Journal of Hazardous Materials 181 (2010) 931–937

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Sequential extraction and availability of copper in Cu fungicide-amended vineyard soils from Southern Brazil

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ARTICLE INFO

Article history:
Received 21 January 2010
Received in revised form 19 April 2010
Accepted 23 May 2010
Available online 27 May 2010

Keywords:
Brazilian soils
Extractants
Fractionation
Heavy metals

ABSTRACT

The continuous use of cupric fungicides in vineyards, mainly copper sulfate (as a component of the bordeaux mixture), has increased Cu concentration in soils to levels near or even above the maximum established by the Commission of Soil Chemistry and Fertility of the States of Santa Catarina and Rio Grande do Sul, Brazil. Besides the total content, the fractions of the element along the soil profile must be known, because the total content of Cu in the soil is not sufficient to express its environmental impact. The objective of this study was to evaluate the variation of Cu contentes along the soil profile and its speciation and partitioning in 29 soil samples from vineyards in the state of Rio Grande do Sul, Brazil. Samples were collected in areas cropped with vineyards older than 15 years that had been frequently treated with the bordeaux mixture. These samples were from Nitosols, Acrisols, Cambisols and Leptosols and were analysed by sequential extractions and several chemical extractors. Soils had diverse chemical and physical attributes: clay content in the plowed layer (0–0.2 m) ranged from 120 to 610 g kg^{-1}, pH ranged from 5.3 to 7.3 and organic carbon contents varied from 2.9 to 51 g dm^{-3}. Among the 29 samples, 16 had the total Cu above the maximum limit allowed by the European Community regulations (140 mg kg^{-1}). The average amount of Cu bonded to the oxide fraction accounted for 49.5% of the total Cu.

1. Introduction

Although copper (Cu) is a plant micronutrient, a high concentration of the element in the soil solution may cause biochemical plant imbalances [1]. The main symptoms of an excess of Cu in plant tissues are an increase in leaf thickness and a decrease in leaf surface and the folding of leafs, resulting in appreciable losses in crop yields.

The State of Rio Grande do Sul (RS) has the largest vineyard area in Brazil. The main regions are the Serra Gaucha and Campanha Gaucha, where approximately 31,000 ha are cultivated with grapevine (*Vitis vinifera*) [2]. The continuous use of cupric fungicides in these vineyards, mainly copper sulfate (to make the bordeaux mixture), has increased Cu concentration in soils to levels near or even above the maximum established by the Comission of Soil Chemistry and Fertility of the states of Santa Catarina and Rio Grande do Sul [3]. The amount of Cu potentially available in vineyard soils where the bordeaux mixture is used may reach up to 60% of the total Cu in the upper soil layer, decreasing towards the subsoil [4].

The concentration, distribution and partition either of natural or anthropogenic Cu in soil is not well known, in spite of its agricultural and environmental relevance. Since the total content of Cu in the soil does not correctly express its environmental impact, the sequential extraction is one of the chemical procedures most commonly used to investigate the Cu partition in soil [5]. By this prodecure, the concentration of the element in each soil fraction is measured. Further, it is possible to correlate the content in each fraction with the bioavailable contents to plants [6]. Several extractors are used to evaluate the metals' availability in soils, more frequently DTPA pH 7.3 [7], Mehlich-III [8] and 0.1 M HCl [9], but few studies were carried out to assess the efficacy of such extractors for Cu in soils from the humid tropic [10].

The environmental impacts of the continuous use of cupric fungicides in these vineyards can negatively affect the production of grape and wine. So, the objective of this study was to evaluate the variation of Cu contentes along the soil profile and its speciation and fractionation in soil samples collected in Brazilian agricultural areas cultivated with vineyards that had been frequently treated with the bordeaux mixture.

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doi:10.1016/j.jhazmat.2010.05.102

Fig. 1. Serra Gaucha and Campanha Gaucha regions, where soil samples were collected from vineyard soils in the state of Rio Grande do Sul, Brazil.

2. Materials and methods

Samples from soils with high Cu content were collected in the Serra Gaucha and Campanha Gaucha regions, state of Rio Grande do Sul, Brazil (Fig. 1). Nitosols, Acrisols, Cambisols and Leptosols are the dominant soils in the Serra Gaucha region, and the most planted grape cultivars are Isabel, Bordeaux and Niagara. Acrisols prevails in the Campanha Gaucha region, and Cabernet is the predominant cultivar.

Twenty-nine soil samples were collected: 26 in vineyards from the Serra Gaucha and three from the Campanha Gaucha region. Vineyards older than 15 years and with frequent application of bordeaux mixture were prefferencialy sampled. To asses the general soil characterization of the plowed layer samples were collected at the 0–0.2 m depth. Soil organic carbon content was determined after oxidation with potassium dicromate and sulfuric acid, and excess dichromate was titrated with ferrous ammonium sulfate [11]. Clay content was determined by the densimeter method [12]. The pH was measured in a water (soil:solution ratio of 1:2.5). Iron and aluminum were extracted with 9 M H_2SO_4, and silicia with NaOH 30% in order to calculate the weathering indexes (Ki and Kr) and estimate the mineralogical composition of the soil samples. Contents of Fe and Al determined using atomic absorption spectrophotometry (AAS), and Si by gravimetry [13]. The Ki weathering index was calculated by the molar relation method where Ki ($Ki = 1.7 \times SiO_2/Al_2O_3$) and Kr [$Kr = 1.7\ (SiO_2/(Al_2O_3 + 0.64\ Fe_2O_3)$] were then calculated.

To assess Cu fractions, soils were sampled at the following layers: 0–0.05, 0.05–0.1, and 0.1–0.2 m. Such samples were manually collected with a plastic hammer and PVC cylinders (height = 0.05 m; diameter = 0.1 m) from each thin specific layer. The chemical extractors were: (i) DTPA (diethylene triamine pentaacetic acid) pH 7.3 [7]; (ii) Mehlich-III (0.015 M NH_4F + 0.2 M HOAc + 0.25 M NH_4NO_3 + 1 M HNO_3 + 0.001 M EDTA) [8], and (iii) 0.1 M HCl bioavailable Cu was extracted with a 0.1 M HCl solution, pH 1.2, with a soil:solution ratio of 1:4, plus an agitation for 30 min and decantation for 16 h, as described by Tedesco et al. [9]. DTPA is the extractor standard used in the State of São Paulo, Brazil, for the extraction of bioavailable contents of micronutrients in soils. The extractor Mehlich-III is commonly used in studies regarding heavy metals, mainly when soil contamination is evaluated. HCl is the extractor used in southern Brazilian region, where the soil samples had been collected. Total Cu was extracted by the US-EPA 3052 method, by digesting samples with HNO_3 + HF + deionized water (DI water) in a microwave oven [14].

The sequential extraction followed the simplified method of Silveira et al. [15] for tropical soils. In the method described by Silveira et al. [15], oxide fraction were splitted in Mn, poor crystalline Fe and crystalline Fe fractions. Conversely, we have not discriminated such fractions in our study, and only one fraction was considered, named oxide-bonded fraction. The determined Cu fractions were: exchangeable (F1), organic matter-bonded (OM) (F2), oxide-bonded (F3) and residual (F4). The F1 fraction was extracted with 15 mL of a 0.1 M $Sr(NO_3)_2$ solution, according to Ahnstrom and Parker [16]. Following F1, F2 was extracted by 5 mL of a 5% NaOCl solution at pH 8.5. Afterwards, F3 was extracted with 0.2 M ammonium oxalate + 0.2 M oxalic acid + 0.1 M ascorbic acid at pH 3 [17]. The residual fraction (F4) was extracted by total digestion with HNO_3 + HF + deionized water in a microwave oven, according to the EPA 3052 method [14].

Between each successive extraction, the solid residues were suspended in 5 mL of a 0.1 M NaCl solution, shaken by hand, and centrifuged to displace the extracting solution remaining from the previous step. This procedure reduces sample dispersion and minimizes re-adsorption of the metal [16]. All suspensions were centrifuged at $1225 \times g$ during 10 min and filtered. Cu concentration in the extracts was determined by AAS, and the recovering index was calculated using the maximum error acceptable as ±20%. Percent recovery was calculated by the ratio of Cu concentration in each fraction and Cu total concentration, and multiplying the result by 100.

Cu concentrations were interpreted by the *F*-test (ANOVA), and regression analysis were obtained when the differences in Cu concentration in the extractor were significant.

3. Results and discussion

3.1. Initial characterization of the soils

Clay content in the plowed layer (0–0.2 m) of the samples ranged from 120 to 610 g kg^{-1}, and organic carbon contents varied from 2.9 to 51 g dm^{-3} (Table 1). Based on the weathering indexes Ki and Kr (Table 2) and the Embrapa [18] scores, all soils were classified either as kaolinitic non-sesquioxidic or kaolinitic ($Ki > 0.75$, $Kr > 0.75$). Among the sampling sites, 16 vineyards (59%) had Cu concentrations above the maximum allowed by the European Community (140 mg kg^{-1}) [19]. Copper concentrations above 0.4 mg kg^{-1} are considered high for grapevine [3].

In a French vineyard Brun et al. [20] reported Cu concentration ranging from 31 to 250 mg kg^{-1} in the 0–0.2 m layer; in a similar forest soil, they observed lower concentration, from 14 to

Table 1
Chemical characterization of the plowed layer (0–0.2 m) of 29 soil samples from vineyards of the state of Rio Grande do Sul, Brazil.

Soil	Geographic coordinates (latitude/longitude)	Location (city)	Cultivar	pH H_2O	OC ($g\,dm^{-3}$)	Clay ($g\,kg^{-1}$)
Acrisol	29°15′/51°32′	Garibaldi	Isabel	6.2	21.0	330
Acrisol	29°15′/51°32′	Garibaldi	Isabel	5.7	10.9	320
Acrisol	29°26′/51°48′	Livramento	Cabernet	7.3	8.4	145
Acrisol	29°26′/51°48′	Livramento	Cabernet	6.8	8.0	120
Acrisol	29°26′/51°48′	Livramento	Cabernet	6.6	2.9	125
Nitosol	29°10′/51°44′	Santa Tereza	Isabel	6.0	41.9	250
Nitosol	29°10′/51°44′	Santa Tereza	Isabel	6.1	17.8	260
Nitosol	29°15′/51°32′	Garibaldi	Isabel	5.6	19.6	360
Nitosol	29°10′/51°10′	Caxias do Sul	Isabel	6.6	23.5	300
Nitosol	29°10′/51°10′	Caxias do Sul	Bordeaux	6.7	23.2	280
Nitosol assoc. Cambisol	29°13′/51°20′	Pinto Bandeira	Isabel	5.9	29.3	230
Nitosol assoc. Cambisol	29°13′/51°20′	Pinto Bandeira	Isabel	6.4	23.8	240
Nitosol assoc. Leptosol	29°10′/51°10′	Caxias do Sul	Bordeaux	5.8	37.3	380
Cambisol	29°10′/51°44′	Santa Tereza	Isabel	6.3	23.4	300
Cambisol	29°01′/51°10′	Flores da Cunha	Niágara	6.2	5.3	270
Cambisol	29°13′/51°20′	Farroupilha	Isabel	6.3	39.3	350
Cambisol assoc. Leptosol	29°13′/51°20′	Farroupilha	Isabel	5.4	38.7	240
Cambisol assoc. Leptosol	29°13′/51°20′	Farroupilha	Isabel	7.0	40.9	250
Cambisol assoc. Leptosol	29°13′/51°20′	Pinto Bandeira	Isabel	5.7	24.2	210
Cambisol assoc. Leptosol	28°58′/51°05′	São Marcos	Isabel	5.5	19.5	390
Cambisol	29°13′/51°20′	Pinto Bandeira	Isabel	5.6	49.9	610
Leptosol	29°01′/51°18′	Nova Pádua	Isabel	5.1	41.9	480
Leptosol	29°10′/51°31′	Bento Gonçalves	Isabel	5.5	50.9	393
Leptosol assoc. Cambisol	28°58′/51°05′	São Marcos	Isabel	6.4	35.6	270
Leptosol assoc. Cambisol	28°58′/51°05′	São Marcos	Isabel	5.9	23.7	300
Leptosol	29°01′/51°10′	Flores da Cunha	Isabel	5.3	27.3	310
Leptosol assoc. Nitosol	29°01′/51°10′	Flores da Cunha	Isabel	6.3	41.6	320
Leptosol	29°01′/51°18′	Nova Pádua	Isabel	5.9	31.0	460
Leptosol	29°01′/51°18′	Nova Pádua	Isabel	5.5	17.7	270

pH H_2O = pH was measured in water (soil:solution ratio of 1:2.5); OC = organic carbon, measured by oxidation with potassium dichromate plus sulfuric acid prior to titration with ferrous ammonium sulfate; Clay = clay contents measured by the densimeter method.

29 mg kg^{-1}. Pietrzak and McPhail [4] found total Cu concentration up to 249 mg kg^{-1} in a vineyard Australian soil. However, the content was lower than 10 mg kg^{-1} in a sample of the same soil collected outside the vineyard area. Total Cu concentration reached 304 mg kg^{-1} in vineyard soils from New Zealand [21], and it ranged from 24.9 to 432.8 mg kg^{-1} in Serbia soils, the concentration in the uncontaminated soil reaching 30 mg kg^{-1} [22]. In Spain, the total Cu concentration in a vineyard soil ranged from 96 to 583 mg kg^{-1} [23].

The pH of the 29 sampled soils ranged from 5.3 to 7.3 (average 6.1). High total Cu concentration was observed even in soils with pH between 6 and 7. This condition was probably the cause of the lack of correlation between soil pH and soil Cu in the 0–0.2 m layer. Heavy metals can precipitate by reacting with carbonate (CO_3^{2-}), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}) and hydroxide (OH^-), among others, and also co-precipitate with low-soluble Ca, Mg, Fe and Al compounds. Yet, it is possible that heavy metals may be adsorbed to the surface of those low-soluble amorphous phases, or be covalently bonded during the crystallization process phase [24].

3.2. Copper availability along the soil profile

Total Cu contents decreased with depth in the majority of soils (Fig. 2). Leptosols had the highest concentration of total Cu, while Cambisols had, in average, the largest levels of available Cu. Acrisols had the smallest average of Cu concentration as determined by all the extractors.

In the Acrisols, total Cu was found to range from 24 to 674 mg kg^{-1} (Fig. 2); bioavailable Cu ranged from 0.6 to 336 mg kg^{-1} in the DTPA extracts, from 0.9 to 415 mg kg^{-1} in the Mehlich-III extracts, and from 0.9 to 347.2 mg kg^{-1} in the HCl extracts. In the Nitosols, total Cu varied from 51.6 to 1648.5 mg kg^{-1}; Cu concentration ranged from 4.7 to 550 mg kg^{-1} in the DTPA extracts, from 6.2 to 710 mg kg^{-1} in Mehlich-III extract,

Table 2
Amounts of Si, Al, Fe, Mn, and Ti oxides, and Ki and Kr indexes in soil samples from Cambisols, Nitosols, Leptosols and Acrisols in vineyards from the state of Rio Grande do Sul, Brazil.

Soil	Layer (m)	SiO_2 ($g\,kg^{-1}$)	Al_2O_3 ($g\,kg^{-1}$)	Fe_2O_3 ($g\,kg^{-1}$)	MnO ($g\,kg^{-1}$)	TiO_2 ($g\,kg^{-1}$)	Ki	Kr
Acrisols	0–0.05	59.6	39.2	40.5	0.7	3.9	2.6	1.6
	0.05–0.1	63.0	35.9	40.8	0.8	3.9	3.0	1.7
	0.1–0.2	62.8	42.6	39.7	0.7	3.9	2.5	1.6
Nitosols	0–0.05	147.9	84.7	96.1	2.2	9.4	3.0	1.7
	0.05–0.1	160.6	92.1	100.8	2.2	10.2	3.0	1.7
	0.1–0.2	141.1	90.4	100.9	2.1	10.5	2.7	1.5
Cambisols	0–0.05	119.8	77.4	79.9	2.1	8.1	2.6	1.6
	0.05–0.1	124.8	76.7	80.5	2.0	8.0	2.8	1.7
	0.1–0.2	130.3	82.0	81.0	1.9	8.2	2.7	1.7
Leptosols	0–0.05	124.8	79.7	94.1	2.0	9.6	2.7	1.5
	0.05–0.1	130.0	86.7	94.7	1.9	9.4	2.5	1.5
	0.1–0.2	147.5	88.3	96.7	1.8	9.5	2.8	1.7

Ki = 1.7 (SiO_2/Al_2O_3) e Kr = 1.7 [$SiO_2/(Al_2O_3 + 0.64\,Fe_2O_3)$]; SiO_2. Al_2O_3. Fe_2O_3. MnO and TiO_2 are the amounts of Si. Al. Fe. Mn e Ti extracted with a 9 M H_2SO_4 solution.

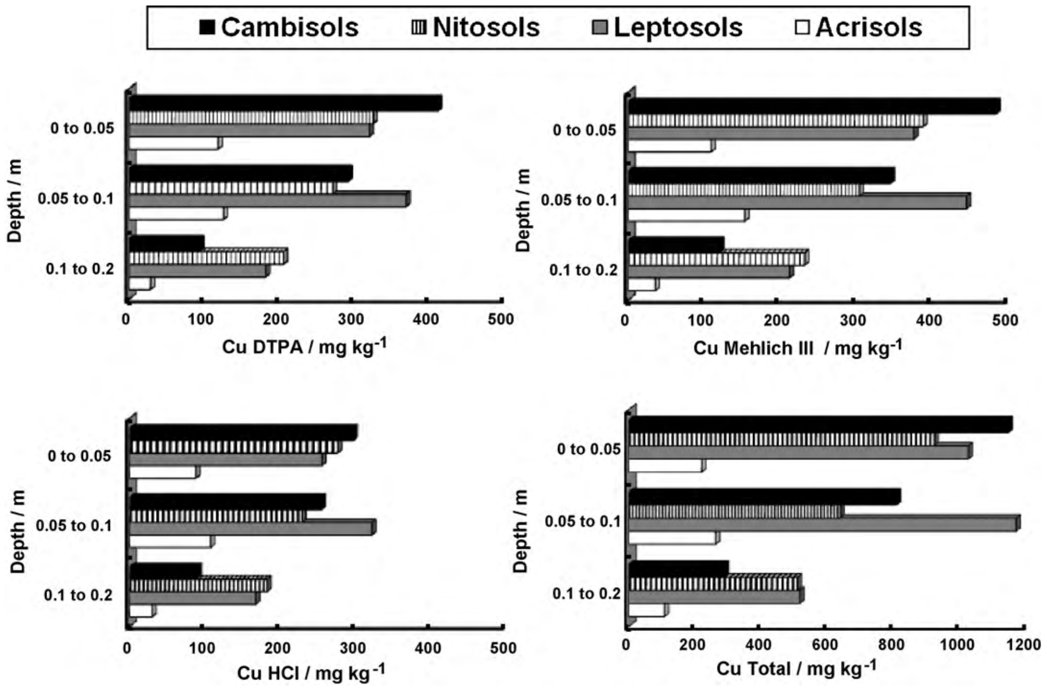

Fig. 2. Average amount of Cu extracted with DTPA, Mehlich-III, 0.1 M HCl, and total Cu in soil samples from Acrisols, Nitosols, Cambisols, and Leptosols at 0–0.05, 0.05–0.1, and 0.1–0.2 m layers from vineyard regions of the state of Rio Grande do Sul, Brazil.

and from 1.8 to 556.6 mg kg^{-1} in HCl extracts. In the Cambisols total Cu ranged from 68.3 to 1844.4 mg kg^{-1}; bioavailable Cu concentration ranged from 8.3 to 550 mg kg^{-1} in the DTPA extracts, from 11 to 644 mg kg^{-1} in Mehlich-III extract, and from 2.6 to 424 mg kg^{-1} in HCl extracts. In the Leptosols, total Cu ranged from 91.4 to 2850 mg kg^{-1}; the Cu concentration in the DTPA extracts ranged from 6.6 to 744 mg kg^{-1}, from 7.5 to 904 mg kg^{-1} in Mehlich-III extract, and from 2.2 to 634.5 mg kg^{-1} in HCl extracts.

In all soil samples, Mehlich-III extracted the greater amount of Cu, probably because acid solutions tend to extract the total Cu amount by dissolving phases containing Cu [25]. Although Mehlich-III is a successful heavy metal extractor, it may overestimate available Cu mainly by dissolving metals retained by carbonates. The chelating capacity of DTPA extractor, on the other hand, may be exhausted in soils with a large amount of heavy metals. A positive linear relationship among Cu concentrations in the DTPA, Mehlich-III and HCl extracts was observed ($p < 0.05$) (Fig. 3). Brun et al. [20] also found a positive correlation between total Cu and DTPA-Cu, while Simonete and Kiehl [26] found positive correlations between Mehlich-III and DTPA for Fe, Mn, Cu and Zn. Positive correlation was also found by Oliveira and Mattiazzo [27] between 0.1 M HCl, Mehlich-III and DTPA extractants to assess the availability of Cu and Zn in a Ferralsol amended with sewage sludge.

Concentration of Cu in DTPA pH 7.3 correlated positively with the cationic exchange capacity of the soils (CEC). It is well known that CEC depends on organic matter and clay contents, as well as varies with pH in variable-charge soils. The lack of correlation between Cu concentration in the extractants and the OM content in these soils is related to their low OM contents. In France, Brun et al. [20] detected total Cu contents ranging from 30 to 250 mg kg^{-1}, depending on vineyard age, soil type and climate. Among the extractors tested by Brun et al. [20], Cu extracted with a 0.01 mol L^{-1} $CaCl_2$ solution was the best to correlate with soil pH and CEC. In Brazil, surveys on Cu content in soils are rare [28]. For tropical and temperate soils, Jackson [29] proposed total Cu content from 5 to 40 mg kg^{-1} as acceptable, because his survey revealed Cu contents ranging from 2 to more than 100 mg kg^{-1}. Until the present time, São Paulo is the only state in Brazil that adopts a table of background values for heavy metals in the soil, and the São Paulo State Environmental Agency (Cetesb) suggests 35 mg kg^{-1} as a background values for Cu [30].

3.3. Sequential extraction

Concentrations from 0.3 to 9.3 mg kg^{-1} were found in the exchangeable fraction, from 4.5 to 1254 mg kg^{-1} in the organic fraction, from 0 to 585 mg kg^{-1} in the oxide fraction and 3.4 to 313.7 mg kg^{-1} in the residual fraction. The total Cu ranged from 24 to 2250 mg kg^{-1}. Table 3 shows the average amount of Cu in the fractions in soil samples from Acrisols, Nitosols, Cambisols, and Leptosols. In 16 of 29 soils, the concentrations were above the maximum allowed at the present by the European Community (140 mg kg^{-}) [19] and by Cetesb (60 mg kg^{-}) [30].

In the Acrisols, 59% of the total Cu was bonded to the oxide fraction, whereas this proportion was of 43% and 42% in the Nitosols and Cambisols, respectively (Fig. 4). Copper transfer from one soil fraction to other is slow, suggesting that Cu may stay active in soils for a long time, being a source for leaching and translocation to deeper layers and horizons, becoming a potential contaminant to ground waters [4]. Cu is mainly in immobile fractions in uncontaminated soils, because the potentially available Cu is about only 10% of the total Cu along the whole soil profile. On average, the organic fraction presented higher values in the Leptosols, highlighting the importance of OM in these soils. Because of their high weathering degree, Acrisols had most of the Cu bonded to the oxide fraction.

The average amount of Cu bonded to the oxide fraction accounted for 49.5% of the total Cu, while 28.5% was bonded to the organic fraction, and 21.6% was bonded to the residual fraction. The exchangeable fraction was less than 1% of the total Cu (Table 3). These results are in agreement with those reported by Sposito [31], i.e. Cu in the exchangeable form was less than 4% of the total Cu. Bradley and Cox [32] found 0.2 to 10% (average 1.5%) of the total Cu in the exchangeable fraction. Although these are small values,

Table 3
Average amount of Cu in the fractions: exchangeable (F1), bonded to organic matter (F2), bonded to oxides (F3), residual (F4), and total Cu in soil samples from Acrisols, Nitosols, Cambisols, and Leptosols at 0–0.05, 0.05–0.1 and 0.1–0.2 m layers from vineyard regions of the state of Rio Grande do Sul, Brazil (values in parenthesis represent the percentage of the total).

Soil	Layer (m)	F1 (mg kg^{-1})	F2 (mg kg^{-1})	F3 (mg kg^{-1})	F4 (mg kg^{-1})	Total (mg kg^{-1})
Acrisols	0–0.05	2.8 (1.0%)	46.9 (17%)	185.0 (67%)	40.9 (15%)	275.6
	0.05–0.1	1.0 (0.4%)	41.0 (18%)	149.0 (65%)	38.4 (17%)	229.4
	0.1–0.2	0.6 (0.7%)	9.0 (10%)	54.0 (63%)	21.5 (26%)	85.1
Nitosols	0–0.05	2.5 (0.3%)	311.4 (41%)	316.3 (42%)	134.5 (17%)	764.7
	0.05–0.1	2.2 (0.4%)	147.3 (24%)	314.4 (52%)	143.8 (24%)	607.7
	0.1–0.2	2.0 (0.4%)	96.1 (21%)	230.6 (50%)	135.4 (29%)	464.1
Cambisols	0–0.05	2.1 (0.2%)	385.9 (44%)	375.0 (42%)	120.8 (14%)	883.8
	0.05–0.1	2.9 (0.4%)	147.0 (22%)	370.6 (56%)	137.4 (22%)	657.9
	0.1–0.2	2.2 (0.9%)	37.3 (15%)	144.4 (57%)	68.9 (27%)	252.8
Leptosols	0–0.05	1.6 (0.2%)	394.1 (55%)	211.3 (29%)	114.2 (16%)	721.2
	0.05–0.1	1.8 (0.2%)	462.9 (51%)	274.4 (30%)	175.4 (19%)	914.5
	0.1–0.2	1.4 (0.3%)	106.0 (25%)	168.8 (41%)	141.9 (34%)	418.1

they are important because this species is an available form to the plants.

In more than half of the samples Cu was dominantly associated with the oxide fraction (Table 3 and Fig. 4). Comparing vineyard soils developed from limestone with those rich in oxides and OM. Parat et al. [33] also observed that most of the Cu was bonded to the oxide fraction. In two Ferralsols from São Paulo State, Brazil, Silveira et al. [15] observed the largest amount of Cu bonded to the iron oxide or in the residual fractions, because these soils were in advanced degree of weathering and had small OM contents. Oxides

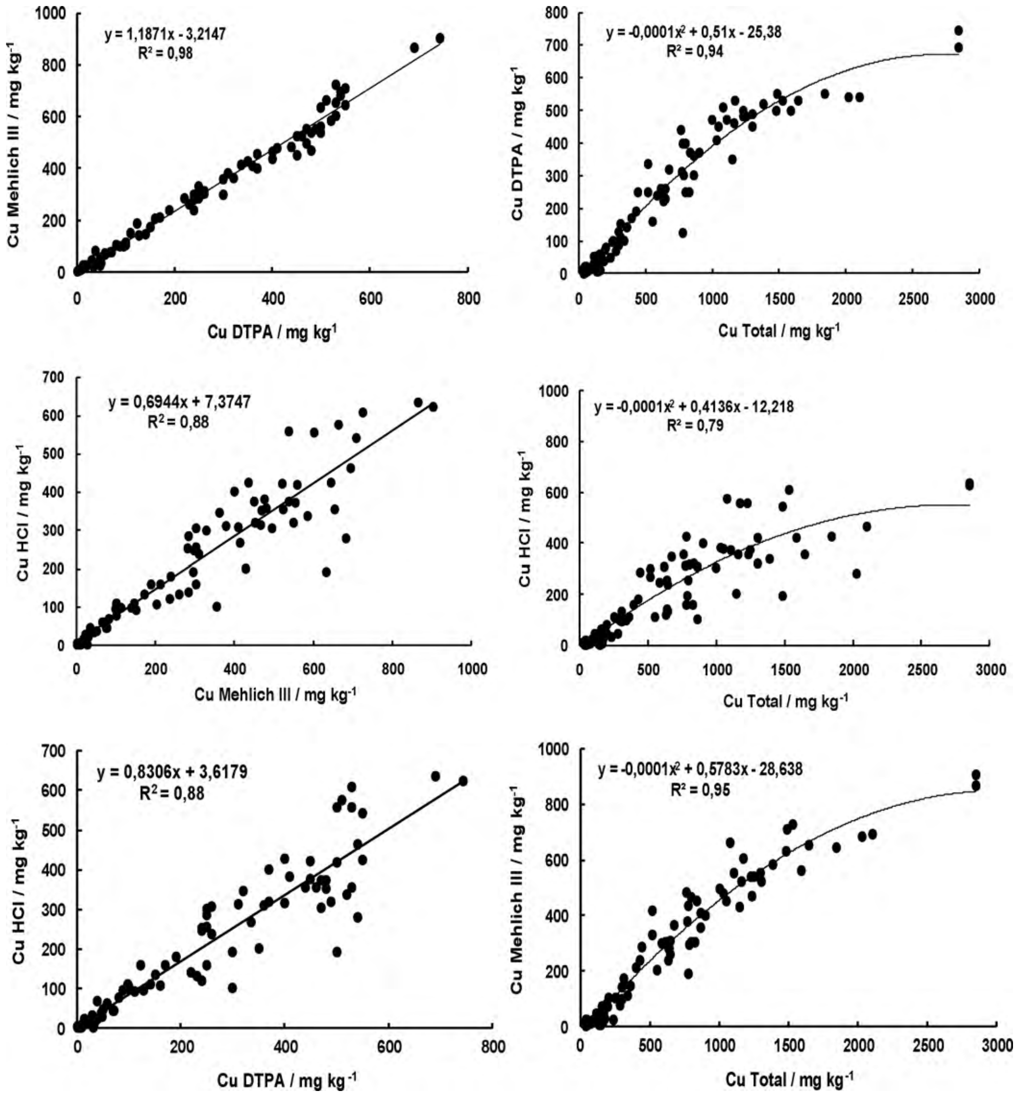

Fig. 3. Relationship between Cu extracted by DTPA, Mehlich-III, 0.1 M HCl, total amount of Cu and Cu extracted with DTPA, Mehlich-III, and 0.1 M HCl solution from 29 soil samples collected in vineyard regions of the state of Rio Grande do Sul, Brazil.

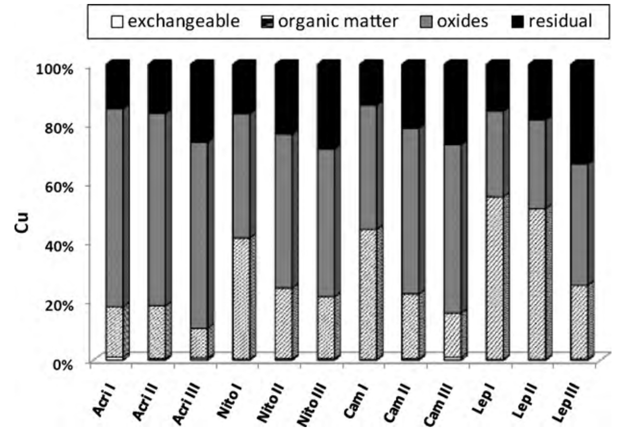

Fig. 4. Percent average distribution of Cu fractions (exchangeable, bonded to organic matter, bonded to oxides and residual Cu) in soil samples from Acrisols (Acr), Nitosols (Nit), Cambisols (Cam), and Leptosols (Lep) rom vineyard regions of the state of Rio Grande do Sul, Brazil (I: 0–0.05 m; II: 0.05–0.1 m; III: 0.1–0.2 m).

have very reactive hydroxyl groups at their surface that may form outer and inner complexes with Cu. These oxides are the main cause of Cu accumulation in the clay fraction, mainly due to Cu inclusion in the Fe oxihydroxides and clay-humus complexes [34].

Copper immobility is usually attributed to the formation of stable, low soluble organo-metalic complexes [35]. In addition to the complexation by organic substances, Cu immobility can be also related to the non-exchangeable fraction of soil. Ma and Rao [36] evaluated nine soils contaminated by four sources of pollutants and observed the following partitioning sequence: residual > organic > Fe and Mn oxides > carbonates > exchangeable > soluble.

The relevance of OM in Cu fractionation seems to be more evident in temperate than in tropical soils. Perez-Novo et al. [37] found total Cu ranging from 166 to 359 mg kg^{-1} in vineyard soils from Spain, the organic fraction being the main containing Cu. In agreement with such results, Viti et al. [38] observed Cu values of 176.6 mg kg^{-1} in vineyard soils from Toscana (Italy) mainly bonded to the humic fraction. In 26 samples from vineyard soils of Northeast Iberian Peninsula, Ferdinandez-Calviño et al. [23] reported total Cu content eight times greater (246 mg kg^{-1}) than in pastures (30 mg kg^{-1}); approximately, 50% of this amount was bonded to the organic fraction. In the region of Champagne (France), Chopin et al. [39] observed 27.8% of the total Cu bonded to the oxide fraction of soil.

4. Conclusions

The Mehlich-III method extracted the largest amount of Cu from soils when compared to DTPA and 0.1 M HCl. The content of total Cu decreased along the soil profile in the majority of the soils studied. Most of the Cu was bonded to the organic matter and oxide fractions, reinforcing the importance of such attributes in soils from the humid tropics. Contents of total Cu exceed the maximum limit allowed by the European Community regulations (140 mg kg^{-1}) in 16 of the 29 soil samples (55% of evaluated samples), representing a serious challenge to be taken into account by growers and researchers.

Acknowledgements

To FAPESP, for the MSc grant to the first author. To CNPq, for the research grant for the second author.

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