Soil contamination by heavy metals in vineyard of a semiarid region: An approach using multivariate analysis

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ARTICLE INFO

Article history:

Received 18 July 2016

Accepted 18 November 2016

Available online 22 November 2016

Keywords:

Tropical soils

Trace elements

Fungicides

Arid environments

Entisols

Ultisols

ABSTRACT

Contamination of vineyard soils by the continuous use of cupric fungicides and fertilizers has been a worldwide concern. The objective of this study was to determine the total concentration of Cu, Zn, Fe, Mn, Ni and Pb from vineyard soils of a semiarid region in Brazil. Soil samples at 0–20 and 20–40 cm depth were collected in areas under 5, 6, 8, 10, 12, 15, 16 and 30 years of cultivation, and compared with native vegetation areas. Samples were digested and total metal concentrations were determined by atomic absorption spectrophotometer. In general, concentrations of Mn, Ni, Fe and Pb were similar to those values found in the reference area, being regarded as background concentrations. On the other hand, Zn and Cu were mainly derived from the widespread use of fertilizers (e.g., phosphate application) and cupric fungicides, respectively. Discriminant analysis clearly demonstrated higher metal accumulation in surface soil samples, chiefly Zn and Cu owing to Zn and Cu-containing chemicals and accumulation of organic matter. This tool was also useful to differentiate between natural and anthropogenic inputs of metals into soils. The high enrichment factor values for Cu and Zn showed that both were mainly derived from anthropogenic sources.

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1. Introduction

The use of cupric fungicides for diseases control of vine has significantly contributed to increase the content of this metal in soils (Mihaljević et al., 2006; Komárek et al., 2010). Different cupric fungicides have been extensively applied to vineyards (Mackie et al., 2012; Navel and Martins, 2014). The harmful effects of this contamination reflect in the agricultural activity, including phytotoxicity by high concentrations, maintenance of microbiological processes as soil microorganisms can also be affected by contamination, and transfer of heavy metals at toxic levels to humans and animals via food chain (Nicholson et al., 2003; Fernández-Calviño et al., 2010; Fernández-Calviño et al., 2011; Fernández-Calviño et al., 2012; Wightwick et al., 2013).

Cu accumulation in soils cultivated with vine is directly related to the frequency of fungicide application, which, in turn, depends on local

climate conditions. For example, contents of up to 1500 mg kg⁻¹ Cu have been reported in France, where soil fungicides were applied for over 130 years (Flores-Veles et al., 1996). The level of soil contamination in vineyards of Galicia (NW Spain) also depended on the length of cultivation, which implies in repeated applications of Cu-based fungicides (Fernández-Calviño et al., 2008a). The total Cu content in vineyard soils from Spain ranged from 55 to 730 mg kg⁻¹ (Nóvoa-Muñoz et al., 2007; Fernández-Calviño et al., 2008b; Fernández-Calviño et al., 2009a; Fernández-Calviño et al., 2009b; Gómez-Armesto et al., 2015). In southern Brazil, with subtropical climate and annual average rainfall of 2000 mm, it was found values of up to 3200 mg kg⁻¹ Cu (Mirlean et al., 2007). In addition to Cu, other metals may be present in fungicide mixtures, other pesticides, or fertilizers, which can increase their contents in the soil (Mirlean et al., 2007; Jiao et al., 2012; Santos et al., 2013; Silva et al., 2014).

The São Francisco Valley, the largest center of irrigated fruit production in Brazil, is one of the most important segments for national agricultural production (Lacerda et al., 2004). Fruit production moves approximately US\$ 300 million per year in this region, and is currently the largest grape producer center in the country, contributing with over 90% of the Brazilian exports (Freitas et al., 2011; Brasil, 2014).

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Despite the importance of the region, studies on soil contamination by heavy metals in vineyard areas are scarce. Most works on contamination of vineyard soils have been carried out in temperate climate (Parat et al., 2002; Chaignon and Hinsinger, 2003; Komárek et al., 2010; Duplay et al., 2014). Thus, there is very little information available for tropical or subtropical climate (Mirlean et al., 2007; Amaral et al., 2011), and no information for vineyard soils in the semi-arid climate, where most of the Brazilian table grapes production occurs.

In this scenario, due to the importance of grape production in the São Francisco Valley, and to the expansion of this activity in the region, this study aimed to: (1) determine the contents of Cu, Zn, Fe, Mn, Ni and Pb accumulated in soils under different cultivation time in the semi-arid climate; and (2) to compare the data obtained for this climate zone in

Northeastern Brazil with other vineyard regions in the country and in the world.

2. Material and methods

The soil samples used for analysis were taken from areas cultivated with vine, located in the municipality of Petrolina, Pernambuco, Brazil (Fig. 1). Soil samples were collected in vineyards with cultivation times of 5, 6, 8, 10, 12, 15, 16 and 30, in two different environments: crop row of the cultivated area (CA), and reference area (RA); and at two depths: 0–20 and 20–40 cm. According to Soil Taxonomy, soils were classified as Entisols (areas under 5, 8, 10, 12 and 16 year of cultivation) and Ultisols (areas under 6, 15 and 30 years of cultivation). The

Fig. 1. Location of the study areas (1 = Colinas do Vale; 2 = Embrapa; 3 = Vale das Uvas; 4 = Fazenda Andorinha; 5 = Frutex; 6 = Bebedouro 1; 7 = Fruit Fort e 8 = Bebedouro 2).

reference areas (Caatinga natural vegetation) had the same soil and were from the vineyards neighboring area. Soil samples were air-dried and sieved (mesh size 2 mm). The pH values were analyzed in H₂O (1:2.5 soil:solution ratio); K⁺ and Na⁺ by flame emission photometry after extraction with Mehlich-1; Ca²⁺, Mg²⁺ and Al³⁺ by titration after extraction with 1 mol L⁻¹ KCl solution; H + Al by the calcium acetate method (0.5 mol L⁻¹, pH 7.0); and available P by colorimetry after extraction with Mehlich-1 (Embrapa, 1999). Organic carbon (OC) was determined by the Walkley-Black method. Cations concentrations were used to calculate base saturation, cation exchange capacity, and aluminum saturation percentage. The particle size distribution was determined using Calgon for chemical dispersion (Embrapa, 1997) (Table 1).

The study areas include five agricultural companies focused mainly on grapes export (Colinas do Vale, Vale das Uvas, Fazenda Andorinha, Frutex, and Fruit Fort), an experimental field of Semi-Arid Embrapa, and a settler of irrigation public projects (Bebedouro 1 and Bebedouro 2). These areas have different fertilization and liming histories. In general, the fertilization of the agricultural companies annually account for 40 m³ ha⁻¹ of organic matter (OM), 120 kg ha⁻¹ N, 150 kg ha⁻¹ K₂O, 210 kg ha⁻¹ magnesium sulfate, and P₂O₅ doses between 100 and 200 kg ha⁻¹; however, due to high contents of P in the soil (Table 1), in recent years, agricultural companies and Embrapa have not used this element. In the experimental field of Embrapa, 30 m³ ha⁻¹ OM, 100 kg ha⁻¹ N, 300 kg ha⁻¹ K₂O, and 222 kg ha⁻¹ magnesium sulfate are annually applied to the soil. The settler applies, on average, lower contents of OM (20 m³ ha⁻¹); for the other nutrients, annual doses are 140 kg ha⁻¹ (N), 125 kg ha⁻¹ (P₂O₅), 230 kg ha⁻¹ (K₂O), and 100 kg ha⁻¹ magnesium sulfate. Liming occurred according to the needs indicated by the soil analysis, and ranged between 500 and 1000 kg ha⁻¹. The K₂O applications are especially high due to the fact that this nutrient is one of the most required by vines. The addition of magnesium sulfate is necessary in these areas due to the high content of Ca, and thus there is the need to provide Mg in suitable contents in the exchange complex.

Three composite samples of each vineyard and of each reference area were collected. For sample collection, the cultivated area was divided into three equal plots. In each plot, 20 points randomly chosen in the rows were sampled in order to form the composite sample. Samples were air dried, destorroadas, sieved in a 2 mm mesh, and kept at room temperature. The total concentration of Cu, Fe, Zn, Mn, Ni and Pb in soil was measured in soil samples that were dried, sieved (2 mm) and ground in a mill with agate balls. Then, 0.25-g samples of

powdered soil were digested in a 10 mL acid solution (H₂O:HF:HClO₄:HNO₃, 2:2:1:1). The solutions were heated to 250 °C until fuming on a hot plate to complete dryness. Next, 4 mL of 50% HCl was added to the residue and the mixture heated for additional 10 min. After cooling, the solutions were increased to 10 mL in volume with the addition of 5% HCl (Ure, 1990). The total heavy metals contents were determined by an atomic absorption spectrophotometry device.

The enrichment factor (EF) was calculated to discriminate the source of heavy metals in the soils. To compensate the difference between the samples composition and the particle size, Fe was used for geochemistry standardization, although other elements, such as Li and Al, can also be used (Thuong et al., 2013; Dung et al., 2013). The concentration of a given chemical element is considered of natural occurrence when EF is less than or close to the unity; values greater than one indicate human influence.

$$EF = \frac{\left(\frac{\text{Metal}}{\text{Fe}}\right)_{\text{sample}}}{\left(\frac{\text{Metal}}{\text{Fe}}\right)_{\text{reference sample}}} \quad (1)$$

Laboratory analyses were carried out in factorial arrangement 8 × 2 × 2 (eight cultivation times, two environments, two depths) with three replications, totaling 96 experimental units. Experimental results were analyzed by applying the F test for the analysis of variance, and by correlation analysis and Tukey test ($p < 0.05$). The discriminant analysis was used to answer the following question: can the combination of heavy metals be used to discriminate areas with high concentrations of these metals, especially due to fertilizers and fungicides application?

3. Results and discussion

Cu contents in the soil ranged from 3.1 to 37.8 mg kg⁻¹, and from 2.5 to 19 mg kg⁻¹ at 0–20 and 20–40 cm depth, respectively, in the cultivated areas (CA); in reference area – Caatinga (RA), these values ranged from 0 to 13.7 mg kg⁻¹, and from 0.9 to 7.8 mg kg⁻¹, at 0–20 and 20–40 cm depth. Significant differences in relation to their RA were observed only for areas with 5, 8 and 10 years of cultivation at 0–20 cm depth, while at 20–40 cm depth, only the areas with 5 and 6 years of cultivation presented differences (Fig. 2a and b).

The highest Cu contents were found on the surface and decreased with the depth. The same result was reported by other authors (Pietrzak and McPhail, 2004; Mirlean et al., 2007; Komárek et al., 2008), and indicates the low mobility of Cu in the soil profile, probably due to fungicides application and to metal interaction with the organic matter. The Cu concentration in vineyard soils of the São Francisco Valley is lower than observed in soils from Spanish vineyards, which can be higher than 100 mg kg⁻¹ (Nóvoa-Muñoz et al., 2007; Fernández-Calviño et al., 2009a; Fernández-Calviño et al., 2009b; Gómez-Armesto et al., 2015). Copper is mainly associated with soil organic matter (Nascimento and Fontes, 2004; Komárek et al., 2008), and forms inner-sphere complexes, which results in lower phytotoxicity when compared with free Cu²⁺ (Komárek et al., 2008). In soils with shorter cultivation times, Cu content decreases more rapidly with the increase in depth than in the long-term cultivation soils (Fig. 2a and b). Similar result was observed by Pietrzak and McPhail (2004).

High Cu contents were found in areas with 5, 6, 8, 10, 12 and 16 years of cultivation, where grape production is intended to export, and which intensively uses agrochemicals (Fig. 2a and b). Areas with 15 and 30 years of cultivation, which had the lowest Cu concentrations, are small farms, whose production is commercialized only in the domestic market, and use fertilizers and pesticides in a less intensive way. The increase of Cu content in vineyard soils is associated with the use of different fungicides, especially cupric-based ones (Mirlean et al., 2007; Wightwick et al., 2010; Fernández-Calviño et al., 2012;

Table 1
Chemical and physical characteristics of soil samples from a vineyard region in Petrolina, Brazil.

Characteristics	Vineyards (cultivation times)							
	5	6	8	10	12	15	16	30
pH	7.26	5.98	7.13	6.35	6.74	6.33	6.71	6.5
P (mg dm ⁻³)	1035	238	378	535	374	77	290	248
K (cmol _c dm ⁻³)	0.27	0.26	0.3	0.23	0.49	0.44	0.18	0.46
Ca ²⁺ (cmol _c dm ⁻³)	4.67	3.52	6.05	4.1	5.02	2.55	4.12	3.28
Mg ²⁺ (cmol _c dm ⁻³)	1.32	1.00	1.73	1.6	1.52	1.13	1.68	1.08
Al ³⁺ (cmol _c dm ⁻³)	0.05	0.13	0.00	0.08	0.07	0.07	0.07	0.05
H + Al (cmol _c dm ⁻³)	0.69	0.85	0.93	1.79	1.02	1.29	0.96	1.07
Na ⁺ (cmol _c dm ⁻³)	0.28	0.24	0.27	0.31	0.31	0.24	0.2	0.29
OC (g kg ⁻¹)	9.15	10.23	10.47	13.32	10.05	6.41	9.84	7.34
OM (g kg ⁻¹)	15.77	17.63	18.05	22.96	17.32	11.05	16.97	12.66
CEC _{effective} (cmol _c dm ⁻³)	6.59	5.15	8.36	6.32	7.4	4.43	6.25	5.17
CEC _{total} (cmol _c dm ⁻³)	7.22	5.87	9.29	8.03	8.35	5.66	7.14	6.19
SB (cmol _c dm ⁻³)	6.54	5.02	8.36	6.24	7.34	4.36	6.18	5.12
V (%)	90.4	84.67	89.84	77.9	87.76	77.47	86.68	82.68
m (%)	0.77	2.43	0.00	1.34	0.93	1.51	1.06	0.97
Sand (%)	89.1	88.1	80.1	75.1	76.6	87.1	83.1	86.6
Silt (%)	4.00	5.00	9.00	10.00	10.5	5.00	5.00	5.5
Clay (%)	6.9	6.9	10.9	14.9	12.9	7.9	11.9	7.9

Fig. 2. Comparison of the means of the total content of Cu, Zn, and Mn in the cultivated areas (CA) and reference area - Caatinga (RA) in two soil depths in function of the cultivation times (CT). Means followed by the same letters are not significantly different. Tukey test (Alpha = 0.05).

Gómez-Armesto et al., 2015). The application of these pesticides, including the Bordeaux mixture ($\text{CuSO}_4 + \text{Ca}(\text{OH})_2$), which is commonly used in the study area, has resulted in increased Cu concentrations in vineyard soils (Komárek et al., 2008).

The interaction of Cu with organic matter in the soil can be inferred by significant correlations found in the cultivated area at both depths ($r = 0.55^{**}$ and 0.45^* , respectively- ** for $p < 0.01$ and * for $p < 0.05$). However, it should be noted the difference in the behavior for the relationship Cu and organic matter between CA and RA, which was positive for CA, and negative for RA ($r = -0.40^*$). In this context, Ramos (2006) suggests that the positive relationship between Cu and organic matter indicates that OM is the main metal reservoir of these soils. Several studies reported that organic matter is the major pool of Cu in soil as it accounts for roughly 50% of total Cu retention (Fernández-Calviño et al., 2008a; Fernández-Calviño et al., 2009b; Gómez-Armesto et al., 2015). This fact corroborates the lack of correlation between Cu and Fe in the cultivated area, suggesting that the binding of Cu to Fe oxides is not as significant as its binding to organic matter. Studies on agricultural soils show that, on average, >90% of the whole Cu added to the soil system bind to soil organic matter (Lofts and Tipping, 1998; Weng et al., 2001). On the other hand, the lower content of organic matter in Caatinga soils can justify the negative relationship with the total content of Cu in the soil.

Komárek et al. (2008) found high Cu concentrations ($168 \text{ mg Cu kg}^{-1}$) in non-active vineyard soil in the Czech Republic, which exceeded the critical limits set by the European Commission ($140 \text{ mg Cu kg}^{-1}$). Similar Cu concentrations were reported by Chaignon and Hinsinger (2003), Pietrzak and McPhail (2004), Rusjan et al. (2007), Wightwick et al. (2008), Fernández-Calviño et al. (2008a) and Gómez-Armesto et al. (2015) in vineyard soils of other countries. Mirlean et al. (2007) found extremely high concentrations of Cu (exceeding 3000 mg kg^{-1}) on the soil surface of vineyards in Bento Gonçalves-RS, significantly exceeding the maximum value of 1500 mg kg^{-1} reported in the literature, which was found in vineyard soils in France by Flores-Veles et al. (1996). Mirlean et al. (2007) consider the climate and the application of high fungicide volumes as a crucial factor for the high contamination by Cu in vineyard soils. The annual average rainfall in Bento Gonçalves-RS region is close to 2000 mm yr^{-1} . This fact stimulates the use of the Bordeaux mixture to decrease the attack of vine powdery mildew. At least $60 \text{ kg ha}^{-1} \text{ yr}^{-1}$ Cu sulfate is applied to the soil, which is an amount 2 to 4 times higher than that applied in other growing areas of the world (Pietrzak and McPhail, 2004).

In this work, the observed Cu concentrations are lower than those recorded by Mirlean et al. (2007), Flores-Veles et al. (1996), and Komárek et al. (2008). Unlike Bento Gonçalves region, which is located in southern Brazil, the Lower São Francisco Valley, located in the

Northeast, presents predominant semi-arid climate, characterized by rainfall scarcity and irregularity, with rainfall in the summer and strong evaporation as a consequence of high temperatures. This fact makes the proliferation of fungal disease in the vineyard areas be considerably smaller. Thus, the need for copper fungicides application is also reduced, and so is Cu concentration in vineyards soils of this region.

Zinc levels in the soil ranged from 29.1 to 161.8 mg kg⁻¹, and from 20.4 to 136.3 mg kg⁻¹ at 0–20 and 20–40 cm depths, respectively, in CA, while in RA, these values ranged from 5.9 mg kg⁻¹ and 8.1 to 40.1 mg kg⁻¹, at 0–20 and 20–40 cm depths, respectively (Fig. 2c and d). The total Zn content at 0–20 cm depth showed significant differences between CA and RA areas for almost every year of cultivation. However, at the depth of 20–40 cm, only the total contents of the areas with 5, 8, 10 and 12 years showed significant difference. In vineyard stands located in northwest Spain, the total Zn content in soils ranged from 60 to 149 mg kg⁻¹ (Fernández-Calviño et al., 2012). The authors reported that most of the element (76% on average) appeared as residual Zn; therefore, the potential environmental impact of Zn was reduced.

Similar trend to that of Cu was observed for Zn. The highest contents were found in the surface, and decreased with the depth (Fig. 2c and d). The considerable increase in the total content of Zn in cultivated areas, when compared with the contents in the references areas, suggests human influence on the addition of this metal to the soil, which is similar to what was observed for Cu. Zinc concentration in cultivated soils increases annually between 0.5 and 1 mg kg⁻¹ due to the use of fungicides and fertilizers containing zinc (Weingerl and Kerin, 2000). The high contents of P observed in the soil (Table 1) resulting from superphosphate application may be responsible for the increase of Zn with the soil cultivation.

In references areas soils, high correlations observed between the Zn and OM contents ($r = 0.48^*$ and $r = 0.65^{**}$), Fe ($r = 0.77^{**}$ and $r = 0.64^{**}$) and Mn ($r = 0.86^*$ and $r = 0.84^{**}$), at both depths, respectively, indicate high Zn affinity by the adsorption sites of the organic matter and of the Fe and Mn oxides. In the cultivated area, despite the increase of the total Zn content in the soil between the years of cultivation no significant correlation was observed between the Zn contents and the soil OM.

In this study, the total content of Mn exceeded those obtained by Oliveira and Nascimento (2006), who found maximum values in soils of Petrolina-PE of 70.5 mg dm⁻³ in the surface layer, and 50.7 mg dm⁻³ in the subsurface layer. However, by analyzing the reference soils of Pernambuco dryland (Sertão), in general, it was found values > 900 and 400 mg dm⁻³ in the surface and subsurface layers, respectively. Manganese content in the soil ranged from 112.6 to 145.2 mg kg⁻¹, and from 80.5 to 131.4 mg kg⁻¹ at 0–20 and 20–40 cm depths, respectively, in the cultivated areas; in references areas, these values ranged from 29.6 to 137.0 mg kg⁻¹, and from 28.6 to 136.3 mg kg⁻¹ at 0–20 and 20–40 cm depths, respectively (Fig. 2e and f). There were significant differences in areas with 5, 8, 10 and 16 years of cultivation for both depths analyzed.

Similar to Cu and Zn, the highest Mn contents were observed in the CA. This significant increase in total Mn content in the soil in these cultivated areas can be attributed to management practices used in vineyard cultivation. When metal is added to the soil using residues, agrochemicals, or simply by atmospheric deposition, if that metal is not removed from the environment by leaching or by culture removal, its content in the soil tends to increase (Revedo and Melo, 2006). In areas with 12, 15 and 30 years of cultivation at both depths (Fig. 2e and f), the total content of Mn should be considered as the natural influence of the geochemistry of the region, which does not evidence the presence of polluting sources (Pereira and Kawamoto, 2016), since there were no significant changes in the contents between the CA and its respective RA.

Nickel in soils is highly dependent on the content in the source material, and in soils of arid and semiarid regions, its content is high. Therefore, Ni concentration in the soil surface reflects both contamination processes and the soil formation (Kabata-Pendias and Pendias, 2001).

Nickel levels in the soil ranged from not detected to 8.97 mg kg⁻¹, and from not detected to 7.82 mg kg⁻¹, at 0–20 and 20–40 cm depths, respectively, in CA, while in RA, these values ranged from not detected to 7.40 mg kg⁻¹, and from not detected to 10.00 mg kg⁻¹, at 0–20 and 20–40 cm depth, respectively (Fig. 3a and b). The area with five years of cultivation, at 0–20 cm depth, was the only one which statistically differed, with significant increase in the total content of Ni with the cultivation of this area, when compared with reference area. At 20–40 cm depth, only the areas with 5 to 8 years of cultivation showed significant difference, and Ni content in the area with 5 years of cultivation increased, and in the area with 8 years of cultivation, Ni content decreased in relation to the reference area. The high nickel content in reference area, referring to 8 years of cultivation, may indicate the contribution of the source material of this soil. Ni removal by the cultures or by leaching to deeper layers in the soil profile can justify the reduction of this element in this cultivated area, given its high mobility (Antoniadis and Tsadilas, 2007).

The total content of Ni showed highly significant positive correlation with the soil organic matter content in the cultivated area at 0–20 cm depth ($r = 0.54^{**}$). This correlation indicates that the contribution of the organic matter on the surface of the cultivated soil contributed to Ni retention in the soil. Nickel such as other heavy metals forms complexes with several organic constituents of the soil (Mellis et al., 2004). According to Egreja Filho (2000), usually 50% of Ni in soil is in the residual fraction, 20% is bound to Fe and Mn oxides, and the rest is distributed in other fractions without an explicit preference. In this study, no relationship was found between Ni and Fe and Mn oxides.

Iron levels in the soil ranged from 1939.5 to 5725.5 mg kg⁻¹, and from 1458.7 to 7543.3 mg kg⁻¹ at 0–20 and 20–40 cm depths, respectively, in the CA, while in RA these values ranged from 732.17 to 5676.33 mg kg⁻¹, and from 870.17 to 6978.33 mg kg⁻¹, at 0–20 and 20–40 cm depths, respectively (Fig. 3c and d). Significant differences between the total content of Fe in the CA and in the RA were observed only in areas with 5, 10 and 16 years of cultivation at 0–20 cm depth, which is similar behavior to that of Mn. Oliveira and Nascimento (2006), working with Pernambuco soils, found in Petrolina maximum content of 3072.1 mg dm⁻³ in the surface layer, and of 971.7 mg dm⁻³ in the subsurface layer. In Sertão soils of the state of Pernambuco, it was obtained values > 7000 mg dm⁻³ Fe in the surface layer, and > 6000 mg dm⁻³ in the subsurface layer.

Pb content in the soil ranged from 5.7 to 52.6 mg kg⁻¹, and from 6.1 to 50.7 mg kg⁻¹ at 0–20 and 20–40 cm depths, respectively, in the CA. In reference area, these values were not detected at 52.6 mg kg⁻¹ and not detected at 50.7 mg kg⁻¹, at 0–20 and 20–40 cm depths, respectively (Fig. 3e and f). Total content of Pb in the area with 5 years of cultivation significantly differ from the RA at 0–20 cm depth, while for 20–40 cm depth, differences were noted in the areas with 5, 6 and 16 years of cultivation. Similarly to Ni, Mn and Fe in some areas, Pb contents were observed for both CA and RA, which again suggests the influence of the source material of these soils in the total content. In general, the metals presented similarities in their distribution between the depths, except for Cu, which remained on the soil surface. Homogeneous concentration with the depth can result either from leaching or from agricultural practices (Pietrzak and McPhail, 2004; Mirlean et al., 2007).

No significant correlation was found between Pb and soil organic matter contents. Different results are reported by Sipos et al. (2005), who claim that most part of Pb was bound to the organic matter instead of clay minerals. Unlike these authors, the relation of the total Pb content with the total Fe content was negative ($r = -0.44^*$). Possibly, Pb is in the form of precipitates with P, which may explain the high positive correlation between Pb and P in the soil of cultivated areas at both depths ($r = 0.65^{**}$ and 0.48^*), respectively. In fact, phosphorus has been widely used to ameliorate soils contaminated with Pb due to the strong affinity between these elements (Cao et al., 2003; Paim et al., 2003; Pierangeli et al., 2004; Lin et al., 2005). Moreover, high P contents available in the soils (Table 1) can influence Pb retention in the soils.

Fig. 3. Comparison of the means of the total content of Ni, Fe, and Pb in the cultivated areas (CA) and reference area - Caatinga (RA) in two soil depths in function of the cultivation times (CT). Means followed by the same letters are not significantly different. Tukey test (Alpha = 0.05).

It should be mentioned that high Pb contents were also observed in the references areas, but these contents may not be related to P due to the absence of correlation between Pb and P in these areas. Possibly, the quantized Pb in the soils of the RA is from the aluminosilicate crystalline structure, which suggests the contribution of the source material. In this case, as shown by [Pereira and Kawamoto \(2016\)](#), the Pb content can be considered as of natural influence of the geochemistry of the region, not evidencing the presence of contaminant sources.

It is noteworthy the negative and significant correlation between P and Fe in the cultivated areas ($r = -0.60^{**}$), which suggests the binding of P to Fe oxides. High positive correlations were also observed between the total content of Fe and Mn in the CA and in the RA at 0–20 cm depth ($r = 0.55^{**}$ and $r = 0.65^{**}$), evidencing the combination of these elements in the structure of the oxides. Fe also presented positive correlation with the soil organic matter content in the RA at both depths ($r = 0.49^{*}$ and $r = 0.50^{**}$, respectively). According to [Oliveira and Nascimento \(2006\)](#), the largest Fe content potentially available is bound to the organic matter both in surface and subsurface horizons. Similarly, the total concentration of Mn presented positive and highly significant correlations with the soil organic matter content in RA at two depths ($r = 0.70^{**}$ and $r = 0.57^{**}$, respectively). According to [Moreira et al. \(2006\)](#), Mn retained by the organic matter can be associated with its functional groups in the form of outer- and inner-sphere complexes.

Table 2

Enrichment factor Cu, Zn, Mn, Ni and Pb in the cultivated areas (CA) and reference area - Caatinga (RA) in two soil depths in function of the cultivation times (CT).

Enrichment factor					
Years	Cu	Zn	Mn	Ni	Pb
0–20 cm					
5	18.14	3.66	5.91	1.47	17.84
6	3.18	1.54	2.35	0.13	4.69
8	11.83	2.44	2.36	0.48	1.56
10	13.16	7.67	6.07	2.70	12.47
12	6.85	4.10	3.01	1.73	1.28
15	0.97	2.11	2.52	0.00	0.84
16	8.72	9.74	3.18	0.00	0.92
30	1.08	1.89	2.32	0.00	2.95
20–40 cm					
5	8.63	3.42	5.62	1.96	22.88
6	2.31	1.23	1.92	0.07	4.95
8	7.33	2.67	2.04	1.06	1.77
10	12.38	7.96	5.46	2.20	13.12
12	4.48	3.71	2.78	1.55	0.92
15	0.58	1.63	1.63	0.00	0.87
16	5.39	8.70	2.87	0.00	1.05
30	1.42	1.86	2.05	0.09	1.35

Enrichment factor based on the quality reference values for heavy metal established for Pernambuco State ([CPRH, 2014](#)). 5 = Colinas do Vale; 6 = Embrapa; 8 = Vale das Uvas; 10 = Fazenda Andorinha; 12 = Frutex; 15 = Bebedouro 1; 16 = Fruit Fort e 30 = Bebedouro 2.

3.1. Enrichment factor

To calculate the enrichment factor, it was adopted soil quality reference values (QRVs) of the state of Pernambuco regarding the presence of chemical substances for the environmental management of contaminated areas (CPRH, 2014). The high EF values for Cu and Zn confirmed the anthropic origin of these elements, as previously discussed by the mean comparison test between the CA and RA. QRVs apparently overestimate the EF values of Pb, both on the surface and on the subsurface (Table 2). Silva et al. (2015) highlight that despite the extreme importance of QRVs established for the state of Pernambuco, they cannot be adopted without a prior evaluation of their representativeness for specific conditions. It is also noteworthy that, unlike Zn, which showed almost similar values at both depths, Cu presented higher enrichment factor on the surface, indicating low mobility, particularly by the strong interaction with the soil organic matter (Nascimento and Fontes, 2004; Komárek et al., 2008).

Copper concentrations exceeded the QRV of this element for the soils of the state of Pernambuco (5 mg kg^{-1}) in areas under 5, 6, 8, 10, 12 and 16 years of cultivation, with values ranging from 11.27 to 46.50 mg kg^{-1} , and from 5.24 to 28.60 mg kg^{-1} , at 0–20 and 20–40 cm depth, respectively. In the areas under 15 to 30 years of cultivation, the concentrations at both depths were below the QRV for the state. In general, the same pattern was observed for Zn, which showed over 90% of the values above the QRV (35 mg kg^{-1}).

3.2. Discriminant analysis

The integrated use of univariate and multivariate techniques, such as discriminant analysis, assists in identifying areas with accumulation of metals from the application of fertilizers and fungicides. The combination of variables previously correlated with multivariate normal distribution was used to evaluate the accumulation of heavy metals in vineyard soils cultivated for over 30 years.

Fig. 4. Discriminant analysis in function of the cultivation time. (a) Soil depth of 0–20 cm; (b) soil depth of 20 to 40 cm.

The areas cultivated for 5, 6, 8, 10, 12 and 16 years indicated greater accumulation of metals in the soil, and were correctly classified (100% accuracy). The cultivated areas for 15 and 30 years had two samples reclassified: one sample of the area cultivated for 15 years was reclassified in the area cultivated for 30 years, and vice versa (Fig. 4a), confirming the different behavior of these cultivation times with lower concentrations of Cu and Zn at both depths, since it is a small rural farm, and consequently uses less fertilizers and fungicides. The total content of Mn, Ni, Fe and Pb found in the CA were similar to those of the RA, and did not increase with the cultivation time, and therefore the anthropic influence on these contents was not taken into account. The highest concentrations of Cu and Zn were observed in soil surface very probably due to the high application of fungicides (Valladares et al. 2009; Brunetto et al. 2014) and great accumulation of organic matter (Duplay et al. 2014).

The accumulation of metals was lower at 20–40 cm depth (Fig. 4b), where the highest classification errors were observed. The Wilks' lambda values indicated significant difference for the concentrations of metals at 0–20 cm and 20–40 cm depth in function of the cultivation time ($p < 0.05$). For areas with 5, 6, 8, 10, 12 and 16 years of cultivation, all centroids were close (group mean) to the respective points, showing the dimension in which these cultivation years may differ in relation to 15 to 30 years of cultivation, which had the lowest heavy metals concentration, especially Cu and Zn.

The comparison between metals concentration in the reference area (RA) and in the cultivated area (CA) confirmed the anthropic influence and the increase of metal concentration over time. Both at 0–20 cm and 20–40 cm depth, RA were correctly classified (100% accuracy). However, there was 25% classification error in the CA, especially those with 15 and 30 years of cultivation, which showed low Cu and Zn concentrations, and therefore is more similar to RA (Fig. 5a and b). Santos et al. (2013) also discriminated cultivated areas in function of the use of the soil. In regions of the State of São Paulo, vineyard areas presented higher Cu and Zn concentrations than non-cultivated areas. The increase of Zn contents seems to be associated with the large amount of phosphate fertilizers in the fields, while for Cu, this increase is probably due to fungicides application (Mackie et al., 2012).

The combined use of techniques provided better understanding to discriminate soils under influence of pesticide application in the São Francisco Valley. In practical terms, the use of discriminant analysis showed greater accumulation of metals, such as Cu and Zn, in function of the application of phosphate fertilizers and fungicides, which can assist in the monitoring of vineyards soils.

4. Conclusions

Copper and Zn contents, in most cultivated areas, showed increase with the years of cultivation. The increase of Zn contents seems to be associated with the large amount of phosphate fertilizers used in the areas, while for Cu this increase is probably due to the application of fungicides. On the other hand, Mn, Ni, Fe and Pb contents found in the cultivated areas were similar to those of the reference areas. Compared with other viticulture regions of the world and Southern Brazil, located in more humid climates, the soils of the São Francisco Valley have relatively low Cu contents.

Discriminant analysis clearly demonstrated higher metal accumulation in surface soil samples, chiefly Zn and Cu owing to Zn and Cu-containing chemicals and accumulation of organic matter. This tool was also useful to differentiate between natural and anthropogenic inputs of metals into soils. The high EF values for Cu and Zn showed that both were mainly derived from anthropogenic sources.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version <http://dx.doi.org/10.1016/j.geodrs.2016.11.002>. These data include the Google map of the most important areas described in this article.

Fig. 5. Discriminant analysis in function of the soil usage. (a) Soil depth of 0–20 cm; (b) soil depth of 20 to 40 cm.

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