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Heavy Metal-Based Fungicides Alter the Chemical Fractions of Cu, Zn, and Mn in Vineyards in Southern Brazil

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Abstract: This study aimed to evaluate Cu, Zn, and Mn fractions in vineyard soils in two important wine-growing regions in Latin America, which have soils with different soil organic matter (SOM) and clay contents. Soils were collected from vineyards aged 35, 37, and 39 years (Serra Gaúcha) and 13, 19, and 36 years (Campanha Gaúcha). In each region, soils were collected from a non-anthropized area, and in the oldest vineyards, the collection was conducted on and between the planting lines. The available and total Cu, Zn, and Mn contents were analyzed in addition to the chemical fractions. The ΔCu, ΔZn, and ΔMn were also calculated by subtracting the contents of each fraction of the vineyards from the reference areas. The use of fungicides promotes increased metal contents in vineyard soils. In soils with high SOM contents, Cu tended to increase in the organic fraction in surface and depth. In contrast, Zn increased in the residual fraction, and Mn increased in most bioavailable fractions. Cu and Zn increased their contents in soils with low SOM and clay contents in the organic and mineral fractions. Mn accumulated in the mineral and residual fractions.

Keywords: heavy metals; *Vitis vinifera*; environmental contamination; chemical contamination; pesticides

Citation: Ferreira, G.W.; Bordallo, S.U.; Meyer, E.; Duarte, Z.V.S.; Schmitt, J.K.; Garlet, L.P.; Kokkonen da Silva, A.A.; Moura-Bueno, J.M.; Bastos de Melo, G.W.; Brunetto, G.; et al. Heavy Metal-Based Fungicides Alter the Chemical Fractions of Cu, Zn, and Mn in Vineyards in Southern Brazil. *Agronomy* **2024**, *14*, 969. https://doi.org/10.3390/agronomy14050969

Academic Editor: Agnieszka Klimkowicz-Pawlas

Received: 2 April 2024
Revised: 24 April 2024
Accepted: 1 May 2024
Published: 5 May 2024

1. Introduction

Soil pollution refers to the presence of a chemical or substance outside its natural environment and/or present at a higher concentration than normal, adversely affecting non-target organisms [1,2].

This issue has become a global concern [3,4]. It has been identified as the third most significant threat to soil functions in Europe and Eurasia, fourth in North Africa, fifth in Asia, seventh in the Northwest Pacific, eighth in North America, and ninth in sub-Saharan Africa and Latin America [1]. Although most pollutants, such as the heavy metals Cu and Zn, have anthropogenic origins, some contaminants can occur naturally in soils as components of minerals and may become toxic at high concentrations [1,5,6].

In the state of Rio Grande do Sul, Southern Brazil, there are two traditional grape-growing regions, Serra Gaúcha and Campanha Gaúcha. These regions have a humid subtropical climate, and frequent precipitation occurs throughout the growing cycle, which tends to favor the emergence of fungal diseases [7].

Thus, grapevines undergo successive applications of Cu-based fungicides, such as Bordeaux mixture [$Ca(OH)_2 + CuSO_4$], copper oxychloride [$CuCl_2.3Cu(OH)_2$], and Zn and Mn, found in Mancozeb ($C_4H_6MnN_2S_4 \times Zn$) [8,9], which can increase the levels of these metals in soils over the years [10–12]. Another factor to consider is that recent studies indicate that the increase in Cu and Zn in vineyard soils tends to increase the absorption of Mn by grapevines, which can be another concerning factor in the management of these agroecosystems [13,14]. This is because, in soils with high levels of Cu and Zn, there are changes in the rhizosphere through the exudation of organic acids, which alters the pH values in this region, favoring the solubilization of insoluble Mn compounds, which, when in solution, can be absorbed by crop plants, potentiating the symptoms of toxicity in these areas [15].

Bordeaux mixture, which is traditionally used as a fungicide and has been applied to vineyards in a proportion of 1 kg CuSO4 + 1 kg CaO in 100 L of water, has resulted in quantities of Cu added to the agroecosystem ranging from approximately 3.13 and 3.28 kg ha^{-1} $year^{-1}$ of Cu in the Bordeaux cultivar (*Vitis labrusca*). For the Cabernet Sauvignon and Isabel cultivars, these values jump to 6.76 and 6.20 kg ha^{-1} $year^{-1}$ of Cu, respectively, as reported for Southern Brazil [16]. On the other hand, Mancozeb has resulted in annual additions of up to 2 kg of Zn ha^{-1} [17]. Therefore, it is expected that, as the age of the vineyard increases, so will the total levels of these elements in the soil.

Although an increase in Cu, Zn, and Mn is expected in these two regions, the contrasting soils present will cause these elements to behave differently. The Serra Gaúcha region has predominantly naturally fertile, acidic soils with medium to high soil organic matter (SOM) content [18], reflecting a high capacity for retaining these metals and a lower potential for environmental contamination. In contrast, the soils of the Campanha Gaúcha region have a sandy texture; a predominance of 1:1 clay; low organic matter content; acidic soil; low natural fertility [18,19]; and, consequently, a low capacity for retaining Cu, Zn, and Mn and a higher potential for environmental contamination [20–22].

Given the above, studies that assess the forms, distribution, and accumulation of heavy metals in soils over the years, in different climate scenarios, soil types, and management systems, are becoming increasingly important in evaluating their impact on agroecosystems [23], especially vineyards. Globally, such research can assist in decision-making for the United Nations' sustainable development agenda [24], which includes ending hunger; achieving food security; improving nutrition; promoting sustainable agriculture; ensuring sustainable consumption and production patterns; protecting, restoring, and promoting the sustainable use of terrestrial ecosystems; the sustainable management of forests; combatting desertification; halting and reversing land degradation; and protecting biodiversity. Therefore, this work presents the following hypotheses: (i) High levels of Cu and Zn in vineyard soils in the southern region of Brazil alter the forms of Mn in these soils and, thereby, increase their bioavailability. (ii) The longer the vineyard cultivation time, the greater the changes in the forms of Cu, Zn, and Mn in the soil, with a more significant increase in the potentially bioavailable forms, contributing to an increased risk of contamination/pollution in these agroecosystems. To test these hypotheses, this study aimed to evaluate the fractions of Cu, Zn, and Mn in vineyard soils in Southern Brazil, which present different levels of SOM, clay, and management systems.

2. Material and Methods

2.1. Characterization of Study Sites and Soil Sampling

Vineyard areas were selected in the regions of Serra Gaúcha (municipality of Bento Gonçalves) and Campanha Gaúcha (municipality of Santana do Livramento) in the state of Rio Grande do Sul, Southern Brazil. The Serra Gaúcha region is located at an altitude of 600–800 m and has annual averages of precipitation, temperature, and relative humidity of 1700 mm, 17.2 °C, and 76%, respectively. The predominant soil is Litholic Entisol [25]. The Campanha Gaúcha region is located at an altitude of 100–300 m and has annual averages of precipitation, temperature, and relative humidity of 1370 mm, 18.4 °C, and 75%, respectively [26]. The predominant soil is Sandy Typic Hapludalf [25]. In each of these regions, three vineyards were selected that had different cultivation times and, consequently, different histories of applications of Cu-, Zn-, and Mn-based fungicides.

In Serra Gaúcha, the three selected vineyards were 35 (V35, 29°09′50″ S and 51°32′03″ O), 37 (V37, 29°09′48″ S and 51°31′45″ W), and 39 years old (V39, 29°09′42″ S and 51°31′44″ W). In addition, soil samples were collected in a forest area (F, 29°09′46″ S and 51°31′49″ W) adjacent to the vineyards. In the V35 and V37 vineyards, soil was collected in the planting rows. In V39, soil was collected in both planting rows and interrows (V39BL). The cultivar in all three vineyards was Isabel (*Vitis labrusca* L.), planted on its roots. The training system was a trellis. The main fungicides used in the vineyards were Delan®, Captan®, Folpan®, Manzate®, and Curzate®. There were 16 annual applications of these products, in addition to 3 applications of the Bordeaux mixture. At the time of soil collection, it had been five years since fertilizers and acidity correctives had been applied to the vineyards.

In Campanha Gaúcha, the three selected vineyards were 13 (V13, 30°46′39″ S and 55°22′35″ W), 19 (V19, 30°46′38″ S and 55°21′59″ W), and 36 years old (V36, 30°46′50″ S and 55°21′07″ W). In addition, soil samples were collected in a native grassland area (NG, 30°47′26″ S and 55°22′04″ W) adjacent to the vineyards. The cultivar in all three vineyards was Cabernet Sauvignon (*Vitis vinifera*), grafted onto rootstock SO4 (*Vitis berlandieri* × *Vitis riparia*). The training system was the Geneva Double Curtain (GDC). The main species found in the NG were *Paspalum notatum*, *Paspalum plicatulum*, *Desmodium incanum*, *Ageratum conyzoides* L., *Chevreulia acuminata* Less, and *Cyperus brevifolius*. In the V13 and V19 vineyards, soil was collected in the planting rows, whereas, in the V36 vineyard, soil was collected in the planting rows and interrows (V36BL). In each vineyard, 9.0 kg ha^{-1} $year^{-1}$ of copper sulfate and 8.0 kg ha^{-1} $year^{-1}$ of copper hydroxide were applied, totaling 8.8 kg ha^{-1} $year^{-1}$ of Cu.

The collection of soil in the oldest vineyards of each region, in the planting rows and interrows, was due to the presence of cover crops in the interrows favoring the immobilization of these metals in the SOM, while in the planting rows, the spontaneous vegetation is desiccated, favoring the mineralization of SOM, consequently keeping these metals in more labile fractions in the soil. Thus, the oldest vineyards were chosen to perform this differentiation, as the differences would be more evident in areas with a long history of vineyard management.

In all areas in Serra Gaúcha, soil samples were collected in July 2017 at layers of 0.00–0.05, 0.05–0.10, 0.10–0.15, and 0.15–0.20 m. In Campanha Gaúcha, soil samples were collected at layers of 0.00–0.05, 0.05–0.10, 0.10–0.20, and 0.20–0.40 m. Soil samples were collected from six points in each area [10].

2.2. Preparation of Soil Samples and Chemical Analyses

The soil samples were air-dried, ground, and passed through a 2 mm mesh sieve. The clay content was determined by the pipette method [27]. The total organic carbon (TOC) content was determined using an auto-analyzer (LECO, TruSpec CHNS, St. Joseph, MI, USA). pH value in water (1:1 soil–water ratio); SMP index; available P, K, Cu, Zn, and Mn contents (extracted by Mehlich-1); and exchangeable Al, Ca, and Mg contents (extracted with 1.0 mol L^{-1} KCl) were determined according to the soil analysis manual used in Southern Brazil [28]. In the obtained solution, the available P content was determined by colorimetry using a UV–visible spectrophotometer (UV—1600, Pró-Análise, Porto Alegre, Brazil) [29]. The K content was determined using a flame photometer (DM-62, DIGIMED, São Paulo, Brazil). All values were obtained by titration with 0.0125 mol L^{-1} NaOH. The Ca, Mg, Cu, Zn, and Mn contents were determined using an Atomic Absorption Spectrophotometer (Aanalyst 200, PERKIN ELMER, Waltham, MA, USA). With the obtained data, the H + Al contents, potential ($CEC_{pH7.0}$) and effective ($CEC_{ef.}$) cation exchange capacity, saturation of $CEC_{pH7.0}$ by Ca + Mg + K, and saturation by Al were calculated [30] (Table A1).

The chemical fractionation of Cu, Zn, and Mn was performed through a sequential extraction, obtaining the following chemical fractions: soluble (extracted with Milli-Q water—Cu_{Sol}, Zn_{Sol}, and Mn_{Sol}); exchangeable (extracted with $MgCl_2$—Cu_E, Zn_E, and Mn_E); extracted with NH_2OHHCl + CH_3COOH, commonly called the fraction associated with clay minerals (Cu_{Min}, Zn_{Min}, and Mn_{Min}); extracted with HNO_3 + H_2O_2, commonly called the fraction associated with SOM (Cu_{OM}, Zn_{OM}, and Mn_{OM}); and residual (extracted with HF + $HClO_4$) [31]. In this study, the same nomenclature was used, although we understand that the extractors used for the extraction of the fractions said to be associated with clay minerals and SOM do not exclusively extract the metals associated with these soil particles.

For this purpose, in triplicate, 1.0 g of soil was weighed in Falcon tubes with a capacity of 50 mL, to which the following extractors were applied in sequence: (1) soluble fraction, extracted with 8 mL of Milli-Q water; (2) exchangeable fraction, bound to the negative charges of the soil, extracted with 8 mL of 1.0 mol L^{-1} $MgCl_2$ solution at pH 7.0; (3) fraction bound to clay minerals, extracted with 20 mL of 0.04 mol L^{-1} NH_2OHHCl solution in 25% (*v*/*v*) CH_3COOH at pH 2.0; (4) fraction bound to SOM, extracted with 3 mL of 0.02 mol L^{-1} HNO_3 solution + 8 mL of 30% H_2O_2 adjusted to pH 2.0 with HNO_3; and (5) residual fraction, which was extracted from total digestion with HF and $HClO_4$. After each extraction, the samples were centrifuged at 3500 rpm for 30 min, and an aliquot of the supernatant was filtered for the determination of Cu, Zn, and Mn contents.

The total Cu, Zn, and Mn contents (Cu_{Total}, Zn_{Total}, and Mn_{Total}) were obtained by digestion with HF and $HClO_4$ in a 5:1 ratio [31] from a new soil sample from the same soil collection carried out in each study area. The Cu_{Sol}, Zn_{Sol}, and Mn_{Sol} contents were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-OES) (Perkin Elmer, Optima 2100 DV, Waltham, MA, USA), and the content of the other fractions (Cu_E, Zn_E, Mn_E, Cu_{Min}, Zn_{Min}, Mn_{Min}, Cu_{OM}, Zn_{OM}, Mn_{OM}, Cu_{Res}, Zn_{Res}, and Mn_{Res}) and the total contents (Cu_{Total}, Zn_{Total}, and Mn_{Total}) were determined by Atomic Absorption Spectrometry (AAS).

2.3. Statistical Analysis

The metal contents in each fraction of the vineyard areas were compared with the fractions of the reference areas with the calculation of ΔCu, ΔZn, and ΔMn using Equation (1):

$$M = MVFraction - MRFraction \tag{1}$$

where ΔM is the metal to be calculated (Cu, Zn, or Mn); MVFraction is the Cu, Zn, or Mn content obtained in the fraction (soluble, exchangeable, associated with SOM, associated with minerals, or residual), in mg kg^{-1}, in each vineyard area; MRFraction is the Cu, Zn, or Mn content obtained in the fraction (soluble, exchangeable, associated with soil organic matter, associated with minerals, or residual), in mg kg^{-1}, in each reference area.

The available Cu, Zn, and Mn contents for each obtained fraction among the areas were subjected to the homoscedasticity test (F-max test). When the variances were homogeneous, they were subjected to parametric analysis for two situations using the t-Student test. The same data, when compared between the evaluated layers, were also subjected to a homoscedasticity test, and the variances, when homogeneous, were subjected to the Tukey mean separation test ($p < 0.05$) using the software Sisvar (v. 5.6).

The data were standardized and subjected to principal component analysis (PCA), with the help of R Statistic software v. 3.6.2 [32], using the packages "FactorMinerR" [33] and "factoextra" [34], to evaluate the similarity between the data for TOC; clay; pH in water; available P; $CTC_{pH7.0}$; available and total Cu, Zn, and Mn levels; and fractions of Cu, Zn, and Mn.

3. Results

3.1. Available and Total Cu, Zn, and Mn Contents in Soils

The soils of Serra Gaúcha showed the highest available Cu contents in the 0.00–0.10 m layer in all evaluated areas, with emphasis on the F and V35 areas (Figure 1A). The same pattern was applied to the highest available Zn contents in the 0.00–0.05 m layer, with the

V39BL area being an exception. In the other layers, the F area had the highest contents (Figure 1B). In the case of available Mn, most vineyards showed the highest contents in the 0.00–0.05 m layer, except for the F area, where the highest contents were observed in the 0.10–0.20 m layer (Figure 1C). For the total Cu and Zn contents, areas V39 and V39BL exhibited higher values across all the soil layers evaluated (Figure 1D or Figure 1E). For total Mn, the highest contents were observed in the deeper layers, with the F area being an exception, having the highest Mn contents compared with the vineyards in all layers, except in the 0.05–0.10 m layer (Figure 1F).

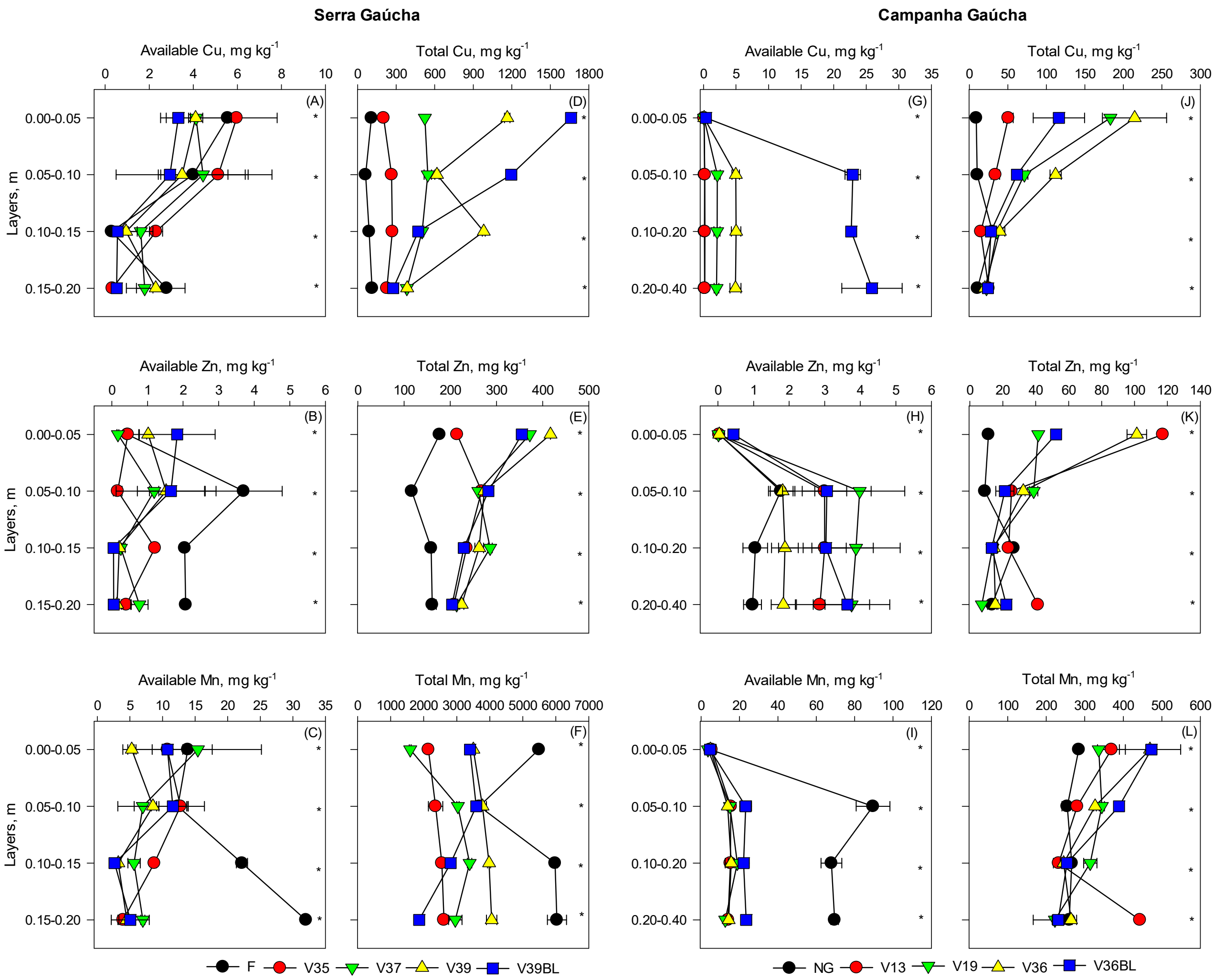


Figure 1. Available contents, extracted by Mehlich-1, and total contents, extracted by digestion with HF and $HClO_4$, respectively, for Cu (**A**,**D**), Zn (**B**,**E**), and Mn (**C**,**F**) in soils of vineyards in the Serra Gaúcha region and Cu (**G**,**J**), Zn (**H**,**K**), and Mn (**I**,**L**) in the soils of vineyards in the Campanha Gaúcha region. F: forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected in planting interrows; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected in planting interrows. * indicates a significant difference between the evaluated depths based on the Tukey test at a 5% significance level. The bars represent the standard error of the mean.

In the Campanha Gaúcha, there was no difference in the available Cu and Zn contents in the 0.00–0.05 m layer. However, in the deeper layers, from 0.05 to 0.40 m, the vineyards showed higher Cu and Zn contents compared with NG (Figure 1G,H). For available Mn, there was no difference between the areas in the 0.00–0.05 m layer, but in the deeper layers, NG presented the highest contents (Figure 1I). The total Cu, Zn, and Mn contents were higher in the 0.00–0.10 m layer in the vineyards, with these values being higher in the older vineyards. In the other layers, the total Cu contents did not show differences between the vineyards and NG (Figure 1J), whereas, for the Zn and Mn contents, there was no difference between vineyards V19, V36, and V36BL and the NG (Figure 1K,L).

3.2. Cu: Contents and Distribution in Fractions

In the Serra Gaúcha areas, the highest percentages of Cu in the reference area (F) were observed in the Cu_{Res} fraction, with percentages ranging from 68% to 79% in the evaluated layers (Figure 2). However, with the cultivation of grapevines over the years, there has been a reduction in the proportion of Cu in the Cu_{Res} fraction, ranging from 44% in V35 to 11% in V39BL in the 0.00–0.05 m layer. Nevertheless, the percentage of Cu in the Cu_{OM} fraction increased, especially in V35 and V37, with values of 43% and 65%, respectively, in the 0.00–0.05 m layer, occurring similarly in the other layers. Therefore, with the increased cultivation time of the vineyards, there has been a reduction in the percentage of Cu in the Cu_{Res} fraction in favor of Cu_{OM} (Figure 2). The Cu contents in the fractions in the soils of Serra Gaúcha can be observed in Table A2.

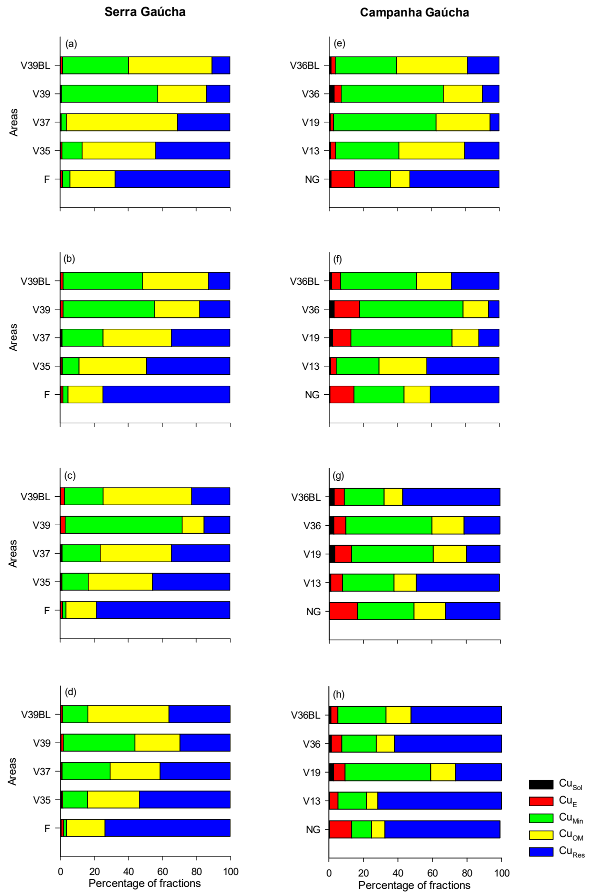


Figure 2. Percentage distribution of Cu fractions for the 0.00–0.05 (**a**), 0.05–0.10 (**b**), 0.10–0.15 (**c**), and 0.15–0.20 m (**d**) layers for the areas of the Serra Gaúcha region and the 0.00–0.05 (**e**), 0.05–0.10 (**f**), 0.10–0.20 (**g**), and 0.20–0.40 m (**h**) layers for the areas of the Campanha Gaúcha region. F: forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Cu_{Sol}: copper in the soluble fraction; Cu_E: exchangeable fraction copper; Cu_{Min}: copper from the fraction bound to clay minerals; Cu_{OM}: copper from the fraction bound to soil organic matter; Cu_{Res}: copper from the residual fraction.

In the Campanha Gaúcha region, a similar behavior was observed, with a reduction in the percentages of Cu in the Cu_{Res} fraction and an increase in the Cu_{OM} fraction. The distribution of Cu fractions observed in the 0.00–0.05 and 0.05–0.10 m layers shows that, for the NG, there is a higher percentage of Cu in the Cu_{Res} fraction (52 and 41%, respectively). In the vineyards, however, the percentage of Cu in the Cu_{Res} fraction decreased, whereas the Cu_{OM} (39, 32, and 23%) and Cu_{Min} (37, 60, and 60%) fractions increased for vineyards V13, V19, and V36 in the 0.00–0.05 m layer. In V36BL, the percentages of Cu in the Cu_{Res} fraction were higher (42 and 28%) than those observed in V36 (23 and 7%) in the 0.00–0.05 and 0.05–0.10 m layers, respectively. In the 0.10–0.20 and 0.20–0.40 m layers, the highest percentages of Cu were observed in the Cu_{Res} and Cu_{Min} fractions (Figure 2). The Cu contents in the fractions in the soils of the Campanha Gaúcha region are shown in Table A2.

The PCAs distinguished the vineyards from reference areas F and NG in Serra Gaúcha and Campanha Gaúcha, with this distinction occurring mainly between the reference areas and V39 and V36, respectively. In the Serra Gaúcha region, Dim1 was responsible for explaining 44.3% of the variance, indicating that clay and the Cu_{Total}, Cu_{Sol}, Cu_{OM}, and Cu_{Res} fractions are positively correlated with each other and have a negative correlation with Cu_{Av} contents. On the other hand, Dim2, which explains 22.1% of the data variation, shows that TOC, pH, $CTC_{pH7.0}$, and P_{Av} are positively correlated with each other (Figure 3a). In Campanha Gaúcha, Dim1, which is responsible for explaining 45.1% of the variance, shows that the Cu_{Min}, Cu_{OM}, and Cu_{Total} fractions are positively correlated. Meanwhile, Dim2, which explained 16.0% of the data variation, showed that the Cu_{Res} and Cu_{Av} contents had a positive correlation but a negative correlation with the contents of clay (Figure 3b).

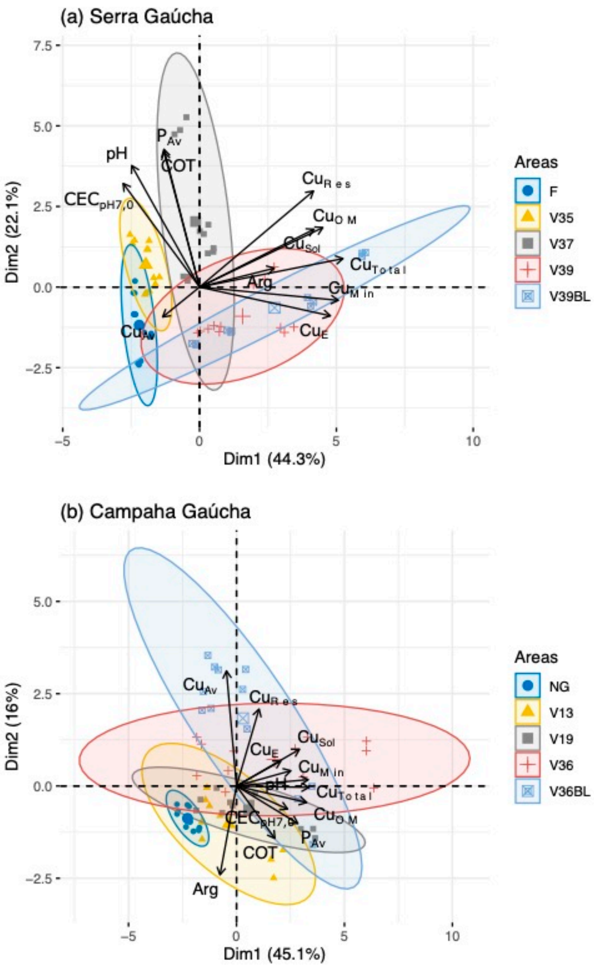


Figure 3. Principal component analysis (PCA) for available and total Cu levels and levels of Cu chemical

fractions in areas of Serra Gaúcha (**a**) and Campanha Gaúcha (**b**) vineyards with different years of cultivation. F: forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Cu_{Sol}: copper in the soluble fraction; Cu_E: exchangeable fraction copper; Cu_{Min}: copper from the fraction bound to clay minerals; Cu_{OM}: copper from the fraction bound to soil organic matter; Cu_{Res}: copper from the residual fraction; Cu_{Total}: total copper; $Cu_{Av.}$: available copper extracted by Mehlich-1 extractor, $P_{Av.}$: available phosphorus extracted by Mehlich-1 extractor; $CEC_{pH7.0}$: cation exchange capacity at pH 7.0; TOC: total organic carbon; clay: clay content.

3.3. Zn: Contents and Distribution in Fractions

In the soils of Serra Gaúcha, the highest percentages of Zn were observed in the Zn_{Res} fraction in all layers of the vineyards and area F (Figure 4a–d). With the increase in the cultivation time of the vineyards, there was a reduction in the percentages of Zn_{Res} and an increase in Zn_{Min} in the 0.00–0.10 m layer (Figure 4a,b). In V39BL, the highest percentage of Zn_{Min} was observed compared with V39, especially in the 0.00–0.10 m layer (Figure 4a,b). The Zn contents in the fractions in the soils of Serra Gaúcha can be observed in Table A3.

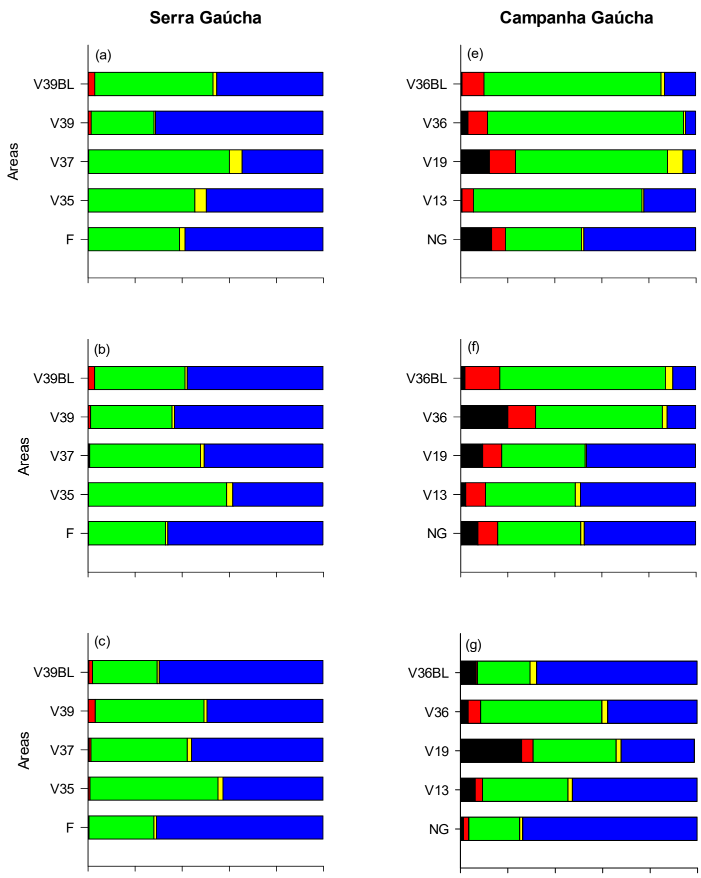


Figure 4. *Cont.*

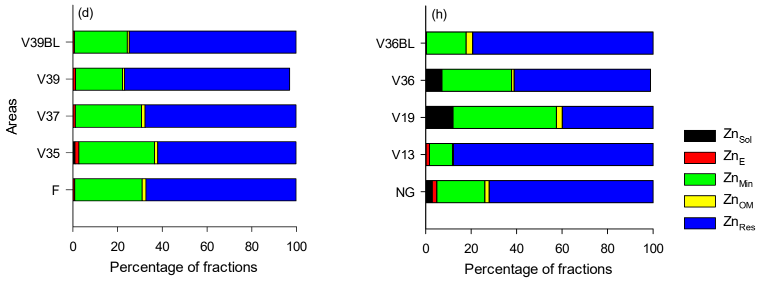


Figure 4. Percentage distribution of Zn fractions for the 0.00–0.05 (**a**), 0.05–0.10 (**b**), 0.10–0.15 (**c**), and 0.15–0.20 m (**d**) layers in the Serra Gaúcha region and the 0.00–0.05 (**e**), 0.05–0.10 (**f**), 0.10–0.20 (**g**) and 0, 20–0.40 m (**h**) layers in the Campanha Gaúcha region. F: forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Zn_{Sol}: zinc from the soluble fraction; Zn_E: exchangeable fraction zinc; Zn_{Min}: zinc from the fraction bound to clay minerals; Zn_{OM}: zinc from the fraction bound to soil organic matter; Zn_{Res}: zinc from the residual fraction.

In Campanha Gaúcha, the highest percentages of Zn were found in the Zn_{Min} and Zn_{Res} fractions in all layers, both in the NG and V13 (Figure 4e–h). With the increase in the cultivation time of the vineyards, there was a reduction in the percentages of Zn_{Res} and an increase in the Zn_{Sol}, Zn_E, and Zn_{OM} fractions, especially in the 0.00–0.10 m layer (Figure 4e,f). In the deeper layers of vineyards V19, V36, and V36BL, the highest percentages of Zn were observed in the Zn_{Min} and Zn_{Res} fractions (Figure 4g,h). The Zn contents in the fractions in the soils of Campanha Gaúcha can be observed in Table A3.

The PCAs conducted for the Zn contents in the areas evaluated in Serra Gaúcha and Campanha Gaúcha helped to explain the total variability of the data (Figure 5). In Serra Gaúcha, there is a strong correlation between the F area and V39. In this area, Dim1, responsible for explaining 36.1% of the variance, shows that TOC, P_{Av}, and Zn_{OM} were positively correlated with each other and negatively correlated with Zn_{Av}. Meanwhile, Dim2, which explained 22.6% of the data variation, showed a positive correlation between the Zn_E, Zn_{Res}, Zn_{Total}, and clay fractions and that these same fractions had a negative correlation with the Zn_{Sol} fraction (Figure 5a). In Campanha Gaúcha, there is no clear distinction between the reference area and the vineyard areas. In this area, Dim1, responsible for explaining 47.2% of the variance, showed that the Zn_E, Zn_{Min}, Zn_{Total}, and $CEC_{pH7.0}$ fractions were positively correlated with each other and negatively with Zn_{Av}. Meanwhile, Dim2, which explained 16.7% of the data variation, demonstrated a positive correlation between clay, TOC, and the Zn_{Res} fraction, and that these same fractions had a negative correlation with the Zn_{Sol} fraction (Figure 5b).

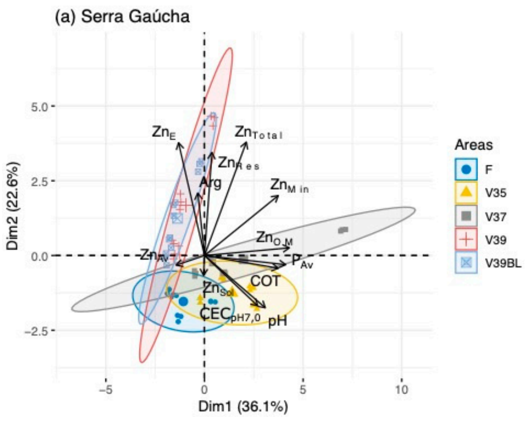


Figure 5. *Cont.*

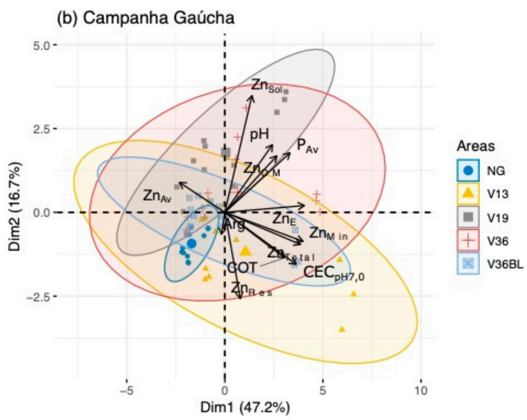
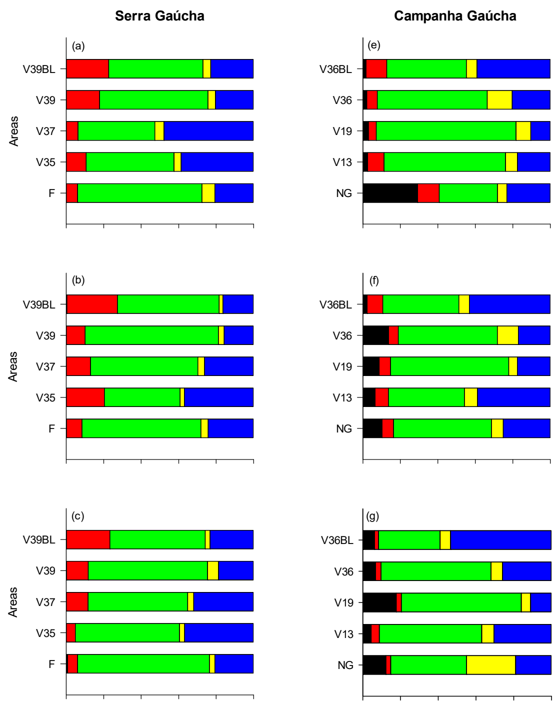


Figure 5. Principal component analysis (PCA) for available and total Zn levels, levels of chemical fractions of Zn, TOC levels, and clay contents in areas of Serra Gaúcha (**a**) and Campanha Gaúcha (**b**) vineyards with different years of cultivation conduction. F: forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Zn_{Sol}: zinc in the soluble fraction; Zn_E: exchangeable fraction zinc; Zn_{Min}: zinc from the fraction bound to clay minerals; Zn_{OM}: zinc from the fraction bound to soil organic matter; Zn_{Res}: zinc from the residual fraction; Zn_{Total}: total zinc; Zn_{Av}: available zinc extracted by Mehlich-1 extractor; $P_{Av.}$: available phosphorus extracted by Mehlich-1 extractor; $CEC_{pH7.0}$: cation exchange capacity at pH 7.0; TOC: total organic carbon; clay: clay content.

3.4. Mn: Contents and Distribution in Fractions

In Serra Gaúcha, the highest percentage of Mn was observed in the Mn_{Min} fraction, followed by Mn_{Res} and Mn_E in all areas and layers. In the V35 and V37 vineyards, it was noted that the proportion of Mn in the Mn_{Res} fraction was higher than in V39 and V39BL, mainly in the 0.00–0.15 m layer (Figure 6). The Mn contents in the fractions in the soils of Serra Gaúcha can be observed in Table A4.



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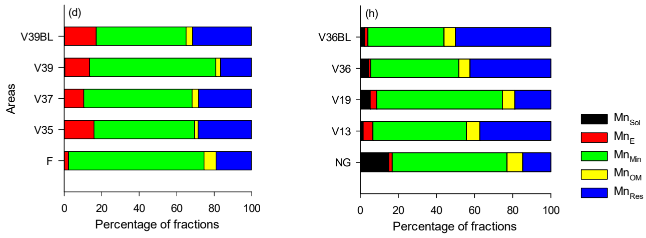
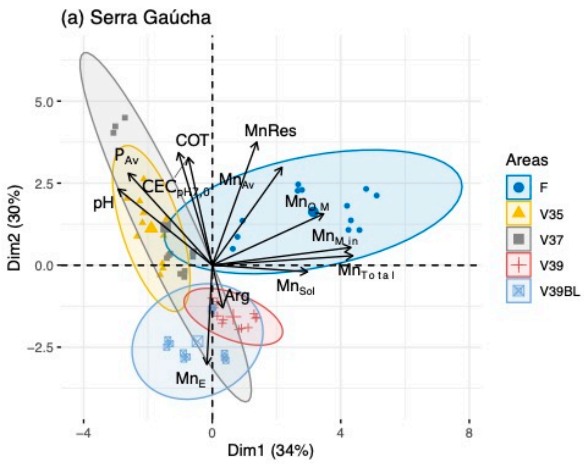


Figure 6. Percentage distribution of Mn fractions for the 0.00–0.05 (**a**), 0.05–0.10 (**b**), 0.10–0.15 (**c**), and 0.15–0.20 m (**d**) layers in the Serra Gaúcha region and the 0.00–0.05 (**e**), 0.05–0.10 (**f**), 0.10–0.20 (**g**) and 0.20–0.40 m (**h**) layers in the Campanha Gaúcha region. F: forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Mn_{Sol} = manganese in the soluble fraction; Mn_E: exchangeable fraction manganese; Mn_{Min}: manganese from the fraction bound to clay minerals; Mn_{OM}: manganese from the fraction bound to soil organic matter; Mn_{Res}: manganese from the residual fraction.

In the Campanha Gaúcha region, the highest percentages of Mn in the vineyards were found in the Mn_{Min} fraction in all layers. In the NG, the highest percentages of Mn were observed in the Mn_{Sol} and Mn_{Min} fractions in the superficial layers and in the Mn_{Min} fraction in the deeper layers. In V36BL, the highest percentage of Mn was verified in the Mn_{Res} fraction, especially in the 0.00–0.05 m layer (Figure 6). The Mn contents in the fractions in the soils of Campanha Gaúcha can be observed in Table A4.

In Serra Gaúcha, PCA separated the F area from the vineyard areas (Figure 7). PCA showed that Dim1 is responsible for explaining 34.0% of the variance and that the Mn_{Sol}, Mn_{Min}, Mn_{OM}, and Mn_{Total} fractions are positively correlated with each other, especially in the F area. Meanwhile, Dim2 explained 30.0% of the data variation and showed that TOC, $CEC_{pH7.0}$, pH, and P_{Av} presented a positive correlation with each other and that they negatively correlated with the clay contents and Mn_E, especially in the V35 and V37 vineyards. In the V39 and V39BL vineyards, the opposite of what was observed in the other vineyards can be observed (Figure 7a). In Campanha Gaúcha, there is no clear distinction between the vineyard areas and the NG. PCA showed that in Dim1, responsible for explaining 38.1% of the variance, pH, TOC, $CEC_{pH7.0}$, and the Mn_E and Mn_{Total} fractions had a positive correlation with each other and a negative correlation with Mn_{Av}. Dim2 explained 16.1% of the total variation of the data and showed that the Mn_{Sol}, Mn_{Min}, Mn_{OM}, and clay fractions were correlated with each other and negatively correlated with the Mn_{Res} fraction (Figure 7b).



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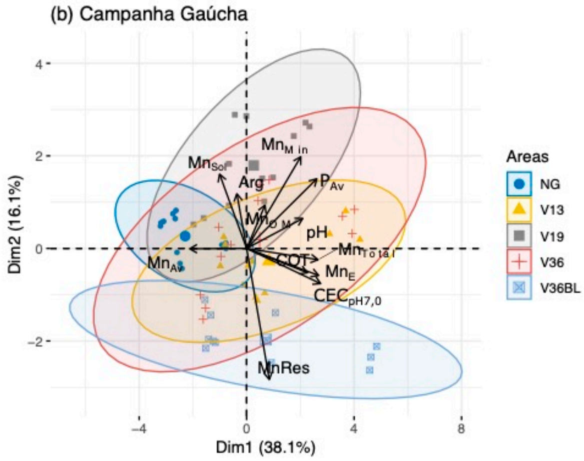

Figure 7. Principal component analysis (PCA) for available and total Mn contents, Mn chemical fraction contents, TOC contents, and clay contents in areas of Serra (**a**) and Campanha Gaúcha (**b**) vineyards with different years of cultivation conduction. F: forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Mn_{Sol}: manganese in the soluble fraction; Mn_E: exchangeable fraction manganese; Mn_{Min}: manganese from the fraction bound to clay minerals; Mn_{OM}: manganese from the fraction bound to soil organic matter; Mn_{Res}: manganese from the residual fraction; Mn_{Total}: total manganese; $Mn_{Av.}$: available manganese extracted by Mehlich-1 extractor; $P_{Av.}$: available phosphorus extracted by Mehlich-1 extractor; $CEC_{pH7.0}$: cation exchange capacity at pH 7.0; TOC: total organic carbon; clay: clay content.

3.5. Values of ΔCu, ΔZn, and ΔMn in Vineyard Soils

In Serra Gaúcha, the values of ΔCu—the difference between the vineyards and the reference area (F)—were positive in all fractions and layers, with a significant increase in the values of Cu_{OM} in V39BL. For the V39 and V39BL areas, there was also an increase in the values of Cu_{Min}, mainly in the 0.00–0.50 and 0.05–0.10 m layers (Figure 8a). In Campanha Gaúcha, the values of ΔCu were positive, especially in the 0.00–0.05 m layer, indicating an increase in Cu in fractions such as Cu_{OM} and Cu_{Res} due to the cultivation of vineyards and the use of Cu-based fungicides over time. The Cu_{Sol}, Cu_E, and Cu_{Min} contents also increased, mainly in the 0.00–0.10 m layer (Figure 8d).

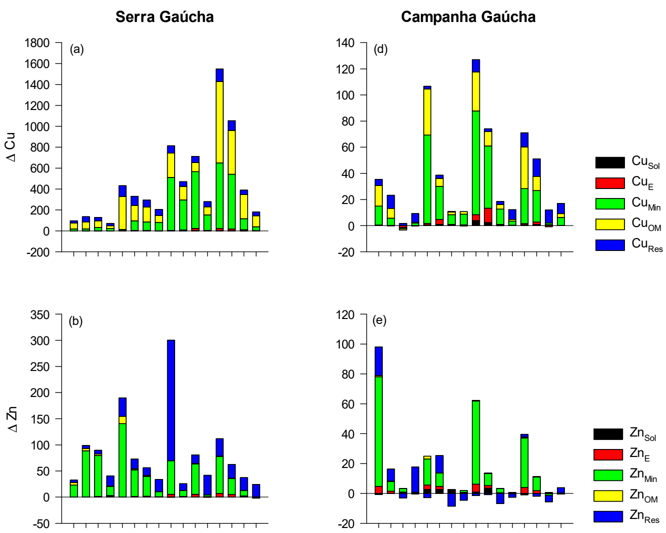

Figure 8. *Cont.*

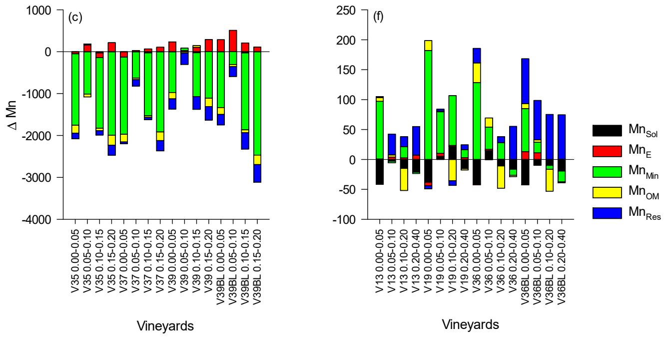

Figure 8. ΔCu, ΔZn, and ΔMn values of the fractions in the soils of the vineyard areas of the Serra Gaúcha region (**a**–**c**) and of Campanha Gaúcha region (**d**–**f**) in comparison with the reference areas. V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Cu_{Sol}: copper in the soluble fraction; Cu_E: exchangeable fraction copper; Cu_{Min}: copper from the fraction bound to clay minerals; Cu_{OM}: copper from the fraction bound to soil organic matter; Cu_{Res}: copper from the residual fraction; Cu_{Total}: total copper; Zn_{Sol}: Zn in the soluble fraction; Zn_E: exchangeable fraction zinc; Zn_{Min}: zinc from the fraction bound to clay minerals; Zn_{OM}: zinc from the fraction bound to soil organic matter; Zn_{Res}: zinc from the residual fraction; Zn_{Total}: total zinc; Mn_{Sol}: manganese in the soluble fraction; Mn_E: exchangeable fraction manganese; Mn_{Min}: manganese from the fraction bound to clay minerals; Mn_{OM}: manganese from the fraction bound to soil organic matter; Mn_{Res}: residual fraction manganese.

The values of ΔZn in the vineyards of Serra Gaúcha were negative only for the Zn_E fraction in V35 (0.00–0.10 m). For V39, ΔZn was negative in the Zn_{OM} fraction in the 0.00–0.05 and 0.15–0.20 m layers, whereas, for the V39BL area, these values were negative for the Zn_{Min} and Zn_{OM} fractions (0.15–0.20 m). The cultivation time influenced the increase in the Zn_{Min} and Zn_{OM} contents in the V39 and V39BL areas (Figure 8b). In Campanha Gaúcha, the values of ΔZn were positive, mainly in the Zn_{Min} fraction. However, some of the other fractions showed negative deltas in all layers evaluated in the vineyards (Figure 8e).

The values of ΔMn in the soils in Serra Gaúcha were negative in almost all fractions, except Mn_E, which showed positive deltas in all areas and layers. The Mn_{Min} fraction was higher in the F area than in the vineyards (Figure 8c). In the soils of Campanha Gaúcha, the Mn_{Min} fraction showed high values of ΔMn in the 0.00–0.05 m layer, along with a slight increase in Mn_{OM} contents. In the older vineyards, there was a significant increase in Mn in the Mn_{Res} fraction, but a decrease in the Mn contents in the Mn_{Sol} (0.00–0.05 m) and Mn_{OM} (0.10–0.20 m) fractions (Figure 8f).

4. Discussion

4.1. Available and Total Cu, Zn, and Mn Contents in Soils

The higher amounts of available and total Cu and Zn in the vineyard soils of Serra Gaúcha and Campanha Gaúcha are a consequence of successive applications of foliar fungicides that contain these elements in their composition [8,35,36]. The same can happen to Mn according to [37]. According to these authors, the use of fungicides that contain Mn in their composition, such as Mancozeb, can increase the availability of this element to the point of becoming toxic to plants over the years. The application of copper fungicides has added approximately 6.76 and 6.20 kg ha^{-1} $year^{-1}$ of Cu to the Cabernet Sauvignon and Isabel cultivars, respectively, in Southern Brazil [16]. For Zn, the use of fungicides such as Mancozeb [($C_8H_{12}MnN_4S_8Zn$)] and Propineb [($C_5H_8N_2S_4Zn$)] has resulted in annual additions of up to 2 kg ha^{-1} of Zn [17]. Therefore, it is expected that, with the increase in vineyard cultivation time, the available and total contents of these elements in the soil will also increase.

The higher amounts of available Cu, Zn, and Mn in areas with cover crops grown in the interrows (V39BL—Serra Gaúcha and V36BL- Campanha Gaúcha) along the soil profile can be attributed to the greater migration of these elements to deeper soil layers. This can happen because cover crops can accumulate these elements in the root system tissue [38], which grows in depth. Thus, as the roots senesce, they are decomposed, and with mineralization, these elements tend to pass into the soil, increasing their contents in depth. Also, the roots of cover crops can create biopores in the soil, which can facilitate the downward flow of water [39], stimulating the migration of these elements to subsurface layers. Another point is that plants stressed by excess metals can exude low-molecular-weight organic acids, such as citric, oxalic, and succinic acids, which can play a critical role in alleviating the phytotoxicity of these elements [40]. In other cases, the stresses caused by the excess of these metals in the soil are so great that the plants can release other types of root exudates, such as phytosiderophores and phenolic compounds [41], which increase the concentration of these metals in solution, stimulating migration along the soil profile.

The available Cu, Zn, and Mn contents in the 0–0.10 m layer in the vineyards of Serra Gaúcha varied from 3.96 to 5.70; 0.41 to 2.34; and 8.05 to 13.30 mg kg^{-1}, while in Campanha Gaúcha, they varied from 0.14 to 7.10; 0.90 to 2.00; and 9.28 to 47.09 mg kg^{-1} (Table A1). According to the "Manual de Calagem e Adubação para os estados do Rio Grande do Sul e Santa Catarina" (Liming and Fertilization Manual for the States of Rio Grande do Sul and Santa Catarina) from Southern Brazil [30], when Cu, Zn, and Mn contents are above 0.4, 0.5, and 5.0 mg kg^{-1}, respectively, they are considered high.

The total Cu, Zn, and Mn contents in the soils of Serra Gaúcha exceeded the reference values for Cu and Mn established by Brazilian environmental agencies for agricultural soils, which are 200 mg kg^{-1} Cu, 450 mg kg^{-1} Zn, and 400 mg kg^{-1} Mn in the 0.00–0.20 m layer (USEPA 3050b) [42,43]. On the other hand, the continuous application of metal-based fungicides did not increase the total Cu and Zn contents over the years in Campanha Gaúcha, to the point of exceeding the values established by Brazilian environmental legislation and other countries with a tradition of vine cultivation. The USEPA sets a maximum limit of 1500 and 2800 mg kg^{-1} of Cu and Zn, respectively, but does not determine the values for Mn [44]. The European Community allows 50 to 140 mg kg^{-1} of Cu and 150 to 300 mg kg^{-1} of Zn for agricultural soils with a pH between 6.0 and 7.0. However, it does not present values for Mn [45]. In Australia and New Zealand, total contents of 2 to 100 mg kg^{-1} of Cu, 10 to 300 mg kg^{-1} of Zn, and 850 mg kg^{-1} of Mn indicate the need for an environmental assessment [46].

The observed difference between the two regions is associated with the types of soils found in these locations. The Serra Gaúcha region presents a predominance of fertile soils, with organic matter content ranging from medium to high and with higher clay contents [18], leading to the increased adsorption of these elements in the soil's colloidal system, thus reducing the potential for transfer to the environment [10,47]. In contrast, the soils of the Campanha Gaúcha region have a sandy texture, a predominance of 1:1 clay, low organic matter content, and low natural fertility [18,19]. These characteristics result in the metals added via fungicide applications being poorly adsorbed by the colloidal system, thus increasing the potential for transfer to the environment, especially by surface runoff [20].

4.2. Cu, Zn, and Mn Contents in Chemical Fractions

The highest Cu contents bound to the Cu_{Res} fraction, extracted with HF + HCl_2O_2, in the superficial layers in the vineyards in the Serra Gaúcha region may occur due to the presence of recalcitrant organic carbon, given that Cu has an electronic configuration of [Ar] $3d^{10}$ $4s^1$ and, thus, high reactivity with the functional groups of SOM that contain S, N, carboxylic, and phenolic groups [48,49]. This phenomenon decreases the desorption of Cu and, consequently, its mobility in the soil [50]. In the deeper layers, the highest contents occur due to clay and silt, amorphous organic matter, and clay minerals, as well as reaction time [31]. In the soils of Campanha Gaúcha, this occurs more quickly. Due to the lower SOM contents, and as a consequence a rapid saturation of the SOM functional groups, other fractions become responsible for absorbing this element, such as the Cu_{Min}, Cu_E, and Cu_{Sol} fractions (Figure 2).

The highest Cu contents in V39 were observed in the Cu_{Min} fraction, extracted with NH_2OHHCl + CH_3COOH, followed by Cu_{OM} and Cu_{Res}, which agrees with results obtained in other studies conducted in vineyards [17,51]. With the increase in Cu contents in the soil, the saturation of the functional groups of reactive particles, such as SOM, is expected, and redistribution of Cu in the soil tends to occur, causing clay minerals to be responsible for adsorbing the largest amounts of this element. When this occurs, the remaining Cu can be adsorbed by functional groups of inorganic particles, mainly clay and 2:1 and 1:1 minerals, such as the OH group of kaolinite and oxides of Fe, Al, and Mn; oxyhydroxides and hydroxides; and amorphous silicates [52,53]. In a study conducted in the Campanha Gaúcha region, the authors, when evaluating two vineyards of 14 and 31 years in an Ultisol, reported that the largest proportion of Cu was present in SOM [17]. Similar results were obtained in vineyards located in Spain [54,55] and Italy [56].

In this study, part of the total Cu was found in the residual fraction in the vineyard soil profile, possibly as a Cu-containing precipitated mineral [57]. When Cu or Zn are applied to agroecosystems, in this case, through metal-based fungicide use, and enter the soil system, they first occupy the most avid loading sites and are then retained in forms with lower binding energy. Although studies indicate that, over time, ionic forms assume greater stability in the soil [58], which suggests that the Cu applied at the beginning of vineyard cultivation more than a century ago would be more recalcitrant, and the capacity of the soil to retain this ion with higher binding energy is limited. The redistribution of subsequent fractions occurs with lower binding energy in the mineral and organic phases of the soil and then in highly soluble and bioavailable phases.

The highest Zn contents in the Zn_{Min} and Zn_{Res} fractions in the soils of vineyards in Serra Gaúcha can be attributed to the greater affinity and reactivity between Zn and the constituents of the mineral solid phase of the soil [7,19]. Among these clay minerals, we can mention the OH group of kaolinite; oxides of Fe, Al, and Mn; oxyhydroxides and hydroxides; and amorphous silicates [50]. Similar results have been observed by other authors in Southern Brazil [10,51]. The Zn present in the Zn_{Min} and Zn_{Res} fractions has low mobility and a low potential for toxicity to grapevines and cover crop species that cohabit the vineyards [7]

In the soils of Campanha Gaúcha, which have low SOM contents, the mineral fraction is very important to retaining this metal since there has been an increase in Zn levels in this fraction over the years of vineyard cultivation. This can be attributed to the high affinity and reactivity between Zn and the mineral solid phase constituents of the soil [19,59]. These fractions are more stable and feature low Cu availability and mobility in the soil [60]. In the presence of high Cu content, Zn remains mainly adsorbed on the functional groups of the mineral fraction as iron oxides and at the edges of phyllosilicate clay minerals [61]. Such a feature can minimize the environmental contamination potential of Zn in the soil but also reduce its toxicity in plants, for example, in grapevines or even in implanted soil cover species or in species coexisting in vineyards [7,10]. However, Zn has migrated to the deepest layers (0.10–0.40 m), and it has mainly increased in the Zn_{Res} fraction.

The Increase in the contents of bioavailable Cu and Zn (Cu_{Sol} + Cu_E and Zn_{Sol} + Zn_E) observed in the 0–0.10 m layer in the vineyards of Serra Gaúcha and Campanha Gaúcha can enhance the transfer of these elements to surface waters adjacent to the vineyards, reducing water quality, the contamination/pollution of aquatic environments, and a loss of biodiversity [18,62]. The decrease in Cu and Zn contents in the residual fraction may have occurred due to redistribution to the soluble (Cu_{Sol} and Zn_{Sol}) and exchangeable (Cu_E and Zn_E) fractions, which are bioavailable, and the fraction associated with clay minerals (Cu_{Min} and Zn_{Min}), which is potentially bioavailable [31], especially in the most superficial layer of the soil. The highest percentage of these elements in the residual fraction occurred in the NG, which has the lowest total Cu and Zn content, corroborating the data obtained in Spain in [54]. According to these authors, the accumulation of Cu in the residual fraction occurs through occlusion and/or co-precipitation processes, especially in vineyard soils with a long history of management, where a large amount of Cu accumulates in soils with pH $\geq$ 6.0.

The lower Mn contents in all fractions in the vineyard soils may occur because excess Cu and Zn can enhance the exudation of organic acids from plants, such as grapevines and cover crop species. In addition, plants can modify the pH of rhizosphere soil [63]. All of this can enhance the solubilization of compounds containing Mn in their composition. As a result, there is an increase in Mn in the soil solution, enhancing its absorption by cultivated plants, such as grapevines and cover crops [13,15,64,65]. If the concentrations of Mn are high in the aerial part, this could enhance the toxicity of the element to the plants, especially if it is not compartmentalized in organelles like vacuoles, which are the organelles responsible for compartmentalizing excess metals in plants [66].

In both Serra and Campanha Gaúcha, there was a higher availability of Mn extracted by Mehlich-1 in the V39BL and V36BL areas. In these areas, we find the presence of spontaneous plants that form a large amount of root mass. Thus, there is a greater release of root exudates, characterized by low-molecular-weight organic solutes, which, among their functions, act as phytoavailability agents for Mn [67].

When observing the behavior of ΔMn, it can be seen that, in-depth, both in Serra and Campanha Gaúcha, there is a reduction in Mn bound to SOM in the vineyards. This can occur because of the lower TOC content of this layer when compared with reference areas F and NG (Table A1). In addition, the organic complexes formed with Mn are of low stability since the complex formed with humic acid has an entirely electrostatic character, and fulvic acids have a limited number of specific complexation sites for the element [67].

In general, the results show that metal-based fungicides increase the levels of Cu, Zn, and Mn in soils over the years. In soils with lower levels of organic matter and clay, this represents a potential risk to the health of the soil and plants and the sustainability of agroecosystems. Thus, future studies should evaluate products that allow for more targeted and judicious use. However, more sustainable practices can be adopted to minimize this problem, such as the rotation and alternation of active ingredients to prevent the excessive accumulation of a single metal in the soil; dosage optimization, always applying the minimum dose of the product and respecting the number of applications per harvest; soil monitoring to understand the levels of these metals over the years; and the use of biodegradable and less persistent products when possible.

5. Conclusions

The continuous use of Cu, Zn, and Mn-based fungicides in vineyard soils in Southern Brazil, both clayey and sandy, with different ages, results in an increase in the total contents of these elements and in changes in the distribution of the soil's chemical fractions, mainly in the superficial layers.

In Serra Gaúcha, where the training system is the trellis, the increases in Cu especially occurred in the planting interrows, while in Campanha Gaúcha, where the training system is Geneva Double Curtain (GDC), it occurred in the planting rows. For Zn and Mn, regardless of the training system, the highest contents for these elements occurred in the planting rows.

In soils rich in organic matter, Cu tends to accumulate in fractions bound to SOM, in the superficial layers, and in fractions associated with clay minerals, in-depth, reducing the risk of toxicity to plants. In soils with low SOM and clay contents, the Cu contents associated with the residual fraction decrease, while the fractions bound to SOM and clay minerals increase. For Zn, in soils with high SOM contents, the highest concentration of these metals is in the residual fraction, reducing the pollution potential. In soils with low

SOM and clay contents, there is a reduction in the contents in the residual fraction and an increase in the fractions bound to organic matter and clay minerals.

The behavior of Mn in vineyard soils is influenced by various factors, including the use of metal-based fungicides. In soils rich in SOM, Mn becomes more available to plants, potentially representing a risk of toxicity, in addition to Cu and Zn. In soils with low SOM and clay contents, there is a reduction in the available fractions, such as the soluble fraction, in favor of more stable fractions, such as the fraction bound to clay minerals and residual.

Author Contributions: Conceptualization, G.W.F., S.U.B., E.M., Z.V.S.D., J.K.S., L.P.G., A.A.K.d.S., J.M.M.-B., G.W.B.d.M., G.B., T.T. and C.R.L.; resources, G.B., T.T. and C.R.L.; methodology, G.W.F., S.U.B., E.M., Z.V.S.D., J.K.S., L.P.G., A.A.K.d.S. and J.M.M.-B.; Investigation, G.W.F., S.U.B., E.M., Z.V.S.D., J.K.S., L.P.G., A.A.K.d.S., J.M.M.-B., G.B., T.T. and C.R.L.; writing—original draft preparation, G.W.F. and C.R.L.; writing—review and editing, G.W.F., G.B., T.T. and C.R.L.; supervision, C.R.L.; project administration, C.R.L.; funding acquisition, C.R.L. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Federal University of Santa Catarina (UFSC) and financed by both the Research Support Foundation of the State of Santa Catarina (FAPESC) (grant number 03/2017 and 2021TR728), which awarded a doctoral scholarship to the first author, and the National Council for Scientific and Technological Development (CNPq) (grant number 426453/2016–6).

Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A

Table A1. Physical and chemical characterization of the 0.00–0.10 m and 0.10–0.20 m layers of soils from reference areas and vineyards with different cultivation times in Serra Gaúcha and Campanha Gaúcha.

Area	Layer (m)	Clay	TOC	pH_{H2O}	SMP Index	Cu	Zn	Mn	P	K	Ca	Mg	Al	H + Al	$CEC_{ef.}$	$CEC_{pH7.0}$	m	V
		$g\ kg^{-1}$				$mg\ kg^{-1}$					$cmol_c\ kg^{-1}$						%	
Serra Gaúcha																		
F	0.00–0.10	261	62.27	6.32	6.75	4.38	2.34	13.12	12.46	215.33	3.74	0.99	0.00	1.77	5.19	6.96	0.00	74.49
	0.10–0.20		24.72	6.20	6.77	0.27	2.06	27.09	4.40	48.00	2.62	0.37	0.00	2.05	3.05	5.10	0.00	59.42
V35	0.00–0.10	182	51.64	6.94	7.24	5.70	0.41	12.56	232.47	339.50	2.99	1.02	0.00	1.11	4.79	5.89	0.00	81.15
	0.10–0.20		28.49	6.76	6.83	1.34	0.84	6.35	79.20	264.83	2.32	1.16	0.00	1.71	4.23	5.93	0.00	70.92
V37	0.00–0.10	342	73.20	7.00	7.16	5.16	0.68	13.30	212.11	233.17	4.87	1.42	0.00	1.23	6.94	8.17	0.00	84.65
	0.10–0.20		23.83	6.87	6.87	1.94	0.48	6.31	13.19	180.33	3.00	1.41	0.00	1.68	4.80	6.48	0.00	74.01
V39	0.00–0.10	301	29.24	6.23	6.73	3.96	1.94	8.05	26.95	176.84	1.62	1.09	0.00	1.95	1.75	3.70	0.00	40.22
	0.10–0.20		13.97	6.17	6.92	1.35	0.12	4.06	14.11	109.34	1.33	1.08	0.00	1.54	1.46	2.99	0.00	39.71
Campanha Gaúcha																		
NG	0.00–0.10	74	25.55	5.57	6.71	0.14	0.90	47.09	6.69	36.09	0.61	1.05	0.13	1.95	1.88	3.70	7.35	47.10
	0.10–0.20		18.28	5.02	6.47	0.16	1.05	67.90	4.19	25.50	0.27	0.33	0.50	2.55	1.17	3.23	42.69	20.86
V13	0.00–0.10	80	30.75	6.30	6.86	0.16	1.51	10.46	66.47	47.84	3.16	4.60	0.03	1.67	7.90	9.54	0.68	78.24
	0.10–0.20		16.79	5.70	6.64	0.22	3.00	15.22	45.81	37.67	1.34	1.73	0.10	2.14	3.27	5.30	3.14	59.93
V19	0.00–0.10	74	22.84	6.33	6.86	1.11	2.00	9.28	89.51	45.25	1.91	1.49	0.03	1.70	3.55	5.21	1.01	67.95
	0.10–0.20		15.72	6.07	6.88	2.09	3.88	18.79	69.15	27.33	1.22	1.16	0.05	1.65	2.50	4.10	2.16	60.30
V36	0.00–0.10	54	23.71	6.24	6.89	7.10	1.34	11.63	60.45	48.29	2.57	4.36	0.07	1.61	7.11	8.66	2.79	77.34
	0.10–0.20		14.08	5.96	6.89	13.85	20.49	37.81	30.97	28.84	1.29	1.86	0.06	1.64	3.27	4.85	1.98	66.27

F: forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; contents of total organic carbon (TOC); pH_{H2O} (1:1 *v*/*v*); available P, K^+, Cu^{+2}, Zn^{+2}, and Mn^{+2} contents extracted with Mehlich-1 extractor; exchangeable Al^{+3}, Ca^{+2}, and Mg^{+2} contents extracted by 1 mol L^{-1} KCl [28]. With the obtained data, the potential cation exchange capacity ($CEC_{pH7.0}$) and effective ($CEC_{ef.}$) saturation by Ca + Mg + K+Na and Al were calculated [30].

Appendix B

Table A2. Cu fractions in vineyard soils with different cultivation times in Serra Gaúcha and Campanha Gaúcha.

Layer (m)	Serra Gaúcha					CV, %	Layer (m)	Campanha Gaúcha					CV, %
	F	V35	V37	V39	V39BL			NG	V13	V19	V36	V36BL	
	mg kg^{-1}							mg kg^{-1}					
							Cu_{Sol}						
0.00–0.05	0.3 aE	0.5 cD	1.0 bC	2.4 aB	2.6 aA	6.6	0.00–0.05	0.09 aD	0.32 aC	0.69 bB	3.94 aA	0.84 aB	7.49
0.05–0.10	0.3 aE	1.3 aC	2.3 aA	0.8 cD	2.0 bB	9.7	0.05–0.10	0.03 bE	0.26 bD	0.90 aB	2.35 bA	0.84 aC	2.96
0.10–0.15	0.2 aD	1.0 abC	2.6 aA	1.9 bB	1.2 cC	12.8	0.10–0.20	0.03 bB	0.12 cB	0.82 aA	0.89 cA	0.78 aA	14.71
0.15–0.20	0.3 aC	0.9 bB	1.1 bA	0.8 cB	0.7 dB	15.0	0.20–0.40	0.03 bC	0.08 dC	0.50 cA	0,30 dB	0.29 bB	15,39
CV, %	17.15	11.36	13.21	6.18	6.40		CV, %	7.70	9.44	5.44	4.52	15.02	
							Cu_{E}						
0.00–0.05	0.9 bE	1.3 aD	2.1 aC	3.7 cB	20.8 aA	1.68	0.00–0.05	1.1 bC	1.3 aC	2.2 bB	5.6 bA	2.0 bB	6.7
0.05–0.10	0.8 cC	1.3 aC	2.1 aC	8.7 bB	18.3 bA	12.01	0.05–0.10	1.1 bD	1.0 bD	5.0 aB	12.2 aA	3.1 aC	15.5
0.10–0.15	0.9 bC	1.1 abC	1.7 bC	21.9 aA	10.6 cB	15.66	0.10–0.20	2.4 aA	0.9 cC	2.5 bA	2.3 cA	1.5 bcB	11.1
0.15–0.20	1.1 aD	1.0 bD	1.7 bC	5.2 cA	2.6 dB	3.92	0.20–0.40	1.0 bA	0.8 cB	1.2 cA	1.2 cA	1.0 cA	8.9
CV, %	3.9	4.5	3.9	12.4	7.0		CV, %	3.1	4.8	8.2	14.8	10.2	
							Cu_{Min}						
0.00–0.05	3.6 aD	21.6 bC	15.8 cC	508.8 bB	632.1 aA	1.40	0.00–0.05	1.7 cE	16.2 aD	69.2 aB	81.1 aA	28.4 aC	5.7
0.05–0.10	2.0 bE	20.0 bD	95.9 aC	290.2 cB	524.4 bA	5.66	0.05–0.10	2.3 bD	7.8 bC	27.6 bB	49.9 bA	26.3 aB	9.5
0.10–0.15	1.6 cE	32.4 aD	84.2 bC	544.7 aA	106.8 cB	5.94	0.10–0.20	4.8 aC	3.9 cC	12.2 cB	16.6 cA	6.0 bC	17.9
0.15–0.20	1.3 cE	22.1 bD	80.2 bB	150.4 dA	38.3 dC	7.01	0.20–0.40	0.9 dE	2.8 cD	9.1 cA	4.0 dC	6.9 bB	7.6
CV, %	7.8	4.7	5.0	2.9	3.7		CV, %	3.0	8.3	7.9	7.0	9.3	
							Cu_{OM}						
0.00–0.05	22.2 aE	78.0 aD	336.7 aB	256.2 aC	800.8 aA	1.67	0.00–0.05	0.9 cD	16.8 aC	36.3 aA	30.9 aB	32.9 aB	7.6
0.05–0.10	14.0 cD	81.2 aC	160.5 bB	142.7 bB	433.2 bA	7.69	0.05–0.10	1.2 bD	8.7 bB	7.3 bC	12.2 bA	12.1 aA	9.0
0.10–0.15	14.1 cE	78.4 aD	156.0 bB	101.6 cC	244.7 cA	3.87	0.10–0.20	2.7 aC	1.7 cD	4.9 bcB	6.2 cA	2.8 bC	14.0
0.15–0.20	17.6 bE	45.6 bD	83.2 cC	94.6 cB	124.1 dA	4.44	0.20–0.40	0.6 dE	1.1 cD	2.6 cB	2.1 dC	3.6 bA	3.3
CV, %	2.1	7.2	1.4	2.2	3.8		CV, %	4.6	12.0	11.0	8.2	8.8	
							Cu_{Res}						
0.00–0.05	56.4 abc	79.1 bC	160.1 aA	126.0 aB	175.1 aA	13.29	0.00–0.05	4.2 abD	8.9 abB	6.2 aC	13.5 aA	15.0 abA	2.8
0.05–0.10	51.1 cC	100.7 aB	137.9 abA	96.8 aB	144.2 bA	6.33	0.05–0.10	3.2 bC	13.3 aB	5.7 aC	5.4 dC	16.7 aA	19.5
0.10–0.15	62.4 aC	94.9 aB	129.7 abA	121.7 aA	106.5 cB	7.50	0.10–0.20	4.7 abD	6.3 bBC	5.1 aCD	7.1 cB	14.8 abA	10.9
0.15–0.20	57.4 abD	80.6 bC	117.4 bA	106.5 aA	94.1 dB	7.18	0.20–0.40	5.2 aB	12.1 aA	4.9 aB	12.5 bA	13.0 bA	5.7
CV, %	6.8	5.9	9.4	14.8	3.2		CV, %	16.3	17.8	11.3	3.5	6.1	

Table A2. *Cont.*

Layer (m)	Serra Gaúcha F	V35	V37	V39	V39BL	CV, %	Layer (m)	Campanha Gaúcha NG	V13	V19	V36	V36BL	CV, %
	mg kg^{-1}							mg kg^{-1}					
							Sum of fractions						
0.00–0.05	83.5 aE	180.5 bD	515.6 aC	897.1 aB	1631.3 aA	2.88	0.00–0.05	8.0 bE	43.5 aD	114.7 aB	135.0 aA	79.0 aC	4.4
0.05–0.10	68.2 bE	204.4 aD	398.7 bC	539.2 cB	1122.0 bA	1.63	0.05–0.10	7.8 bE	31.0 bD	46.4 bC	81.9 bA	59.0 bB	7.4
0.10–0.15	79.1 aE	207.9 aD	374.2 bC	791.9 bA	469.8 cB	3.83	0.10–0.20	14.6 aC	13.0 dC	25.5 cB	33.0 cA	25.8 bB	8.3
0.15–0.20	77.7 abE	150.2 cD	283.5 cB	357.4 dA	259.8 dC	4.07	0.20–0.40	7.8 bE	16.9 cD	18.3 cC	20.0 dB	24.8 bA	3.3
CV, %	4.9	2.7	3.6	3.7	3.3		CV, %	7.1	5.3	7.1	5.4	4.4	

F: Forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Cu_{Sol}: copper in the soluble fraction; Cu_E: copper in the exchangeable fraction; Cu_{Min}: copper in the fraction bound to clay minerals; Cu_{OM}: copper in the fraction bound to soil organic matter; Cu_{Res}: copper in the residual fraction. CV: coefficient of variation. Means followed by the same lowercase letter in the column do not differ from each other in the Tukey test ($p < 0.05$). Means followed by the same uppercase letter in the row and within each region do not differ from each other in the t-test (LSD) ($p < 0.05$).

Appendix C

Table A3. Zn fractions in vineyard soils with different cultivation times in Serra Gaúcha and Campanha Gaúcha.

Layer (m)	Serra Gaúcha F	V35	V37	V39	V39BL	CV, %	Layer (m)	Campanha Gaúcha NG	V13	V19	V36	V36BL	CV, %
	mg kg^{-1}							mg kg^{-1}					
							Zn_{Sol}						
0.00–0.05	0.00 aC	0.00 cC	0.02 dB	0.07 cA	0.00 cC	31.94	0.00–0.05	1.3 aC	0.6 bD	3.9 aA	2.2 bB	0.3 bD	13.0
0.05–0.10	0.00 aE	0.32 bB	0.61 bA	0.04 dD	0.25 bC	8.83	0.05–0.10	0.5 bC	0.5 bC	3.0 bB	3.9 aA	0.3 bC	20.6
0.10–0.15	0.00 aE	0.18 bcD	0.80 aA	0.28 aC	0.46 aB	13.07	0.10–0.20	0.2 cE	1.0 aB	2.5 cA	0.4 cD	0.7 aC	5.9
0.15–0.20	0.00 aB	1.40 aA	0.15 cB	0.10 bB	0.00 cB	26.33	0.20–0.40	0.3 cC	0.0 cD	0.9 dA	0.6 cB	0.0 cD	18.7
CV, %	0.00	20.39	12.33	10.70	14.21		CV, %	9.7	9.2	4.5	24.5	17.3	
							Zn_E						
0.00–0.05	0.03 dC	0.00 cC	0.08 cC	5.19 aB	6.84 aA	8.44	0.00–0.05	0.58 aD	5.22 aAB	3.53 aC	5.85 aA	4.51 aB	12.23
0.05–0.10	0.23 cD	0.21 cD	0.63 bC	1.48 bB	4.82 bA	7.17	0.05–0.10	0.57 aC	1.95 bB	2.63 bA	2.31 bAB	2.40 bA	10.15
0.10–0.15	0.49 bD	1.47 bC	1.21 aC	5.66 aA	2.33 cB	10.98	0.10–0.20	0.35 bC	0.50 cB	0.47 cB	0.64 cA	0.07 cD	14.76
0.15–0.20	0.82 aD	2.41 aA	1.34 aC	1.68 bB	0.85 dD	11.11	0.20–0.40	0.21 bB	0.46 cA	0.03 cCD	0.01 dD	0.05 cC	9.92
CV, %	13.70	9.15	17.26	7.83	6.92		CV, %	14.92	4.41	12.17	10.12	28.01	

Table A3. *Cont.*

Layer (m)	Serra Gaúcha F	V35	V37	V39	V39BL	CV, %	Layer (m)	Campanha Gaúcha NG	V13	V19	V36	V36BL	CV, %
			mg kg^{-1}							mg kg^{-1}			
							Zn_{Min}						
0.00–0.05	49.9 aE	72.9 cD	190.4 aA	114.2 aC	120.7 aB	2.4	0.00–0.05	3.2 bE	76.7 aA	20.6 aD	59.0 aB	36.5 aC	4.5
0.05–0.10	38.4 bE	127.0 aA	89.6 bB	49.6 cD	69.0 bC	2.9	0.05–0.10	2.4 cC	8.8 bB	11.4 bA	10.5 bA	11.4 bA	9.3
0.10–0.15	30.6 cE	109.3 bA	68.7 cC	88.7 bB	40.9 cD	2.3	0.10–0.20	3.4 aB	5.8 bcA	3.4 Bc	6.3 cA	2.4 cC	10.4
0.15–0.20	31.4 cD	49.0 dA	40.9 dB	34.9 dC	29.9 dD	3.9	0.20–0.40	2.3 cE	2.8 cB	3.5 cA	2.6 dC	2.4 cD	2.1
CV, %	4.2	2.3	1.9	3.4	3.2		CV, %	2.7	6.0	13.4	3.0	7.5	
							Zn_{OM}						
0.00–0.05	3.0 aC	8.0 aB	17.2 aA	2.7 aC	3.7 aC	9.2	0.00–0.05	0.1 bE	0.9 aB	2.1 aA	0.6 aD	0.7 aC	4.1
0.05–0.10	1.1 cE	5.5 bA	3.1 bB	1.5 bB	1.8 bC	4.7	0.05–0.10	0.1 bE	0.5 bA	0.2 bC	0.4 bB	0.5 bA	4.7
0.10–0.15	1.3 cD	4.3 cA	3.1 bB	2.5 aC	1.3 cD	5.6	0.10–0.20	0.2 bB	0.3 cA	0.2 bAB	0.3 cA	0.3 cA	20.4
0.15–0.20	1.7 bB	2.1 dA	2.0 bA	1.2 bC	1.1 cC	4.8	0.20–0.40	0.2 aB	0.1 dC	0.2 bBC	0.1 dC	0.4 bcA	20.4
CV, %	5.5	4.6	10.6	8.4	5.29		CV, %	7.0	9.2	2.8	11.5	13.5	
							Zn_{Res}						
0.00–0.05	75.5 aD	80.0 aC	110.6 aB	306.3 aA	109.1 aB	1.6	0.00–0.05	4.7 cB	23.9 aA	1.8 bB	3.2 bcB	6.5 aB	7.,0
0.05–0.10	77.6 aD	83.4 aCD	96.2 bAB	90.6 bBC	103.9 aA	6.1	0.05–0.10	3.3 cB	11.4 aA	14.9 aA	2.4 cB	1.6 bB	57.9
0.10–0.15	79.0 aD	85.8 aCD	93.8 bBC	95.1 bB	103.8 aA	4.9	0.10–0.20	11.7 aA	8.6 aB	3.0 bC	4.8 abC	7.4 aB	16.2
0.15–0.20	70.1 aC	89.7 aB	93.5 bAB	107.4 bA	94.2 aAB	9.6	0.20–0.40	7.5 bC	24.4 aA	3.1 bD	5.1 aCD	11.0 aB	14.5
CV, %	8.6	5.5	2.0	5.2	5.9		CV, %	11.2	47.7	10.8	17.1	26.7	
							Sum of fractions						
0.00–0.05	128.4 aE	160.9 cD	318.3 aB	428.4 aA	240.3 aC	1.2	0.00–0.05	9.9 bE	107.2 aA	31.9 aD	70.8 aB	48,5 aC	13.2
0.05–0.10	117.4 abD	216.4 aA	190.3 bB	143.2 cC	179.8 bB	3.8	0.05–0.10	6.8 cD	23.1 bB	32.2 aA	19.5 bAB	16,2 bC	18.9
0.10–0.15	111.4 abD	201.1 bA	167.7 cB	192.3 bA	148.7 cC	3.2	0.10–0.20	15.8 aA	16.1 bA	9.7 bB	12.3 cB	10,8 bAB	9.7
0.15–0.20	104.0 bC	144.6 dA	137.9 dAB	145.3 cA	126.1 dB	6.9	0.20–0.40	10.4 bC	27.7 bA	7.7 bD	8.5 dCD	13.8 bB	10.9
CV, %	5.9	2.6	1.5	3.6	4.3		CV, %	7.0	19.6	7.9	4.8	11.4	

F: Forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Zn_{Sol}: Zinc in the soluble fraction; Zn_E: zinc in the exchangeable fraction; Zn_{Min}: zinc in the fraction bound to clay minerals; Zn_{OM}: zinc in the fraction bound to soil organic matter; Zn_{Res}: zinc in the residual fraction. CV: coefficient of variation. Means followed by the same lowercase letter in the column do not differ from each other in the Tukey test ($p < 0.05$). Means followed by the same uppercase letter in the row and within each region do not differ from each other in the t-test (LSD) ($p < 0.05$).

Appendix D

Table A4. Mn fractions in vineyard soils with different cultivation times in Serra Gaúcha and Campanha Gaúcha.

Layer (m)	Serra Gaúcha					CV, %	Layer (m)	Campanha Gaúcha					CV, %
	F	V35	V37	V39	V39BL			NG	V13	V19	V36	V36BL	
	mg kg^{-1}							mg kg^{-1}					
							Mn_{Sol}						
0.00–0.05	3.8 bA	0.2 cC	0.1 cC	2.1 cB	0.2 cC	20.6	0.00–0.05	47.5 aA	5.8 cBC	9.3 cB	6.4 bC	4.8 bC	11.4
0.05–0.10	4.0 bB	0.5 abD	0.4 cD	1.9 cC	8.7 aA	20.7	0.05–0.10	15.5 cC	12.4 aC	20.6 bB	30.3 aA	5.5 bD	14.3
0.10–0.15	31.2 aA	0.4 bC	1.2 bBC	5.6 bB	3.0 bBC	33.4	0.10–0.20	22.6 bB	7.6 bC	44.0 aA	11.8 bC	12.9 aC	15.1
0.15–0.20	5.1 bB	0.7 aD	2.4 aC	10.1 aA	2.6 bC	20.4	0.20–0.40	24.0 bA	2.9 dD	8.7 cB	8.2 bB	4.6 bC	3.7
CV, %	28.2	16.4	15.1	18.4	19.9		CV, %	7.3	8.7	15.2	19.2	9.8	
							Mn_E						
0.00–0.05	222.8 bC	175.1 cD	95.8 cE	457.2 aB	511.5 bA	6.2	0.00–0.05	18.8 aB	19.7 aB	13.0 aC	17.1 aB	31.9 aA	8.9
0.05–0.10	231.8 aC	390.2 aB	261.0 aC	259.1 dC	735.9 aA	5.6	0.05–0.10	9.4 bD	13.3 bB	14.3 aB	11.4 bC	20.2 bA	5.7
0.10–0.15	207.0 abD	93.4 dE	274.2 aC	310.3 cB	416.2 cA	3.2	0.10–0.20	4.8 cB	7.3 cA	6.6 bA	5.0 cB	4.4 cB	7.9
0.15–0.20	94.1 cD	310.1 bB	202.6 bC	380.2 bA	205.3 dC	8.0	0.20–0.40	2.8 dD	9.9 cA	5.8 bB	2.2 cE	3.4 cC	5.5
CV, %	4.0	10.8	9.5	3.2	3.3		CV, %	5.6	8.5	14.8	12.9	3.1	
							Mn_{Min}						
0.00–0.05	2465.6 bA	766.7 bD	623.0 cE	1494.1 bB	1135.8 bC	3.5	0.00–0.05	50.5 cE	146.6 aC	232.7 aA	179.0 aB	122.2 aD	4.0
0.05–0.10	1782.9 cA	773.9 bD	1153.9 bC	1842.7 bA	1478.6 aB	5.2	0.05–0.10	79.6 bD	76.8 bD	149.1 bA	116.8 bB	97.6 bC	8.3
0.10–0.15	2756.2 abA	1071.4 aD	1254.3 aC	1712.2 abB	917.0 cE	3.6	0.10–0.20	73.8 bBC	92.5 bBC	157.2 bA	101.5 bcB	67.3 dC	17.9
0.15–0.20	3045.6 aA	1053.8 aC	1131.9 bC	1935.9 aB	582.3 dD	12.6	0.20–0.40	96.0 aB	93.5 bBC	109.4 bA	85.8 cCD	77.8 cD	5.8
CV, %	8.6	4.5	3.0	5.3	4.3		CV, %	4.0	6.5	13.2	5.1	4.0	
							Mn_{OM}						
0.00–0.05	256.2 aA	62.6 aD	74.6 aC	103.2 bB	93.5 aB	5.4	0.00–0.05	8.1 dE	14.4 aD	24.6 aB	40.9 aA	16.5 aC	3.5
0.05–0.10	107.9 bA	42.2 bD	73.1 aB	78.9 cB	56.3 bC	6.8	0.05–0.10	9.6 cD	13.1 aB	10.9 cC	24.7 bA	13.4 bB	3.4
0.10–0.15	111.7 bB	53.1 aD	75.6 aC	156.9 aA	47.1 cD	8.9	0.10–0.20	48.0 aA	11.0 bC	12.3 bB	10.8 cC	11.5 bC	2.4
0.15–0.20	271.1 aA	36.2 bD	68.9 aB	72.2 cB	42.7 cC	3.3	0.20–0.40	13.1 bA	13.4 aA	10.8 cB	10.9 cB	11.6 bB	5.0
CV, %	5.4	7.8	5.9	5.6	3.8		CV, %	1.6	5.0	3.06	2.9	5.6	

Table A4. *Cont.*

Layer (m)	F	V35	V37	V39	V39BL	CV, %	Layer (m)	NG	V13	V19	V36	V36BL	CV, %
	Serra Gaúcha mg kg^{-1}							Campanha Gaúcha mg kg^{-1}					
							Mn_{Res}						
0.00–0.05	768.6 aA	633.6 aB	727.2 aA	525.8 aC	516.7 aC	6.4	0.00–0.05	37.8 aC	39.6 bC	32.7 abD	62.1 bB	112.6 aA	4.7
0.05–0.10	682.0 aA	707.9 aA	527.1 bB	407.2 bB	445.5 bB	13.0	0.05–0.10	38.3 aC	73.4 aB	41.7 aC	37.7 cC	104.2 aA	10.6
0.10–0.15	812.0 aA	709.2 aA	753.3 aA	503.6 aB	418.8 bcB	9.1	0.10–0.20	35.1 aC	52.1 bB	27.4 bC	45.1 cB	110.2 aA	9.1
0.15–0.20	805.1 aA	561.3 aB	555.1 bB	478.4 abC	383.2 cD	6.3	0.20–0.40	23.5 bC	71.0 aB	31.5 abC	78.7 aB	97.4 aA	11.3
CV, %	10.8	12.0	2.0	6.1	3.5		CV, %	8.6	11.0	15.97	6.2	7.0	
							Sum of fractions						
0.00–0.05	3717.0 aA	1638.2 bD	1520.6 cE	2582.3 bB	2257.7 bC	2.0	0.00–0.05	162.7 bD	226.2 aC	312.2 aA	305.4 aA	288.0 aB	2.9
0.05–0.10	2808.5 bA	1914.7 aC	2015.6 bC	2589.9 bB	2725.0 aAB	4.9	0.05–0.10	152.3 cC	189.0 bB	236.5 bA	221.0 bA	240.9 bA	5.8
0.10–0.15	3918.0 aA	1927.5 aD	2358.6 aC	2688.5 abB	1802.0 bE	2.7	0.10–0.20	184.2 aBC	170.4 bC	247.5 abA	174.2 cBC	206.3 cB	10.0
0.15–0.20	4221.0 aA	1962.0 aC	1960.9 bC	2876.8 aB	1216.0 cD	8.6	0.20–0.40	159.4 bcB	190.7 bA	166.1 cB	185.8 cA	194.9 cA	5.4
CV, %	6.6	3.9	1.9	4.0	3.7		CV, %	2.2	5.0	10.4	2.9	3.6	

F: Forest; V35: vineyard with 35 years of cultivation; V37: vineyard with 37 years of cultivation; V39: vineyard with 39 years of cultivation; V39BL: vineyard with 39 years of cultivation, with soil collected between planting lines; NG: native grassland; V13: vineyard with 13 years of cultivation; V19: vineyard with 19 years of cultivation; V36: vineyard with 36 years of cultivation; V36BL: vineyard with 36 years of cultivation, with soil collected between planting lines; Mn_{Sol}: manganese in the soluble fraction; Mn_E: manganese in the exchangeable fraction; Mn_{Min}: manganese in the fraction bound to clay minerals; Mn_{OM}: manganese in the fraction bound to soil organic matter; Mn_{Res}: manganese in the residual fraction. CV: coefficient of variation. Means followed by the same lowercase letter in the column do not differ from each other in the Tukey test ($p < 0.05$). Means followed by the same uppercase letter in the row and within each region do not differ from each other in the t-test (LSD) ($p < 0.05$).

References

1. FAO; ITPS. *Status of the World's Soil Resources (SWSR)—Main Report*; Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils: Rome, Italy, 2015; ISBN 9789251090046.
2. Gautam, K.; Sharma, P.; Dwivedi, S.; Singh, A.; Gaur, V.K.; Varjani, S.; Srivastava, J.K.; Pandey, A.; Chang, J.-S.; Ngo, H.H. A Review on Control and Abatement of Soil Pollution by Heavy Metals: Emphasis on Artificial Intelligence in Recovery of Contaminated Soil. *Environ. Res.* **2023**, *225*, 115592. [CrossRef] [PubMed]
3. Mahlungulu, A.; Kambizi, L.; Akinpelu, E.A.; Nchu, F. Levels of Heavy Metals in Grapevine Soil and Leaf Samples in Response to Seasonal Change and Farming Practice in the Cape Winelands. *Toxics* **2023**, *11*, 193. [CrossRef] [PubMed]
4. Liu, J.; Kang, H.; Tao, W.; Li, H.; He, D.; Ma, L.; Tang, H.; Wu, S.; Yang, K.; Li, X. A Spatial Distribution—Principal Component Analysis (SD-PCA) Model to Assess Pollution of Heavy Metals in Soil. *Sci. Total Environ.* **2023**, *859*, 160112. [CrossRef] [PubMed]
5. Sánchez-Castro, I.; Molina, L.; Prieto-Fernández, M.-Á.; Segura, A. Past, Present and Future Trends in the Remediation of Heavy-Metal Contaminated Soil—Remediation Techniques Applied in Real Soil-Contamination Events. *Heliyon* **2023**, *9*, e16692. [CrossRef] [PubMed]
6. Wang, J.; Ma, T.; Wei, M.; Lan, T.; Bao, S.; Zhao, Q.; Fang, Y.; Sun, X. Copper in Grape and Wine Industry: Source, Presence, Impacts on Production and Human Health, and Removal Methods. *Compr. Rev. Food Sci. Food Saf.* **2023**, *22*, 1794–1816. [CrossRef] [PubMed]
7. Tiecher, T.L.; Ceretta, C.A.; Tiecher, T.; Ferreira, P.A.A.; Nicoloso, F.T.; Soriani, H.H.; Rossato, L.V.; Mimmo, T.; Cesco, S.; Lourenzi, C.R.; et al. Effects of Zinc Addition to a Copper-Contaminated Vineyard Soil on Sorption of Zn by Soil and Plant Physiological Responses. *Ecotoxicol. Environ. Saf.* **2016**, *129*, 109–119. [CrossRef] [PubMed]
8. Tiecher, T.L.; Soriani, H.H.; Tiecher, T.; Ceretta, C.A.; Nicoloso, F.T.; Tarouco, C.P.; Clasen, B.E.; De Conti, L.; Tassinari, A.; de Melo, G.W.B.; et al. The Interaction of High Copper and Zinc Doses in Acid Soil Changes the Physiological State and Development of the Root System in Young Grapevines (Vitis Vinifera). *Ecotoxicol. Environ. Saf.* **2018**, *148*, 985–994. [CrossRef]
9. Pesce, S.; Mamy, L.; Sanchez, W.; Artigas, J.; Bérard, A.; Betoulle, S.; Chaumot, A.; Coutellec, M.-A.; Crouzet, O.; Faburé, J.; et al. The Use of Copper as Plant Protection Product Contributes to Environmental Contamination and Resulting Impacts on Terrestrial and Aquatic Biodiversity and Ecosystem Functions. *Environ. Sci. Pollut. Res.* **2024**. [CrossRef] [PubMed]
10. Brunetto, G.; Benedet, L.; Ambrosini, V.G.; Comin, J.J.; de Melo, G.W.B.; dos Santos, M.A.; Lourenzi, C.R.; Loss, A.; Belli Filho, P.; Schmitt, D.E.; et al. Copper and Zinc Fractions in the Profile of an Inceptisol Cultivated with Apple in Southern Brazil. *Bragantia* **2018**, *77*, 333–347. [CrossRef]
11. Sonoda, K.; Hashimoto, Y.; Wang, S.-L.; Ban, T. Copper and Zinc in Vineyard and Orchard Soils at Millimeter Vertical Resolution. *Sci. Total Environ.* **2019**, *689*, 958–962. [CrossRef]
12. Hummes, A.P.; Bortoluzzi, E.C.; Tonini, V.; da Silva, L.P.; Petry, C. Transfer of Copper and Zinc from Soil to Grapevine-Derived Products in Young and Centenarian Vineyards. *Water Air Soil. Pollut.* **2019**, *230*, 150. [CrossRef]
13. De Conti, L.; Ceretta, C.A.; de Melo, G.W.B.; Tiecher, T.L.; Silva, L.O.S.; Garlet, L.P.; Mimmo, T.; Cesco, S.; Brunetto, G. Intercropping of Young Grapevines with Native Grasses for Phytoremediation of Cu-Contaminated Soils. *Chemosphere* **2019**, *216*, 147–156. [CrossRef]
14. Trentin, E.; Cesco, S.; Pii, Y.; Valentinuzzi, F.; Celletti, S.; Feil, S.B.; Zuluaga, M.Y.A.; Ferreira, P.A.A.; Ricachenevsky, F.K.; Stefanello, L.O.; et al. Plant Species and PH Dependent Responses to Copper Toxicity. *Environ. Exp. Bot.* **2022**, *196*, 104791. [CrossRef]
15. Trentin, E.; Facco, D.B.; Hammerschmitt, R.K.; Ferreira, P.A.A.; Morsch, L.; Belles, S.W.; Ricachenevsky, F.K.; Nicoloso, F.T.; Ceretta, C.A.; Tiecher, T.L.; et al. Potential of Vermicompost and Limestone in Reducing Copper Toxicity in Young Grapevines Grown in Cu-Contaminated Vineyard Soil. *Chemosphere* **2019**, *226*, 421–430. [CrossRef] [PubMed]
16. Tiecher, T.L.; Girotto, E.; Ceretta, C.A.; Tassinari, A.; Almeida, H.; Rupp, L.C.D.; Reffatti, L.; Mortele, D.F.; Almança, M.A.K. Fontes de Entrada de Nitrogênio, Fósforo, Cobre e Zinco Em Sistemas de Produção Frutícola. In *Contaminação em Solos de Pomares e Vinhedos: Causas, Efeitos e Estratégias de Manejo*; Brunetto, G., Trentin, E., de Melo, G.W.B., Girotto, E., Eds.; Sociedade Brasileira de Ciencia do Solo—Núcleo Regional Sul: Santa Maria, CA, USA, 2022; pp. 49–81. ISBN 978-65-89469-23-0.
17. Brunetto, G.; Miotto, A.; Ceretta, C.A.; Schmitt, D.E.; Heinzen, J.; de Moraes, M.P.; Canton, L.; Tiecher, T.L.; Comin, J.J.; Girotto, E. Mobility of Copper and Zinc Fractions in Fungicide-Amended Vineyard Sandy Soils. *Arch. Agron. Soil. Sci.* **2014**, *60*, 609–624. [CrossRef]
18. de Melo, G.W.B.; Zalamena, J. Retrato Da Fertilidade de Solos Cultivados Com Videira Nas Regiões Da Serra e Campanha Gaúcha. *Comun. Técnico* **2016**, *181*, 1–9.
19. Hammerschmitt, R.K.; Tiecher, T.L.; Facco, D.B.; Silva, L.O.S.; Schwalbert, R.; Drescher, G.L.; Trentin, E.; Somavilla, L.M.; Kulmann, M.S.S.; Silva, I.C.B.; et al. Copper and Zinc Distribution and Toxicity in 'Jade'/'Genovesa' Young Peach Tree. *Sci. Hortic.* **2020**, *259*, 108763. [CrossRef]
20. Fernández-Calviño, D.; Pateiro-Moure, M.; Nóvoa-Muñoz, J.C.; Garrido-Rodríguez, B.; Arias-Estévez, M. Zinc Distribution and Acid–Base Mobilisation in Vineyard Soils and Sediments. *Sci. Total Environ.* **2012**, *414*, 470–479. [CrossRef] [PubMed]
21. Gupta, N.; Yadav, K.K.; Kumar, V.; Kumar, S.; Chadd, R.P.; Kumar, A. Trace Elements in Soil-Vegetables Interface: Translocation, Bioaccumulation, Toxicity and Amelioration—A Review. *Sci. Total Environ.* **2019**, *651*, 2927–2942. [CrossRef]
22. Kumar, V.; Pandita, S.; Singh Sidhu, G.P.; Sharma, A.; Khanna, K.; Kaur, P.; Bali, A.S.; Setia, R. Copper Bioavailability, Uptake, Toxicity and Tolerance in Plants: A Comprehensive Review. *Chemosphere* **2021**, *262*, 127810. [CrossRef]

23. Ferreira, G.W.; Lourenzi, C.R.; Comin, J.J.; Loss, A.; Girotto, E.; Ludwig, M.P.; Freiberg, J.A.; de Oliveira Camera, D.; Marchezan, C.; Palermo, N.M.; et al. Effect of Organic and Mineral Fertilizers Applications in Pasture and No-Tillage System on Crop Yield, Fractions and Contaminant Potential of Cu and Zn. *Soil. Tillage Res.* **2023**, *225*, 105523. [CrossRef]
24. Mikhailova, E.A.; Post, C.J.; Nelson, D.G. Integrating United Nations Sustainable Development Goals in Soil Science Education. *Soil. Syst.* **2024**, *8*, 29. [CrossRef]
25. U.S. Department of Agriculture. *Soil Survey Staff Keys to Soil Taxonomy*, 13th ed.; Natural Resources Conservation Service, U.S. Department of Agriculture: Washington, DC, USA, 2022. Available online: https://www.nrcs.usda.gov/resources/guides-and-instructions/keys-to-soil-taxonomy (accessed on 2 July 2023).
26. Monteiro, J.E.B.; Tonietto, J. *Condições Meteorológicas e Sua Influência Na Vindima de 2013 Em Regiões Vitivinícolas Sul Brasileiras*; Comunicado; Embrapa Uva e Vinho: Bento Goncalves, Brazil, 2013.
27. EMBRAPA. *Manual de Métodos de Análise de Solo*; Embrapa Solos: Rio de Janeiro, Brazil, 2011.
28. Tedesco, M.J.; Gianello, C.; Bissani, C.A.; Bohnen, H.; Volkweiss, S.J. *Análises de Solo, Plantas e Outros Materiais*, 2nd ed.; Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H., Volkweiss, S.J., Eds.; Departamento de Solos, Universidade Federal do Rio Grande do Sul: Porto Alegre, Brazil, 1995.
29. Murphy, J.; Riley, J.P. A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. *Anal. Chim. Acta* **1962**, *27*, 31–36. [CrossRef]
30. CQFS-RS/SC. *Manual de Calagem e Adubação Para Os Estados Do Rio Grande Do Sul e Santa Catarina*; Sociedade Brasileira de Ciência do Solo—Núcleo Regional Sul, Ed.; Comissão de Química e Fertilidade do Solo—RS/SC: Santa Maria, Brazil, 2016.
31. Tessier, A.; Campbell, P.G.C.; Bisson, M. Sequential Extraction Procedure for the Speciation of Particulate Trace Metals. *Anal. Chem.* **1979**, *51*, 844–851. [CrossRef]
32. R Core Team R. *A Language and Environment for Statistical Computing*; R Core Team: Vienna, Austria, 2020.
33. Lê, S.; Josse, J.; Husson, F. FactoMineR : An R Package for Multivariate Analysis. *J. Stat. Softw.* **2008**, *25*, 1–18. [CrossRef]
34. Kassambara, A.; Mundt, F. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. 2020. Available online: https://rpkgs.datanovia.com/factoextra/ (accessed on 2 July 2023).
35. Vázquez-Blanco, R.; Nóvoa-Muñoz, J.C.; Arias-Estévez, M.; Fernández-Calviño, D.; Pérez-Rodríguez, P. Changes in Cu Accumulation and Fractionation along Soil Depth in Acid Soils of Vineyards and Abandoned Vineyards (Now Forests). *Agric. Ecosyst. Environ.* **2022**, *339*, 108146. [CrossRef]
36. García-Navarro, F.J.; Jiménez-Ballesta, R.; Garcia-Pradas, J.; Amoros, J.A.; Perez de los Reyes, C.; Bravo, S. Zinc Concentration and Distribution in Vineyard Soils and Grapevine Leaves from Valdepeñas Designation of Origin (Central Spain). *Sustainability* **2021**, *13*, 7390. [CrossRef]
37. La Pera, L.; Dugo, G.; Rando, R.; Di Bella, G.; Maisano, R.; Salvo, F. Statistical Study of the Influence of Fungicide Treatments (Mancozeb, Zoxamide and Copper Oxychloride) on Heavy Metal Concentrations in Sicilian Red Wine. *Food Addit. Contam. Part A* **2008**, *25*, 302–313. [CrossRef]
38. Regus, F.; Laffont-Schwob, I.; Hamrouni, R.; Dupuy, N.; da Silva, A.-M.F. Using Bibliometrics to Analyze the State of Art of Pesticide Use in Vineyard Agrosystems: A Review. *Environ. Sci. Pollut. Res.* **2022**, *29*, 80123–80136. [CrossRef]
39. Blanco-Canqui, H. Cover Crops and Water Quality. *Agron. J.* **2018**, *110*, 1633–1647. [CrossRef]
40. Meier, S.; Alvear, M.; Borie, F.; Aguilera, P.; Ginocchio, R.; Cornejo, P. Influence of Copper on Root Exudate Patterns in Some Metallophytes and Agricultural Plants. *Ecotoxicol. Environ. Saf.* **2012**, *75*, 8–15. [CrossRef] [PubMed]
41. Borges, F.; Guimarães, C.; Lima, J.L.F.C.; Pinto, I.; Reis, S. Potentiometric Studies on the Complexation of Copper(II) by Phenolic Acids as Discrete Ligand Models of Humic Substances. *Talanta* **2005**, *66*, 670–673. [CrossRef] [PubMed]
42. CETESB. *Relatório de Estabelecimento de Valores Orientadores Para Solos e Águas Subterrâneas*; CETESB: São Paulo, Brazil, 2005; p. 4.
43. CONAMA—Conselho Nacional do Meio Ambiente. *Resolução No 420, de 28 de Dezembro de 2009*; MMA: Brasília, Brazil, 2009.
44. USEPA. *Standards for the Use or Disposal of Swage Sludge*; USEPA: Washington, DC, USA, 1993.
45. EC Protection of the Environment, and in Particular of the Soil, When Sewage Sludge Is Used in Agriculture; Official Journal of the European Communities: 4, p. 6–12: European Union, 1986. Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1986:181:0006:0012:EN:PDF (accessed on 2 July 2023).
46. ANZECC; NHMRC (Eds.) *Australian and New Zealand Guidelines for the Assessment and Management of Contaminated Sites*; The Councils: Canberra, Australia, 2021. Available online: https://www.wa.gov.au/system/files/2023-05/guideline-assessment-and-management-of-contaminated-sites.pdf (accessed on 2 July 2023).
47. Huang, B.; Li, Z.; Huang, J.; Guo, L.; Nie, X.; Wang, Y.; Zhang, Y.; Zeng, G. Adsorption Characteristics of Cu and Zn onto Various Size Fractions of Aggregates from Red Paddy Soil. *J. Hazard. Mater.* **2014**, *264*, 176–183. [CrossRef] [PubMed]
48. Croué, J.-P.; Benedetti, M.F.; Violleau, D.; Leenheer, J.A. Characterization and Copper Binding of Humic and Nonhumic Organic Matter Isolated from the South Platte River: Evidence for the Presence of Nitrogenous Binding Site. *Environ. Sci. Technol.* **2003**, *37*, 328–336. [CrossRef] [PubMed]
49. Refaey, Y.; Jansen, B.; Parsons, J.R.; de Voogt, P.; Bagnis, S.; Markus, A.; El-Shater, A.-H.; El-Haddad, A.-A.; Kalbitz, K. Effects of Clay Minerals, Hydroxides, and Timing of Dissolved Organic Matter Addition on the Competitive Sorption of Copper, Nickel, and Zinc: A Column Experiment. *J. Environ. Manag.* **2017**, *187*, 273–285. [CrossRef] [PubMed]
50. Morais, G.P. *Fracionamento e Quantificação de Limites Críticos de Transferência de Cobre e Zinco Para a Solução de Solos Com Diferentes Teores de Argila e Matéria Orgânica*; Universidade Federal de Santa Catarina: Florianópolis, Brazil, 2020.

51. Korchagin, J.; Moterle, D.F.; Escosteguy, P.A.V.; Bortoluzzi, E.C. Distribution of Copper and Zinc Fractions in a Regosol Profile under Centenary Vineyard. *Environ. Earth Sci.* **2020**, *79*, 439. [CrossRef]
52. Bradl, H.B. Adsorption of Heavy Metal Ions on Soils and Soils Constituents. *J. Colloid. Interface Sci.* **2004**, *277*, 1–18. [CrossRef] [PubMed]
53. Telkapalliwar, N.G.; Shivankar, V.M. Adsorption of Zinc onto Microwave Assisted Carbonized Acacia Nilotica Bark. *Mater. Today Proc.* **2018**, *5*, 22694–22705. [CrossRef]
54. Fernández-Calviño, D.; Nóvoa-Muñoz, J.C.; Díaz-Raviña, M.; Arias-Estévez, M. Copper Accumulation and Fractionation in Vineyard Soils from Temperate Humid Zone (NW Iberian Peninsula). *Geoderma* **2009**, *153*, 119–129. [CrossRef]
55. Gómez-Armesto, A.; Carballeira-Díaz, J.; Pérez-Rodríguez, P.; Fernández-Calviño, D.; Arias-Estévez, M.; Novoa Muñoz, J.C.; Álvarez-Rodríguez, E.; Fernández-Sanjurjo, M.J.; Núñez-Delgado, A. Copper Content and Distribution in Vineyard Soils from Betanzos (A Coruña, Spain). *Span. J. Soil. Sci.* **2015**, *5*, 60–61. [CrossRef]
56. Viti, C.; Quaranta, D.; de Philippis, R.; Corti, G.; Agnelli, A.; Cuniglio, R.; Giovannetti, L. Characterizing Cultivable Soil Microbial Communities from Copper Fungicide-Amended Olive Orchard and Vineyard Soils. *World J. Microbiol. Biotechnol.* **2008**, *24*, 309–318. [CrossRef]
57. Bortoluzzi, E.C.; Korchagin, J.; Moterle, D.F.; Santos, D.R.; Caner, L. Accumulation and Precipitation of Cu and Zn in a Centenarian Vineyard. *Soil Sci. Soc. Am. J.* **2019**, *83*, 492–502. [CrossRef]
58. Arias-Estévez, M.; Nóvoa-Muñoz, J.C.; Pateiro, M.; López-Periago, E. Influence of aging on copper fractionation in an acid soil. *Soil. Sci.* **2007**, *172*, 225–232. [CrossRef]
59. Brunetto, G.; Comin, J.J.; Miotto, A.; de Moraes, M.P.; Sete, P.B.; Schmitt, D.E.; Gatiboni, L.C.; de Melo, G.W.B.; Morais, G.P. Copper and Zinc Accumulation, Fractionation and Migration in Vineyard Soils from Santa Catarina State, Brazil. *Bragantia* **2017**, *77*, 141–151. [CrossRef]
60. Formentini, T.A.; Mallmann, F.J.K.; Pinheiro, A.; Fernandes, C.V.S.; Bender, M.A.; da Veiga, M.; dos Santos, D.R.; Doelsch, E. Copper and Zinc Accumulation and Fractionation in a Clayey Hapludox Soil Subject to Long-Term Pig Slurry Application. *Sci. Total Environ.* **2015**, *536*, 831–839. [CrossRef] [PubMed]
61. Pérez-Novo, C.; Bermúdez-Couso, A.; López-Periago, E.; Fernández-Calviño, D.; Arias-Estévez, M. Zinc Adsorption in Acid Soils: Influence of Phosphate. *Geoderma* **2011**, *162*, 358–364. [CrossRef]
62. Yadav, M.R.; Parihar, C.M.; Kumar, R.; Jat, S.L.; Yadav, R.K.; Ram, H.; Singh, A.K.; Singh, M.; Meena, R.K.; Yadav, N.; et al. Conservation Agriculture and Soil Quality—An Overview. *Int. J. Curr. Microbiol. Appl. Sci.* **2017**, *6*, 707–734. [CrossRef]
63. Lambers, H.; Mougel, C.; Jaillard, B.; Hinsinger, P. Plant-Microbe-Soil Interactions in the Rhizosphere: An Evolutionary Perspective. *Plant Soil.* **2009**, *321*, 83–115. [CrossRef]
64. Lambers, H.; Wright, I.J.; Pereira, C.G.; Bellingham, P.J.; Bentley, L.P.; Boonman, A.; Cernusak, L.A.; Foulds, W.; Gleason, S.M.; Gray, E.F.; et al. Leaf Manganese Concentrations as a Tool to Assess Belowground Plant Functioning in Phosphorus-Impoverished Environments. *Plant Soil.* **2021**, *461*, 43–61. [CrossRef]
65. Facco, D.B.; Trentin, E.; Drescher, G.L.; Hammerschmitt, R.K.; Ceretta, C.A.; da Silva, L.S.; Brunetto, G.; Ferreira, P.A.A. Chemical Speciation of Copper and Manganese in Solution of a Copper-Contaminated Soil and Young Grapevine Growth with Amendment Application. *Pedosphere* **2023**, *33*, 496–507. [CrossRef]
66. Dinkelaker, B.; Romheld, V.; Marschner, H. Citric Acid Excretion and Precipitation of Calcium Citrate in the Rhizosphere of White Lupin (Lupinus Albus L.). *Plant Cell Environ.* **1989**, *12*, 285–292. [CrossRef]
67. McBride, M.B. *Environmental Chemistry of Soils*; Oxford University Press: New York, NY, USA, 1994.