symptoms of phytotoxicity such as reduced growth (especially of the root system), chlorosis and necrosis in the leaves.

On the basis of the Cu values found, one can expect shortening and thickening of the apex of the vine root (Ambrosini et al. 2015) along with an increase in the number of lateral roots and plasmolysis in the epidermis of some cells, thus reducing the density of the root hairs (Chen et al. 2013; Zhang et al. 2014). In addition, the presence of excess Cu in plant shoots can lead to a decrease in the concentration of photosynthetic
pigments and the fixation rate of C. These changes may in turn increase oxidative stress at the cellular level (Cambrellé et al. 2013). However, these symptoms were not observed in the studied area, because Cu-fungicide applications are minimal to achieve desired effects. The farmer is aware of this and avoids incorporating products that contain Cu.

Conclusions
The vineyard soils display a heterogeneous concentration in terms of elemental composition in the study area, with Ca being one of the most abundant major elements and S below the average world background values. The small differences between the concentrations of the elements with depth can be explained by assuming a common source that is primarily geogenic and pedogenic in nature followed by secondary factors of ploughing and agricultural practices.

Regarding the pedogeochemical baseline levels, and despite the fact that the use of fertilizers and pesticides in the vineyard is common, we can describe the trace element contents as normal for the investigated area, that is, trace element values associated with potential toxicity in soils were not found. Indeed, a risk assessment of the elementary composition of the soils indicated that this does not currently pose a high risk and that there are no adverse effects caused by PTEs accumulation in the soils. This is because the farmers are very sensitive to the environment and they aim to achieve only moderate yields of grapes per hectare.

The relationships between concentrations of elements in soil versus leaves indicate that the higher soil contents in Sr, Pb and Cr imply higher contents of these elements in the leaves, while the V content decreases in leaf as it increases in the soil. It was found that some elements were not taken up by the Vitis vinifera, even though elements like Zr and Rb are present in relatively high concentrations in the soil.

In conclusion, the present study provides a reference for future analogous studies in other areas, particularly in a Mediterranean environment, but additional research on trace element contents in grape clusters is required to understand better the transfer of trace elements from soil to products (grapes and wine).

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