Research Article

Assessment of Copper and Zinc in Soils of a Vineyard Region in the State of São Paulo, Brazil

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This soil acidification may increase the bioavailability of copper (Cu) and zinc (Zn) in soils. The objective of this study was to verify the concentrations of Cu and Zn in soils of a vineyard region, including sample acidification, to simulate acid rain. The study was developed in an area of vineyard cultivation, with an adjacent land having other crops grown, in the state of São Paulo, Brazil. Soil samples were collected and GPS located under different uses and coverings. The extracted solutions used to determine the available Cu and Zn forms were diethylenetriaminepentaacetic acid (DTPA), pH 7.3, and calcium chloride 0.01 M. The total forms were obtained by HNO₃ digestion. The amounts of Cu and Zn extracted using DTPA were considered high in most of the samples and were greater in the areas cultivated with vineyards that had received fungicide applications for several decades. The total forms were higher in vineyard soils. The amounts of Cu and Zn extracted using CaCl₂ did not have good correlation with vineyards or with other metals' forms. The results confirmed that the soil was enriched with Cu and Zn due to the management of the vineyards with chemicals for several decades.

1. Introduction

Soil conservation is fundamental for the sustainable development and preservation of ecosystems and biodiversity. The soil is exposed to contamination through several anthropic activities, mainly agriculture. The contamination of soil by heavy metals results in a high risk of its productive capacity and of the balance of the ecosystems [1].

Soil has a diverse heavy-metal concentration that is dependent on the parent material from which it is formed, the formation processes, and the composition and proportion of the components of the solid phase [2]. This concentration may be affected by several anthropic activities, including irrigation, fertilizer and chemical applications, and industrial or urban sewage incorporation [3, 4]. Moreover, the concentration, distribution, and bioavailability of heavy metals in the environment are influenced by the soil type, topography, geology, and erosive processes [5].

Cultivation may cause soil contamination by heavy metals, specifically copper in vineyard areas [1, 6, 7]. The intensive use of agrochemicals with Cu and Zn in their composition may pollute the soil [8–10]. Historical and current applications have resulted in Cu accumulation in the soil, and total Cu quantities have been measured in vineyards worldwide. High concentrations of fungicide-derived copper in orchard and vineyard soils have been reported from around the world, for example, Brazil, 36–3,215 mg kg⁻¹ [11]; Champagne/France, 100–1,500 mg kg⁻¹ [12]; India, 29–131 mg kg⁻¹ [13]; and Australia, 1–223 mg kg⁻¹ [14]. It has been suggested that areas with greater humidity and precipitation, such as Brazil or Champagne, France, exhibit higher Cu concentrations than dry environments, due to higher Cu use [10].

The acidification of soil and surface water is a serious concern in society today [15–17]. The acid rain and resulting soil acidification are a real problem in São Paulo state due to

TABLE 1: Descriptive statistics of soil attributes in the vineyard and other uses in the region studied.

Variable	Unit	Vineyard				Other uses			
		Minimum	Maximum	Average	STD	Minimum	Maximum	Average	STD
Organic matter	g L^{-1}	17.0	55.0	34.4	10.9	13.0	82.0	33.0	14.2
CEC	cmolc L^{-1}	6.07	31.2	12.2	6.49	4.55	15.1	8.05	2.64
Base saturation	%	37.0	95.0	67.5	16.8	10.0	93.0	42.9	21.0
Clay	g kg^{-1}	150	313	223	41.8	125	450	254	66.9
Silt	g kg^{-1}	75.0	226	142	38.7	74.0	243	154	41.7
Sand	g kg^{-1}	562	729	635	41.9	346	788	591	82.9
CuT	mg kg^{-1}	10.0	40.5	20.7	8.41	4.65	80.3	13.4	11.7
ZnT	mg kg^{-1}	14.3	243	53.4	43.0	6.48	225	45.7	40.2
pH CaCl_2		4.20	6.50	5.16	0.62	3.70	6.40	4.70	0.63
pH CaCl_2 after acidified		3.63	6.97	4.96	0.92	3.33	6.60	4.31	0.70
CuDTPA	mg kg^{-1}	2.80	15.5	6.79	3.27	1.30	15.4	2.69	2.18
CuDTPA after acidified	mg kg^{-1}	2.59	22.1	9.11	4.89	1.26	20.0	3.47	2.82
ZnDTPA	mg kg^{-1}	4.80	25.0	11.8	5.63	1.40	24.8	5.39	4.75
ZnDTPA after acidified	mg kg^{-1}	5.02	29.9	13.7	6.47	0.97	28.0	5.64	5.37
CuCaCl	mg kg^{-1}	0.01	0.12	0.08	0.03	0.01	0.61	0.06	0.10
CuCaCl after acidified	mg kg^{-1}	0.03	0.08	0.05	0.01	0.02	1.23	0.07	0.19
ZnCaCl	mg kg^{-1}	0.01	2.58	0.73	0.73	0.01	8.97	1.17	1.67
ZnCaCl after acidified	mg kg^{-1}	0.01	3.11	1.37	1.11	0.03	4.54	1.09	1.05

STD: standard deviation.

the intense industrialization [18], and the soils in the region studied here are considered the most sensitive to acidification in Brazil [19].

Statistical and geostatistical techniques can assist in the interpretation of research in soil pollution by heavy metals [1, 20, 21].

The objective of this study was to verify the concentrations of Cu and Zn in the soils of a vineyard region, including the acidification of the samples, with the objective of simulating acid rain.

2. Materials and Methods

The study was performed in a 59.8 ha catchment area in Jundiá, São Paulo state, Brazil ($23^{\circ}11' \text{ S}$, $46^{\circ}53' \text{ W}$), which is occupied by 2.9 ha vineyard (Figure 1) that is ten to sixty years old, has natural vegetation, pastures, and other types of orchards in the vicinity, and is 672–755 m altitude. The landscape is rolling and hilly in a geomorphological province that is dominated by a “half-orange” type of relief. The soil types in the area are inceptisols, ultisols, and oxisols [22], and the main original rock is the schist. The descriptive statistics of soil attributes are presented in Table 1.

The spatial data were uniform made and were GPS located based on satellite images with high-spatial resolution. An interpretation of land use and cover was made with detailed field checking based on an IKONOS II mosaic (orbital point 159539, on July 4, 2001, at 13:19 PM and on November 08, 2001, at 13:24 PM). The land use/land cover map and the topographic data were utilized to plan the soil sampling, ultimately establishing a total of one hundred sample points. These points were georeferenced and integrated in a vectorial format in the geographic information system (GIS).

FIGURE 1: Area of study in a vineyard region of São Paulo state, Brazil.

At this stage, the area was traversed. With the help of an auger, 67 georeferenced perturbed soil samples at 0–0.15 m depth were collected, including 37 from the vineyard and 30 from the land under other crops grown. The samples were air dried, crushed, and passed through a 2 mm sieve.

The 67 soil samples (100 g each), after being dried and sieved, were assembled in glass percolation columns and treated with 20 mL of HNO_3 0.1 mol L^{-1} and 100 mL of

deionized water to eliminate the H^+ excess and alter their pH, as described by Camargo and Raji [23]. The samples were incubated for 15 days for a complete acidification reaction and were then dried, sieved, and chemically analyzed for the bioavailability of Cu and Zn. The CuT and ZnT concentrations were determined using HNO_3 according to the procedure described in the U.S. EPA 3051 method [24]; the available form was determined using DTPA pH 7.3 (CuDTPA and ZnDTPA) [25] and $CaCl_2$ 0.01 mol L^{-1} (CuCaCl and ZnCaCl) [6, 26] extractions. The Cu and Zn levels in the digestion extracts were determined by ICP-OES.

A descriptive statistical analysis was used for calculation. The discriminate analysis (DA) is a method of dependence analysis and is a special case of canonical correlation. In this study, the DA was first used to reveal whether the land use patterns differed significantly in terms of Cu and Zn concentrations. To analyze the spatial variability, the geostatistical analysis [27, 28] was used through the elaboration and adjustment of semivariograms and data interpolation by kriging for mapping.

3. Results and Discussion

3.1. General Soil Characteristics. The physicochemical characteristics and element contents are summarized in Table 1. The selected soils were naturally acidic to slightly acidic (1 M CaCl_2 pH range 3.7–6.5), with an average value of 5.1, with textures that varied from loam (common in soils developed from schist or slate) to sandy loam (typical for granite soils). The soil samples taken in this region had large sand contents ($346\text{--}788\text{ g kg}^{-1}$), and the loam or sandy loam textures that are typical of soils were developed over granites.

Cation exchange capacities (CEC) ranged from 6.07 to $31.2\text{ cmol}_c\text{ L}^{-1}$ (Table 1). The highest values of CEC were found in vineyard soil.

Soil organic matter contents were generally high but varied widely, ranging from 13 to 82 g L^{-1} (Table 1). The soil organic matter content is useful to give an idea of the texture of the soil, with values up to 15 g L^{-1} for sandy soils, between 16 and 30 g L^{-1} for medium texture and $31\text{--}60\text{ g L}^{-1}$ for clay soils. Values above 60 g L^{-1} indicate the accumulation of organic matter in the soil, generally by poor drainage or high acidity. The highest values of organic matter were found in forest soils (82 g L^{-1}) and vineyard soils (55 g L^{-1}).

3.2. Total Cu and Zn Concentrations. The total Cu content in the vineyard and in soil under other uses in the region varied between 10 and 40.5 mg kg^{-1} and between 4.7 and 80.3 mg kg^{-1} , respectively (Table 1).

The total Cu values are generally higher than or similar to those reported by Kabata-Pendias and Pendias [29] for natural soils, whose typical values ranged from 13 to 24 mg kg^{-1} , depending on the soil type. On the other hand, the total Cu values are generally lower than or similar to those reported by Brun et al. [26] in Southern France (30 to 250 mg kg^{-1}) and by Fernández-Calviño et al. [7] in the Northwest Iberian Peninsula (25 to 666 mg kg^{-1}). The differences could reflect

different application rates as well as different physical and chemical parameters in the soils, but we do not have enough information to discern diverse possibilities.

The total Zn content in the vineyard and in soil under other uses in the region varied between 14.3 and 243 mg kg^{-1} and between 6.48 and 225 mg kg^{-1} , respectively (Table 1). The total Zn values are generally higher than or similar to those reported by Alloway [30] for natural soils, whose typical values ranged from 10 to 300 mg kg^{-1} , depending on the soil type. Only 25% of samples exceeded the average Zn concentrations reported by Alloway [30] for soils (50 mg kg^{-1}). The total Zn values are generally higher than or similar to those reported by Fernández-Calviño et al. [31] in vineyard soils (60 to 149 mg kg^{-1}).

The total concentrations of Cu and Zn in the area compared to the soil reference values (mg kg^{-1}) from São Paulo state range from 35 to 60 mg kg^{-1} and from 60 to 300 mg kg^{-1} [32], respectively. It was observed that only three topsoil samples (3%) had a concentration higher than the reference value for Cu and that only one sample presented a concentration higher than the prevention values. The higher total Cu concentrations were observed in soils under vineyard, palm, and *Typha*. The area with palm had previously been cultivated with vineyards for decades, and the soil under *Typha* vegetation represents a wetland that is in a lower altitude position of the study area, which most likely received contaminated sediments from the higher parts of the landscape. These results clearly demonstrate the risk of contaminating the adjacent areas and the power of Cu permanency in the soil.

Because many vineyards are located on steep slopes, intensive erosion further influences Cu mobility in these soils and thus increases the risks associated with groundwater contamination.

The effect of landscape on copper levels in soils is unclear. Sediments in Galicia (Spain) river valley were found to have higher copper levels than nearby vineyard soils [33]. Rusjan et al. [34] found that amongst plains, plateaus, and terraces, copper levels were highest on terraces.

The copper content in vineyard soils is attributed to factors such as frequency of pesticide application and local climatic conditions. Studies from France and Italy demonstrate that vineyard soils in wet regions contain more copper than in dry regions [26, 35]. Deluisa et al. [35] attributed this effect to differences in soil type and precipitation, the latter encouraging greater use of copper. However, Pietrzak and McPhail [36] did not see a difference in copper application between humid regions and drier regions. The vineyards situated in the region where humid climate dominates (Gippsland region) generally do not receive greater annual application of copper-based fungicides than vineyards located in sub-humid conditions (Rutherford region). The local factors of each vineyard (e.g., slope, exposure, amount of sun received, surrounded vegetation, and wind breakers) play important roles in controlling any outbreaks of diseases and at the same time influence the use of fungicides. Other authors have suggested that erosion, leaching, and plowing are the main determinants [37].

For the Zn total concentration, 14 topsoil samples (21%) presented concentrations higher than the reference values in both vineyard and soil used to grow pears. These results indicate that agricultural management contributed to soil contamination with Cu and Zn.

3.3. Available Cu and Zn Concentrations and Soil Attributes. Although the total Cu and Zn soil concentrations are a fair indication of availability, including deficiency or oversupply, they do not provide conclusive information about the environmental impact of this availability. The Cu and Zn availability to the biota, when used as nutrients or toxic elements, and their mobility are important factors to consider when investigating the effect of these metals on the environment. In this context, the available concentrations of Cu and Zn seem to be the best indicator to make inferences about the potential environment impacts of these elements.

The concentrations of Cu and Zn extracted using DTPA may provide information about the elements' availability for plant nutrition [38] and their potential to pollute the soil [39]. The Cu concentration in topsoil varied from 1.3 to 15.5 mg kg⁻¹, with a mean of 4.3 mg kg⁻¹ (CuDTPA), and from 0.01 to 0.61 mg kg⁻¹, with a mean of 0.06 mg kg⁻¹ (CuCaCl) (Table 1). The highest concentrations of Cu extracted using DTPA occurred in soils under the same use as that used to obtain the total Cu. However, for Cu extraction with CaCl₂, the highest Cu concentration which occurred in soils under experimental vineyards differs from the highest total and DTPA Cu concentrations which occurred in experimental and commercial vineyards.

The Zn concentration in topsoil varied from 1.4 to 25.0 mg kg⁻¹, with a mean of 8.1 mg kg⁻¹ (ZnDTPA), and from 0.01 to 8.97 mg kg⁻¹, with a mean of 1.0 mg kg⁻¹ (ZnCaCl) (Table 1). The higher concentration of available Zn occurred in soils under vineyards and natural vegetation, indicating that the high available Zn concentration may be explained by anthropogenic pollution. Despite the application of agrochemicals, the same finding was observed for the total Zn, that is, the natural concentration of the element once it also occurs in some natural vegetation land uses (Table 1).

According to Student's *t*-test ($P < 0.05$), topsoils in vineyards had higher concentrations of the total form of Cu as well as that extracted by DTPA and CaCl₂ than did the other land uses. The same result was observed for Zn extracted by DTPA but not for Zn extracted by CaCl₂ or for its total form. These results show a reasonable soil contamination in vineyard areas by Cu and a probable elevation in the available Zn concentration.

The average concentrations of available forms of Cu and Zn in vineyard soils tend to be higher than the concentrations observed in soils under other uses, with exception of CaCl₂-extractable Cu and Zn, where the higher mean concentrations (1.23 and 4.54 mg kg⁻¹) were observed in soils under natural vegetation (Table 1).

Generally, the vineyard soils have higher pH and CEC than what is observed in soils under other uses. High CEC encourages metals to bind with soil aggregates, depending on the organic matter and the clay content of the soil. High pH

enhances the dissociation of organic acids and, therefore, the formation of complexes with metals, altering metal speciation and reducing bioavailability [40].

3.4. Available Forms of Cu and Zn after Acidification. Considering all of the surface samples acidified (Table 1), there is a slight increase in Cu content in the soil, which ranged from 1.26 to 22.1 mg kg⁻¹, with a mean of 5.7 mg kg⁻¹ (CuDTPA), and from 0.02 to 1.23 mg kg⁻¹, with a mean of 0.06 mg kg⁻¹ (CuCaCl). As observed in the natural, nonacid samples, the maximum values of Cu were found in areas that were occupied by vineyards and *Typha*.

The soil acidification revealed higher concentrations of DTPA-extractable Cu and Zn, which were not observed for CaCl₂-extractable Cu and Zn. These results indicate that the DTPA method was more sensitive to soil acidification for simulating acid rain than was CaCl₂. These results disagree with those obtained by Brun et al. [26] that indicated the CaCl₂ extractant as the best for the available forms of Cu in vineyard soils. Such disagreement is probably due to differences between the original pHs of these soil regions.

The statistical method used to compare the Cu and Zn concentrations extracted both with and without acidification was linear regression ($Y = b_0 + b_1X$), as suggested by J. C. Miller and J. N. Miller [41]. The null hypotheses were that the declivity (b_1) would not be different from one (1) and that the intercept (b_0) would not be different from zero (0). These hypotheses were tested by calculating the confidence limits at 95% for both coefficients. The results were also submitted to an analysis of variance (*F*-test) and to correlation.

According to the regression analyses of Cu and Zn extracted by DTPA, the intercept is equal to zero (0), and the angular coefficient is higher than the unit (1). These results indicate that the acidification promoted more metal extraction (Table 2). Then, the soil acidification increased the availability of Cu and Zn, thus increasing the risk of environmental contamination. Moreover, in Jundiá, an undulated relief is predominant [22, 42], and the soil erosion potential is high which is reflected by the slope and, in some cases, the texture gradient of the ultisols. In this landscape, erosion may thus transport sediments that have been contaminated with Cu to the lower regions, thus increasing the environmental impact of Cu contamination. Excessive amounts of hydrogen ions, introduced into the soil by acid rain or fertilization, can release Cu through cation exchange. The metal mobilized in the soil can thus eventually enter aquatic systems through rainwater or can enter groundwater, rivers, and lakes. Rivers draining cultivated areas with high soil Cu concentrations can also have high Cu concentrations [43]. In the case of vineyard soils, which are the most easily eroded cultivated soils [44], applied Cu can reach water bodies not only in water-soluble forms but also, due to erosion, in colloid-bound forms that can accumulate below the water body in sediments. In vine growing areas, evaluating the risk of water quality that is posed by the use of copper-based fungicides therefore requires determining the Cu levels and the distribution of Cu among its more and less bioavailable forms in both vineyard soils and the sediments of local water bodies.

TABLE 2: Regression coefficients for the available forms of Cu and Zn extracted by DTPA and CaCl₂; natural (independent variable) and acidified (dependent variable) samples.

Element/method	r^2	Intercept			P	Angular coefficient			P
		Min.	Average	Max.		Min.	Average	Max.	
CuDTPA	0.95	-0.61	-0.18	0.24	0.39	1.29	1.37	1.44	<0.01
ZnDTPA	0.94	-0.88	-0.17	0.53	0.62	1.06	1.13	1.20	<0.01
CuCaCl	0.15	0.028	0.033	0.038	<0.01	0.053	0.126	0.200	<0.01
ZnCaCl	0.59	0.401	0.611	0.820	<0.01	0.480	0.605	0.730	<0.01

TABLE 3: Correlation coefficients between forms of Cu and Zn and other soil attributes.

	CuT	ZnT	CuDTPA acidified	ZnDTPA acidified	CuCaCl acidified	ZnCaCl acidified
Organic matter	0.49*	0.15	0.27	0.57*	0.12	-0.28
pH	0.37*	-0.09	0.33	-0.25	-0.01	-0.87*
CEC	0.52*	0.08	0.22	-0.01	0.10	-0.60*
Bases saturation	0.45*	-0.05	0.35	-0.06	0.00	-0.88*
CuDTPA	0.72*	0.52*	0.97*	0.44*	0.14	-0.18
ZnDTPA	0.49*	0.63*	0.47*	0.94*	0.06	0.25
CuCaCl	0.00	-0.01	-0.49*	0.12	0.20	0.25
ZnCaCl	-0.16	0.22	-0.25	0.37	0.11	0.88*
CuT	1.00	0.54*	0.70*	0.44*	0.20	-0.29
ZnT		1.00	0.47*	0.63*	-0.01	0.21
CuDTPA acidified			1.00	0.33	0.11	-0.27
ZnDTPA acidified				1.00	0.06	0.23
CuCaCl acidified					1.00	-0.02
Clay			0.45*	-0.17	-0.24	-0.18
Silt			-0.30	0.01	0.26	0.20
Sand			-0.17	0.16	-0.01	-0.01

* $P < 0.05$.

The regression analyses of Cu and Zn extracted by CaCl₂ show that the intercepts are higher than zero (0) and that the angular coefficients are lower than one (1). These results indicate a dual behavior in the samples. Conversely, in samples with low metal concentrations, the acidification increases the concentration of the metals, and in samples with high concentrations, the acidification promoted less extraction.

The Cu concentration extracted by CaCl₂ was compared to soils under either vineyard or other land uses, using Student's t -test. For both acidified and nonacidified soils, the CuCaCl₂ concentration was higher in vineyard soils, though the difference was greater among the nonacidified soils, with a mean of 0.076 mg kg⁻¹ in vineyard soils and a mean of 0.041 mg kg⁻¹ in soil under other uses. For the acidified soils, the mean in vineyard soils was 0.046 mg kg⁻¹, and it was 0.037 mg kg⁻¹ for soil under other uses; the results were significant at the 0.05 level. For Zn extracted by CaCl₂, the results showed no difference between soils under vineyard

or other uses. These results indicate that the CaCl₂ method could not efficiently show Zn contamination by agricultural management: the higher concentrations of Zn extracted by CaCl₂ occurred in soils under natural vegetation, which differ from the Zn concentrations determined by total Zn and Zn extracted by DTPA.

3.5. Relationship between Cu and Zn and Soil Attributes. Table 3 presents the correlation coefficients obtained between the Cu and Zn total and acidified forms and for the other soil properties in 27 samples of vineyard topsoil. The total Cu was correlated with soil organic matter ($r = 0.49$), pH ($r = 0.37$), cation exchange capacity (CEC) ($r = 0.52$), base saturation ($r = 0.45$), CuDTPA ($r = 0.72$), ZnDTPA ($r = 0.49$), and total Zn ($r = 0.54$), whereas total Zn was correlated only with CuDTPA ($r = 0.52$) and ZnDTPA ($r = 0.63$).

Brun et al. [26], comparing various soil extractors, observed that Cu extracted by 0.01 mol L⁻¹ CaCl₂ was correlated well with the soil pH (i.e., it was decreased with increasing

TABLE 4: Confusion matrix of land use classifications based on discriminant analysis.

Land use	To natural vegetation	To nonvineyard	To vineyard	Sum
From natural vegetation	3 60%	2 40%	0 0.00%	5 100%
From nonvineyard	0 0%	32 94%	2 6%	34 100%
From vineyard	0 0.00%	2 7%	25 93%	27 100%
Sum	3	36	27	66

soil pH), which is an important property controlling the bioavailability of Cu. However, Cu extracted by DTPA at pH 7.3 was correlated only with the CEC.

High positive correlation between CuDTPA and ZnDTPA content, pH, organic matter, and base saturation was found in the same area by Valladares et al. [45] (i.e., the Cu content increases with increasing pH, organic matter, and base saturation).

Copper in soils is strongly immobilized by the composition of the soil sorption complex [10, 46] (i.e., organic matter, Fe-, Mn-oxyhydroxides, and nature of the humic substances). Soluble humic and fulvic acids may increase the solubility and mobility of the elements; once in a neutral to alkaline reaction environment, they form stable complexes with the carboxyl, hydroxyl, and amino groups of these compounds [46, 47].

The acidified forms of Cu and Zn extracted by DTPA were highly correlated with total Cu and Zn, whereas Zn DTPA showed a higher correlation with soil organic matter than did Cu. These results differ from those observed in the literature [46, 47], which correlates Cu with soil organic matter. The acidified Cu DTPA was correlated with clay content.

Unlike the Cu and Zn forms extracted by DTPA, the behaviors of the acidified forms of Cu and Zn that were extracted by CaCl_2 were very different. The acidified Cu extracted by CaCl_2 was not correlated with other soil attributes; this result should reflect the Cu forms that were released by the weathering of shale because the soils are poorly developed in the vineyards (inceptisols). No correlation between acidified and nonacidified Cu extracted by CaCl_2 was observed. The acidified and nonacidified Zn extracted by CaCl_2 had good intercorrelation and were high-negatively correlated with pH, CEC, and base saturation.

In order to determine whether the three different land uses, native vegetation, vineyard, and other agriculture activities, differed significantly in terms of Cu and Zn soil concentrations before and after acidification, discriminate analysis was used. In this way, the three land uses were entered in the calculations as the grouping variables and the soil Cu and Zn concentration forms were entered as the independent variables.

The discriminate analysis results are displayed in Figure 2, which present the groups of land uses formed according to the Cu and Zn concentrations in soil. The obtained results indicate that the three land uses had different levels of Cu and Zn concentrations in the top layer of soil, both before and after acidification. For each extracted element concentration, a high degree of between-groups variation

FIGURE 2: Factors of discriminant analysis showing land use groups formed according to Cu and Zn concentrations in soil.

TABLE 5: Factor loadings generated by discriminant analysis.

Variable	F_1	F_2
CuDTPA	0.798	-0.206
ZnDTPA	0.510	-0.642
CuCaCl	0.427	-0.242
ZnCaCl	-0.301	-0.853
CuT	0.661	-0.087
ZnT	0.124	0.172
CuDTPA acidified	0.768	-0.209
ZnDTPA acidified	0.559	-0.586
CuCaCl ₂ acidified	0.369	-0.083
ZnCaCl ₂ acidified	0.057	-0.621

exists, with canonical correlation values varying between 0.63 and 0.88. The Wilks' lambda statistics indicate that the difference among the land uses is significant at $P = 0.05$. The statistically significant results also show a very high percentage of correct classification, ranging from 60% to 94% (Table 4). The analysis of Mahalanobis distances indicates a

FIGURE 3: Continued.

FIGURE 3: Spatial distributions of the Cu and Zn extracted by DTPA and CaCl_2 in natural and acidified samples and the total content of Cu and Zn in natural soil samples. (a) and (c) Cu and Zn extracted by DTPA in natural samples; (b) and (d) Cu and Zn extracted by DTPA in acidified soil samples; (e) and (f) total content of Cu and Zn in soil; (g) Cu extracted by CaCl_2 in natural samples.

TABLE 6: Semivariograms parameters for spatial distributions of Cu and Zn in soils from a vineyard region of São Paulo state: nugget effect (C_0), sill (C), range (A), and dependency degree (GD).

Variable	C_0	C	A (m)	GD (%)	Model
CuDTPA	0.94	6.85	125.8	86	Spherical
CuDTPA acidified	2.36	12.80	125.6	82	Spherical
ZnDTPA	22.00	59.34	966.0	63	Gaussian
ZnDTPA acidified	29.70	107.40	1204.0	72	Exponential
CuT	51.66	102.18	290.5	49	Spherical
ZnT	890.00	5890.00	1666.0	85	Gaussian
CuCaCl	0.0008	0.0026	854.0	69	Spherical
CuCaCl acidified	0.0002	0.0002	—	—	Nugget effect
ZnCaCl	0.64	0.64	—	—	Nugget effect
ZnCaCl acidified	0.13	0.13	—	—	Nugget effect

highly significant probability of differences among the land uses, varying from 0.0001 to 0.003, though vineyard differed from the other land uses more significantly.

Eigenvalues explained 84.05% of variance in the first factor (Figure 2). Factor loadings generated by the discriminate analysis are presented in Table 5, which shows the groups formed by the metals' forms, indicating correlation among them. The first group with high values for factor 1 is formed by the two forms of Cu extracted by DTPA and by the total Cu. A second group is formed by the two forms of Zn extracted by DTPA. These groups indicate the high contaminations of Cu and Zn in the vineyard soils. Other forms of the metals did not have good correlations and may thus describe natural high concentrations of these metals. These results confirm the low correlation between the acidified and nonacidified forms of Cu and Zn extracted by CaCl_2 .

Discriminate analysis shows strong differences between the Cu and Zn concentrations in the investigated land uses and that the behaviors of the forms are variable.

3.6. Spatial Distribution of Cu and Zn. Copper and Zinc concentrations extracted by DTPA, which was obtained from 67 topsoil georeferenced samples, both natural and acidified, were analyzed to determine their spatial dependence using geostatistical and semivariograms analyses (Table 6), kriging interpolation of data, and map building isolines. The spatial dependence of the samples was strong for Cu and moderate for Zn, according to the ratio of spatial dependence (GD) (Table 6). Spherical model for Cu had a good adjustment, with a nugget effect near 0 (zero). For Zn, the nonacidified soil had the best adjustment using the Gaussian model, and the acidified soil had better adjustment in the exponential model.

A change occurred in the spatial behavior of the Cu and Zn concentrations, extracted by DTPA, in natural and acidified samples (Figures 3(a), 3(b), 3(c), and 3(d)), indicating that an occurrence of acid rain or an acid fertilizer reaction would increase the amount of copper available in the area. This effect is even more pronounced in the vineyard

soils, where an increased Cu concentration (Figure 1) was observed.

High total Cu concentration coincides with the areas under vines (Figures 1 and 3(e)). Thus, a risk of contamination may be occurring in these areas. Considering such factors as high Cu concentrations in vineyard soils, soil acidification, and slope, erosion effects may contaminate the lower lands in the landscape. The total Zn concentration coincides with vineyards and natural vegetation, indicating a natural high concentration and contamination in the vineyard (Figure 3(f)).

Analyzing spatial dependence of Cu and Zn extracted by CaCl_2 , only nonacidified Cu had moderate dependence and displayed good adjustment to the spherical model (Figure 3(g)). Acidified extraction of the Cu and Zn forms did not show spatial dependence and had verified the “nugget effect.” The forms extracted by CaCl_2 did not have a spatial correlation with the DTPA and total forms and instead showed different behaviors. These results suggest that CaCl_2 extraction was less efficient than DTPA in representing the bioavailability of Cu and Zn.

4. Conclusions

The results confirmed the enrichment of the soil with Cu and Zn due to the use and management of the vineyards with chemicals for several decades.

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