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Impact of vineyard abandonment and natural recolonization on metal content and availability in Mediterranean soils

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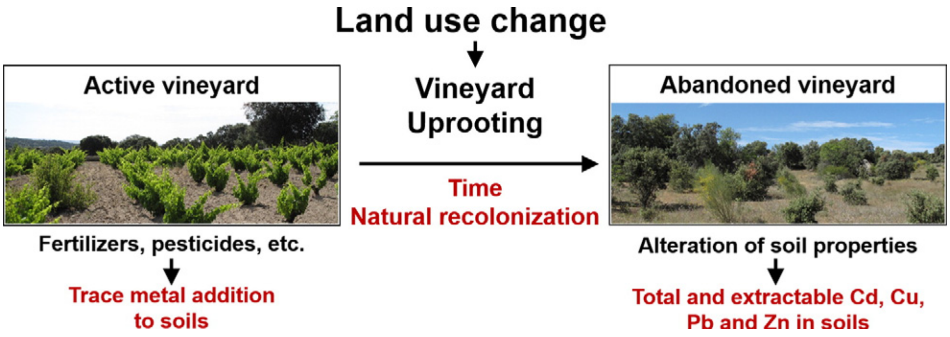
HIGHLIGHTS

- We studied the contribution of vineyard abandonment to metal concentration patterns.
- Age abandonment enhances soil metal total content and extractability.
- The impact of land use change depended on the type of vegetation cover.
- Metal concentration patterns are better explained when considering soil properties.
- Clay and organic fractions are key players in soil extractable metals.

GRAPHICAL ABSTRACT



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ABSTRACT

Abandonment of vineyards after uprooting has dramatically increased in last decades in Mediterranean countries, often followed by vegetation expansion processes. Inadequate management strategies can have negative consequences on soil quality. We studied how the age and type of vegetation cover and several environmental characteristics (lithology, soil properties, vineyard slope and so on) after vineyard uprooting and abandonment contribute to the variation patterns in total, HAc (acetic acid-method, HAc) and EDTA-extractable (ethylenediaminetetraacetic acid-method) concentrations of Cd, Cu, Pb and Zn in soils. We sampled 141 points from vineyards and abandoned vineyard Mediterranean soils recolonized by natural vegetation in recent decades. The contribution of several environmental variables (e.g. age and type of vegetation cover, lithology, soil properties and vineyard slope) to the total and extractable concentrations of metals was evaluated by canonical ordination based on redundancy analysis, considering the interaction between both environmental and response variables. The ranges of total metal contents were: 0.01–0.15 (Cd), 2.6–34 (Cu), 6.6–30 (Pb), and 29–92 mg kg^{-1} (Zn). Cadmium (11–100%) had the highest relative extractability with both extractants, and Zn and Pb the lowest. The total and EDTA-extractable of Cd, Pb and Zn were positively related to the age of abandonment, to the presence of *Agrostis castellana* and *Retama sphaerocarpa*, and to the contents of Fe-oxides, clay and organic matter (OM). A different pattern was noted for Cu, positively related to vineyard soils. Soil properties

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Abbreviations: Am-Fe, amorphous Fe-oxides; ANOVA, analysis of variance; CEC, cation exchange capacity; EDTA, ethylenediaminetetraacetic acid; HAc, acetic acid; ICP-MS, inductively coupled plasma mass spectrometry; OM, organic matter; RDA, redundancy analysis; RP, recalcitrant pool organic C; SPSS, Statistical Package for the Social Sciences; TCd, TCu, TPb, and TZn, total Cd, Pb, Cu, and Zn, respectively; TOC, total organic C.

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successfully explained HAc-extractable Cd, Cu, Pb and Zn but the age and type of vegetation cover lost significance. Clay content was negatively related to HAc-extractable Cu and Pb; and OM was positively related to HAc-Cd and Zn. In conclusion, the time elapsed after vineyard uprooting, and subsequent land abandonment, affects the soil content and availability of metals, and this impact depended on the colonizing plant species and soil properties.

1. Introduction

Spain is one of the world's largest wine producers, surpassed only by France and Italy (Spanish Wine Market Observatory, 2014). However, in recent decades an overall decrease has been reported in the number of hectares of Spanish vineyards (~10,000 ha per year), and a displacement from European Union countries – which have lost 30,000 ha per year – to other countries (e.g. Chile, Argentina and China), with increases of 10,000 ha per year (Lissarrague García-Gutiérrez and Martínez de Toda Fernández, 2010). The most significant effects have been seen in Spain, France, Italy, Portugal, Bulgaria and Hungary (International Organisation of Vine and Wine, 2013). There are several reasons for this decline: loss of vineyard productivity due to soil depth, slope, parent material, water availability and other factors; population migration to the cities and the lack of generational replacement; and the control of wine production to suit market needs caused by the European Union strategies implemented in the most recent reforms of the organisation of the common market (Martínez-Casasnovas et al., 2010). These consist of i) promoting the restructuring and conversion of vineyards to increase production; ii) regulating the number of cultivated hectares; and – in the case at hand – iii) subsidising the uprooting of vines in non-productive vineyards, thus encouraging abandonment.

The abandonment of agricultural soils is generally followed by one of two trends, with contrasting effects depending on environmental conditions and management: land degradation and desertification due to the spread of overgrazing, steep slopes, climate, and the parent material, which seriously hinders the establishment of natural vegetation (Dunjó et al., 2003); or vegetation expansion processes that facilitate ecosystem recovery if proper management is applied (Escribano-Avila et al., 2014; Pardini et al., 2003). The recovery of the vegetation cover in abandoned agricultural soils often results in the improvement of key soil properties and characteristics such as organic matter – OM – (content and composition), aggregation stability, infiltration, and cation exchange capacity (Kosmas et al., 2000; Lesschen et al., 2008).

However, the alteration of soil properties under land abandonment could affect patterns of total content and availability of trace metals (Fernández-Calviño et al., 2008, 2012) potentially accumulated in agricultural soils through anthropogenic activities, including repeated applications of fertilizers, pesticides, sewage sludge and animal manure (Bai et al., 2010; de Santiago-Martín et al., 2015a). Vegetation colonizing the soils can significantly reduce soil pH through a variety of mechanisms directly related to plant strategies, including rhizodeposition (increasing labile organic fractions), fixation of atmospheric N, etc., which could increase trace metal availability and leaching (Chantigny, 2003; Li et al., 2011; Strobel et al., 2005). In contrast, vegetation cover enhances aggregation stability and prevents erosion processes (Boix-Fayos et al., 2001), contrary to that typically observed in agricultural soils (Fernández-Calviño et al., 2012). This can favour metal retention in soils and should be considered if soils are reused for cultivation, as previously reported (Komárek et al., 2008). Plant cover may also reduce the metal content in surface soils through direct uptake by plants, as observed by Duplay et al. (2014) studying Cu and Zn uptake by grass roots in vineyard soils. The possible scenarios are highly determined by the time elapsed after the land abandonment and the type of subsequent potential vegetation cover (Lesschen et al., 2008). Studies contributing to a better understanding of the effects of different land management strategies after vineyard uprooting and abandonment (reforestation, directed revegetation, semi-natural vegetation, use of amendments, maintenance of certain structures such as terraces, etc.) are essential to minimize negative consequences on soil quality. However, the studies on this subject are scarce (Fernández-Calviño et al., 2008; Michaud et al., 2007).

To ensure proper soil management it is therefore essential to gain greater knowledge of the way in which the type and age of the colonizing vegetation cover and the soil and other environmental characteristics contribute to the concentration patterns in total and available fractions of trace metals in abandoned vineyards. With this aim we selected as our study area a typical Mediterranean scenario that had undergone major changes in land use in recent decades, evolving from a landscape primarily of vineyards with patches of natural vegetation, to its current situation in which most of the area is dominated by sclerophyllous Mediterranean vegetation with occasional vineyards. We sampled 141 points including both vineyard soils and abandoned vineyard soils recolonized by natural vegetation. The contribution of several environmental variables (age and type of vegetation cover, lithology, soil properties, vineyard slope and so on) to the total, HAc and EDTA-extractable concentrations of Cd, Cu, Pb, and Zn in soils was interpreted statistically and discussed.

2. Material and methods

2.1. Study area, sampling design, and soil characteristics

The survey was taken in a 2.5 × 2 km area in the municipality of Navas del Rey located in the Alberche valley (western Madrid region, Spain) at an altitude of 709 m (Fig. 1). The site is typical of a Mediterranean pluviseasonal-oceanic bioclimate in an upper meso-Mediterranean low dry bioclimatic belt (Worldwide Bioclimatic Classification System, 2009). Average annual temperature is 13.2 °C (23.5 °C in the warmest month of the year, July, and 4.6 °C in the coldest month, January) and total annual rainfall is 401 mm year^{-1} (11 mm in the driest month, July, and 48 mm in the month of highest rainfall, May) (Climate-data, 2014). The area is in the contact zone between metamorphic rocks (mainly schist) from the pre-Cambrian period, and igneous rocks (mainly granites). The cultivation of vineyards, along with livestock, has been the main activity in the area, especially from the 19th century. However, in recent decades the area has seen drastic changes in land use, from a landscape of mainly vineyards with scattered areas of natural vegetation to its current situation in which most of the area is dominated by natural vegetation with occasional vineyards. The landscape is composed of Holm oak in mosaic with rain-fed crops (vineyards) and pastures, accompanied by isolated stands of juniper and pine trees, and several species of shrubs such as broom, lavender and others. Soils are characterized by having coarse texture and good drainage, predominantly Lithosols and Regosols according to FAO (Consorcio Sierra Oeste, 2009). Table 1 shows the main soil characteristics. Soil pH values varied from slightly acid to neutral, and reactive soil fractions were in the following range: 2 to 10% (clay), 0.3 to 9.7% (total organic C, TOC), and 83 to 1707 g kg^{-1} (crystalline Fe oxides).

The sampling design consisted of an aggregate survey in 19 plots and a 500-m grid overlaid on the first one. Out of the 141 points sampled in 2010, 102 belong to one of the 19 plots considered, while 39 points belong to the 500 m grid (Fig. 1). Soil samples were taken on each plot under six different types of vegetation cover, selected according to the

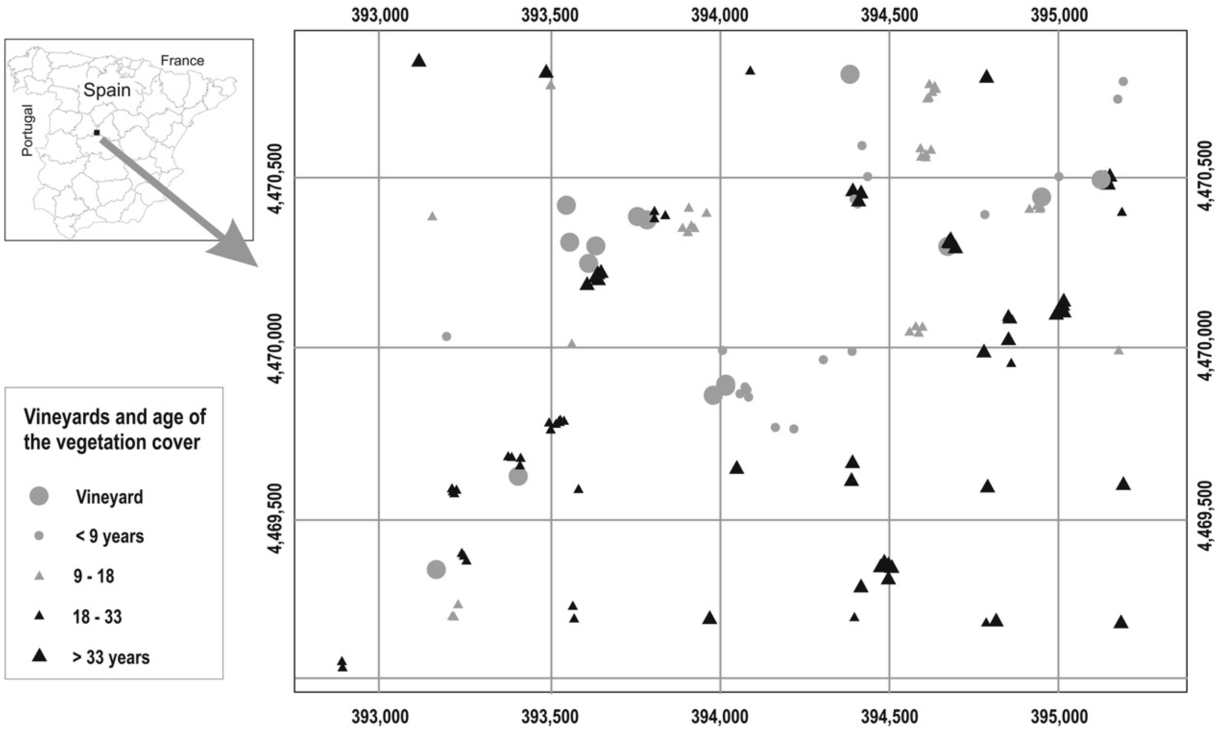

Fig. 1. Map of the study area showing the location of the soil sampling points (n = 141): vineyards (large grey dots) and abandoned vineyard soils with different ages of natural vegetation cover.

dominant species in the landscape: for woody plants we distinguished Holm oak, conifers (pine and juniper trees), broom, and lavender; and for herbaceous communities we distinguished perennial bentgrass and annual grassland. If there was a vineyard nearby, we also took a soil sample. In general, we sampled 3 to 5 points per plot, except in one in which we took 2 samples and another in which we took 8. Approximately 2.5 kg of soil sample was taken from the surface horizon (0–5 cm) at each sampling point. Field characteristics such as topographic description (slope and aspect), lithology and the plant species under which the soil sample was taken were recorded at each location: slope (≤5%, 5–10%, 10–15%, or >15%), aspect (flat, north, east, south, or west), lithology (schist, granite, or transition) and the vegetation cover (herbaceous communities dominated by therophytes and *Agrostis castellana* Boiss. & Reut., *Lavandula stoechas* L., *Retama sphaerocarpa* L., *Quercus rotundifolia* Lam., the conifers *Pinus pinea* L. and *Juniperus oxycedrus* Sibth. & Sm., and vineyard).

Table 1
Physicochemical characteristics of soil samples (n = 141).

Parameter		Unit	Mean	Median	SE	Min	Max
pH			6.30	6.29	0.03	5.46	7.10
Clay		%	5.27	5.15	0.14	1.57	10.05
CEC		$cmol_+ kg^{-1}$	4.63	4.21	0.17	0.26	9.71
Organic fraction	TOC	%	2.03	1.92	0.10	0.28	5.24
	LPI		0.29	0.27	0.01	0.06	0.60
	LPII		0.25	0.25	0.01	0.10	0.39
	RP		0.48	0.49	0.01	0.24	0.94
	N		0.13	0.13	0.01	0.01	0.35
	C/N		14.89	14.92	0.27	9.14	29.00
Oxide fraction	Cry-Fe	$mg kg^{-1}$	626.92	528.40	35.89	82.96	1707.38
	Cry-Mn		164.93	149.10	7.35	29.67	368.90
	Am-Fe		16.00	12.68	1.15	0.05	52.91
	Am-Mn		0.67	0.59	0.05	0.03	1.91

SE = standard error; Min = minimum; Max = maximum; CEC = cation exchange capacity; TOC = total organic C; LPI = labile pool I organic C; LPII = labile pool I organic C; RP = recalcitrant pool organic C; Cry-Fe = crystalline Fe oxide; Am-Fe = amorphous Fe oxide; Cry-Mn = crystalline Mn oxide; and Am-Mn = amorphous Mn oxide.

To investigate the influence of the age of abandonment we consider a chrono-sequence of abandoned vineyard soils. We used aerial photographs of the sampled plots in the study area on different dates to quantify how many years each plot has been covered by natural vegetation and/or vineyards. The time span between the first aerial photograph (1957) and the sampling date (2010) is 53 years. The age range of the vegetation cover in years was: 0, <9, 9–18, 18–33, and >33 years. For the >33 year range it cannot be confirmed whether the plots were once vineyards.

2.2. Analytical methods

Soil samples were air-dried, sieved at 2 mm, and stored until analysis. Soil physicochemical analyses were done following International Soil Reference and Information Center (ISRIC)-methods (2002). The composition of the OM was characterised by determining three organic pools from low to high degrees of polymerization following a modification of the two-step sulphuric acid hydrolysis procedure proposed by Rovira and Vallejo (2000), as previously described in detail (de Santiago-Martín et al., 2015b).

The available concentrations of Cd, Cu, Pb and Zn were estimated by one-step chemical extractions of different strengths (de Santiago-Martín et al., 2013): acetic acid (HAc)-method (0.11 mol L^{-1} HAc solution, 1:40 w/v, 16 h) (Rauret et al., 1999); and ethylenediaminetetraacetic acid (EDTA)-method (0.05 mol L^{-1} EDTA solution, 1:10 w/v, 1 h) (Quevauviller et al., 1996) respectively. The soil samples and corresponding extraction solution were shaken in a vibrator agitator (Vibromatic, Selecta) at 400 oscillations per minute (opm), then the supernatant was centrifuged (3500 rpm, 15 min) and filtered (low ash filters, 5–7 μm).

The pseudo-total content (thereafter called total content) of Cd, Cu, Pb and Zn was determined by a certified laboratory (Eurofins Analytico, The Netherlands). The samples were subjected to a pseudo-total destruction with a pre-treatment consisting of *aqua regia* and microwave digestion. The Cd, Cu, Pb and Zn concentrations in the extracts (EDTA, HAc and digestions) were quantified by inductively coupled plasma mass spectrometry (ICP-MS) by Eurofins Analytico laboratories (protocol NEN-EN-ISO 17294–2). These tests are accredited by the Dutch Accreditation Council (RvA). Quality assurance and quality control (QA/QC) measures performed by Eurofins Analytico included blank samples, laboratory control samples (standard reference samples), device controls (calibration, sensitivity, interference), internal standards, and so on (TerrAttesT® soil method).

All chemicals were obtained from analytic grade reagents from Merck (Germany) and Sigma-Aldrich (St. Louis, MO, USA). All glassware used was pre-washed with an aqueous solution of HNO_3 0.1% for 24 h and rinsed with deionized type I water (Water Purification System, Younglin, Aqua MAX-Basic 360 series).

2.3. Statistical analysis

The total, HAc and EDTA-extractable concentrations of Cd, Cu, Pb and Zn were analysed using one-way and two-way analysis of variance (ANOVA), with age and type of vegetation cover as factors. The homogeneity of variances was verified by the Levene test. These analyses were done using the Statistical Package for the Social Sciences v.17 (SPSS, Inc.) software.

The contribution of environmental factors to the concentration patterns of metals was evaluated by redundancy analysis (RDA), a type of canonical ordination. In these analyses, the canonical axes obtained in the ordination are a linear combination of the explanatory variables (Ter Braak, 1994). We performed a standardised RDA based on a correlation matrix in which the axes 1 and 2 are canonical and the axes 3 and 4 are free. The relationship between the eigenvalues of these two types of axes can indicate the relationship between the response and the explanatory matrices. These kinds of analyses have demonstrated their utility in other soil interactions and metal availability analyses (de Santiago-Martín et al., 2015b; González et al., 2007). Explanatory variables, associated with different units, were standardised with mean 1 and variance 0. As the response dataset, in the three analyses performed, we used the total, HAc and EDTA-extractable concentrations of Cd, Cu, Pb and Zn. As explanatory, or environmental, variables we used quantitative and also qualitative, or nominal, variables (Table 2), such as the age and type of vegetation cover, lithology, aspect and slope. In addition, soil parameters and properties currently considered to play a key role in metal availability (de Santiago-Martín et al., 2013) were included: pH, clay, OM (total content and pools), Fe and Mn oxides and cation exchange capacity (CEC). When the extractable concentration of trace metals was the response variable the total contents of these metals were considered in the dataset of environmental variables. The environmental variables were selected and ranked by the forward selection method according to their importance in explaining the response variables for each ordination, and were validated by Monte Carlo permutation tests ($n = 499$). These analyses were made using CANOCO 4.5 software, and the biplots were drawn with Canodraw (Ter Braak and Smilauer, 2002). In the correlation biplots, each vector points towards the maximum variation in the variable values. The length of the vectors indicates the strength of the correlation and vectors pointing in the same direction indicate correlation between both. Pearson's correlation analyses were also performed using SPSS for supporting the data interpretation.

Table 2
Dataset of the environmental variables.

Category	Environmental variables	Statistical code
Quantitative environmental variables		
	Age of vegetation cover	Years
	Aspect	Aspect
	Slope	Slope
	pH	pH
	Clay	Clay
	Total organic C	TOC
	Labile pool I organic C	LPI
	Labile pool II organic C	LPII
	Recalcitrant pool organic C	RP
	Crystalline Fe oxides	Cry-Fe
	Amorphous Fe oxides	Am-Fe
	Crystalline Mn oxides	Cry-Mn
	Amorphous Mn oxides	Am-Mn
	Cation exchange capacity	CEC
	Total Cd	TCd
	Total Cu	TCu
	Total Pb	TPb
	Total Zn	TZn
Nominal environmental variables		
Lithology	Schist	Schist
	Granite	Granite
	Transition	Transition
Vegetation cover	*Quercus rotundifolia*	Quercus
	Juniperus oxycedrus and *Pinus pinea*	Conifer
	Retama sphaerocarpa	Broom
	Lavandula stoechas	Lavender
	Agrostis castellana	Agrostis
	Annual grassland	Grass
	Vineyard	Vineyard

3. Results and discussion

3.1. Total content and availability of trace metals

Fig. 2 shows the spatial distribution of the total, HAc and EDTA-extractable concentrations of Cd, Cu, Pb and Zn in soils of the study area. The total metal contents (Fig. 2a) were representative of the common contents reported for Mediterranean agricultural and forest soils (de Santiago-Martín et al., 2015a; Gandois et al., 2010) and periurban soils in Madrid (Vázquez de la Cueva et al., 2013), with a range of 0.01 to 0.15 mg kg^{-1} (Cd), 2.6 to 34 mg kg^{-1} (Cu), 6.6 to 30 mg kg^{-1} (Pb) and 29 to 92 mg kg^{-1} (Zn). It is noteworthy that mean Cu contents (12 mg kg^{-1}) were in general lower than those usually found in Mediterranean vineyard soils: 104 to 632 mg kg^{-1} (Nóvoa-Muñoz et al., 2007); 14 to 700 mg kg^{-1} (Komárek et al., 2010); and 38 to 251 mg kg^{-1} (Brun et al., 2001; Michaud et al., 2007). This may be attributed to leaching processes favoured by the slightly acidic pH values of the soils.

EDTA and HAc-extractable Cd, Cu, Pb and Zn concentrations were in all cases lower than previously found in periurban agricultural soils in Madrid (de Santiago-Martín et al., 2013). The data range was as follows for EDTA (Fig. 2b): 0.01–0.07 mg kg^{-1} (Cd), 0.26–1.60 mg kg^{-1} (Cu), 0.76–7.20 mg kg^{-1} (Pb) and 0.52–5.10 mg kg^{-1} (Zn); and for HAc (Fig. 2c): 0.01–0.04 mg kg^{-1} (Cd), 0.17–3.76 mg kg^{-1} (Cu), 0.08–0.34 mg kg^{-1} (Pb) and 0.68–5.20 mg kg^{-1} (Zn). In general EDTA-extractable metal concentrations were higher or similar than that of HAc-extractions, except for Cu. A similar result with Cu extractions in low contaminated soils was previously reported (de Santiago-Martín et al., 2013). Some authors have attributed this result to readsorption processes of Me-EDTA complexes in the soil OM (Ettler et al., 2007). Further studies by comparing with soil pore water extractions or DGT-method (diffusive gradients in thin films) would be of great interest. The relative extractability was calculated for each metal (% extractable metal vs total metal content). Overall, the highest range of variation among samples and the highest percentages were found for the relative extractability of Cd, and the lowest for Zn. The sequences for EDTA values, from lowest to highest, were as follows: 1–9% (Zn) < 1–33% (Cu) < 7–46% (Pb) < 11–100% (Cd); and for HAc: 0.5–4% (Pb) < 1–11% (Zn) < 1–76% (Cu) < 11–100% (Cd). A higher Cd extractability than Zn, for example with HAc (the first step of the BCR sequential extraction method), has been previously reported by other authors, such as Žemberyová et al. (2006). A lack of correspondence between the total content and the HAc-extractable fraction of metals was revealed by Pearson's correlations – according to the literature (Kelepertzis et al., 2015) – except in the case of Cd (positive correlation at $p < 0.001$).

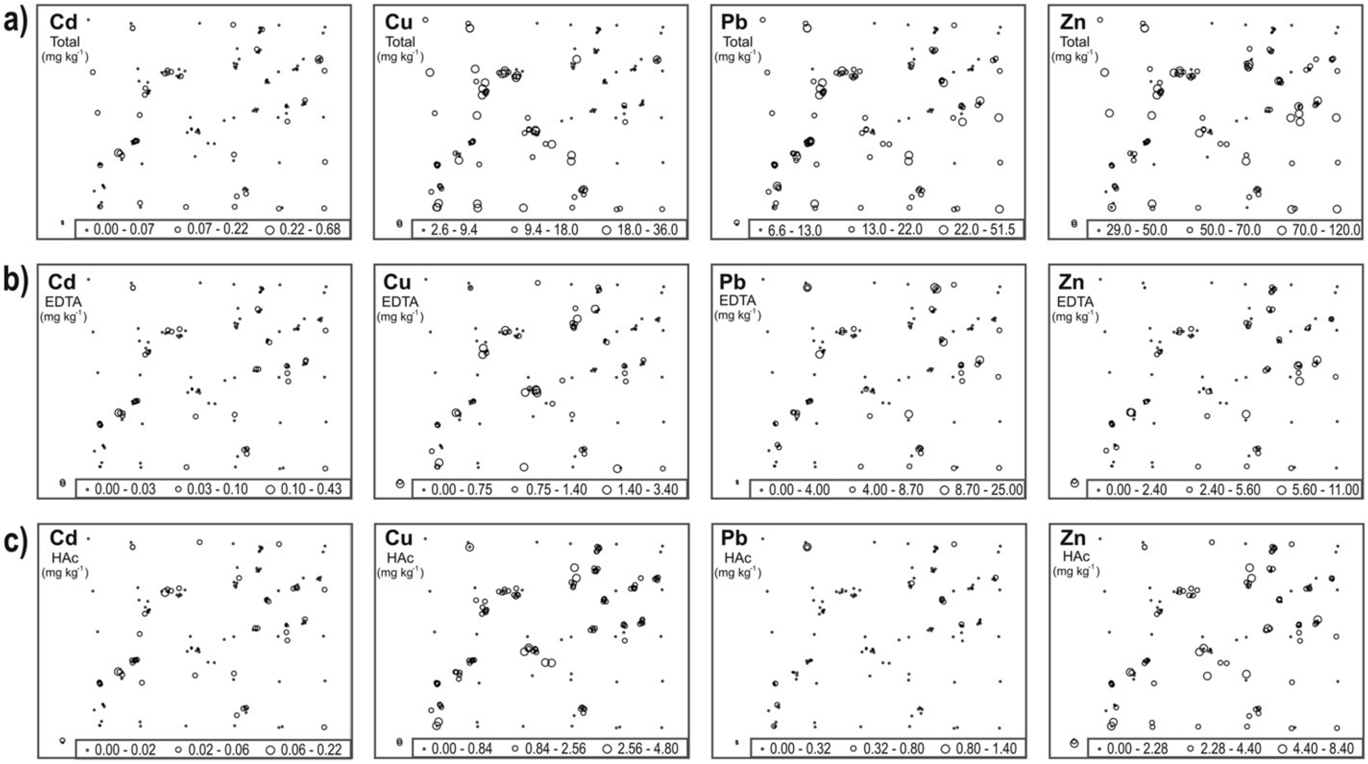

Fig. 2. Maps of the spatial distribution of the total (a), EDTA (b), and HAc (c) extractable concentration ranges (mg kg^{-1}) of Cd, Cu, Pb, and Zn in soils. Vineyard sampling points are marked in black.

Conversely, EDTA-extractable metals were positively and significantly correlated with total metal content ($p < 0.001$), except Zn.

3.2. Contribution of age and type of vegetation cover to metal content and availability

Fig. 3a shows the total, HAc and EDTA-extractable concentrations of metals grouped according to the plant species where the soil samples were collected; and Fig. 3b shows them grouped according to the age of the vegetation cover. Table 3 shows the results of the two-way ANOVA. Total Cd and Pb contents were significantly affected by the age of the vegetation cover ($p < 0.05$ and $p < 0.001$, respectively), but no significant relation was found with the type of vegetation (Table 3). In both cases, after ~9 years, the longer the time elapsed since the abandonment of the vineyard, the higher the total Cd and Pb contents (Fig. 3b). However, as shown in Fig. 3b not significant differences were obtained between abandoned plots after 33 years and vineyards. Neither the numbers of years nor the vegetation cover significantly contributed to the concentration patterns of the total Cu and Zn content in soils, contrary to previous reports (Komárek et al., 2008).

Both age and type of vegetation cover substantially affected EDTA-extractable Cd and Pb (Table 3). Significantly lower EDTA-Cd and Pb concentrations were found in vineyard soils (and in grassland in the case of Pb) than in soils taken under broom (case of Cd and Pb) (Fig. 3a). Overall, higher EDTA-Cd and Pb values were observed as the number of years increased (Fig. 3b). It is worth noting that there was no significant *Species* × *Years* interaction (Table 3). The plant species showed no effect on HAc-Pb, although the reverse was true for HAc-Cd, which was lower in vineyard soils. Low or null effect of both years and species was observed on Cu extractability, in line with the findings for total content (Table 3). Zinc extractability was significantly lower in vineyard soils than in soils under broom (HAc-Zn) and agrostis (EDTA- and HAc-Zn). The age of the vegetation cover was not observed to have any significant effect, although an increasing trend was noted for EDTA- and HAc-Zn with time. The wide range of variation observed in each group of data may account for the lack of significant differences in some cases and could indicate that other environmental factors such as soil properties or competing metals (in addition to and/or in interaction with age and type of vegetation cover) play an important role in governing metal concentration patterns.

3.3. Role of environmental variables in metal concentration patterns

A RDA was conducted to study the interaction of environmental variables (Table 2) in explaining metal concentration patterns (Table 4). Fig. 4 shows the biplots from total metal contents (Fig. 4a), EDTA-metal concentrations (Fig. 4b) and HAc-metal concentrations (Fig. 4c). The Monte Carlo permutation test indicated that both the first canonical axis and the sum of axes were in all cases statistically significant ($p < 0.01$) (Table 4). The results therefore support the idea that the patterns of total and extractable metal concentrations are better explained by considering the interaction among environmental variables as well as among the response variables.

Lithology and age and type of vegetation cover were the most important environmental variables in the ordination of the concentration patterns of total Cd, Cu, Pb and Zn (Fig. 4a). The most explanatory variables chosen by the forward selection method were ranked as follows, from most to least: granite, years, agrostis, am-Fe, broom, vineyard and clay. Overall, the total metal content was negatively related to plots dominated by granite, which suggests that metal content is higher in soils developed on schist, as usually reported (Alloway, 2013). As shown in the biplot (Fig. 4a), the vectors for total Cd, Pb and Zn contents had the same direction as the number of years (age of vegetation cover) and were positively influenced by the presence of broom and agrostis, as previously observed (Fig. 3a). Total content of Cd, Pb and Zn was also positively related to am-Fe oxides and clay contents. This result is consistent with the hypothesis that the progressive recovery of vegetation cover minimizes clay dispersion and erosion processes, which may

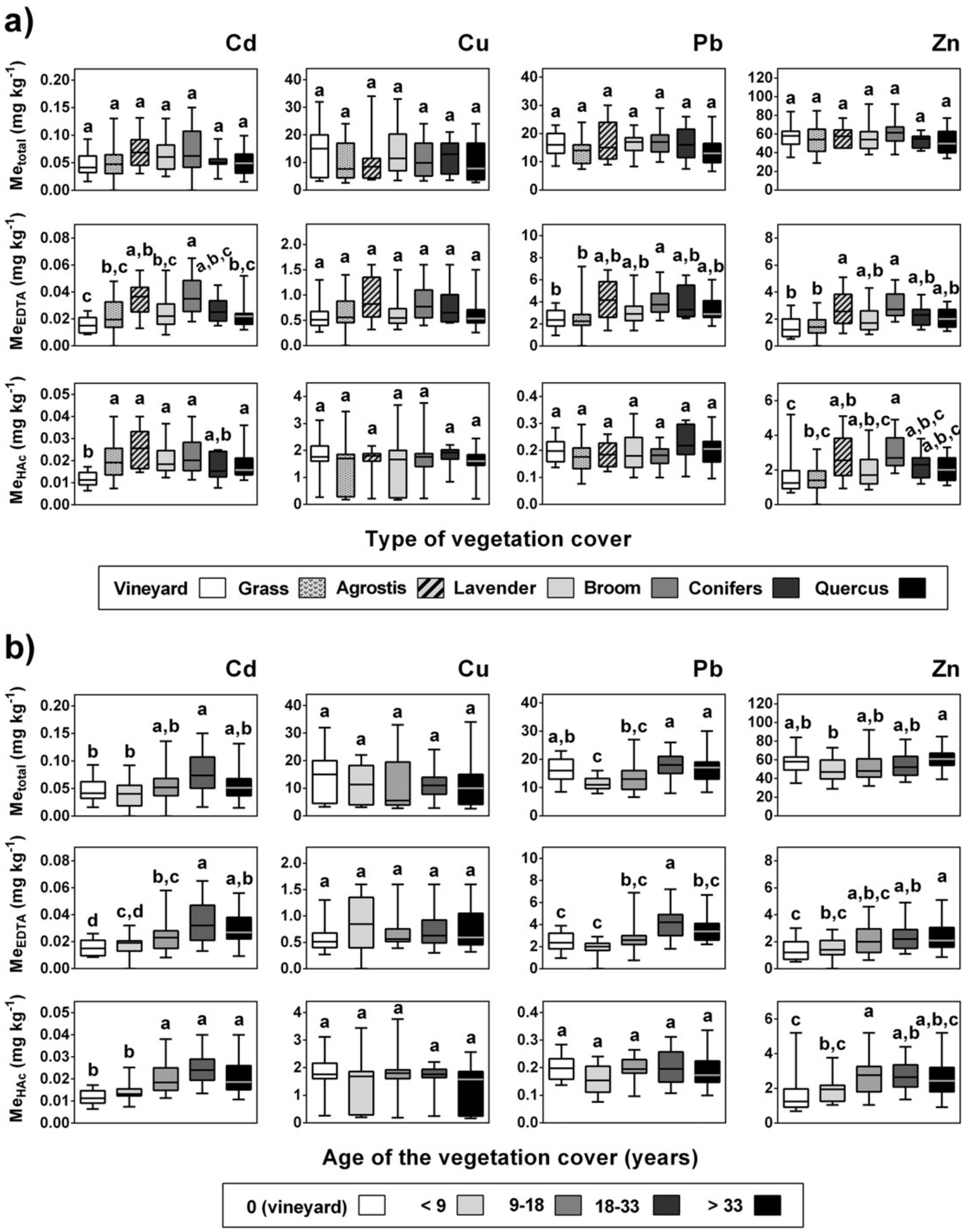

Fig. 3. Total, EDTA and HAc-extractable concentrations of Cd, Cu, Pb and Zn (mg kg^{-1}) in soils grouped according to the plant species where soil samples were collected (a) or age of vegetation cover (b). The boxplots show the lower, median, and upper quartiles, with whiskers extending to the most extreme data point. Different letters indicate significant differences among levels at $p < 0.05$ after one-way ANOVA.

favour metal accumulation in fine mineral fractions, contrary to what is often observed in agricultural soils (Lesschen et al., 2008; Vázquez de la Cueva et al., 2013). The positive and highly significant correlations ($p < 0.001$) between clay content and both the age of vegetation cover and the total content of Cd, Pb and Zn support this result. The RDA revealed that total Cu content was enhanced in vineyard soils (not revealed by ANOVA, Table 3), which can be attributed to the widespread use of fungicides based on Cu salts including sulphates. Fernández-Calviño et al. (2008) studied Cu content and distribution in young, old and abandoned acid vineyard soils. They reported much higher Cu contents in abandoned than in young vineyard soils (up to 2-fold), which was ascribed to: 1) the history of Cu treatments for protecting grapes, subsequent inputs into the soils, which are strongly influenced by the number of years elapsed since the vines were planted; and 2) the Cu accumulation in the organic and Fe-oxide fractions. Both factors are consistent with the pattern of total soil Cu contents determined in our study, although the relationship of Cu with soil colloids was weaker than for Cd, Pb and Zn. Similarly, Bech et al. (2008) didn't find significant relations between total Cu and clay fraction when they studied the levels and distribution of Cu, Ni, and Cr in soils in the province of Barcelona.

Similarities were found between biplots from total (Fig. 4a) and EDTA-metal contents (Fig. 4b) according to the previously observed correlations. The age of the vegetation cover and the presence of broom were important factors. More specifically, the rank of the most important explanatory variables for EDTA-extractable Cd, Cu, Pb and

Table 3
Results of two-way ANOVA calculated for the study variables (total, EDTA, and HAc concentrations of Cd, Cu, Pb, and Zn) considering the type of vegetation colonizing soils (*Specie*), the age of vegetation cover (*Years*), and their interaction (*Specie* × *Years*).

Factor	Variable	Total			EDTA			HAc		
		F	*p*		*F*	*p*		*F*	*p*	
Specie	Cd	2.232	0.056	ns	5.985	0.000	***	3.052	0.013	*
Years		3.844	0.012	*	6.775	0.000	***	6.834	0.000	***
Specie × *Years*		1.258	0.249	ns	0.755	0.705	ns	1.088	0.377	ns
Specie	Cu	1.139	0.344	ns	2.882	0.018	*	0.570	0.723	ns
Years		0.165	0.920	ns	1.637	0.185	ns	0.931	0.428	ns
Specie × *Years*		0.731	0.729	ns	1.600	0.096	ns	0.491	0.926	ns
Specie	Pb	1.656	0.151	ns	3.267	0.009	**	1.722	0.136	ns
Years		14.102	0.000	***	11.641	0.000	***	3.798	0.012	*
Specie × *Years*		1.167	0.313	ns	0.855	0.601	ns	1.304	0.223	ns
Specie	Zn	1.224	0.303	ns	5.538	0.000	***	3.898	0.003	**
Years		2.668	0.051	ns	1.173	0.324	ns	1.332	0.268	ns
Specie × *Years*		0.755	0.705	ns	1.028	0.431	ns	1.051	0.409	ns

EDTA = ethylenediaminetetraacetic acid; HAc = acetic acid. * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$ statistical significance at these probability levels; ns = no significant.

Table 4
Eigenvalues obtained for the canonical and the free axes in the RDA analyses performed with each of the response variable dataset (total, EDTA, or HAc concentrations of Cd, Cu, Pb, and Zn) and significance of the first and all canonical axes based on the Monte Carlo permutation test.

Dataset	Eigenvalues				*p* value (Monte Carlo)	
	Canonical axes		Free axes		First canonical axis	All canonical axes
	1	1 + 2	3	3 + 4		
Total	0.247	0.310	0.329	0.523	0.002	0.002
EDTA	0.348	0.418	0.264	0.397	0.002	0.002
HAc	0.205	0.374	0.250	0.409	0.002	0.002

EDTA = ethylenediaminetetraacetic acid; HAc = acetic acid.

Zn was TOC, CEC, years, broom, TCu, TPb, TCd, RP and pH. It was noteworthy that the significance of agrostis declined and that clay and Fe-oxide contents were replaced by TOC, CEC, and RP. Assuming vegetation cover enhances aggregate stability, the presence of broom may not only minimize clay dispersion but also increase OM content; both these colloids are directly related to CEC (Boix-Fayos et al., 2001; Kosmas et al., 2000). The positive and highly significant correlations found between years, clay, TOC and CEC ($p < 0.001$ in all cases) – as well as the fact that the highest TOC was quantified in soils taken under broom and agrostis (~1.4-fold) – reinforces this result (data pending publication). Frequent disturbances to agricultural soils have been signalled as a factor impeding the formation of stable organo-metal complexes, which are more abundant in forest soils (Chantigny, 2003). Under this scenario, clay and organic fractions in recolonized soils may determine the potential availability of metals (Luo et al., 2011). EDTA is a strong chelating agent and weak acid commonly reported to remove metals that are tightly bound to organic, oxide, and clay fractions (Feng et al., 2005). On the other hand, earthworm activity favoured in forest soils (due to the higher OM content) can increase the availability of metals (Ruiz et al., 2011). It is worth noting that the total metal content

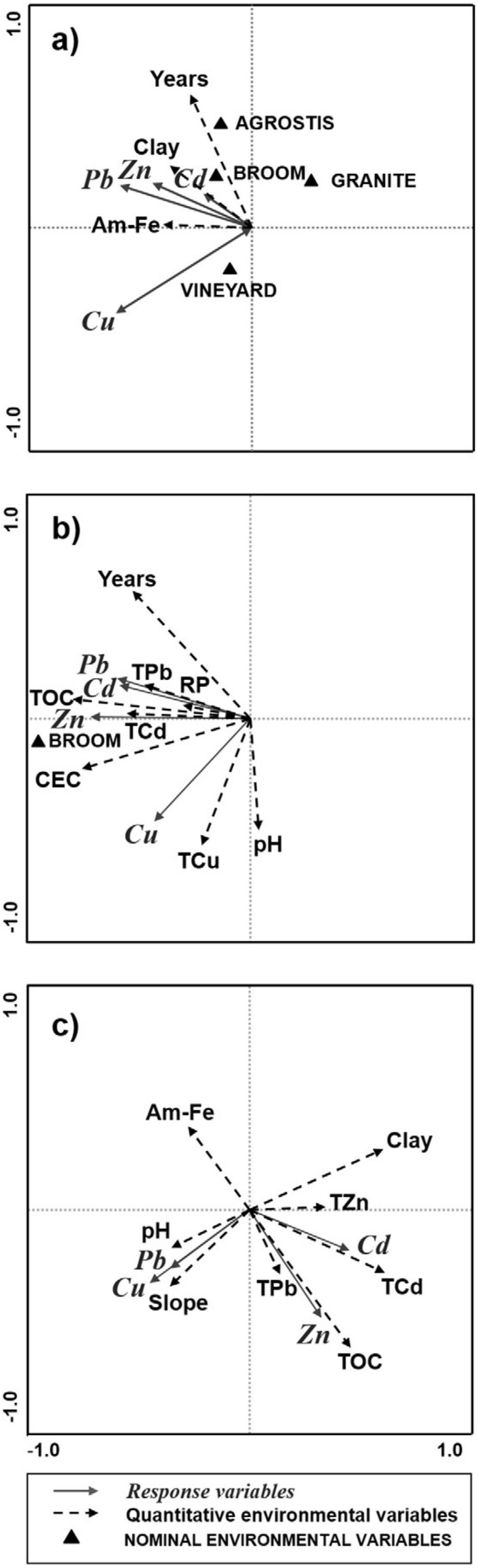

Fig. 4. Ordination diagrams from correlation-based analysis with the first two axes for total (a), EDTA (b), and HAc (c) extractable concentrations of Cd, Cu, Pb and Zn in soils as a function of environmental variables. Each diagram shows the response variables (grey italics) and the most statistically significant quantitative (black scripts, dashed arrows) and nominal (black scripts, triangle symbols) environmental variables. Am-Fe = amorphous Fe-oxides; TOC = total organic C; TCd, TCu, TPb, and TZn = total Cd, Pb, Cu, and Zn, respectively; and CEC = cation exchange capacity.

(Cd and Zn) contributed to the ordination diagram, but to a lesser extent. As previously reported, the relationship between total metal contents and metal (bio)availability in the Mediterranean area is improved when soil properties are considered (de Santiago-Martín et al., 2015b, 2013). EDTA-extractable Cu was more substantially affected by total soil Cu, concurring with the above. The negative and significant correlations found between pH and the age of the vegetation cover ($p < 0.001$) and TOC ($p < 0.05$) may account for the positive relation between pH and EDTA-Cu shown in the biplot.

In the case of HAc-extractions, the rank of the most important explanatory variables was TCd, clay, TOC, slope, pH, am-Fe, TPb and TZn. There was a marked loss of significance of the number of years and the species colonizing the soils, and a notable inclusion of the slope and the total metal content (Cd, Pb, and Zn); total Cd was the most important explanatory variable. Pearson's correlation analysis showed significant positive correlations between the number of years of abandonment and the slope ($p < 0.05$). A higher abandonment rate of vineyards in plots with higher slope could explain the inclusion of the variable slope in the biplot. Although the biplot (Fig. 4c) was different to those of total- and EDTA-metals, similarities can be found. Clay and TOC appeared in the ordination diagram. Two main patterns were observed: clay content opposite HAc-Cu and Pb, and TOC in the same direction as HAc-Cd and Zn. Although the Pearson's correlations showed no direct and positive relationship between total metal content and extractable fractions (except for Cd), the content of weakly adsorbed metals is related to metal competition processes for the adsorption sites in the soil and to the nature of the metal and the soil properties. Cadmium and Zn are generally mobile in soils (Pueyo et al., 2004), but the sorption–desorption processes regulating their degree of availability largely depend on the composition of the organic fraction. As concluded by Wong et al. (2007), the effect of OM on Cd and Zn sorption is closely linked to soil characteristics such as pH and clay, but also to competing cations. In the case of low or moderate metal contents, Cu and Pb are retained more in soils due to weaker competitive interactions with other metals for adsorption sites such as the clay fraction (Jalali and Moradi, 2013). In this situation, the clay content may decrease the HAc-extractable concentration of Cu and Pb, while increasing that of Cd and Zn. This could explain why despite the significant differences in the HAc-extractable concentrations of Cd and Zn with the age and type of vegetation cover (Table 3), the total metal content and soil fractions with higher affinity for Cu and Pb are more important in the overall explanation of HAc-extractable metal concentration patterns (Fig. 4c). As a result, the same soil parameters (clay and TOC) govern both the HAc and EDTA fractions of metals, albeit in a different manner.

4. Conclusions

A correspondence was generally observed between total and EDTA-extractable concentrations of Cd, Cu and Pb in soils, while only total Cd was correlated with HAc-extractable Cd. The contribution of age and type of vegetation cover to the concentration patterns of metals was successfully explained by the interaction among the environmental variables (including soil characteristics) and the response variables. The total content and EDTA-extractable concentrations of Cd, Pb and Zn were positively related to the age of the vegetation cover and to the presence of *Agrostis castellana* and *Retama sphaerocarpa*, which was attributed to the role played by vegetation in: i) minimize clay dispersion and erosion processes, and ii) enhance aggregation stability and organic matter contents. Total and EDTA-extractable Cu concentrations were favoured in vineyard soils. The HAc-extractable Cd, Cu, Pb and Zn fractions were significantly governed by the same soil fractions (clay and organic matter), but differently and to a lesser extent by the age and type of vegetation cover. In the (abandoned) Mediterranean vineyard low-polluted soils we studied, the change in land use (vineyard uprooting and land abandonment) affected the content and availability of Cd, Cu, Pb and Zn, and this impact depended on the time elapsed after the land abandonment, the colonizing plant species and soil properties. These findings should be taken into account in the management of these soils. Since it has been provided evidence that recolonizing species and lithology play an important role, further studies in this direction would be of great interest, e.g. plant metal content assessment and mineralogical or geochemical research.

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