



Assessment of metal bioavailability in the vineyard soil-grapevine system using different extraction methods



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ABSTRACT

This study was focused on the assessment of single and sequential extraction methods to predict the bioavailability of metals in the vineyard soil-grapevine system. The modified BCR sequential extraction method and two single-step extraction methods based on the use of EDTA and acetic acid were applied to differently amended vineyard soils. The variety effect was studied on the uptake of metals by leaves and grapes. Most of the elements studied (Ca, Mg, Cu, Fe, Mn, Zn and Pb) were weakly mobilized from vineyard soils, with the exception of Cu and Mn. The determination of total metal content in leaves and grapes showed a different accumulation pattern in the two parts of the vine. A significant relationship was observed, for all the elements studied except for Fe, between the content bioavailable in the soil and the accumulated in both leaves and grapes ($R = 0.602\text{--}0.775$, $p < 0.01$).

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1. Introduction

Wine is one of the most traditional and widely-consumed alcoholic beverages worldwide. The presence of trace metals in the wine is important from a toxicological and organoleptic point of view (Pyrzyńska, 2004). The contents of trace metals depend on environmental and anthropogenic factors including the type of vineyard soil, the metal intake from the soil into the grapes, grape variety and maturity, climatic conditions, viticulture management practices, environmental pollution, winemaking additives, winery equipment, wine preservation and bottling (Grindlay, Mora, Gras, & de Loos-Vollebregt, 2011; Pohl, 2007; Pyrzyńska, 2004). Despite the variability associated with viticultural and winemaking practices, recent studies have reported a successful discrimination of wines produced in different growing areas based on the content of trace metals (González, Llorens, Cervera, Armenta, & de la Guardia, 2009; Martin, Watling, & Lee, 2012; Rodrigues et al., 2011). Therefore, soil and climatic conditions are primary factors determining the elemental composition of grapes and wines. Furthermore, the accumulation patterns of trace metals depend on the grape cultivar (Ko et al., 2007; Vystavna, Rushenko, Diadin, Klymenko, & Klymenko, 2014).

It is well known that mineral nutrients and other trace metals are absorbed by plants, and the mobility and extractability from soils influence their bioavailability (Kabata-Pendias, 2004). The total metal content is a poor indicator of metal bioavailability because it basically depends on the chemical association with the different soil components. Therefore, the distribution of metals among soil components is important to assess the soil potential for supplying enough mineral nutrients and to evaluate the toxicity to plants. Various single and sequential extraction procedures have been proposed to assess the mobility and bioavailability of metals in soils.

Many multistep extraction procedures have been widely used for sediment and soil analysis (Gleyzes, Tellier, & Astruc, 2002), but the establishment of standard and unified sequential extraction criteria for non-polluted agricultural soils is required. So, the three-step sequential extraction procedure proposed by the Community Bureau of Reference (BCR, now superseded by the Standards, Measurement and Testing Programme) was initially developed for sediment analysis, but it has been also applied to soils (Rauret et al., 2000; Sahuquillo, Rauret, Rehenert, & Muntau, 2003). The BCR sequential extraction procedure was modified in order to improve the reproducibility of the results obtained (Sahuquillo et al., 1999). Both BCR sequential extraction methods (original and modified) allow the differentiation of extractable metals (available for plants) according to the following fractions:

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acid soluble, reducible or associated with Fe-Mn oxides, and oxidizable or associated with organic matter.

To considerably simplify the experimental task associated with the use of multistep extraction procedures for estimating the labile fraction of metals, leaching tests based on single extractions have been developed. In this sense, the use of ethylenediaminetetraacetic acid (EDTA) and acetic acid single extraction procedures is widely extended, and even certified reference materials with EDTA and acetic acid extractable contents of metals are available for quality control purposes (Pueyo et al., 2001; Quevauviller et al., 1997).

Although numerous attempts have been made to establish correlations between metal fractionation in soils and their uptake by plants (Álvarez, López-Valdivia, Novillo, Obrador, & Rico, 2006), the problem still remains unsolved because a general behavior has not been found. In fact, the absorption of metals by plants depends on several factors including the type of plant and the soil properties and, therefore, each soil-plant system can be different. There are a few published studies on the bioavailability of trace metals in the vineyard soil-vine-grape system (Orescanin, Katunar, Kutle, & Valkovic, 2003; Tokalioglu, Kartal, & Güneş, 2004; Vystavna et al., 2014) in which the acid soluble and exchangeable fractions were determined using dilute hydrochloric or nitric acids and ammonium acetate, respectively, as single extractants. Particularly, the application of organic wastes to improve the physical properties of soil affects the total content and extractable fractions of metals in amended vineyard soils (Ramos, 2006).

Therefore, the present work was focused on: i) the determination of the extractable contents of some macronutrients (Ca and Mg), micronutrients (Cu, Zn, Mn and Fe) and heavy metals (Pb) in vineyard soils amended with different organic fertilizers (cattle and sheep manures) using the modified BCR sequential extraction method as well as EDTA and acetic acid single extractions; ii) the determination of total metal contents in leaves and grapes of two Galician white grapevine varieties (Treixadura and Loureira) grown in the above mentioned soils; iii) the establishment of correlations between extractable metal contents from soils (using single and sequential procedures) and those accumulated in leaves and grapes. This study could strongly contribute to the development of empirical models to predict the bioavailability of Ca, Mg, Cu, Fe, Mn, Zn and Pb in the vineyard soil-vine-grape system because the different responses of the grapevine varieties to the intake of essential and non-essential elements from soils amended with different animal manures are considered.

2. Materials and methods

2.1. Study area

The study area is located in the Ribeiro region (Ourense province), in North-West Spain (42° 21' 41" N; 8° 7' 2.5" W). This region has an Atlantic climate characterized by abundant rainfalls, particularly in winter and spring seasons. The annual precipitations are about 600 mm. Daily average temperature varies between 7 and 13 °C in the cold season and ranges from 15 to 24 °C in the hot season. The high intensity rainfall causes significant erosion, which leads to nutrient losses and alterations of the soil surface. Therefore, the application of organic amendments is a common practice to improve the physical properties of the soil and to enrich it in mineral nutrients.

Two Galician white Treixadura and Loureira varieties (*Vitis vinifera* L.), grown in the same vineyard, were studied. The vineyard soil was amended with different organic fertilizers (cattle and sheep manures) at a dose of 50 Mg/ha. The cattle manure was applied four years before sampling to some Treixadura vines of

twelve years old (sample 1). The sheep manure was applied one and four years before sampling to other Treixadura vines of six years old (samples 2 and 3, respectively) and one year before sampling to all Loureira vines of six years old (sample 4). In February, a mineral amendment (600 kg/ha of Lithothamne 400, Timac Agro, Navarra, Spain) to provide Ca and Mg, and an organo-mineral fertilizer composed of N, P, K and humic/fulvic acids (1000 kg/ha of VITALOR ATB K, Timac Agro, Navarra, Spain) were applied to the soil. Grapevine treatments were also used to control vine fungal diseases. When the vines began to sprout, non-phytotoxic organic fungicides were applied as treatment against mildew at intervals of 12–15 days. At the end of spring, organo-cupric fungicides were used, whereas in summer, when the vegetation growth slowed, copper-based fungicides (oxychlorides and sulfates) were preferred against mildew. Anti-oidium treatments were also used: powdered sulfur was applied at the beginning of May, and organic fungicides were then applied in May–July at intervals of 25–30 days. Finally, a foliar fertilizer was applied in August (400 mL/hL of Fertileader, Timac Agro, Navarra, Spain).

2.2. Sample collection and pretreatment

The vineyard topsoil was sampled at the depth interval 0–20 cm using a handle steel soil sampler. At the four sampling locations (samples 1–4), soil samples (samples S) were collected in two consecutive seasons (winter, samples S1A–S4A; spring, samples S1B–S4B) to evaluate the influence of the season on the mobility of nutrients and heavy metals. The soil samples were diagonally collected from three rows representing the one-fourth, middle and three-fourths rows into each sampling location of the vineyard. The three subsamples were combined to obtain a composite sample (approximately 3 kg). In the same sampling zones (samples 1–4), leaf samples (samples H) were also collected in spring (samples H1B–H4B) and at harvest (samples H1C–H4C), and grapes (samples G) were sampled at harvest (samples G1C–G4C). Thirty leaves and ten bunches per sample were randomly picked up from ten plants.

Once in the laboratory, soil samples were initially air-dried and then oven dried at 40 °C. The dry soil samples were sieved using a 2 mm stainless-steel sieve. Leaf samples were oven dried at 40 °C and cut in small pieces (1–3 cm). Afterwards, the soil and leaf samples were separately ground using a S-100 ball-mill (Retsch, Haan, Germany) and sieved with a 70 µm nylon-sieve. All the berries of each cluster were manually separated from the stalk, and the skins and seeds were manually removed from the pulp using a plastic spatula. Skins, seeds and pulps were separately oven dried at 40 °C, powdered using an agate mortar and a ball-mill, and sieved with a 500 µm stainless steel-sieve. All samples were stored in polyethylene bottles in a desiccator until analysis.

2.3. Reagents and standards

All chemicals were of analytical-reagent grade and purchased from Panreac (Barcelona, Spain) and Merck (Madrid, Spain). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, United Kingdom). The stock standard solutions of metals (1000 mg/L) were prepared by dissolving the appropriate amount of the pure metal or a high purity salt in dilute acid and stored in a refrigerator at 4 °C. The stock standard solution of mercury (1000 mg/L) was supplied by Merck. The working standard solutions for each individual element were prepared daily by appropriately diluting the stock standard solutions.

The stannous chloride (100 g/L) reducing agent for mercury determination was prepared daily by dissolving the appropriate amount of stannous chloride dihydrate (Panreac) in a minimum

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