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Article

Leached Copper Correlation with Dissolved Organic Carbon in Sloped Vineyard Soil

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Abstract: The solubility and mobility of copper (Cu) in soil is strongly influenced by the presence of dissolved organic carbon (DOC); however, the interactions between Cu and DOC are complex and not yet fully understood. In this study, Cu and DOC concentrations were measured monthly for two years in leachates from self-constructed lysimeters installed at inter- and intra-row vineyard hilltop, backslope, and footslope areas at the SUPREHILL Critical Zone Observatory, Croatia. The aim was to quantify Cu and DOC leaching from the hilltop towards the backslope and the footslope. The assumed strong relationship between Cu and DOC in the leachates was statistically analyzed and explained using chemical equilibrium software. Leachates were analyzed for pH, EC, DOC, Cu, and major ion concentrations. The highest Cu concentrations found in leachates from the intra-row footslope suggested Cu downhill transport. Although not strong, a significant positive correlation between Cu and DOC in footslope leachates confirmed the relevance of Cu complexation by DOC. Speciation confirmed that more than 99.9% of total Cu in leachates was found as a Cu-DOC complex. Data implied the role of soil water flow pathways in explaining Cu downhill transport. Critical timing for applying Cu fungicides at sloped vineyards was highlighted.

Keywords: trace metal mobility in soil; hillslope landscape; soil organic carbon; copper oxychloride; vineyard management

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1. Introduction

Copper-based pesticides are commonly used in conventional and organic winegrowing to control vine fungal diseases such as, e.g., downy mildew [1], thus fairly high amounts of copper (Cu) have been applied in winegrowing areas of Europe for decades. Moreover, about 86% of total fungicides used in Europe are used in viticulture [2], therefore the European Union regulation [3] decreases the threshold for the allowed amount of Cu which can be applied in viticulture [4]. This fact clearly shows that the issue of increasing Cu concentrations in the environment is well recognized, at least in Europe, and therefore studies focusing on defining and quantifying processes that govern Cu mobility and transport in soil are gaining importance.

Copper is present in soil almost exclusively in its divalent form because the monovalent form is considered unstable [5]. The major factors that affect Cu sorption in soil, and thus mobility, are soil organic matter (SOM) and pH. Charged sites on SOM retain cations in a non-leachable exchangeable form. Cu sorption (covalent bond formation) is stronger than that of base cations and other divalent transition metals [6]. Additionally, inner-sphere complexes which Cu forms with SOM [7] are considered a stronger bond than outer-sphere complexes formed by alkaline earth metals, such as Ca or Mg [8]. Generally, Cu concentration in the soil solution decreases as pH increases due to the stronger adsorption of Cu. However, it has been found that the connection between Cu and SOM is not as straightforward as it appears. This is because the increase in SOM particulates will consequently increase dissolved organic matter (DOM), which is determined as dissolved organic carbon (DOC) in the soil solution, due to the microbial decomposition of organic compounds. Copper in the soil solution again has a strong affinity to organic molecules; therefore, it is predominantly present as the Cu-DOC complex, with the proportion of other Cu species increasing with the Cu concentration, but only if the DOC concentration does not also increase [9]. The concentration of DOC in the soil solution is directly related to the quantity and quality of SOM, which also strongly affects microbial activity [10]. This suggests that by increasing SOM, more Cu would be retained in the soil; however, a certain increase in Cu mobility due to complexation with DOC can also be expected, although complexation process with solid SOM seems to have a greater effect on Cu mobility in soil than complexation with DOC [9]. Microbial decomposition of SOM, influenced by many factors such as microbial biomass, diversity, and activity, as well as the quantity and quality of SOM and environmental factors (e.g., water/air ratio in soil, temperature, etc.), can affect Cu mobility in soil. Therefore, the interconnectedness of processes between soil pH, SOM, and Cu mobility in soil is of great significance for Cu flux in soil. For example, an increase in soil solution pH dominantly decreases Cu mobility because of its adsorption to the soil solid phase (the concentration of dissolved Cu and its free ionic activity in the solution decreases due to complexation with SOM [11]). However, although not to a greater extent, a certain increase in Cu mobility may occur with an increase in soil solution pH, because the proportion of Cu-DOC complexes in the soil solution also increases, which decreases Cu free ionic activity due to complexation [12]. Furthermore, the decomposition of SOM is generally considered to lower the soil pH by releasing hydrogen ions, thus this effect would presumably increase Cu mobility in soil, even though a pH change of less than a half pH unit will unlikely induce significant changes in Cu mobility in soil [13]. Another indirect effect of increasing Cu mobility is possible through improvements in the soil structure by adding SOM, and thus water/air relations in soil, further stimulating the microbial community, which in turn affects the SOM decomposition. The same processes that govern Cu mobility and leaching in soil affect Cu fractionation in soil and speciation in the solution. Copper fractionation in soil (e.g., by using several sequential chemical extractants) refers to defining soil fractions that contain Cu, such as a readily soluble and easily exchangeable fraction, fraction bound to carbonates or Fe-Mn oxides, organically-bound fraction, precipitated fraction and residual forms. Copper speciation in the soil solution describes an actual chemical form of Cu found in the soil solution under specific conditions (e.g., Cu^{2+}, $CuOH^{+}$, $CuCO_3$, and Cu-DOC). At hillslopes, it can be expected that under field conditions, even relatively immobile cations such as Cu are transported in specific chemical forms: vertically through soil water flow pathways and laterally along the slope.

The aim of this study was to quantify leaching of Cu and DOC from a hillslope vineyard (i.e., from the hilltop towards the backslope and the footslope) by measuring the concentrations of Cu and DOC in the leachates from self-constructed lysimeters installed at the inter- and intra-row area of the vineyard hilltop, backslope and footslope. In order to test the hypothesis that Cu in leachates will be present mainly in a complexed form with DOC, the assumed strong relationship between Cu and DOC concentrations in leachates was statistically analyzed using significance tests and geochemically explained using chemical equilibrium software.

2. Materials and Methods

2.1. Experimental Site

The sloped agricultural (vineyard) experimental site is a field-scale critical zone observatory SUPREHILL (https://sites.google.com/view/suprehill (accessed on 15 February 2023)), located in Zagreb, Croatia (45°51′24″ N 16°00′22″ E), established in 2020, with all field experiments operative since the end of 2020. The experimental plot is part of a 15-year-old hillslope vineyard with southwest exposure. The vines are planted along the slope in rows with a spacing of 1.2 m between the vines (intra-row area). The interrow area is a 2 m wide grassed spacing between the rows. The experimental plot is separated into three segments: hilltop, backslope, and footslope, each with lysimeters installed in the vineyard inter- and intra-row area (Figure 1). The slope between the segments was determined using an unmanned aerial vehicle (UAV) and Agisoft Metashape software (Agisoft LLC., ver. 1.7.5), with a slope between the hilltop and backslope lysimeters being 17.5%, and between backslope and footslope lysimeters 25.4%. The texture of the soil at the site is silt loam (at a soil depth of 0–30 cm and 30–60 cm for all segments), and the soil type is classified as Dystric Luvic Stagnosol [14]. More details on soil classification and soil hydraulic properties can be found in [15]. The average annual precipitation and the average annual air temperature recorded at the nearby meteorological station Maksimir (Zagreb, Croatia) were 857 mm and 11.2 °C (1970–2020), respectively. A copper-based pesticide during the studied years was applied in April 2021 and April 2022 by spraying colloid-sized particles of copper oxychloride-based fungicide (Cuprablau Z 35 WP).

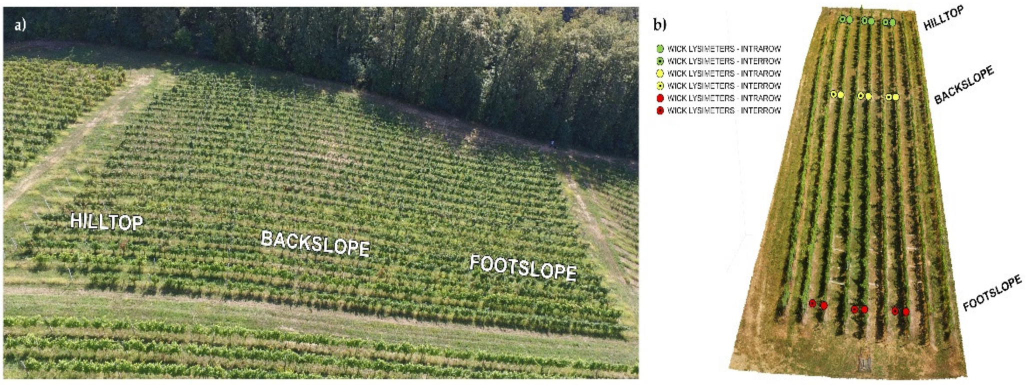

Figure 1. (**a**) Segments (i.e., hilltop, backslope, and footslope) of the SUPREHILL critical zone observatory experimental vineyard plot, and (**b**) 3D model of the SUPREHILL observatory with installation points of self-constructed lysimeters.

2.2. Field Equipment

Self-constructed lysimeters were installed at the hilltop, backslope, and footslope in triplicates, in the inter- and intra-row area at 40 cm of soil depth. A total of 18 lysimeters were installed, i.e., two lysimeters (one at the inter-, and one at the intra-row area; Figure 2b) per segment (hilltop, backslope, and footslope), all in three repetitions. The lysimeters were self-constructed out of 1.5 mm thick stainless steel (250 mm × 250 mm × 40 mm) with a drain extension at the center (100 mm length, 30 mm diameter). Filter mesh (1 mm × 1 mm) was placed at the bottom of the lysimeter to prevent clogging, and a fiberglass rope was spread to ensure a small negative pressure that enables drainage seepage (Figure 2a). The lysimeters were filled with dried soil taken from the same soil profile, applying a layer of quartz flour (mean particle size of 71 µm) on top. The lysimeters were placed in a razor-sharp groove with a hammer and raised to ensure contact with the upper soil with solid boards and metal brackets. Lysimeters were connected to leachate tanks placed 50 cm below the lysimeter in the soil to ensure that the solution was continuously collected in leachate tanks (Figure 2b).

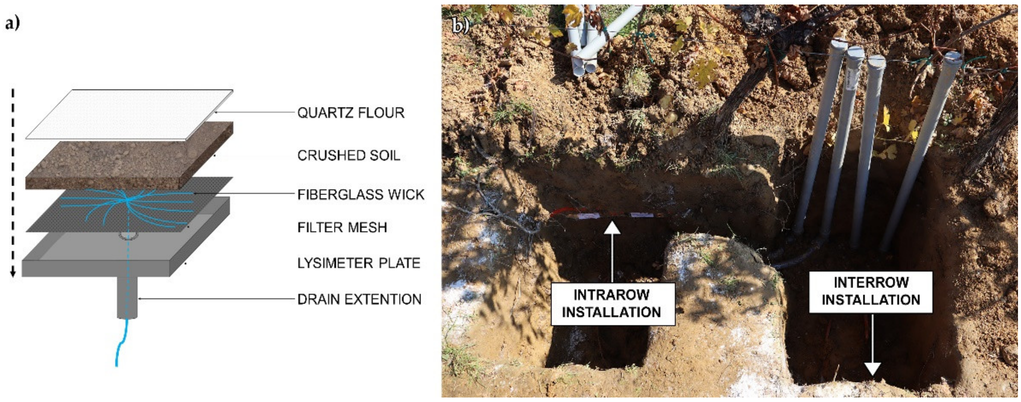

Figure 2. (**a**) Scheme of self-constructed lysimeters, and (**b**) installation in-field points of lysimeters at the SUPREHILL site.

2.3. Soil Sampling and Chemical Analyses

Soil samples were taken from two soil depths (0–30 and 30–60 cm) at the location of lysimeters placement in the vineyard (i.e., at the hilltop, backslope and footslope). Lysimeters were installed in a way that a large amount of soil was excavated first, and this is when soil samples were taken (19 October 2020); therefore, no separation of soil samples into inter- or intra-row was carried out (inter- and intra-row samples were pooled). Here it should be noted that after a few months of measurements, it was recognized (using statistical tools) that leachate data from vineyard inter- and intra-row area cannot be considered as duplicates. Thus, although measured separately from the beginning of the experiment, inter- or intra-row placement of lysimeters was, after a few initial months, recognized as a separate factor for leachate chemical data processing and analyses.

Soil samples were air-dried, sieved through a 0.5 mm mesh, and analyzed for pH_{H2O} and electrical conductivity (EC) in a 1:5 soil:water ratio, and soil organic carbon (OC) by sulfochromic oxidation (ISO 14235:1998). The soil samples were then digested in aqua regia (ISO 11466:1995) by applying the microwave technique on the MARSXpress system for the detection of Cu total concentration in soil using inductively coupled plasma optical emission spectroscopy (Vista MPX AX, Varian). Concentrations were calculated based on the dry weight of samples (105 °C, 24 h). Quality control procedures consisted of reagent blanks, duplicate samples, and referenced soil samples with a similar matrix from the interlaboratory calibration program [16].

Calcium chloride extraction [17] was used for the determination of the available Cu fraction in soil. More specifically, 5 g of soil was extracted with 50 mL of $CaCl_2$ 0.01 M for 2 h at 20 °C under stirring, prior to being filtered. Cu concentrations in $CaCl_2$ extracts were determined using inductively coupled plasma-optical emission spectroscopy (Vista MPX AX, Varian).

2.4. Leachate Sampling and Chemical Analyses

The sampling of lysimeter leachates was performed using a submersible pump for a period of two years (2 November 2020–25 November 2022). Sampling was carried out monthly as part of ongoing monitoring of leachate chemical quality. A total of 312 leachate samples were collected during 19 sampling events, with a total number of samples collected per sampling event depending on soil moisture and rainfall. After the sampling, leachate samples were transported to the laboratory in a cooling container and stored at 4 °C until analyses.

Leachate samples were analyzed for pH, electrical conductivity, and ionic composition, i.e., Cu^{2+}, DOC, $NH_4{}^+$, $NO_3{}^-$, $PO_4{}^{3-}$, Na^+, K^+, Cl^-, $SO_4{}^{2-}$, Ca^{2+} and Mg^{2+} concentrations. The pH and electrical conductivity of the samples were measured using pH/EC-meter

(MPC 227, Mettler Toledo). Copper, Ca^{2+} and Mg^{2+} concentrations were measured using inductively coupled plasma optical emission spectroscopy (Vista MPX AX, Varian), while Na^+ and K^+ concentrations were determined using atomic emission spectroscopy (Atomic Absorption Spectrometer 3110, Perkin Elmer). The concentrations of NH_4^+, NO_3^-, PO_4^{3-}, Cl^-, and SO_4^{2-} were measured using segmented flow analysis (San++ Continuous Flow Auto-Analyzer, Skalar). Dissolved organic carbon (DOC) was measured using a TOC Analyzer (vario TOC cube, Elementar). The quality control procedure consisted of duplicate samples and referenced water samples from the interlaboratory calibration program (IFA-Proficiency Testing Scheme, Tulln, Austria). Data for ionic composition were used to estimate Cu species distribution in the soil solution using chemical equilibrium software (see the next section).

2.5. Geochemical Modeling, Data Processing and Statistical Analysis

The distribution (%) of different Cu species in the soil solution was estimated using the Visual MINTEQ version 3.1 chemical equilibrium software [18]. The non-ideal competitive adsorption (NICA)-Donnan model for the adsorption of cations onto DOC and software database on equilibrium constants were applied [19]. The speciation was calculated using the results of leachate ionic analyses. For all calculations, the measured pH value of leachates was used, ionic strength was set to be calculated, CO_2 partial pressure was set at 0.00038 atm, and the temperature to 15 °C. The ratio of active dissolved organic matter (DOM) to DOC was set at 1.65, and humic acids were selected in the model as dominant over fulvic acids. Other default settings of the software were not modified. Cu speciation was calculated as the percentage of total Cu concentration in leachate.

Leached DOC and Cu cumulative masses for the studied two years were summed from DOC and Cu masses in the leachate sample, calculated from measured concentrations and leachate volumes.

Statistical analysis was performed using Statistical Analysis Software (SAS Institute Inc., Version 8.3 Update 1, Cary, NC, USA, 2019–2020). The position on the slope (hilltop, backslope, and footslope) was selected as the main factor, with a grouping of data according to the position of the lysimeter in vineyard inter- or intra-row. Analysis of variance was carried out using one-way ANOVA, with the significance of differences between the means determined using Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$. For the graphical overlay of copper by dissolved organic carbon concentrations in leachates, JMP software was used (JMP® Pro 16.0.0, SAS Institute Inc., 2021), presented with polynomial line of fit of linear degree.

3. Results

No statistically significant differences in pH, EC, total Cu and OC concentrations were found (Table 1) between the hilltop, backslope, or footslope at two soil depths (0–30, and 30–60 cm) for the pooled data of inter- and intra-row samples. However, a significantly higher concentration of available Cu in soil was found at the hilltop compared to the backslope and footslope at a soil depth of 0–30 cm, while no difference in concentration of available Cu was found at a soil depth of 30–60 cm between the hilltop, backslope, and footslope (Table 1).

Only Cu concentrations in leachates collected from vineyard intra-row were affected by the position of the lysimeter on the slope during the studied period (Table 2). The highest Cu concentration was observed in leachates from lysimeters installed at the intra-row footslope compared to the hilltop and backslope, but without statistically significant differences between them (Table 2). The electrical conductivity of leachates collected from the intra-row area was significantly affected by the position of the lysimeter on the slope, with the highest value measured at the footslope. Leachate pH and DOC concentrations were not significantly different between the hilltop, backslope, or footslope, at either the inter- or intra-row area (Table 2).

Table 1. Mean values of soil pH, electrical conductivity (EC), organic carbon (OC), total (Cu_{total}) and available copper (Cu_{cacl2}) concentrations in soil samples taken from two soil depths (0–30 and 30–60 cm) at the location of lysimeters placement in the vineyard, i.e., at the hilltop, backslope and footslope (inter- and intra-row samples were pooled).

Position on the Slope	pH_{H2O}	EC mS m^{-1}	Cu_{total} mg kg^{-1}	Cu_{CaCl2} mg kg^{-1}	OC g kg^{-1}
			0–30 cm		
Hilltop	5.3 a	3.1 a	50.4 a	0.15 a	11.9 a
Backslope	5.5 a	2.6 a	40.7 a	0.09 b	9.8 a
Footslope	5.5 a	3.4 a	54.8 a	0.09 b	12.4 a
			30–60 cm		
Hilltop	5.1 a	3.7 a	35.4 a	0.14 a	5.9 a
Backslope	5.3 a	4.3 a	30.2 a	0.09 a	6.1 a
Footslope	5.3 a	3.2 a	57.0 a	0.22 a	8.1 a [1]

[1] Means in the same column and for the same soil depth marked with the same letter are not statistically significant at $p < 0.05$.

Table 2. Mean values of pH, electrical conductivity (EC), copper (Cu) and dissolved organic carbon (DOC) concentrations in leachates collected monthly for two years (2 November 2020–25 November 2022) from lysimeters installed at the hilltop, backslope and footslope of the vineyard inter- and intra-row.

Position on the Slope	pH 25 °C	EC mS m^{-1}	Cu mg L^{-1}	DOC mg L^{-1}
		Hillslope interrow		
Hilltop	6.3 a	14.7 a	12.4 a	8.2 a
Backslope	6.4 a	15.4 a	12.8 a	7.9 a
Footslope	6.4 a	14.8 a	18.8 a	8.6 a [1]
		Hillslope intra-row		
Hilltop	6.4 a	17.0 ab	12.9 b	8.7 a
Backslope	6.5 a	14.4 b	13.8 b	8.8 a
Footslope	6.5 a	19.0 a	24.8 a	9.6 a [1]

[1] Means in the same column and for the same row position marked with the same letter are not statistically significant at $p < 0.05$.

The statistical correlation of Cu × DOC (Table 3) was significant only in leachates from the intra-row footslope and interrow hilltop ($p < 0.05$), and highly significant at the interrow footslope ($p < 0.01$). However, although Pearson coefficients indicated a positive correlation between Cu and DOC (i.e., when there is an increase in Cu concentration in leachates, an increase in DOC concentration is also observed), these coefficients were not high (Table 3). This suggests that although a positive relationship between Cu and DOC in leachates exists, it is not strong, but rather low to moderate at best (Table 3). Thus, certain data points were close to the line of the Cu and DOC linear relationship, but other data points were not (Figure 3), further showing only a low to moderate linear relationship. Here, it should be noted that a significant correlation does not relate to the causality, which in the context of obtained Cu and DOC data means that other factor(s) could be influencing their concentrations in leachates, and often in a same way, i.e., when Cu concentration was increased, an increase in DOC concentration was more or less frequently also recorded (Table 3).

In Figure 3, it can be easily observed that various Cu concentrations were connected to the same DOC concentration during the studied two years, and that the highest Cu concentrations recorded were not linked, for example, to the highest DOC concentrations and vice versa (Figure 3), illustrating only a positive relationship of low strength (Table 3).

Table 3. Correlations between copper and dissolved organic carbon concentrations in leachates collected monthly for two years (2 November 2020–25 November 2022) from lysimeters installed at the hilltop, backslope and footslope of the vineyard inter- and intra-row.

Position on the Slope	Pearson Correlation Coefficients for Cu × DOC Correlation
	Hillslope interrow
Hilltop	0.271 *
Backslope	0.126
Footslope	0.441 **
	Hillslope intra-row
Hilltop	0.235
Backslope	0.196
Footslope	0.364 *

* Statistically significant at $p < 0.05$; ** Statistically significant at $p < 0.01$.

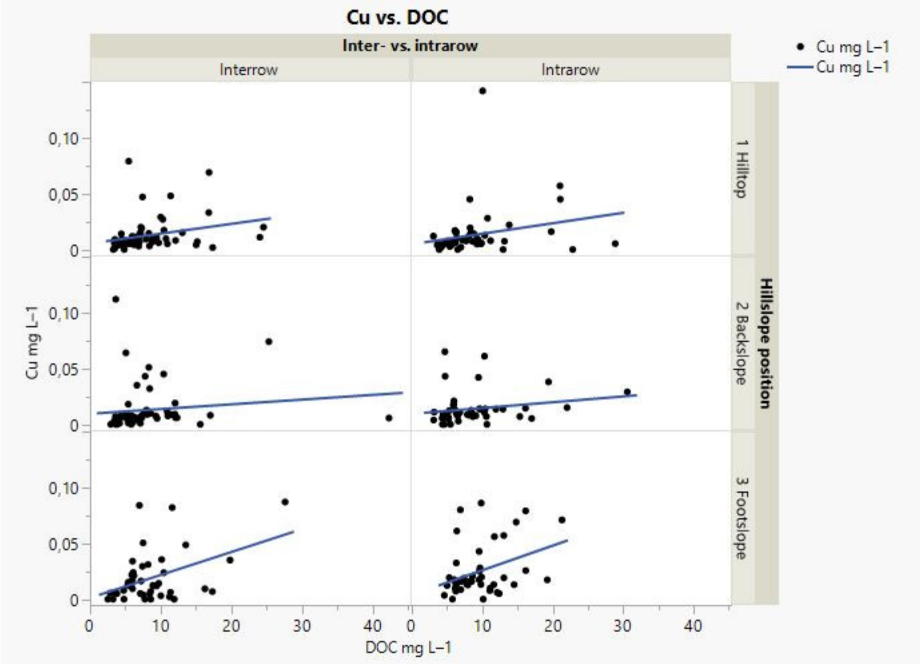

Figure 3. Graphical overlay of copper by dissolved organic carbon concentrations in leachates collected monthly for two years (2 November 2020–25 November 2022) from lysimeters installed at the hilltop, backslope and footslope of vineyard inter- and intra-row.

To further investigate copper mobility along the hillslope and its relationship with DOC concentration during this process, Cu species distribution in leachates was estimated using Visual MINTEQ software and the NICA-Donnan model [18], with separate estimations for leachates from lysimeters installed at the hilltop, backslope, and footslope of the vineyard inter- or intra-row (Table 4). Of all the leachate scenarios tested (i.e., hilltop intra-row, hilltop interrow, backslope intra-row, etc.) that differed in Cu and DOC concentrations, as well as in pH and other observed ion concentrations, Cu was found as only two chemical species; that is, almost exclusively as organically complexed Cu (Cu-DOC), and in a very low proportion as a free ion (Cu^{2+}).

Dissolved organic carbon cumulative mass (mg) in leachates was higher at the hilltop as compared to the backslope and footslope, at both, inter- and intra-row (Figure 4). However, Cu cumulative mass (μg) in leachates was the highest at the footslope at inter- and intra-row, compared to the hilltop and backslope (Figure 5). It should be noted that Cu oxychloride-based fungicide was applied to the vines (intra-row) in April 2020 and 2021.

Table 4. Average distribution of copper species in leachates collected monthly for two years (2 November 2020–25 November 2022) from lysimeters installed at the hilltop, backslope, and footslope of the vineyard inter- and intra-row, estimated using Visual MINTEQ chemical equilibrium software and NICA-Donnan model.

Position on the Slope	Cu Species [1, 2]	Interrow	Intra-Row
		% of Total Concentration	
Hilltop	Cu^{2+} (aq)	0.045	0.043
	HA1-Cu(6)(aq)	30.002	29.147
	HA2-Cu(6)(aq)	69.949	70.806
	Cu-DOC	99.951	99.953
Backslope	Cu^{2+} (aq)	0.054	0.033
	HA1-Cu(6)(aq)	30.137	27.379
	HA2-Cu(6)(aq)	69.804	72.584
	Cu-DOC	99.941	99.963
Footslope	Cu^{2+} (aq)	0.070	0.075
	HA1-Cu(6)(aq)	34.945	30.830
	HA2-Cu(6)(aq)	64.977	69.087
	Cu-DOC	99.922	99.917

[1] HA-Cu: humic acid-complexed Cu via 1-carboxylic and 2-phenolic functional groups; [2] Cu-DOC: total organically complexed Cu, a sum of HA1-Cu and HA2-Cu.

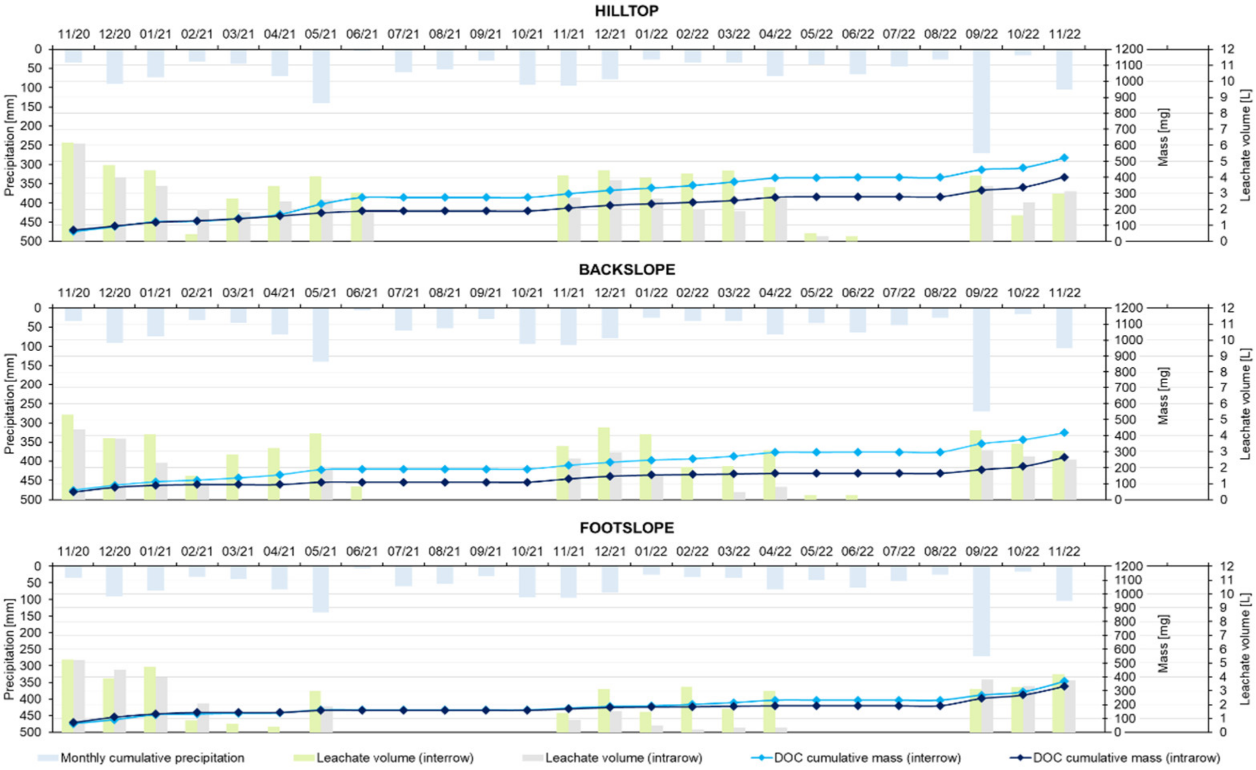

Figure 4. Dissolved organic carbon cumulative mass (mg) in leachates, collected for two years (2 November 2020–25 November 2022), from lysimeters installed at hilltop, backslope and footslope of vineyard inter- and intra-row, presented with monthly cumulative precipitation and leachate volumes per sampling event.

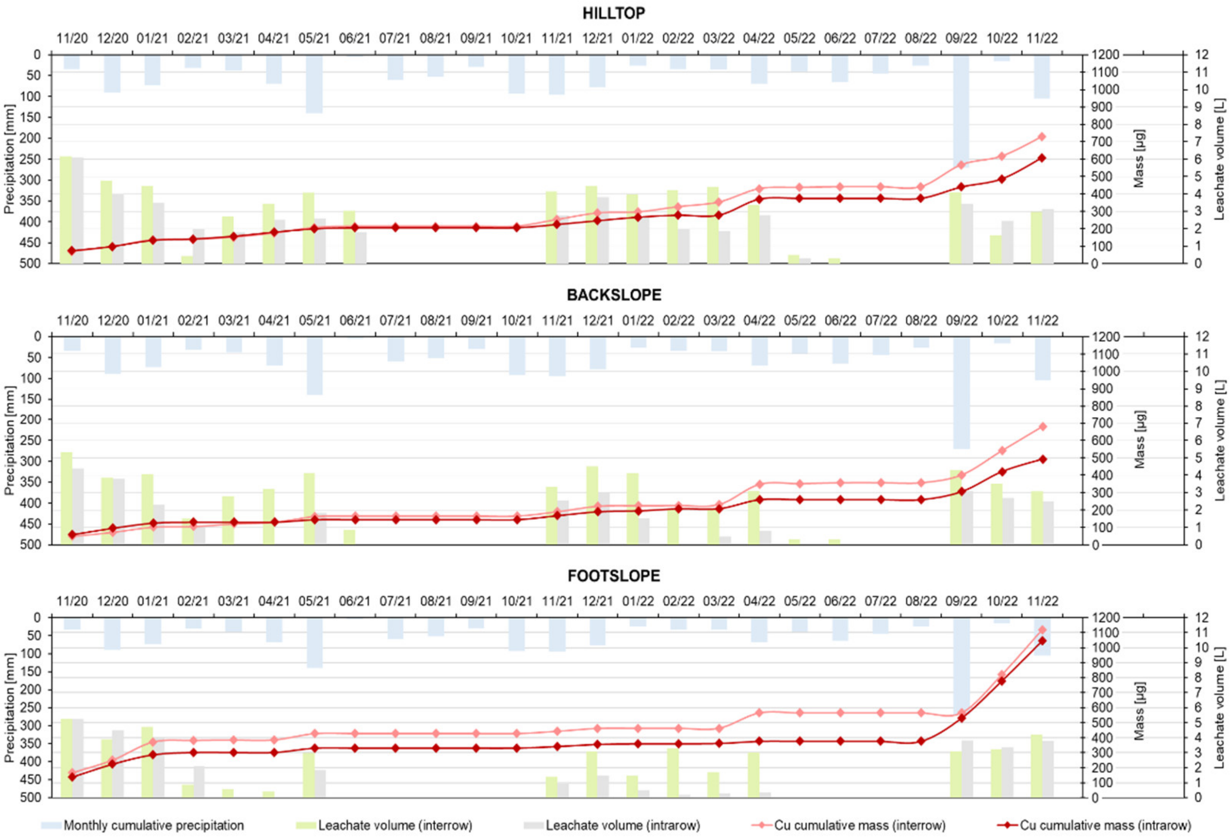

Figure 5. Copper (Cu) cumulative mass (µg) in leachates, collected for two years (2 November 2020–25 November 2022), from lysimeters installed at hilltop, backslope and footslope of vineyard inter- and intra-row, presented with monthly cumulative precipitation and leachate volumes per sampling event.

4. Discussion

Soil data (at 0–30 and 30–60 cm of soil depth) showed that there are no differences in soil pH, EC, total Cu and OC concentrations between the hilltop, backslope, and footslope at the studied vineyard (Table 1). Interestingly, a significantly higher concentration of available Cu in the soil was found at the hilltop compared to the backslope and footslope at the soil depth of 0–30 cm, suggesting that there is more Cu in its available (soluble), and thus mobile, form present in the hilltop topsoil, compared to the topsoil of the backslope and footslope. As Cu is quite immobile in soil, higher total Cu concentrations are frequently reported in upper soil horizons compared to the subsoil [20]. However, no statistically significant difference in total Cu concentration in the topsoil was found in relation to the position on the slope (Table 1), suggesting that other factor(s) besides Cu total concentration affected Cu availability in the soil. Furthermore, these results are somewhat inconsistent with the measurements of Cu concentrations in leachates from lysimeters installed at the hilltop, backslope, and footslope, which showed that Cu concentrations in leachates collected from the intra-row footslope were significantly higher than those collected from the intra-row hilltop and backslope during the studied period (Table 2). It would be expected that when more Cu is present in topsoil (0–30 cm) in its available form (i.e., at the hilltop), more would also be leached to lysimeters installed at 40 cm of soil depth. However, combined data (Tables 1 and 2) possibly suggest that in addition to vertical transport of available Cu from topsoil to subsoil (i.e., lysimeters), lateral leaching of Cu also occurs along the slope (i.e., from the hilltop and backslope to the footslope), and that this transport process is quite significant at the studied sloped vineyard, surprisingly even at 0–40 cm of soil depth. This conclusion possibly supports the premise that in addition to chemical parameters, soil physical and hydraulic parameters affected Cu transport at the hillslope. Regarding this hypothesis, we note that Cu as a divalent cation has an affinity for soil colloids (e.g., clay). More importantly, we note that Cu was applied as colloid-sized particles of Cu oxychloride-based fungicide at the studied site. When Cu-pesticides are sprayed as water suspension of colloid-sized Cu minerals, the Cu in vineyard soil may also be transported via colloid transport mechanisms, which are affected by different interactions with soil surfaces than Cu alone. Paradelo et al. [21] studied the transport of Cu oxychloride-based fungicide (COF) colloidal particles in water-saturated quartz sand columns under varying electrochemical and hydrodynamic conditions and demonstrated that the retention of Cu in the soil occurs due to stagnation zones arising from the physical and chemical heterogeneity of properties at the quartz surface, and that increased flow rate reduces the stagnation zones in which colloids can be retained. This suggests that if the increased water flow rate at the studied hillslope can transport Cu as COF colloidal particles, then this effect was presumably captured in lysimeter leachates. In this study, there is a possibility that the constructed lysimeters did not exclude the particle-facilitated Cu transport. Filter mesh (1 mm × 1 mm) placed at the bottom of the lysimeter would not retain colloid-size particles, which is supported by the fact that sampled leachates frequently had high turbidity. In their subsequent study, Paradelo et al. [22] focused on the effects of soil structure on Cu leaching and showed that the leaching was indeed facilitated by particle (and DOC) transport, and that Cu leaching as attached to particles was more relevant during the first flush than at later times when DOC-facilitated Cu transport became more relevant. The authors obtained a good correlation between Cu leaching and soil macroporosity, and further revealed that the soil with the highest Cu concentration did not pose the highest risk for Cu leaching because water bypassed most of the soil matrix [22]. In addition, their study emphasized that any disruption of soil structure (for example, tillage) could initiate Cu-colloid leaching [22].

The OC concentration in soil samples taken at the location of the lysimeters (0–30 cm and 30–60 cm soil depth) was not affected by the slope position (inter- and intra-row pooled samples; Table 1). However, DOC cumulative mass in leachates at inter- and intra-row was higher at the hilltop compared to the backslope and footslope after two years of measurements (Figure 4), suggesting that DOC is not transported to the footslope. Additionally, DOC concentration in leachates (Table 2) did not show any dependency on the position of lysimeter on the slope. This is interesting considering the major role that SOM has on Cu behavior in soil, i.e., immobilization of Cu by retention mechanisms with SOM and mobilization of Cu through the formation of Cu-DOC complexes [23]. However, the biogeochemistry of SOM is highly complex and dynamic, with only a small proportion of solid SOM representing its potentially dissolved organic fraction, i.e., DOC [24]. Statistical analysis of the Cu × DOC correlation in leachate samples suggested that there is a significant relationship between Cu and DOC in certain leachates, but also that this correlation is only low to moderately strong (Table 3). Moreover, more than 99.9% of the total Cu concentration in leachates was estimated as Cu-DOC, and only less than 0.01% as Cu^{2+} (Table 4). However, a small increase in the proportion of Cu^{2+} is observed at the expense of Cu-DOC species at the vineyard footslope compared to the hilltop and backslope, and this tendency is also slightly more pronounced at the footslope intra-row, compared to the footslope interrow position (Table 4). Such results are usually obtained when under similar conditions an increase in Cu concentration in the soil solution is not accompanied by an adequate increase in DOC concentration [9], which seems to be the case at the vineyard footslope (Table 2). Furthermore, it should be noted that the Cu speciation results presented in Table 4 describe the most probable Cu species occurring in leachates of chemical characteristics defined at the sampling event, but these data are not informative regarding Cu species transported along the hillslope per se. This means that Cu could have been transported to the lysimeter as a species other than complex with DOC, and then under conditions of the lysimeter tank solution, represented as a leachate sample, bond with DOC. Additionally, the stationarity of a solution that occurs at the sampling event by defining the finite point in obtaining the chemical equilibrium probably influences Cu speciation, even if only in a slight way. This supports the need for speciation modeling data interpretation in conjunction with field measurements and by recognizing and defining the possible indications from minor changes in the output data in a broader context.

Therefore, the results presented in Table 4 show that > 99.9% of total Cu in all leachates is complexed with DOC, which clearly indicates that DOC is a major factor governing Cu mobility at the hillslope. However, by observing a slight change in Cu speciation in favor of Cu ion at the footslope and especially by connecting this information with Cu and DOC concentrations in leachates (Table 2) and weak Cu × DOC correlations (Table 3), these results possibly point to yet unknown factors and processes affecting Cu leaching along the hillslope. Moreover, if soil pH, total Cu and OC concentration (Table 1) and leachate pH data (Table 2) are considered in this hypothesis, combined with information on Cu application such as Cu oxychloride-based fungicide at the studied site, it is suggested that presumably soil water movement along the preferential pathways was significant for Cu mobility in soils along the slope. Thus, data confirm that DOC is an important factor for Cu leaching (Table 4), and that Cu-complexation with DOC affects Cu transport (Table 3). In addition, it is suggested that the influence of soil water flow via, but possibly not exclusively, Cu-colloidal transport mechanisms are relevant on hillslopes, especially if DOC is not increased in relation to Cu concentration. This hypothesis is possibly supported by the Cu cumulative mass in leachates (Figure 5), which showed a substantial increase in Cu cumulative mass after September 2022, i.e., after the application of Cu-based fungicide (April 2022) and a heavy rainfall event (September 2022), further implying the role of soil water flow in Cu downhill transport.

Overall, two years of measuring Cu concentration in leachates from lysimeters installed at the vineyard hillslope show that Cu is transported to the footslope (Figure 5) significantly at the intra-row area (Table 2). Considering only chemical processes, even at sloped soils, Cu leaching is generally not expected unless the total Cu concentration in soil is increased such that it exceeds the soil capacity to retain cations, or the soil pH and SOM content are relatively low [9]. By addressing these two soil chemical parameters, more Cu could be retained in the soil. In this study, soil pH was well below neutral (5.3–5.5 at 0–30 cm depth, and 5.1–5.3 at 30–60 cm depth; Table 1), thus the agricultural practice of adjusting soil pH (e.g., liming) and possibly also by increasing the SOM content, could reduce Cu leaching here. However, increasing SOM could initially increase Cu leaching due to an increase in the concentration of DOC in the soil solution, but this effect will presumably have a short-term effect until the soil solution reaches equilibrium and the particulate SOM likely becomes the main retention mechanism of Cu in the soil [9].

A similar trend between the concentration of leachate Cu at the hilltop, backslope, and footslope is observed at the vineyard inter- and intra-row (i.e., the highest leached Cu at the footslope), but it was statistically significant only at the intra-row. This was probably because the data from the interrow were not consistent in expressing this trend, i.e., they showed more variability in the concentration of leachate Cu. Still, this may be an indication of different physicochemical processes in the soil affecting Cu mobility at intra-row compared to that at the interrow position (Table 2). However, it is also possible that significant leaching of Cu only at the intra-row lysimeters is simply because Cu-pesticides are applied at the intra-row; that is, to the vines. This also means that more decomposed vine leaves treated with Cu will be present at the intra-row positions. Peralta et al. [25] showed spatially variable distribution in vineyard polluted soils, which was attributed to a non-homogeneous and repetitive application of the Bordeaux mixture. In addition, the grass cover present at interrow lysimeters may affect Cu transport and leaching, either directly or indirectly, by influencing soil water flow. In their soil column study, Ortega et al. [26] studied the effects of cover crops in vineyards introduced to prevent the leaching of Cu and organic fungicides to groundwater. They showed that cover crops reduced the leaching of fungicides, but only when the fungicides were applied 24 h before the rain event, and not when a rain event occurred after the application of the fungicide. Therefore, if rainfall immediately follows Cu-pesticides application to the vines, certain leaching of Cu may be expected regardless of the soil cover. Considering that the data from this study implied the role of soil water flow pathways in explaining Cu downhill transport, critical timing for applying Cu fungicides at sloped vineyards is highlighted.

5. Conclusions

The highest Cu concentrations found in leachates from the intra-row footslope suggested Cu is transported downhill. Although not strong, a significant positive correlation between Cu and DOC in footslope leachates confirmed the relevance of Cu complexation by DOC. Speciation further suggested that more than 99.9% of total Cu in leachates was found as a Cu-DOC complex. However, data also suggested that in addition to chemical parameters, soil physical and hydraulic parameters probably affected Cu transport at the hillslope. At the studied site, Cu was applied as colloid-sized particles of Cu oxychloride-based fungicide (COF), thus transport via colloid transport mechanisms possibly occurred. Therefore, if increased water flow rate at the studied hillslope can transport Cu as COF colloidal particles, critical timing for applying Cu fungicides is highlighted.

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References

1. Peña, N.; Antón, A.; Kamilaris, A.; fantke, P. Modeling ecotoxicity impacts in vineyard production: Addressing spatial differentiation for copper fungicides. *Sci. Total Environ.* **2018**, *616*, 796–804. [CrossRef] [PubMed]
2. Nadin, P. *The Use of Plant Protection Products in the European Union Data 1992–2003*; Office for Official Publications of the European Communities: Luxembourg, 2007.
3. European Commission 2018/1981. Commission implementing regulation (EU) 2018/1981 of 13 December 2018 renewing the approval of the active substances copper compounds, as candidates for substitution, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. *Off. J. Eur. Union.* **2018**. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32018R1981 (accessed on 25 January 2023).
4. Droz, B.; Payraudeau, S.; Rodríguez Martín, J.A.; Tóth, G.; Panagos, P.; Montanarella, L.; Borrelli, P.; Imfeld, G. Copper Content and Export in European Vineyard Soils Influenced by Climate and Soil Properties. *Environ. Sci. Technol.* **2021**, *55*, 7327–7334. [CrossRef] [PubMed]
5. Reichman, S.M. *The Responses of Plants to Metal Toxicity: A Review Focusing on Copper, Manganese and Zinc*; Australian Minerals and Energy Environment Foundation: Melbourne, Australia, 2002; p. 54.
6. Degryse, F.; Smolders, E.; Parker, D.R. Partitioning of metals (Cd Co, Cu, Ni, Pb, Zn) in soils: Concepts, methodologies, prediction and applications—A review. *J. Soil Sci.* **2009**, *60*, 590–612. [CrossRef]
7. Boudesocque, S.; Guillon, E.; Aplincourt, M.; Marceau, E.; Stievano, L. Sorption of Cu(II) onto vineyard soils: Macroscopic and spectroscopic investigations. *J. Colloid Interface Sci.* **2007**, *307*, 40–49. [CrossRef]
8. Tessier, A.; Fortin, D.; Belzile, N.; DeVitre, R.R.; Leppard, G.G. Metal sorption to diagenetic iron and manganese oxyhydroxides and associated organic matter: Narrowing the gap between field and laboratory measurements. *Geochim. Cosmochim. Acta* **1996**, *60*, 387–404. [CrossRef]
9. Matijević, L.; Romić, D.; Romić, M. Soil organic matter and salinity affect copper bioavailability in root zone and uptake by *Vicia faba* L. plants. *Environ. Geochem. Health* **2014**, *36*, 883–896. [CrossRef] [PubMed]
10. Yang, F.; Tian, J.; Fang, H.; Gao, Y.; Xu, M.; Lou, Y.; Zhou, B.; Kuzyakov, Y. Functional soil organic matter fractions, microbial community, and enzyme activities in a Mollisol under 35 years manure and mineral fertilization. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 430–439. [CrossRef]

11. Liao, M. Mechanisms of Copper Uptake and Transport in Plants. Ph.D. Thesis, Massey University, Palmerston North, New Zealand, 2000.
12. Kim, K.-R.; Owens, G.; Naidu, R.; Kwon, S. Influence of plant roots on rhizosphere soil solution composition of long-term contaminated soils. *Geoderma* **2010**, *155*, 86–92. [CrossRef]
13. Tao, S.; Liu, W.X.; Chen, Y.J.; Xu, F.L.; Dawson, R.W.; Li, B.G.; Cao, J.; Wang, X.J.; Hu, J.Y.; Fang, J.Y. Evaluation of factors influencing root induced changes of copper fractionation in rhizosphere of a calcareous soil. *Environ. Pollut.* **2004**, *129*, 5–12. [CrossRef]
14. International Union of Soil Sciences (IUSS) Working Group WRB. World reference base for soil resources. International soil classification system for naming soils and creating legends for soil maps. *Exp. Agric.* **2014**, *43*, 264. [CrossRef]
15. Filipović, V.; Defterdarović, J.; Krevh, V.; Filipović, L.; Magdić, I.; He, H.; Haghverdi, A.; Gerke, H.H. Estimation of Stagnosol Hydraulic Properties and Water Flow Using Uni- and Bimodal Porosity Models in Erosion-Affected Hillslope Vineyard Soils. *Agronomy* **2022**, *12*, 33. [CrossRef]
16. Houba, V.J.G.; Uittenbogaard, J.; Pellen, P. Wageningen evaluating programmes for analytical laboratories (WEPAL) organization and purpose. *Commun. Soil Sci. Plant Anal.* **1996**, *27*, 421–431. [CrossRef]
17. Houba, V.J.G.; Temminghoff, E.J.M.; Gaikhorst, G.A.; van Vark, W. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Commun. Soil Sci. Plant Anal.* **2000**, *31*, 1299–1396. [CrossRef]
18. Gustafsson, J.P. *Visual MINTEQ Version 3.1*, KTH, SEED: Stockholm, Sweden, 2020. Available online: https://vminteq.lwr.kth.se/(accessed on 15 February 2023).
19. Kinniburgh, D.G.; Milne, C.J.; Benedetti, M.F.; Pinheiro, J.P.; Filius, J.; Koopal, L.K.; Van Riemsdijk, W.H. Metal ion binding by humic acid: Application of the NICA-Donnan model. *Environ. Sci. Technol.* **1996**, *30*, 1687–1698. [CrossRef]
20. Schoffer, J.T.; Sauvé, S.; Neaman, A.; Ginocchio, R. Role of Leaf Litter on the Incorporation of Copper-Containing Pesticides into Soils Under Fruit Production: A Review. *J. Soil Sci. Plant. Nutr.* **2020**, *20*, 990–1000. [CrossRef]
21. Paradello, M.; Šimůnek, J.; Novoa-Muñoz, J.C.; Arias-Estevez, M.; Lopez-Periago, J.E. Transport of Copper Oxychloride-Based Fungicide Particles in Saturated Quartz Sand. *Environ. Sci. Technol.* **2009**, *43*, 8860–8866. [CrossRef]
22. Paradello, M.; Moldrup, P.; Arthur, E.; Naveed, M.; Holmstrup, M.; López-Periago, J.E.; de Jonge, L.W. Effects of Past Copper Contamination and Soil Structure on Copper Leaching from Soil. *J. Environ. Qual.* **2014**, *42*, 1852–1862. [CrossRef]
23. Sauvé, S.; McBride, M.B.; Norvell, W.A.; Hendershot, W.H. Copper solubility and speciation of in situ contaminated soils: Effects of copper level, pH and organic matter. *Water Air Soil Pollut.* **1997**, *100*, 133–149. [CrossRef]
24. Ondrasek, G.; Bakić Begić, H.; Zovko, M.; Filipović, L.; Meriño-Gergichevich, C.; Savić, R.; Rengel, Z. Biogeochemistry of soil organic matter in agroecosystems & environmental implications. *Sci. Total Environ.* **2019**, *658*, 1559–1573. [CrossRef]
25. Peralta, E.; Perez, G.; Ojeda, G.; Alcañiz, J.M.; Valiente, M.; López-Mesas, M.; Sánchez-Martín, M.-J. Heavy metal availability assessment using portable X-ray fluorescence and single extraction procedures on former vineyard polluted soils. *Sci. Total Environ.* **2020**, *726*, 138670. [CrossRef]
26. Ortega, P.; Sánchez, E.; Gil, E.; Matamoros, V. Use of cover crops in vineyards to prevent groundwater pollution by copper and organic fungicides. Soil column studies. *Chemosphere* **2022**, *303*, 134975. [CrossRef] [PubMed]