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Copper Bioavailability and Fractionation in Copper-Contaminated Sandy Soils in the Wet Subtropics (Southern Brazil)

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Abstract We studied the distribution, bioavailability and speciation of copper in subtropical sandy soils exposed to long-term applications of copper-based fungicide. In the surface horizon of the contaminated soils, bioavailable copper surpasses the toxic threshold for plants up to several times. Mobile fractions of copper predominate in top layers, contributing to contamination spreading downward to groundwater. Copper accumulates on the underlying iron hydroxide barrier in quantities comparable to total copper concentrations in the surface horizon. Despite the impediment of the geochemical barrier, most applied copper reaches the aquifer, contaminating the groundwater.

Keywords Copper contamination · Toxicity · Speciation · Soil · Subtropics

Copper concentrations exceeding 60 mg kg^{-1} in soil require environmental risk assessments (Schramel et al. 2000). Very high concentrations of copper in top vineyard soils have been reported for various world regions due to intensive applications of copper-based fungicide (Prasad et al. 1984; Flores-Veles et al. 1996; Pietrzak and McPhail 2004; Vavoulidou et al. 2005). One of the major problems with copper-polluted soils is the bioavailability of Cu to living organisms. The toxicity of Cu is mainly observed in acid sandy soils with low cation exchange capacities (Brun et al. 2001). A number of chemical extractions are used to estimate the bioavailability of Cu in soils (Rao et al. 2008). Copper-based fungicide contamination and related copper toxicity have predominantly been studied in regions with temperate climates and moderate precipitation. Little information is available regarding copper derived from fungicide application in tropical and subtropical wet conditions. In Brazil, grape cultivation was begun about a hundred years ago by Italian immigrants. A fast expansion of vine-growing areas can be currently observed in various states of the country, especially in Rio Grande do Sul state, where a fairly cold winter facilitates grape cultivation. Due to the wet subtropical climate, Brazilian viticulture areas are the dampest in the world. High humidity and rain require frequent applications of copper-based fungicide, which contributes to environmental contamination. As a result, soils accumulate up to 3,200 mg kg^{-1} of copper in the top layer, a quantity several times higher than in other vine-growing areas throughout the world (Mirlean et al. 2007). Winegrowing areas in the southern part of Rio Grande state are situated in the coastal plane zone, which is composed of Quaternary sands. Sandy soils in the region are poor in organic matter and clay minerals and are acid. These soil parameters, together with intense precipitation throughout the year and shallow groundwater, predispose the area to high mobility and toxicity of copper. Besides vineyards, copper-based fungicide is used in vegetable and fruit gardens in the area, which considerably increases the impacted area.

Taking into consideration the increasing agricultural production from wet tropical and subtropical climates in developing countries, we focused on evaluating the possible bioavailability and toxicity of copper from copper-based

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fungicide as well as its mobility and distribution in the profile of subtropical acid sandy soils.

Materials and Methods

We studied three vine-growing plots (A, B and C) with approximately equal areas of 0.3 ha near the town of Rio Grande, in the southernmost part of Brazil. The region is characterized by plain relief, acid sandy soils developed on Quaternary fine sands and humid subtropical climate with average precipitation of 1,300 mm/yr. All studied vineyards were about 50 years old and had been subjected to intensive applications of copper-based fungicide up to the present. A pasture area (Bg) some distance from the vineyards and habitat areas was studied as a representative soil background.

At each vineyard and background area we sampled one soil depth profile and 15 surface soil samples (0–5 cm). Surface soil samples were collected by net 20 × 20 m. For soil profiles, we sampled every 10 cm interval down to the shallow groundwater. Soil samples were collected by plastic shovel and placed in polyethylene bags. Groundwater samples were collected in 1 L polypropylene using a siphon bomb. In the laboratory, water samples were filtered through a membrane of 0.45 µm, acidified by HNO_3 s.p. and stored for later analysis. Soil samples were air-dried and sieved through a 0.2 mm nylon sieve.

Organic matter content, soil pH, cation exchange capacity (CEC), and particle size distribution were determined using common methods (Pansu and Gautheyrou 2006). For total metal analysis, soil samples pulverized in an agate mortar were digested using the $HF/HClO_4/HNO_3$ method: about 1 g of sample was digested with the acid mixture for 12 h at room temperature, then evaporated in a Teflon vial until it reached the wet salt state, diluted to 100 mL with distilled water and filtered. To evaluate copper bioavailability in surface soil samples, we used calcium chloride, ammonium acetate and EDTA single extractions (Brun et al. 1998). For copper fractionation along the soil profile (20 cm interval soil samples) sequential extraction was applied according to Ure et al. (1993).

Copper and iron were analyzed by flame atomic absorption spectrophotometry, and the electrothermic atomization mode was used in copper analysis in water samples and extracts. The maximal relative standard deviation for three replicate analyses of an individual sample was less than 4%.

The accuracy and precision of the total copper analyses were ensured by sequential digestion and analysis of NRCC-PACS-2 Certificate Reference Material (CRM). Good recovery was found within the 95% confidence limits for the CRM.

Results and Discussion

Surface soil samples from the vineyards and background site differed slightly in their physical and chemical proprieties (Table 1).

Surface soil from the background area was somewhat finer in grain size and contained more organic matter than that in the vineyard plots, resulting in a higher cation exchange capacity. Lower concentrations of C_{org} and coarser grain size of the vineyard soil are probably due to soil tillage and partial removal of organic remains over a long cultivation period.

The mean copper concentration in soil from the background area was about three times lower than the average value for soils around the world (Baker and Sentf 1995). Studied vineyard soils were noticeably enriched in copper, containing on average 40–50 times more copper than uncontaminated soil and about eight times the environmental risk threshold (Table 2). Top soil from contaminated plots did not significantly differ in mean total copper concentration, confirming nearly the same duration of copper-based fungicide application in the vineyards.

There are various approaches to assessing copper bioavailability and its negative effect on plants based on copper chemical extractability from soil (Brun et al. 1998; Delas 1984; Chaignon et al. 2003). According to Brun et al. (1998), EDTA-extractable Cu demonstrates high correlations with total Cu, but this extraction method is only significant for predicting the availability of Cu to plants. $CaCl_2$ extractable Cu is considered more appropriate for

Table 1 Selected chemical and physical properties of top layer (0–5 cm) of studied soils

Sampled areas[a]	Clay 0–2 µm (%)	Silt 2–63 µm (%)	Fine sand 63–200 µm (%)	pH (H_2O (KCl))	CEC ($cmol_{c+}$ kg^{-1})	C org. (%)	Fe_2O_3 (%)
Plot A	0.5	3.9	85.3	3.8 (3.7)	1.2	0.9	1.0
Plot B	0.9	4.6	78.6	4.1 (3.9)	1.6	0.8	0.8
Plot C	0.7	4.1	82.4	3.9 (3.7)	1.5	0.6	1.2
Bg area	2.1	6.5	84.1	3.8 (3.6)	2.5	1.4	0.9

[a] 15 surface soil samples of each area combined into one

Table 2 Concentration of total copper (mean ± SD, range in parentheses) and extractable copper (single-step extraction) in surface (0–5 cm) soil

Sampled area	Total Cu (n = 15)	[a]$CaCl_2$ 0.01 M		[a]CH_3COONH_4 1 M		[a]Na_2-EDTA 0.01 M + CH_3COONH_4 1 M	
	mg kg^{-1}	mg kg^{-1}	% of total	mg kg^{-1}	% of total	mg kg^{-1}	% of total
Plot A	468.2 ± 72.5 (324.1–543.2) [a]473.3	5.8	1.2	153.5	32.5	382.4	80.8
Plot B	432.8 ± 84.2 (298.4–563.7) [a]498.5	6.4	1.3	147.2	29.5	369.3	74.1
Plot C	517.3 ± 64.5 (342.4–672.5) [a]549.6	5.5	1.0	206.4	37.6	417.3	75.9
Bg area	11.2 ± 3.5 (8.4–16.1) [a]12.5	0.02	0.2	1.2	9.6	2.1	16.8

[a] Fifteen samples combined into one; n, sample size

assessing copper bioavailability for plants, especially in neutral to acid soils. According to Delas (1984), 25 mg kg^{-1} of Cu extracted with ammonium acetate is the toxic threshold content for sandy soils with pH below 6.

Table 2 shows that copper in the vineyard topsoil is much more bioavailable than in soil from the background area. Over 75% of copper is extractable by EDTA, which is nine times more than was extracted from unpolluted soil. The mean ratio of copper extractable by $CaCl_2$ between polluted and unpolluted soil can be as high as 65:1. Thus, following the above approaches we can consider that copper in the studied vineyards is highly bioavailable and surpasses the toxic threshold for plants by about four times.

A depth pattern of the copper distribution in soil profiles demonstrates a general decrease of total copper concentration from the top soil horizon down to the shallow groundwater level (Fig. 1). However, this distribution was rather different in the background and polluted soils. In the profile of the background soil, the copper concentration decreased steadily, whereas in polluted soil, it was sharper and more irregular (Fig. 1). In all profiles of polluted soil, the peak copper concentration was observed in the depth interval of 50–70 cm. This interval is a transition zone between the upper oxic soil layers and the anoxic shallow groundwater aquifer. It is marked by intense spots of iron hydroxides forming a 10–20 cm thick layer. In this part of the soil profiles, the copper distribution coincided with the iron, which had a concentration peak 2–3 times higher than in the upper soil horizon.

Copper speciation (sequential extraction) differed between background and polluted soils (Fig. 1). In background soil, the residual fraction (extractable by aqua regia) was predominant and gradually increased with depth from 65% in the top layer to 85% in the shallow groundwater aquifer. The exchangeable copper fraction (extractable by ammonium acetate $pH = 7$) varied irregularly along the soil profile, demonstrating the highest portion in Fe-enriched horizons. The organic fraction (extractable by hydrogen peroxide + HNO_3) was the third most common, decreasing from 15% in the top layer to about 1% at the groundwater level. Both carbonate (extractable by sodium acetate $pH = 5$) and reducible (extractable by hydroxylamine hydrochloride, $pH = 2$) fractions varied from 6 to >1% and had irregular distributions. However, the reducible fraction had a tendency to increase in the Fe-enriched soil horizon compared to the upper and lower horizons.

The distributions of copper fractions along soil profiles in all the studied vineyard plots were similar (Fig. 1). Compared to the background plot, the residual fraction in polluted soils was not predominant in all soil horizons and had an irregular distribution. The organic fraction of copper was predominant (about 50%) in the upper horizons and decreased to about 3%–5% in the shallow groundwater aquifer. The character of the distribution of carbonate fraction was analogous to the organic. Higher proportions of carbonate fraction in the upper horizon probably incorporate untransformed copper fungicide, as was previously reported in Schramel et al. (2000). It may also be true for exchangeable copper, which has a rather larger portion in polluted soil than in the background despite the low content of clayey material.

Schramel et al. (2000) noted that the reducible fraction bound to soil oxides and hydroxides is higher in uncontaminated soils. This observation was confirmed for the upper horizons of the studied soils. However the distribution of the reducible fraction downward along the soil profile was rather expressive in contaminated sites than in background area. The reducible fraction of copper drastically increases in the depth range of 50–80 cm, where soil material ferruginization was detected visually and was proved by a Fe concentration peak (Fig. 1). At this horizon, the total copper concentration also increased, reaching about 50% of its value in the top layer. Accumulation of metals in sites of Fe–Mn oxide and hydroxide concretion is well known, and in the geochemistry of hypergenese is referred to as a dual geochemical barrier (Perelman 1989). Geochemical barriers act as filters for metals migrating in natural waters. Thus, a copper accumulation on a geochemical barrier in polluted soils confirms its intense downward migration.

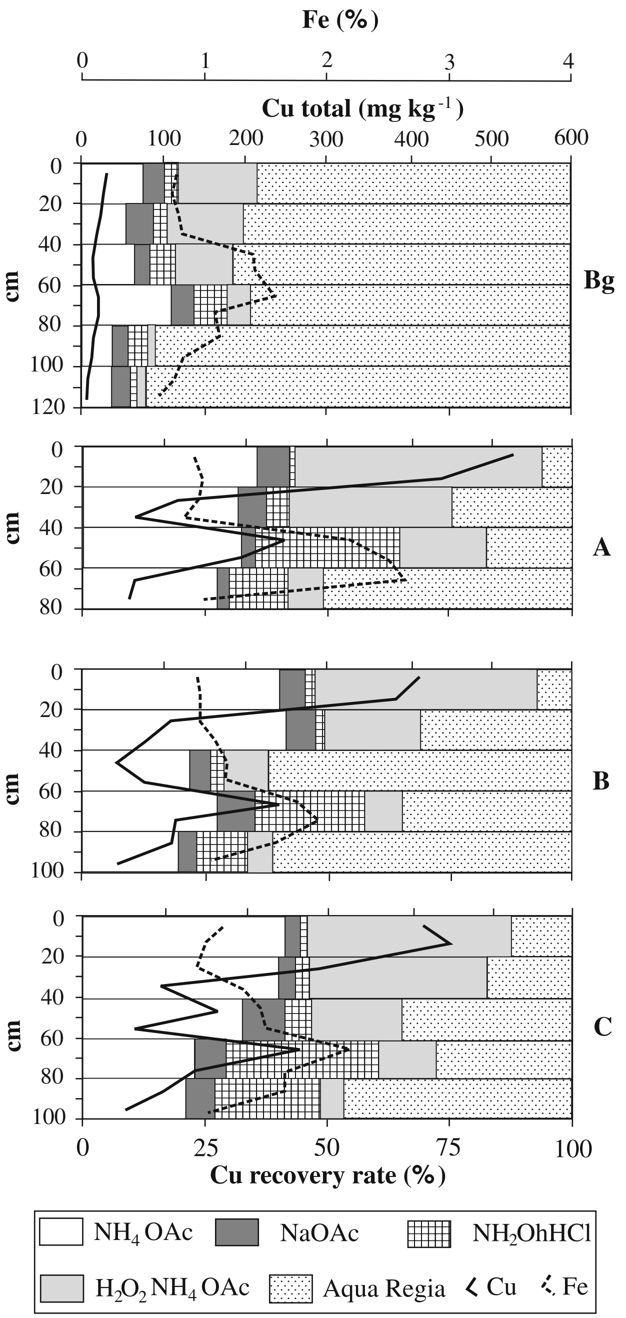

Fig. 1 Total Fe, Cu and copper fraction distributions along the soil profiles (*Bg*, *A*, *B* and *C*). Copper fractions by sequential extraction: "exchangeable and water soluble," NH_4Oac; "carbonate," NaOAc; "reducible," $NH_2OhxHCl$; "organic," H_2O_2 NH_4Oac; "residual," aqua regia

Rain events evenly distributed during the vegetation period and high humidity require grape growers to use copper-based fungicide more frequently to repress vine downy mildew attacks. In this climate, copper sulfate application can reach 80 kg/ha years, which surpasses the dosage of this fungicide in regions with temperate climate by several times (Brun et al. 1998). As a result, during 50 years of fungicide application, about 1,000 kg of Cu per ha should accumulate in vineyard soils. However, our data demonstrate that vineyard soils contain no more than 25% of this value. This negative balance of copper in vineyard soils has already been reported and was attributed to surface soil washout in undulating relief (Ribolzi et al. 2002). The vertical migration of copper was reported as unimportant, as fungicide copper normally does not penetrate below 30–40 cm of soil depth; it was therefore considered that copper normally could not contaminate shallow groundwater (Brun et al. 1998). Nevertheless, the data obtained in the current study testify that copper in sandy acid soils reaches deeper soil horizons, and at the level of groundwater its concentration several times surpasses the background value. Plain relief of the studied plots excludes any significant lateral soil erosion, so the fungicide copper depletion occurs predominantly due to vertical downward migration. The Fe-hydroxide barrier before the aquifer is the only obstacle that could prevent copper penetration in groundwater. However, the balance of copper applied in vineyards and retained in soil testifies that a major quantity of copper should pass through the barrier and enter the groundwater. The analyses of groundwater collected in soil profiles support this assumption. The copper concentration in groundwater from the background site was $4.5\ \mu g\ L^{-1}$, whereas in the vineyard plots it was significantly higher, ranging from 60.1 to $112.\ 3\ \mu g\ L^{-1}$.

The results of this study indicate that copper-based fungicide application in sandy soils in a wet subtropical climate leads to significant pollution of the soil by bioavailable copper, which may several times surpass the toxic threshold for plants. Within the soil profile, copper accumulates on the Fe-hydroxide geochemical barrier, forming a Cu-enriched layer. A high precipitation rates, together with the acidity of sandy soil, favor copper penetration down to the shallow groundwater aquifer and water contamination.

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