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Master Thesis

**Temporal changes in the efficiency of biochar-based soil
amendments on copper immobilization in vineyard soils**

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Statutory Declaration

I declare that I have developed and written this thesis independently and have not used sources or means without declaration in the text and that all material quoted literally or by content has been marked.

Abstract

Copper (Cu) based fungicides have been an important tool against disease in viticulture since the 19th century. However, prolonged use leads to Cu accumulation in the soil, with negative repercussions for soil microbiology and plant growth. As there are currently no viable alternatives for these fungicides in organic viticulture, long-term mitigation strategies are vital. The application of biochar (BC) based amendments has been promising, due to BC's longevity in the soil and its potential to complex Cu. This study investigated the temporal changes in the efficiency of various compost and BC-based amendments on copper immobilization in two contrasting Austrian vineyard soils (calcareous vs. slightly acidic). Batch experiments were treated with six combinations of amendments containing compost and different BCs with and without surface modification, as well as an additional lime treatment for the acidic soil. In one batch, the immobilization of historically accumulated Cu was studied, while in another batch, 250 mg Cu kg⁻¹ was freshly spiked. The amended soils were incubated at 10°C, and soluble Cu was extracted with 0.01 M CaCl₂ after 6 weeks and 3 years, respectively.

The tested amendments were not effective in reducing the mobility of the historically accumulated Cu in the calcareous soil, with pure compost amendment even doubling the soluble Cu. For the acidic soil, pure wood-chip BC was the only organic amendment that led to a reduction (by 20%) of soluble Cu after 6 weeks, while after 3 years, the same amendment reduced soluble Cu by 40% and all other tested amendments were also effective in reducing the mobility of the historically accumulated Cu. Nevertheless, none of the organic amendments reached the immobilization efficiency of the lime treatment (56% reduction). Freshly spiked Cu was strongly immobilized in both soils even without any amendments, with only 0.06% and 0.39% extractable after 6 weeks, and 0.02% and 0.16% extractable after 3 years in the calcareous and slightly acidic soil, respectively. For the calcareous soil, the tested amendments did not effectuate additional Cu immobilization, but in the acidic soil, the soluble Cu was further reduced to between 25 and 50% of the unamended control by the tested organic amendments and to 6% by the lime treatment after 6 weeks of incubation. Overall, the acidic soil exhibited a stronger response to the amendments than did the calcareous soil, suggesting the amendments' liming effect was an important factor for Cu immobilization in this study.

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1. Introduction

In viticulture, the application of copper (Cu) based pesticides and fungicides is highly necessary to fight disease outbreak, such as the downy mildew infection. The application of copper is also used as a preventative measure, requiring a continuous application to the vineyards (Wightwick et al., 2010; Dagostin et al., 2011; Rusjan, 2012, Morgan and Taylor, 2004). However, this results in the accumulation of Cu in the soil, which increases the toxicity of the soil and may have negative repercussions on the soil microbiology (Fernández-Calveño et al., 2010; Oorts et al., 2006, Keiblinger et al., 2018). This accumulation can also threaten the practice of organic farming itself, due to potential future environmental issues as concentrations increase (Komárek et al., 2010). In 2006, the European Commission passed measures to limit the amount of Cu applied, initially at 8 kg per hectare, and reduced to 6 kg after four years (European Commission, 2002). Austria, and many other countries around the world, report increasing levels of copper in the vineyards, which require remediation to reduce potential toxicity and long-term negative consequences. Copper-based fungicides are currently the most effective approach in viticulture, which increases the need to reduce the amount of copper present in soils (Dagostin et al., 2011).

2. Background

2.1 Copper in the Soil

The application of copper to combat downy mildew first began in 1880, when grape vines in Bordeaux, France were coated in a paste of copper sulphate, lime, and water (Rusjan, 2012). Since then, copper has become a staple in horticulture to combat a variety of pests.

Copper is an important microelement that is necessary for optimal plant growth and reproduction. It is naturally derived from the soil parent material and is typically present at an average concentration of approximately 30 mg kg⁻¹ (Wuana and Okieimen, 2011; Rusjan, 2012). However, in agricultural settings, due to its use as a fungicide, total concentrations have been found in the range from 77 to 3200 mg kg⁻¹ within the topsoil (Komarek et al., 2010; Wightwick et al., 2008; Ruyters et al., 2013).

Copper is present in the soil in its divalent form (Cu^{2+}), either incorporated into minerals or adsorbed to inorganic and organic soil particles (Rusjan, 2012; Wang et al., 2012). Most of the Cu is associated with the solid soil particles, but a part is available as free Cu^{2+} ions in soil solution. Specific adsorption occurs with iron, aluminum and manganese oxides and is considered to be inert as it is not easily released (Wightwick et al., 2008, Uchimiya et al., 2011). The Cu complexed with organic ligands, however, is biologically available. This is the form that can be taken up and utilized by organisms, and is of special significance when determining potential toxicity (Wang et al., 2012; Sauvé et al., 1997; Smolders et al., 2009).

The solubility of Cu, like many other metals, is highly influenced by soil properties and the Cu that is complexed to the soil organic matter can be released into the soil solution as conditions change (Rusjan, 2012; Sauvé et al., 1997). Sauvé et al. (1997) suggests that in highly-contaminated soils, the activity of Cu^{2+} is controlled by precipitation-dissolution equilibrium relationships. However, when the concentration is very low, the Cu is more likely to be influenced by specific adsorption mechanisms. The specific soil conditions include factors such as pH and the cation exchange capacity (CEC) (Li et al., 2015; Uchimiya et al., 2011; Chaignon et al., 2003). Increasing pH is associated with a decreasing concentration of Cu^{2+} in the soil solution, although pH has less of an influence on Cu complexes (Rusjan, 2012). Mobility is also affected by soil organic matter, which acts either as sorbent for Cu or by facilitating soluble Cu complexes (Komarek et al., 2010; Mackie et al., 2015; Temminghof et al., 1997). The influences of these various interactions are important to consider in order to employ the most effective methods in reducing the amount of bioavailable Cu in the soil.

2.2 The Effects of Cu Accumulation

Copper is necessary in small quantities for optimal plant growth but becomes damaging to the soil microbiology and crop yields as it accumulates in the soil (Michaud et al., 2007; Fernández-Calveño et al., 2010; Wang et al., 2006). However, soil toxicity is not determined by the total concentration of a contaminant, but rather by its bioavailability, the proportion that can be utilized by organisms (Smolders et al., 2009; Wang et al., 2006; Brun et al., 2001; Maderova et al., 2011). In this study, a distinction between total copper extracted with the 0.01 M CaCl_2 solution and the ecologically toxic Cu^{2+} is made. As metal solubility is influenced by factors such as adsorption-desorption reactions, complexation with dissolved organic and inorganic

ligands, as well as exchange reactions with the organic material, the CaCl₂-extracted Cu provides an overview of the total potentially available Cu (Sauvé et al., 1998; Yin et al., 2002; Harmsen et al., 2005; Pietrzak and McPhail, 2004; Okonokhua, 2014). This chemically mobile fraction is defined as the sum of the element in soil solution and the amount remaining in the solid phase that may be transferred into solution if the required conditions are met, influencing the amount of Cu potentially taken up by plants and microorganisms (Rieuwerts et al. 1998; Michaud et al., 2007). A particular emphasis is placed on Cu²⁺ as this is the form utilized by organisms, and may be used to predict potential toxicity, although there is some evidence that forms such as CuOH⁺ may also contribute to toxicity (Sauvé et al., 1998; Wang et al., 2012; Wang et al., 2009). In a study conducted by Kim et al. (2009), it was found that Cu²⁺ was a more reliable predictor of soil urease, which was used as a measure of enzyme activity, than CaCl₂-extracted Cu. This corresponds to the findings by Wang et al., (2012) that determined that Cu²⁺ concentrations had the most influence on root growth in solution cultures as compared to other species of Cu. Thus, the CaCl₂-extracted Cu provides a general overview of the soluble Cu present in the soil whereas the Cu²⁺ allows for the determination of potential toxicity.

Copper is typically present within the surface layers, due to the adsorption to soil organic matter, so mature grapevines may be safe as their roots penetrate deep enough (Rusjan, 2012). However, increased Cu concentrations may be dangerous for new plants. The effect of elevated Cu in crops includes stunted plant growth and chlorosis (Wyszkowska et al., 2013; Ruyters et al., 2013). Additionally, though Cu is not particularly mobile within the soil, there is still the possibility that leaching can occur if certain conditions are presented (Rieuwerts, 2007).

In the microbial community, Cu toxicity results in decreased populations sizes and reduced enzymatic activities (Wang et al., 2006; Fernández-Calveño et al., 2010; Kim et al., 2008). This results in the decline of soil fertility, reducing plant yields and becoming an environmental concern (Wightwick et al., 2008).

As Cu is not particularly mobile, the dangers of Cu accumulation do not necessarily come from the threat of leaching, but rather the increased risk of potential availability. The Cu bound to organic ligands and particles within the soil may become available when soil conditions change, such as decreasing pH. As Cu-based fungicides are the only viable fungicide currently available, it is necessary to reduce the accumulation in the soil to allow for their continued use (Dagostin et al., 2011). In addition, Cu toxicity is not only a problem in vineyards, but in

agricultural lands in general, as well as former mine and smelting sites (Beesley et al., 2013; Perez-Esteban et al., 2013). As a result, it is important to reduce the amount of Cu in the soil to avoid the potential of re-mobilization in the future.

2.3 Biochar and Compost Amendments

Amendments to the soil in the form of biochars and compost have been found effective at reducing metal toxicity due to the complexation of Cu, rendering it immobile and reducing its bioavailability (Chaignon et al., 2003; Mackie et al., 2015; Ruyters et al., 2013). Biochar is created through the pyrolysis of biomass under low oxygen conditions, resulting in a C rich product that may enhance the sorption of organic and inorganic contaminants. It additionally acts as a soil conditioner, and can improve water holding capacity and soil structure (Ahmad et al., 2013; Beesley et al., 2011; Park et al., 2011). Biochar-induced reduction in Cu mobility is the result of increased interaction with the biochar's surface area and may be influenced by various mechanisms (Uchimaya et al., 2011b; Borchard et al., 2012; Ahmad et al., 2013). One such immobilizing mechanism may be due to the increase in soil pH and CEC as the result of the biochar application (Uchimiya et al., 2010; Karami et al., 2011; Houben et al., 2013). As most biochars typically have a neutral to alkaline pH, their application has a liming effect on the soil (Ahmad et al., 2013; Uchimiya et al., 2011; Yin et al., 2002).

Another mechanism is through the interaction with the dissolved organic carbon (DOC). However, biochars with a high amount of DOC may facilitate the formation of soluble Cu complexes, leading to increased mobility (Ahmad et al., 2013; Beesley et al., 2010; Park et al., 2011; Bolan et al., 2014; Houben et al., 2013), while biochars that are low in DOC may contribute to Cu immobilization (Bolan et al., 2014). The use of compost has the potential effect of reducing Cu availability through the release of dissolved organic ligands to form complexes (Mackie et al., 2015).

A third potential mechanism for immobilization is the influence of oxygen-containing surface functional groups that increase in number as biochar is oxidized (Cheng et al., 2006; Zimmerman, 2010). These surface functional groups may lead to increased copper sorption (Uchimiya et al., 2011).

Compost can additionally lead to an increase in soil fertility and plant growth, increasing the benefits of processes such as phytoremediation in removing copper from the soil (Beesley et al.,

2013; Karami et al., 2011). Additionally, the amendments contain a high C content and a low heavy metal concentration, reducing the potential introduction of other contamination (Beesley et al., 2010; Perez-Esteban et al., 2013). While other organic amendments, such as sewage sludge and other biosolids, also demonstrate the ability to bind Cu, there is the potential that this ability may be reduced during the processing phase that is required for these amendments (Smolders et al., 2012). There is also the benefit of reduced costs associated with biochar and compost amendments, as compared to other organic amendments (Tan et al., 2015; Xu et al., 2016).

The biochar amendments may also undergo additional activation processes to improve specific physiochemical properties, such as increasing the surface area (Cha et al., 2016; Beesley et al., 2011; Brändli et al., 2008). The use of chemical oxidization, such as H₂O₂, or activation through the use of citric acid, increases the presence of surface groups, allowing for enhanced Cu binding (Xu et al., 2016; Rajapaksha et al., 2016). The composting of biochars is another process that modifies the biochars to create bio-activated surfaces (Borchard et al., 2012). However, one consequence of such modification processes is the increase in cost (Tan et al., 2015). Another potential consequence is that while Cu availability may be reduced, other elements, such as Ca and Al, may exhibit increased mobility instead (Rajapaksha et al., 2016; Uchimiya et al. 2010).

2.4 Incubation and Aging

A key characteristic of biochar is the stability of its components, which increases its longevity in the soil (Sohi et al., 2010, Ahmad et al., 2014). This makes it a potentially useful tool in carbon sequestration to reduce the impact of greenhouse gas emissions (Ippolito et al., 2012). However, the long-term impact of biochar amendments on Cu mobility is not yet fully understood. It may be that the initial priming effect of biochar, the mineralization that occurs shortly after application, is a transient one, and declines over time (Singh and Cowie, 2014; Wagner and Kaupenjohann, 2015; Zimmerman et al., 2011). This would follow the decomposition of biochar, which is initially rapid after application, but transitions to a slow and partial decomposition. It is also possible that there is a negative priming effect, in that the application of biochar actually reduces mineralization rates, which would further contribute to the longevity of biochar in the soil (Wang et al., 2016).

Biochar is slowly oxidized in the soil over time, potentially impacting its Cu absorbing capability. This oxidation can lead to the formation of oxygen-containing surface functional

groups on the biochar (Cheng et al., 2006; Zimmerman, 2010; Kuzyakov et al., 2009; Li et al., 2016). Biochar may also initially be hydrophobic and become more hydrophilic over time, due to the increase of these functional groups (Zimmerman, 2010; Verheijen et al., 2009; Joseph et al., 2010; Rechberger et al., 2017).

In addition to the incubation periods, there is also the influence of freshly contaminated soils against historically contaminated ones. As soils age over time, as in the historically contaminated, the bioavailability of metals declines (Ruyters et al., 2013; Lock and Janssen, 2003; Smolders et al., 2012). As a result, freshly contaminated soils may have more mobile Cu as it has not yet been complexed, which may lead to an increased probability of leaching (Oorts, et al., 2006; Ma et al., 2006). However, the exact extent of the effect of aging on Cu toxicity is uncertain due to the inherent variability of soil microbial processes (Ruyters et al., 2013). The difference between bioavailability in fresh and historically contaminated soils may be the result of several factors, such as the length of the aging process, differences in soil properties, or adaption of the soil microbial community (Ruyters et al., 2013; Ma et al., 2006). Due to the impact of soil aging on the bioavailability of copper, freshly spiked copper may be more mobile than in historically contaminated soils and may therefore be more available for the amendments to impact. The influence of aging of the soil, in conjunction with the aging of the biochar, must be taken into account when determining the influence of amendments on mobile/bioavailable Cu concentrations.

2.5 Objectives and Outlines

The objective of this study is to determine the long-term effects of biochar and compost based amendments on mobile copper concentrations in vineyard soils, as this is not yet well understood. In acidic soils, lime was used as an additional control due to the liming effect of the alkaline biochar. This study is a continuation of the study by Deinhofer et al., (2015), which examined the short-term impacts of the amendments.

The hypothesis is that i) the amendments, specifically the compost-based amendments due to the modification of the biochar surface, will reduce soluble Cu concentrations, although with a more pronounced effect in the freshly contaminated soils, and ii) that the soils that have been incubated with biochar in a long-term incubation period will exhibit stronger effects than in the short-term incubation.

A total of seven BC and compost-based amendments were tested on two vineyard soils in Austria to determine their influence on reducing mobile Cu concentrations. Additionally, a comparison between historically and freshly contaminated soils was conducted, as well as a comparison between incubation periods. The results are discussed with an analysis between total soluble Cu and Cu²⁺, as well as the influence of specific soil properties.

3. Materials and Methods

3.1 Soil Sampling and Pre-treatment

In the previous experiment, a total of six Austrian vineyard soils were sampled in early spring 2014. At each site, Cu-containing fungicides had been used, resulting in increased copper concentrations of varying amounts. During the analysis of the short-term effects, focus was primarily on the soils from two of the six sites, Sankt Stefan (Western Styria) and Rossatz (Wachau, Lower Austria), due to the large variation in their soil characteristics. An overview of their soil properties, including texture, pH values, and organic matter content, is given in Table 1. The soil samples were collected from the uppermost soil layer, at a depth of 0-10 cm, at different positions throughout the vineyards and combined to one composite sample per vineyard. The samples were fully homogenized and sieved to < 2 mm while moist, prior to the analysis. In the comparison of the long-term effects of the organic amendments, focus was again concentrated on the same two sites; Sankt Stefan and Rossatz, to allow for the analysis between the short and the long-term effects.

Table 1: Soil properties of the two Austrian vineyard soils focused on for this analysis. eCEC= effective cation exchange capacity, WHC= water holding capacity. Data provided by G. Dersch, AGES and E. Deinhofer (2014).

	pH in CaCl ₂ (1:10)	Organic Matter	Sand	Silt	Clay	Total Cu (mg kg ⁻¹)	eCEC (cmol _c kg ⁻¹)	WHC (%)
Rossatz	7.21	1.5	63	26	11	337	13.1	24.7
St. Stefan	6.15	4.7	44.6	31	21.3	201	18.5	50.1

3.2 Experimental Set-up

Six amendments were applied to the soils, with an additional seventh treatment containing lime for the acidic soil from St. Stefan. The treatments are presented in **Table 2**, below.

Table 2: Amendments tested in the batch experiments

Amendment	Abbreviation	Application Rate (wt%)	Corresponding Field Application Rate (t DM ha ⁻¹)
Control	CO		
Compost	K	1.5	40
Wood-BC	wBC	1.5	40
Compost+Wood-BC (1:3)	K+wBC	1.5	40
Compost + H ₂ O ₂ -oxidized Wood-BC (1:3)	K+H ₂ O ₂	1.5	40
Compost + Citric-acid activated Wood-BC (1:3)	K+CA	1.5	40
Control + Lime (only for the acidic soil of St. Stefan)	L	0.19	5

Compost of class A+ (compost plant Pixendorf, Austria) was applied at a rate of 1.5 wt% (corresponding to 40 t DM ha⁻¹), as a separate treatment and in combination with different BCs. Green garden waste had been the input material for composting. The resulting compost had a Cu concentration of 40.4 mg kg⁻¹ and a pH (in CaCl₂) of 7.4.

The BCs were produced from softwood chips, mostly poplar, and wheat husks (*Sonnenerde*, Riedlingsdorf, Austria). The biochars were carbonized by slow pyrolysis in a Pyreg reactor (PYREG GmbH, 56281 Dörth, Germany) at 560°C and had a Cu concentration of 0.2 mg kg⁻¹. Wood BC was applied in pure form in one amendment, and in two of the other amendments, wood BC was pretreated with H₂O₂, and citric acid (CA) respectively. These modifications are intended to increase specific physiochemical properties with the intended outcome of increased Cu binding as compared to untreated wood BC. To produce the H₂O₂-oxidized BC, an aliquot of 0.1 g C of <2 mm sieved wood BC was shaken with 7 ml 0.01 M

H₂O₂ in a water bath at 80°C for 48 hours. The resulting charcoal was washed four times in distilled water and dried at room temperature for two days (Cross & Sohi, 2013).

The wood BC pretreated with citric acid was prepared following the procedure of Zhu et al. (2008). 1 g of wood BC was mixed with 10 mL 0.6 M CA and dried at 50°C in a forced air oven for fourteen hours. The BC was washed four times with distilled water and tested for alkalinity with 0.1 M lead (II) nitrate. In order to accelerate the thermochemical reaction between acid and wood BC, the oven temperature was increased to 110°C for two hours.

6.25 g CaCO₃ kg⁻¹ soil was added as a liming treatment to the acidic soil of St Stefan.

Table 3: Incubation length and Cu additions for freshly spiked soils

Timing of Cu spike	Abbreviation
Soils were incubated for three years and then spiked with Cu	3yr+Cu
Soils were spiked with Cu and then incubated for three years	Cu+3yr

Two batch incubation experiments, as described in **Table 3**, were conducted to determine the long-term effects of various organic soil amendments on Cu immobilization. In addition, a third batch experiment was conducted where the soil and the amendments were incubated for three years, but no additional Cu was spiked. Prior to incubation, an equivalent amount of 30 g dry matter of each soil was added to 50 mL plastic Erlenmeyer flasks. The amount of the amendment added was determined according to the application rate as described in **Table 2**. A glass rod was used to manually stir the mixture of soil and amendments. Throughout the incubation period, the soils were adjusted to 50% water holding capacity (WHC). The flasks were covered with cotton to ensure gaseous exchange and to prevent contamination or excessive loss of water. The samples were incubated for three years at 10°C.

In 3yr+Cu, amendments were added to the contaminated soil and then incubated for three years. Towards the end of the incubation period (6 weeks prior to sampling), the samples were supplied with 250 mg kg⁻¹ of dissolved CuSO₄ to mimic fresh contamination. In Cu+3yr, amendments were added to the soils, and the mixtures were immediately spiked with fresh copper (250 mg kg⁻¹ of dissolved CuSO₄), and then incubated for three years. In the third batch experiment, amendments were added to the contaminated soil and incubated for three years,

without additional Cu spiking to represent historically contaminated soils. In all experiments, the treatments were set up in triplicates, resulting in a total of 171 incubation samples.

3.3 Laboratory Analysis

3.3.1 Determination of Water Holding Capacity (WHC)

To determine the WHC, sieved soil was filled in a soil cylinder (\varnothing 5 cm, height 5 cm) and closed at the bottom with gauze bandage. The samples were moistened from above and left overnight in a water bath to ensure full saturation. Thereafter, the samples were placed on a sand bed. After 24 h, an aliquot of the moist soil was dried at 105°C to determine WHC.

3.3.2 Preparation of Extracts

After the incubation period, 0.01 M CaCl₂ extracts were prepared to determine the Cu concentration of the soil samples. This was conducted using 3 g of soil extracted with 30 mL solution for a soil: solution ratio of 1:10 using 50 mL centrifuge tubes (Houba et al., 2000). The tubes were transferred to a rotational shaker and shaken at room temperature for two hours and then centrifuged for ten minutes at 2000 rpm (*Rotanta 460 R*, Hettich, Germany). The solutions were filtered through 0.45 μ m cellulose acetate membrane filters, and acidified with three droplets of 65% nitric acid for the copper measurements. The same extraction procedure was used to measure pH and DOC (in non-acidified extracts).

3.3.3 Cu, DOC, and pH measurements

Total CaCl₂-extractable copper of samples with concentrations above 50 μ g/L was determined by flame atomic absorption spectroscopy (flame AAS, Perkin Elmer). For the samples below this concentration, graphite furnace atomic absorption spectroscopy (GF-AAS, Perkin Elmer) and inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700, Agilent Technologies) was used.

Dissolved organic carbon (DOC) was determined using UV absorbance (254 nm) in *BRANDplates*[®] *pureGradeTM* 96-Well Microtiterplates in 2 replicates on a Multimode plate reader (EnSpire[®], Perkin Elmer).

The pH values were measured using a pH-meter (WTW, InoLab).

3.4 Data evaluation and statistical analyses

Speciation of the total CaCl₂-extractable Cu was conducted using *Visual MINTEQ 3.1* (KTH Royal Institute of Technology, Sweden). Measured DOC and pH of the samples were used as input parameters as well as the ionic strength of the background electrolyte CaCl₂, based on concentrations of 400.0 and 709 mg/L for Ca²⁺ and Cl⁻, respectively. The non-ideal competitive adsorption (NICA) and Donnan database were used to determine the amount of Cu complexed with DOC (Bryan et al. 2002; Hoppe et al. 2015). The speciated Cu²⁺ concentrations were converted from µg/L to µg/kg soil, based on the preparation of the extracts, as outlined in section 3.2.3.

Statistical analyses were carried out using the program *SPSS 22* developed by IBM. To determine differences between amendments, a one-way ANOVA was conducted, followed by Tukey's HSD test to specify significant differences. For testing significant differences between incubation times, a Welch test was conducted for historically contaminated soils, while a one-way ANOVA and Tukey's HSD test were conducted for the freshly contaminated soils. Significance was tested for $p < 0.05$ in all cases.

4. Results

4.1 Historically contaminated soils

4.1.1 Effects of amendments on pH and DOC

Soil from site Rossatz

The addition of the amendments had no significant impact on the pH level in either the six-week or the three-year incubation, as demonstrated in **Table 4**.

The DOC increased significantly in the K and the K+CA amendments in the six-week incubation. In the three-year incubation, the K amendment again showed a significant increase. In contrast, the wBC amendment exhibited a significant decrease of 14% in the amount of DOC.

Soil from site St. Stefan

In the six-week incubation period, there was a significant increase in the pH of the K and the L amendments, from 6.06 to 6.39 and 6.84, respectively. In the three-year incubation, there was no significant change in the pH level amongst the amendments.

The DOC increased significantly in nearly all the amendments for the six-week incubation, with the exception of the wBC amendment. The biggest increase was in the K amendment, which increased by 78%, closely followed by the increase in the K+CA amendment, which increased by 41%. K+wBC had the smallest significant increase, increasing by 17%. In the three-year incubation, only the K and the L amendment varied significantly, both increasing by about 30%.

Table 4: pH level and DOC concentration after the incubation of the amendments in historically Cu-contaminated soils. Lowercase letters indicate significant differences between amendments. Uppercase letters indicate significant differences between the incubation times. ($p < 0.05$). \pm values indicate standard deviation, $n=3$.

	pH (in CaCl ₂)				DOC (mg kg ⁻¹)			
	Rossatz		Sankt Stefan		Rossatz		Sankt Stefan	
	6 weeks	3 years	6 weeks	3 years	6 weeks	3 years	6 weeks	3 years
CO	6.65 (± 0.03) a A	6.06 (± 0.36) a A	6.057 (± 0.16) a A	5.95 (± 0.17) ab A	27.3 (± 3.1) ab A	58.7 (± 0.2) b B	37.11 (± 1.9) a A	54.0 (± 2.9) a B
K	6.66 (± 0.07) a B	6.37 (± 0.12) a A	6.39 (± 0.12) b A	6.23 (± 0.07) ab A	59.7 (± 1.95) d A	73.6 (± 3.7) c B	66.0 (± 3.4) d A	70.1 (± 1.7) b A
wBC	6.61 (± 0.22) a A	6.22 (± 0.12) a A	5.99 (± 0.06) ab A	5.99 (± 0.24) ab A	25.5 (± 0.4) a A	50.4 (± 5.5) a B	34.8 (± 0.2) a A	47.9 (± 5.2) a B
K+wBC	6.64 (± 0.28) a B	6.42 (± 0.05) a A	6.14 (± 0.06) ab A	5.99 (± 0.23) ab A	32.4 (± 3.0) ab A	57.6 (± 1.3) ab B	43.4 (± 1.7) b A	54.7 (± 1.5) a B
K+H₂O₂	6.63 (± 0.14) a A	6.53 (± 0.16) a A	6.18 (± 0.18) ab A	6.17 (± 0.08) ab A	34.2 (± 2.1) b A	55.6 (± 0.7) ab B	44.7 (± 2.0) b A	54.1 (± 1.1) a B
K+CA	6.63 (± 0.06) a B	6.26 (± 0.12) a A	6.26 (± 0.01) ab B	5.84 (± 0.10) a A	49.2 (± 5.6) c A	61.8 (± 2.0) b B	52.4 (± 1.1) c A	51.4 (± 1.7) a A
L			6.84 (± 0.11) c B	6.4 (± 0.19) b A			47.3 (± 1.7) bc A	70.5 (± 1.3) b B

4.1.2. Effects of incubation time on pH and DOC

Soil from site Rossatz

There was a significant decrease in pH in the K, K+wBC, and the K+CA amendments from the six-week incubation to the three-year incubation. The largest significant variation was in the K+CA amendment, which decreased from 6.63 to 6.26.

In the comparison of the DOC concentration, all amendments showed significant increases in the three-year incubation, although the CO had the largest variation, with an increase of 115%. Of the amendments, the wBC and the K+wBC had the largest variation, 98% and 78% respectively.

Soil from site St. Stefan

From the six-week incubation period to the three-year incubation, two amendments showed significant decreases in pH level, K+CA from 6.26 to 5.84 and L from 6.84 to 6.4.

In the comparison of DOC, all amendments showed significant increases in the three-year incubation, with the exception of K and K+CA. L had the biggest variation, increasing 49%, whereas K+H₂O₂ increased the least with 21%.

4.1.3 Effects of amendments on CaCl₂-extractable Cu

Soil from site Rossatz

The six-week incubation showed no significant reduction in Cu concentration in any of the amendments, as demonstrated in **Figure 1a**. In contrast, the K and the K+CA amendments exhibited a significant increase, increasing by 88% and 30% respectively.

In the three-year incubation, the K amendment again significantly increased the Cu concentration by 43%. However, this was accompanied by a significant decrease of 21% in the wBC amendment.

Soil from site St. Stefan

The six-week incubation showed a significant reduction in the wBC and the L amendments, as demonstrated in **Figure 1b**. In these amendments, CaCl₂-extractable Cu decreased by 20% and 30% respectively.

In the three-year incubation, all amendments demonstrated a significant reduction in CaCl₂-extractable Cu. The largest reduction was in the L amendment, with a decrease of 56%, whereas K had the smallest reduction with 25%.

4.1.4 Effects of Incubation Time on CaCl₂-extractable Cu

Soil from site Rossatz

As demonstrated in **Figure 1a**, all amendments exhibited a significant decrease in CaCl₂-extractable Cu from the six-week to the three-year incubation, with the exception of the control.

The largest reduction was in the K amendment, which decreased by 34%. In comparison, the wBC demonstrated the least amount of variation, decreasing by 20%.

Soil from site St. Stefan

Most of the amendments showed a significant decrease of CaCl₂-extractable Cu in the three-year incubation. The exception to this was the CO, which increased significantly in the three-year incubation by 17%, and the K and wBC amendments, which showed no significant variations. Although the K amendment did have a reduction of 22%, it was not considered significant due to the large standard deviation. Of the significant decreases, K+CA had the largest variation, decreasing by 28%.

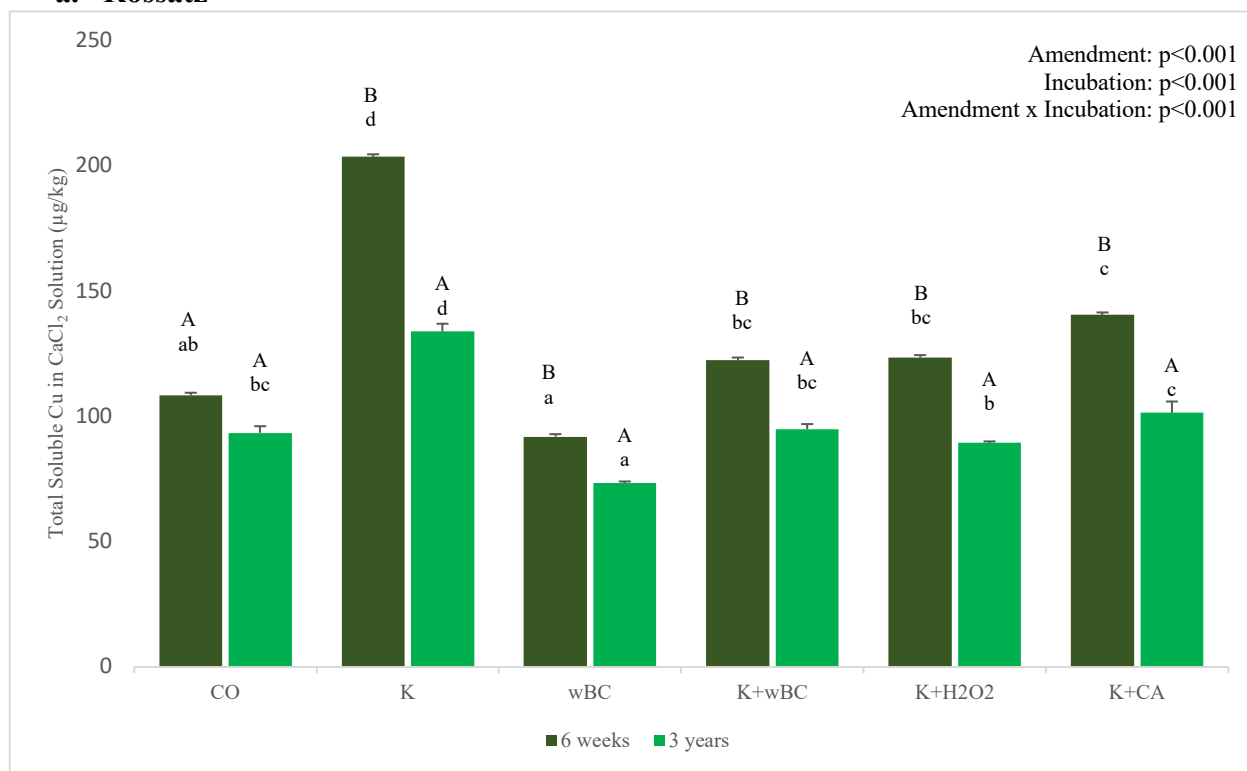
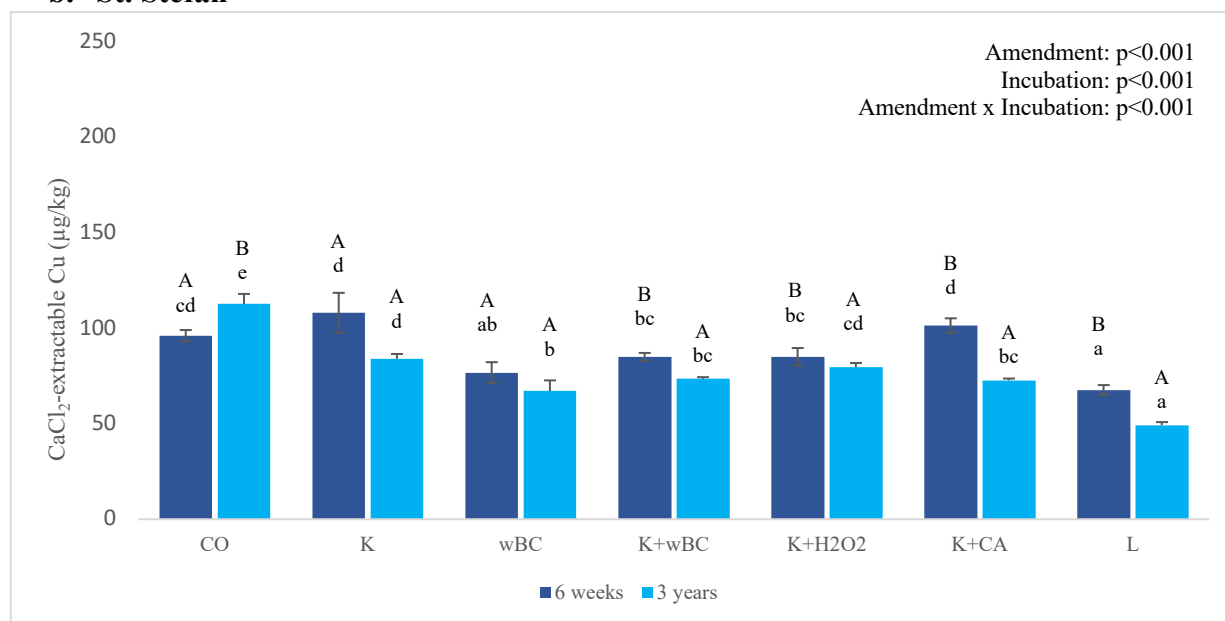
a. Rossatz**b. St. Stefan**

Figure 1(a-b): 0.01 M CaCl₂ – extractable Cu concentration after the incubation of the amendments in historically Cu-contaminated soils. Lowercase letters indicate significant differences between amendments. Uppercase letters indicate significant differences between the incubation times. (p<0.05). Error bars indicate standard deviation, and n=3. A two-way ANOVA was conducted to determine the effect of each variable as well as the interaction between amendments and incubation, as exhibited in the upper right-hand corner.

4.1.5 Effects of amendments on ecologically toxic Cu²⁺

Soil from site Rossatz

In the six-week incubation treatment, none of the amendments exhibited significant changes in the amount of Cu²⁺.

The same result is repeated in the three-year incubation, with no amendment significantly impacting Cu²⁺ concentrations.

Soil from site St. Stefan

In the six-week incubation period, all the amendments, except for the wBC amendment, showed significantly decreased amounts of Cu²⁺. The amendments with the largest decreases were K, which decreased by 83%, and L, which decreased by 97%. In contrast, K+wBC decreased by 26%.

In the three-year incubation period, all the amendments showed significant decreases. The largest decreases, as in the six-week incubation period, were in the K and the L amendments, which decreased by 85% and 97%, respectively. The smallest decrease was found for K+CA, which decreased by 46%.

4.1.6 Effects of incubation time on Cu²⁺ concentrations

Soil from site Rossatz

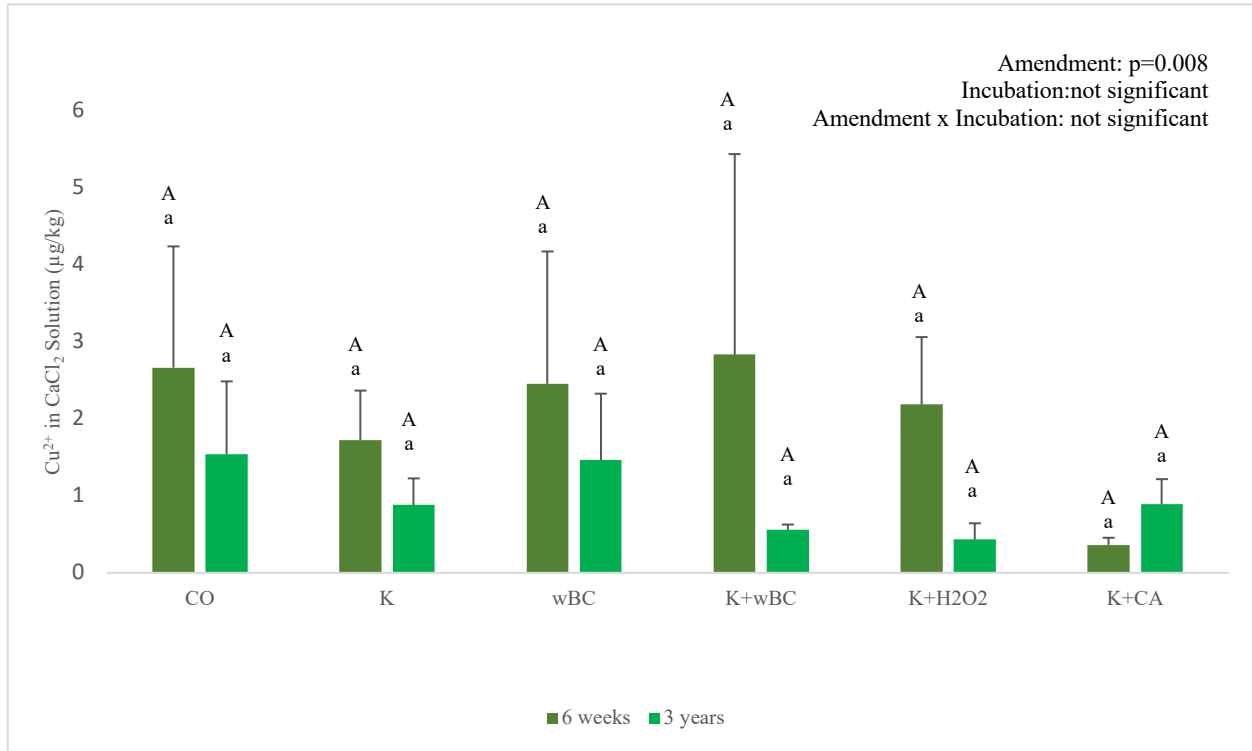
In the comparison of the incubation times, there were no significant differences, as seen in **Figure 2a**.

Soil from site St. Stefan

In the comparison between the six-week incubation period and the three-year incubation period, only one amendment showed a significant difference. In the wBC amendment, the amount of Cu²⁺ decreased by 55% in the three-year incubation, as seen in **Figure 2b**.

Historically Cu-contaminated soils
a. Rossatz

Cu²⁺



b. St. Stefan

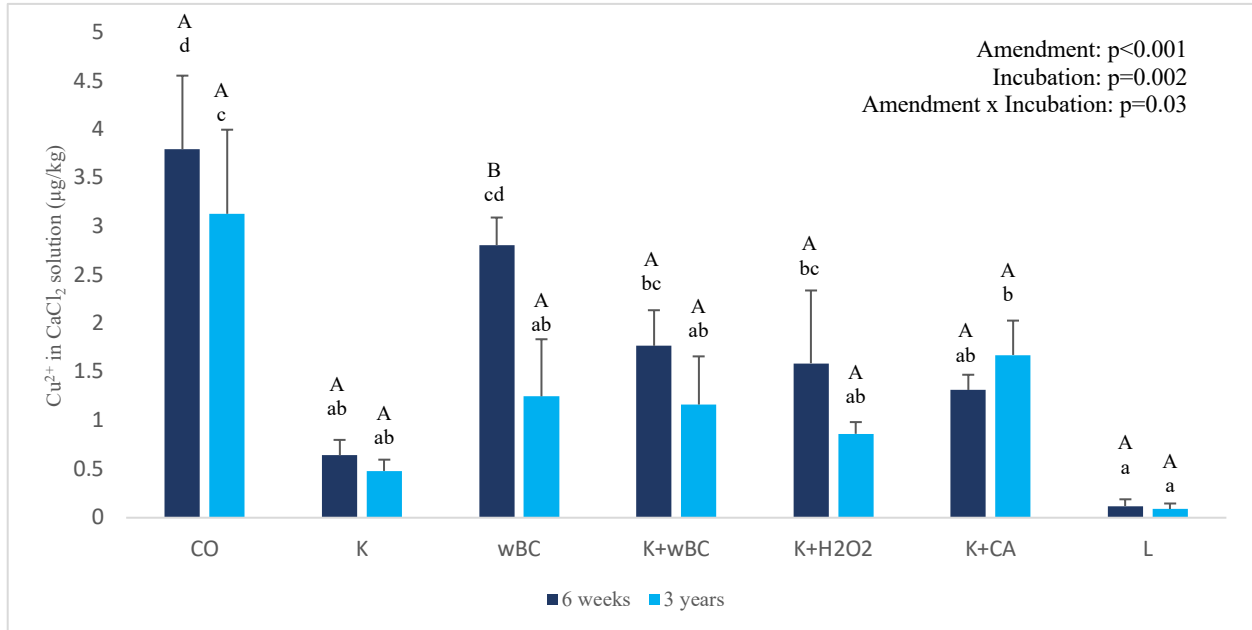


Figure 2(a-b): Cu²⁺ concentrations in CaCl₂ solution (by speciation with *Visual MINTEQ 3.1*) after the incubation of the amendments in historically Cu-contaminated soils. Lowercase letters indicate significant differences between amendments. Uppercase letters indicate significant differences between the incubation times. (p<0.05) Error bars indicate standard deviation, and n=3. A two-way ANOVA was conducted to determine the effect of each variable as well as the interaction between amendments and incubation, as exhibited in the upper right-hand corner.

4.2 Freshly contaminated soils

4.2.1 Effects of amendments on pH and DOC

Soil from site Rossatz

In the comparison of pH in the six-week incubation period, only one amendment, K +CA, showed a significant increase, from 6.46 to 6.79, although observable increases were noted in all amendments. In the 3yr + Cu incubation, there were no significant changes in the pH levels amongst the amendments, although variations were observed. In the Cu + 3yr, there was no significant changes between the treatments, although a decrease in pH level for all the amendments was observed.

In the comparison of DOC in the six-week incubation period, there were significant increases in the K and the K + CA amendments, by 158% and 90%, respectively. In the 3yr + Cu incubation, no significant changes were observed in either of the amendments. In the Cu + 3yr incubation, significant increases occurred in the K and K+CA amendments, by 97% and 40%, respectively.

Soil from site St. Stefan

In the analysis of the pH level in the six-week incubation period, there were no statistically significant changes among the amendments. In the 3yr + Cu incubation, there was a significant increase in the L amendment, from 5.94 to 6.53. In the Cu + 3yr incubation, the K and the L amendments demonstrated significant increases in the pH level. L had the greatest increase, from 5.65 in the control to 6.44, whereas K increased to 6.25.

In the comparison of the DOC in the six-week incubation, there were no significant changes amongst the amendments. Several increases were observed, however, with the largest being the K amendment with a 40% increase. In the 3yr + Cu incubation, there were significant variations in all the amendments. Four of the amendments demonstrated significant decreases, the wBC, the K+wBC, the K + H₂O₂, and the C + CA. The largest decrease was observed for the wBC, by 19%. In contrast, significant increases were exhibited in the K and L amendments, by

12% and 13% respectively. In the Cu + 3yr incubation, only the L amendment had a significant change, increasing by 60%.

Table 5: pH level after the incubation of the amendments in freshly Cu-contaminated soils. Lowercase letters indicate significant differences between amendments. Uppercase letters indicate significant differences between the incubation treatments. ($p < 0.05$). \pm values indicate standard deviation, $n=3$.

	pH (in CaCl ₂)					
	<i>Rosatz</i>			<i>Sankt Stefan</i>		
	6 weeks	3yr+ Cu	Cu +3yr	6 weeks	3yr+ Cu	Cu +3yr
CO	6.46 (± 0.02) a A	6.05 (± 0.38) a A	6.53 (± 0.36) a A	6.00 (± 0.13) a A	5.94 (± 0.14) a A	5.65 (± 0.11) a A
K	6.63 (± 0.14) abcd A	6.55 (± 0.07) a A	6.48 (± 0.06) a A	6.29 (± 0.13) a A	6.09 (± 0.13) a A	6.25 (± 0.21) bc A
wBC	6.53 (± 0.06) abc B	6.32 (± 0.10) a A	6.15 (± 0.19) a A	6.26 (± 0.20) a A	5.93 (± 0.12) a A	6.05 (± 0.12) abc A
K+wBC	6.53 (± 0.05) abc A	6.48 (± 0.07) a A	6.43 (± 0.15) a A	6.33 (± 0.30) a A	6.00 (± 0.07) a A	6.00 (± 0.11) abc A
K+H₂O₂	6.53 (± 0.05) abc A	6.33 (± 0.37) a A	6.49 (± 0.03) a A	6.08 (± 0.06) a A	5.98 (± 0.02) a A	6.00 (± 0.06) abc A
K+CA	6.79 (± 0.09) d B	6.51 (± 0.09) a A	6.26 (± 0.22) a A	6.19 (± 0.09) a A	5.84 (± 0.11) a A	5.89 (± 0.23) ab A
L				6.85 (± 0.12) a A	6.53 (± 0.24) b A	6.44 (± 0.33) c A

Table 6: DOC concentration after the incubation of the amendments in freshly Cu-contaminated soils. Lowercase letters indicate significant differences between amendments. Uppercase letters indicate significant differences between the incubation treatments. ($p < 0.05$). \pm values indicate standard deviation, $n=3$.

	DOC (mg kg ⁻¹)					
	<i>Rossatz</i>			<i>Sankt Stefan</i>		
	6 weeks	3yr+ Cu	Cu +3yr	6 weeks	3yr+ Cu	Cu +3yr
CO	23.9 (± 2.8) a A	24.5 (± 0.9) a A	22.3 (± 0.7) a A	51.4 (± 3.9) a A	52.6 (± 0.9) b A	57.1 (± 1.7) ab A
K	61.6 (± 7.1) e B	39.3 (± 2.5) a A	43.9 (± 1.5) e A	72.0 (± 3.4) a B	59.0 (± 1.3) c A	62.3 (± 3.0) ab A
wBC	24.3 (± 1.9) ab A	33.7 (± 24.1) a A	23.6 (± 0.9) ab A	51.2 (± 12.0) a AB	42.6 (± 0.7) a A	67.5 (± 1.1) b B
K+wBC	30.2 (± 3.2) abc A	27.6 (± 0.2) a A	64.3 (± 0.4) abc A	53.4 (± 3.4) a B	45.5 (± 1.8) a A	47.9 (± 2.2) a AB
K+H₂O₂	31.4 (± 0.9) abc B	25.6 (± 1.1) a A	24.3 (± 2.0) abc A	53.0 (± 3.4) a AB	45.6 (± 1.2) a A	68.9 (± 15.3) b B
K+CA	45.3 (± 7.3) d B	27.4 (± 0.9) a A	31.2 (± 2.6) d A	62.0 (± 2.7) a B	45.3 (± 0.7) a A	74.4 (± 0.9) bc C
L				66.6 (± 14.4) a A	59.6 (± 1.9) c A	91.1 (± 6.6) c B

4.2.2. Effects of timing on pH and DOC

Soil from site Rossatz

In the comparison of pH between the incubation treatments, the 3 yr + Cu incubation had two amendments that demonstrated significantly lower levels as compared to the six-week incubation, wBC and K+CA. A similar trend was repeated in the comparison between the six-week incubation and the Cu + 3yr incubation, where the wBC and the K+CA demonstrated

significantly lower values in the Cu + 3yr incubation. In the comparison between the 3yr + Cu and the Cu + 3yr incubations, there were no significant changes in pH level.

In the comparison of the DOC, the 3yr + Cu incubation was significantly lower than the six-week incubation for the K, K+ H₂O₂, and the K + CA amendments. The largest difference was in the K amendment, which decreased by 36% in the 3yr + Cu incubation, as compared to the six-week incubation. The Cu + 3 yr incubation was also significantly lower for those amendments, K, K + H₂O₂, and K +CA, than in the six-week incubation. The largest difference was again the K amendment, which decreased by 29% in the Cu + 3yr incubation. In the comparison between 3yr + Cu and Cu + 3yr, there was no significant differences in the amount of DOC.

Soil from site St. Stefan

In the comparison of changes in pH level, there were no significant changes from one incubation period to the others.

In the comparison of the DOC, in the six-week incubation to the 3yr + Cu, the K, the K +wBC, and K +CA exhibited significantly lower values in the 3 yr + Cu incubation. The largest difference was found for the K +CA amendment, which decreased by 27% in the 3yr +Cu incubation.

In the comparison of the six-week incubation to the Cu + 3yr, there were significant differences in the C, C +CA, and the L amendments. K and K+CA had lower concentrations of DOC in the Cu + 3 yr incubation, whereas L had higher amounts. In the comparison of the 3yr + Cu to the Cu + 3yr incubations, the DOC for the Cu + 3yr was significantly higher for the wBC, K + H₂O₂, K+CA, and the L amendments. The largest difference was found for the L amendment, which increased by 53% in the Cu + 3yr incubation.

4.2.3. Effects of amendments on CaCl₂-extractable Cu

Soil from site Rossatz

In the six-week incubation period, there was no statistically significant difference amongst the amendments. In the 3yr + Cu incubation, once again there were no significant

changes among the amendments. In the Cu + 3yr incubation, only the wBC amendment demonstrated a significant difference, decreasing by 25%.

Soil from site St. Stefan

In the six-week incubation, all the amendments showed significantly decreased amounts of soluble Cu. The L amendment had the largest decrease, with 94%. In the 3yr + Cu incubation, the K and the L amendments demonstrated significant decreases. In the Cu +3yr incubation, only the L amendment showed a significant decrease of 89%.

4.2.4. Effects of timing on CaCl₂-extractable Cu

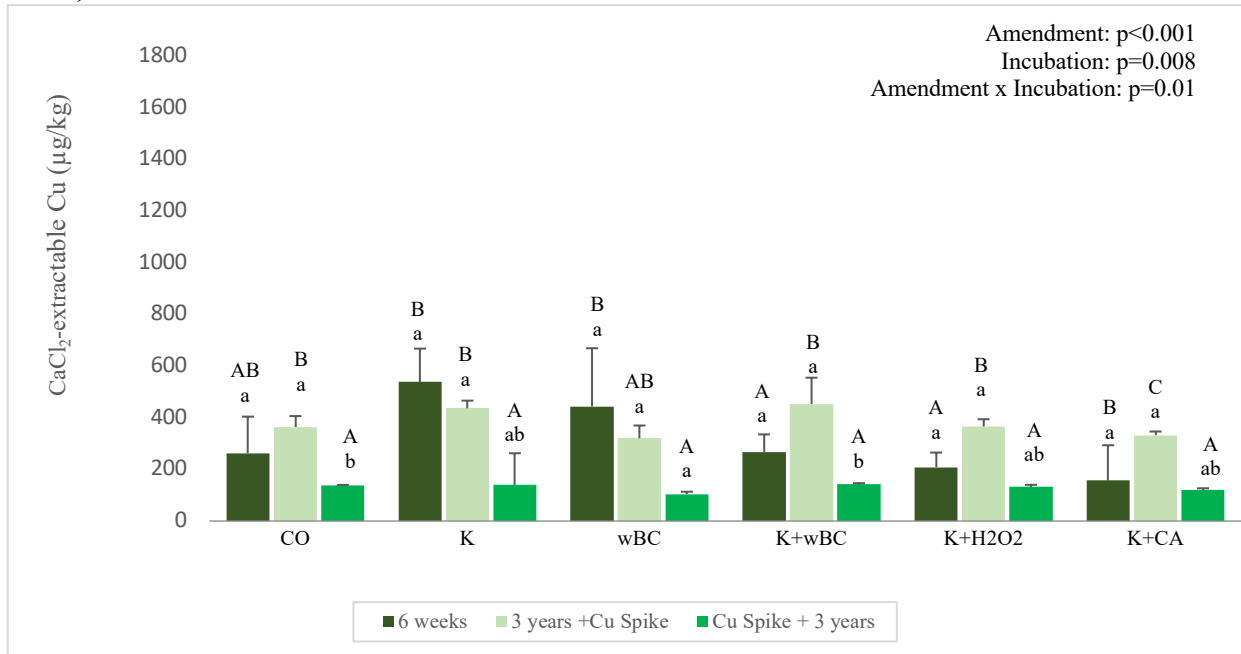
Soil from site Rossatz

In the comparison between the six-week and the 3yr + Cu incubation, three amendments showed significant increases in the 3yr + Cu incubation, the K+wBC, K+ H₂O₂, and K+CA amendments. The largest difference was in the K+CA, which increased by 113%. In the comparison between the six-week and the Cu + 3yr incubations, three amendments had lower concentrations in the Cu + 3yr incubation, K, K+CA, and wBC amendments. The largest difference was found for the K amendment, decreasing by 74%. In the comparison between the 3yr + Cu and the Cu + 3yr incubations, the 3yr + Cu had significantly higher CaCl₂-extractable Cu in all the amendments except for the wBC. The largest difference was found for the K+ wBC amendment, which varied by 91%.

Soil from site St. Stefan

In the comparison between the six-week and the 3yr + Cu incubations, all amendments, except CO and K, had increased Cu amounts in the 3yr+ Cu incubation. In the comparison between the six-week and the Cu + 3yr incubations, the only significant variation was in CO, which decreased in the Cu + 3 yr incubation. Amongst the amendments, there was no discernable differences in CaCl₂-extractable Cu. In the comparison between the 3yr+ Cu and the Cu+ 3yr incubations, all the amendments in the 3yr + Cu incubation had significantly higher CaCl₂-extractable Cu, the only exception being the K +wBC amendment. The largest difference was in the wBC, which varied by 73%.

a.) Rossatz



b.) St. Stefan

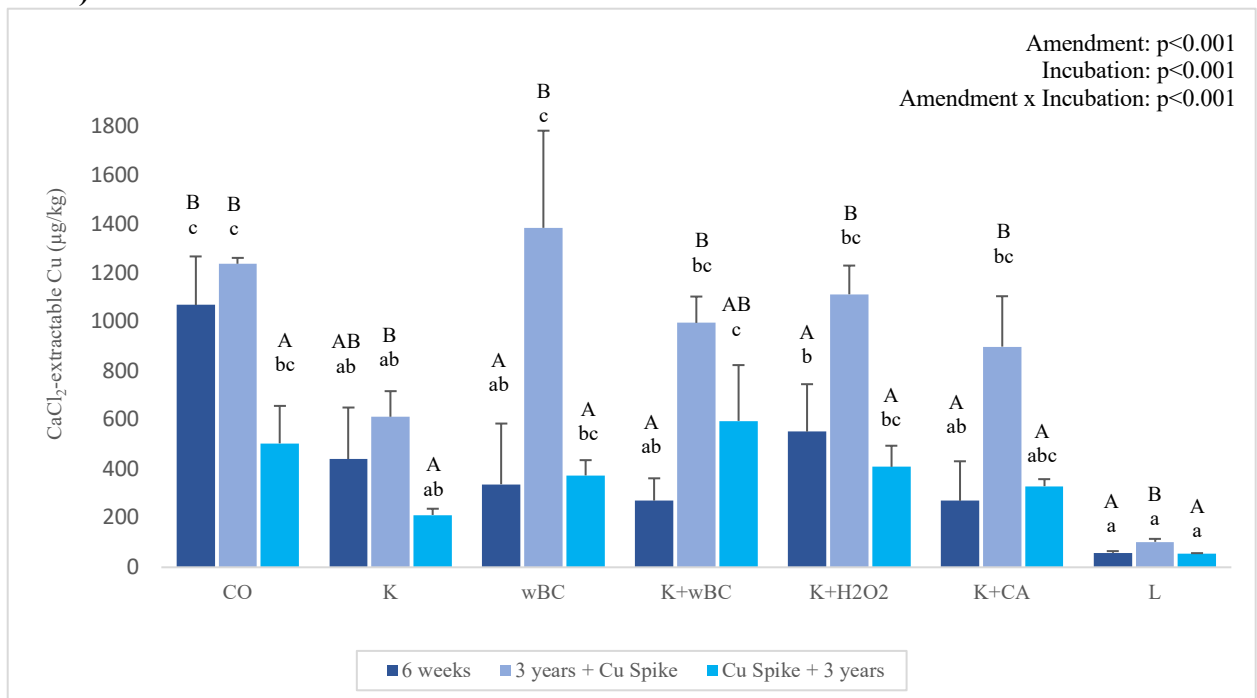


Figure 3(a-b): Total soluble Cu concentrations in CaCl₂ solution after the incubation of the amendments in freshly Cu-contaminated soils. Lowercase letters indicate significant differences between amendments. Uppercase letters indicate significant differences between the incubation treatments. (p<0.05). Error bars indicate standard deviation, n=3. A two-way ANOVA was conducted to determine the effect of each variable as well as the interaction between amendments and incubation, as exhibited in the upper right-hand corner.

4.2.5. Effects of amendments on Cu²⁺ concentrations

Soil from site Rossatz

In the six-week incubation, none of the amendments showed statistically significant differences, with the same trend repeated in the 3yr+Cu incubation and the Cu+3yr incubation.

Soil from site St. Stefan

In the six-week incubation, the Cu²⁺ concentration decreased significantly with all amendments. The largest difference was found for the L amendment, which had a nearly 100% reduction, from 317.7 µg·kg⁻¹ to 0.03 µg·kg⁻¹. The K+wBC and the K+CA amendments also resulted in large decreases, both with 97%. The amendment that had the least amount of variation was K+H₂O₂, which decreased by 79%. In the 3yr+Cu, the L amendment once again nearly had a 100% decrease to 0.5 µg·kg⁻¹. The K amendment also resulted in a significant reduction of 80%. This trend was repeated in the Cu+3yr incubation, with the L amendment decreasing from 88.5 µg·kg⁻¹ to 0.07 µg·kg⁻¹ and the K amendment decreasing to 5.3 µg·kg⁻¹.

4.2.6. Effects of timing on Cu²⁺ concentrations

Soil from site Rossatz

In the comparison between the six-week and the 3yr + Cu incubations, only two amendments showed significant increases in the 3yr + Cu incubation, the K+H₂O₂ and the K+CA. Only the wBC amendment had decreased Cu²⁺ in the 3yr + Cu incubation. In the comparison between the six-week and the Cu+3yr incubation, the wBC amendment showed a significant decrease in the Cu + 3yr, 14.6 µg·kg⁻¹ as compared to 8.8 µg·kg⁻¹. In the comparison between the 3yr+Cu and the Cu+3yr incubations, there was significantly lower Cu²⁺ in the Cu+3yr incubations for all the amendments. Although the control had the largest variation with a 90% decrease, the K+wBC and the wBC amendments also exhibited differences of 88%.

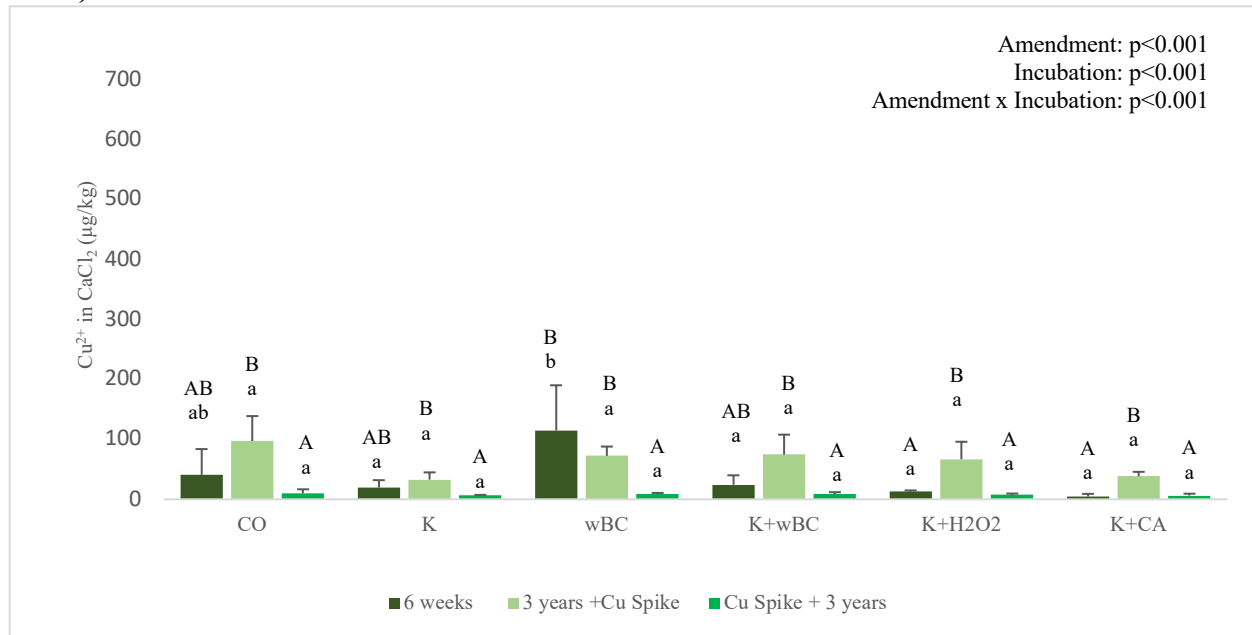
Soil from site St. Stefan

In the comparison between the six-week and the 3yr + Cu incubations, most amendments exhibited significantly increased concentrations of Cu²⁺ in the 3yr + Cu incubation. Only two did not show a significant change, CO and the K amendment. The greatest difference was found for the wBC amendment, which was measured at 15.4 µg·kg⁻¹ in the six-week incubation and 518.1

$\mu\text{g}\cdot\text{kg}^{-1}$ in the 3yr+Cu incubation. The smallest significant variation was found in L, which increased by only 16% in the 3yr+Cu incubation. In the comparison between the six-week and the Cu+3yr incubations, the CO and the K amendment showed significantly lower amounts of Cu^{2+} in the Cu+3yr incubation than in the six-week incubation. In contrast, in the K+wBC amendment, the Cu+ 3yr incubation demonstrated significantly higher amounts than the six-week incubation. In the remaining amendments, there were no significant changes between the two incubation periods. In the comparison between the 3yr+Cu and the Cu+3yr incubations, all amendments demonstrated a significantly decreased amount of Cu^{2+} in the Cu+3yr incubation. The largest decrease was in the wBC amendment, in which Cu^{2+} decreased by 96%.

Freshly Cu-contaminated soils
a.) Rossatz

Cu²⁺



b.) St. Stefan

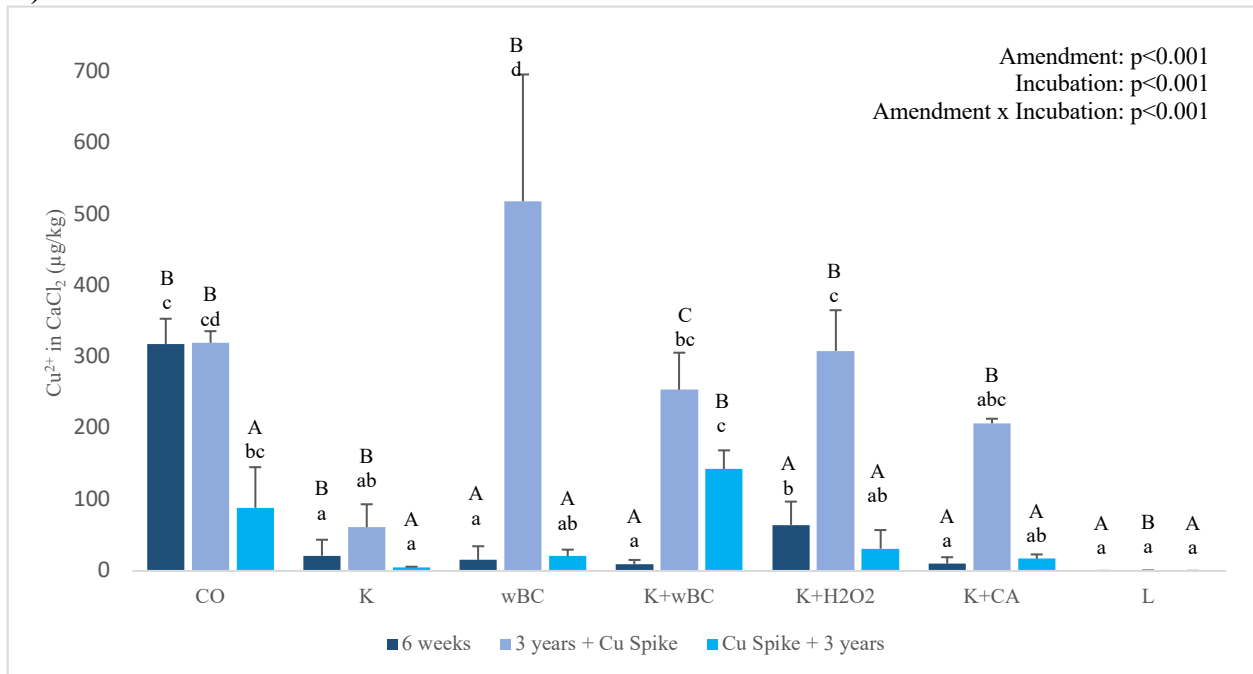


Figure 4(a-b): Cu²⁺ concentrations in CaCl₂ solution (by speciation with *Visual MINTEQ 3.1*) after the incubation of the amendments in freshly Cu-contaminated soils. Lowercase letters indicate significant differences between amendments. Uppercase letters indicate significant differences between the incubation treatments. (p<0.05). Error bars indicate standard deviation, n=3. A two-way ANOVA was conducted to determine the effect of each variable as well as the interaction between amendments and incubation, as exhibited in the upper right-hand corner.

5. Discussion

5.1 CaCl₂-extractable Cu and Cu²⁺

In speciating the CaCl₂-Cu concentrations, amendment effects on parameters such as DOC and pH may result in a changed distribution of soluble Cu species. This allows for the appropriate biochar amendment to be selected, based on the goal of reducing either total soluble Cu or the more eco-toxicologically relevant Cu²⁺. In this experiment, CaCl₂-extractable Cu concentrations at both sites were impacted by amendments and incubation periods, with a more pronounced effect seen for the acidic soil from St. Stefan. However, the results for the Cu²⁺ concentrations were more mixed. In the historically contaminated soils of Rossatz, the CaCl₂-extractable Cu concentration values ranged from 73.5 µg·kg⁻¹ to 203.7 µg·kg⁻¹ across both incubations, with an average value of 114.8 µg·kg⁻¹. Two significant increases are reported in the K and the K+CA amendments. However, this increase was not mirrored in the Cu²⁺ concentrations, in which none of the amendments appeared to have a significant effect. Values for Cu²⁺ ranged from 0.4 µg·kg⁻¹ to 2.8 µg·kg⁻¹, with an average value of 1.5 µg·kg⁻¹. For most of the amendments, Cu²⁺ accounted for less than 1% of the total soluble Cu concentration, with the exception of the wBC amendment, in which Cu²⁺ accounted for about 2%. wBC was also the only amendment to significantly decrease the amount of CaCl₂-extracted Cu. Perhaps the lack of amendment influence can be attributed to a low amount of Cu²⁺. In the European Union, the predicted no effect concentration (PNEC) of the total Cu in soil was set at 20-200 mg·kg⁻¹, with the wide range dependent on various soil properties (Ruyters et al., 2013; Brunetto et al., 2016; Smolders et al., 2009). Toxic effects on microbial communities have been demonstrated at concentrations of around 150-200 mg Cu kg⁻¹ (Fernandez-Calvino et al., 2010; Brunetto et al., 2016). These thresholds correspond to the total Cu values observed in this study, which were 201 mg Cu kg⁻¹ and 337 mg Cu kg⁻¹ based on site location, as noted in Table 1. However, in a similar study conducted by Mackie et al. (2015), biochar amendments did not have a significant influence on Cu immobilization in vineyards that had between 100-170 mg Cu kg⁻¹ and suggested that perhaps the total Cu concentrations were simply too low for the amendments to have a more pronounced effect. The lack of a consistent effect of biochars in our study may be attributed to this as well, although the total Cu concentrations were well above Mackie et al.'s

(2015). A survey by Ruyters et al. (2013), conducted on six established European vineyards, concluded that Cu toxicity was not yet observed in vineyard soil samples at Cu concentrations well above the limits set by the E.U. It was hypothesized that this lack of toxicity was due to the decreased Cu bioavailability in vineyard soils as compared to Cu-spiked soils. This may mean that Cu toxicity limits may be difficult to define, especially in vineyard soils as the vines are Cu-tolerant (Pietrzak and Uren, 2011; Rusjan, 2012).

In the freshly contaminated soils of Rossatz, a similar trend as in the historically contaminated soils is repeated, with the K amendment often increasing the amount of $\text{CaCl}_2\text{-Cu}$, and a decline accompanying the wBC. Due to the Cu spike, the Cu amounts are higher, ranging from $103.6 \mu\text{g}\cdot\text{kg}^{-1}$ to $537.4 \mu\text{g}\cdot\text{kg}^{-1}$, and with an average value of $273.2 \mu\text{g}\cdot\text{kg}^{-1}$ across all the incubation periods. However, as in the historically contaminated soil, there does not appear to be significant effects by the amendments in the reduction of Cu^{2+} , although there is significant variation between the incubation timings. Values ranged from $4.7 \mu\text{g}\cdot\text{kg}^{-1}$ to $114.6 \mu\text{g}\cdot\text{kg}^{-1}$, with an average of $35.9 \mu\text{g}\cdot\text{kg}^{-1}$. This is much higher than in the historically contaminated soils, due to the Cu spike, and demonstrates how Cu spiked soils may not necessarily reflect on field processes (Oorts et al., Okonokhua, 2014). It may represent that freshly applied Cu may be mobile as it not yet had the time to equilibrate or leach as in the historically contaminated soils (Lock and Janssen, 2002; Smolders et al., 2012; Oorts et al., 2006; Ma et al., 2006a). In the six-week incubation, Cu^{2+} represents 2-7% of the total Cu concentration, with peaks of 13% and 18% for the CO and wBC amendments. In the 3 yr+Cu spike incubation, this proportion was even higher, around 7-16%, with CO and K+ H_2O_2 reaching 27% and 18%. This higher percentage of Cu^{2+} , as compared to the historically contaminated soils, may suggest that the formation of Cu complexes may not be a rapid process and the interaction with DOC requires time. The addition of soluble Cu^{2+} may precipitate in the soil as other species which can lead to the overestimation of ecotoxicity immediately after the spiking (Ma et al., 2006b; Smolders et al., 2009). In the Cu spike+3yr incubation, the Cu^{2+} proportion declined again, with all amendments reporting percentages of 5-8%, with no peaks occurring. This decline may be due to the aging of the Cu itself that depletes the bioavailability (Anxiang et al., 2008; Ma et al., 2006a; Houben et al., 2013). In the neutral soil of Rossatz, the amendments did not have a particularly strong effect on either $\text{CaCl}_2\text{-Cu}$ or the Cu^{2+} concentrations. This may be due to the result of the

neutral pH, which was not significantly impacted by the liming effect of biochar (Uchimiya et al., 2011; Soja et al., 2018; Li et al., 2015).

In the acidic soils of St. Stefan, more pronounced results were reported for both the $\text{CaCl}_2\text{-Cu}$ and the Cu^{2+} concentrations. In the historically contaminated soils, two amendments reduced the $\text{CaCl}_2\text{-Cu}$ in the six-week incubation, but all amendments had a significant decrease in the three-year incubation. The $\text{CaCl}_2\text{-Cu}$ concentrations encompassed a range from $49.2 \mu\text{g}\cdot\text{kg}^{-1}$ to $112.6 \mu\text{g}\cdot\text{kg}^{-1}$ across the two incubation periods, and with an average value of $82.7 \mu\text{g}\cdot\text{kg}^{-1}$. These are lower values than what were seen in the historically contaminated soils of Rossatz, perhaps due to the increase in pH, leading to increased Cu immobilization (Uchimiya et al., 2011; Beesley et al., 2010; Perez-Esteban et al., 2013). For the Cu^{2+} , nearly all amendments reduced the amount of Cu^{2+} , with the exception of wBC in the six-week amendment. Values ranged from $0.1 \mu\text{g}\cdot\text{kg}^{-1}$ to $3.8 \mu\text{g}\cdot\text{kg}^{-1}$, with an average of $1.5 \mu\text{g}\cdot\text{kg}^{-1}$. wBC had the highest percentage of Cu^{2+} , accounting for nearly 4% of the total Cu concentration. The other values ranged from less than 1% to 3%, similar to the pattern demonstrated in Rossatz. Although the proportion of Cu^{2+} to the total Cu concentration was nearly the same in both sites, St. Stefan appeared to have stronger responses to the amendments. Whether this is due to the greater increase in pH or the composition of the soil, it is not possible to say. The other factors influencing Cu immobility will be discussed in the following chapters.

In the freshly contaminated soils, there was a large range in the $\text{CaCl}_2\text{-Cu}$ concentrations, with a low of $56.3 \mu\text{g}\cdot\text{kg}^{-1}$ to a peak of $1386.4 \mu\text{g}\cdot\text{kg}^{-1}$, with the average values around $564.8 \mu\text{g}\cdot\text{kg}^{-1}$. These values are much higher than the values in the freshly spiked soils of Rossatz. In the Cu^{2+} concentrations, these values decreased, but still had a wide range, from $0.03 \mu\text{g}\cdot\text{kg}^{-1}$ to $518.1 \mu\text{g}\cdot\text{kg}^{-1}$, with an average of $114.9 \mu\text{g}\cdot\text{kg}^{-1}$. As in Rossatz, the proportion of Cu^{2+} to the total Cu concentration also varies widely, despite the influence of incubation time. The L amendment typically had less than 1% Cu^{2+} , whereas the wBC amendment was often as high as 30%.

The wide range in the proportion of Cu^{2+} to the total Cu concentration, as well as the varied decreases in $\text{CaCl}_2\text{-Cu}$ and Cu^{2+} , may suggest that a reduction in total soluble Cu does not always correlate with a reduction in Cu^{2+} . A study conducted by Pietrzak and McPhail (2004) in the vineyards of Australia concluded that there was no overall relationship between the total amount of copper in the soils and the active fraction. This suggests that the total copper

concentration is not an accurate predictive of copper toxicity. There is also the possibility that the neutral pH levels played a role, as the activity of Cu^{2+} has been linked to pH (Yin et al., 2002; Wang et al., 2012). Wang et al., (2012) concluded that at pH values about 6.6, the contribution of Cu^{2+} to the overall Cu concentration declines sharply. In both soils, the pH ranges were around this value, which may explain the low proportion of Cu^{2+} . Wang et al., (2012) also determined that while Cu^{2+} binds very strongly to soft, or more polarizable, ligands, it shows comparatively weak binding to hard, or less polarizable, ligands.

The lack of correlation between CaCl_2 -Cu and Cu^{2+} may be the result of several factors, such as the length of the aging process, or differences in soil properties, and influences the effectiveness of biochars (Ruyters et al., 2013; Ma et al., 2006). In a study conducted by Dai et al., (2018), the addition of various biochar amendments did decrease CaCl_2 -extractable Cu from .1701 to 0.666 mg kg⁻¹, supporting that biochar does significantly decrease Cu. These factors are described in more detail in the following chapters.

5.2 Evaluation of Amendment Effectiveness

It was hypothesized that the particular amendments chosen in this experiment would reduce Cu concentrations, with some amendments potentially displaying more significant effects than others, as biochars and compost have both been found effective in mitigating metal toxicity (Beesley et al., 2013; Ruyters et al., 2013). However, in the comparison between the soils from Rossatz and St. Stefan, Cu concentrations appeared to be more heavily influenced by factors outside the amendments. For the (calcareous) soil from Rossatz, the amendments did not appear to have significantly decreased either CaCl_2 -extractable Cu or Cu^{2+} concentrations. In fact, the K and K+CA amendments actually significantly increased, despite what was hypothesized. However, it is difficult to draw definitive conclusions from the Cu^{2+} effects, due to the large standard deviations.

Conversely, for the (acidic) soil from St. Stefan, the amendments appeared to have a greater impact on reducing Cu concentrations, especially in the freshly contaminated treatments. This shows that it is difficult to derive universally applicable recommendations, and suggests that other factors, such as incubation length or soil properties, may strongly influence the effectiveness of amendments in reducing soluble Cu concentrations or Cu toxicity. This correlates with the findings of Uchimiya et al., (2011), in which copper sorption was compared

between two different soils, and the mechanisms in sequestering Cu were dependent on the soil properties, which in turn influenced the effect of the biochar.

The decrease in the concentration of soluble Cu also corresponds to the influence of soil properties in Cu solubility and retention, such as pH and DOC (Uchimiya et al., 2011; Beesley et al., 2010; Perez-Esteban et al., 2013). It has been hypothesized that increasing the DOC concentration has an adverse effect on Cu immobilization, resulting in increased mobility (Beesley and Dickinson, 2010; Wagner and Kaupenjohann, 2015; Park et al., 2011). However, the results in this study were not as conclusive. For the soil from Rossatz, the effects of the amendments on pH were negligible, with the exception of K+CA in the historically contaminated soil. However, the elevated pH of the K+CA corresponded to increased DOC as well, and an increased amount Cu concentration. For the soil from St. Stefan, in the historically contaminated treatments, an increase in pH by the K amendment corresponded to an increase in DOC as well. However, this did not correlate to any reduction in Cu concentration. The addition of lime resulted in the increase in both pH and DOC, as well as a decrease in both CaCl₂-extractable Cu and Cu²⁺. However, whether the increase in DOC was a direct result of the lime or rather a secondary effect from improving soil conditions, it is not possible to state. The addition of lime may also promote the uptake of calcium and magnesium of plants, reducing the negative impacts of Cu within the plant structure, making it a potentially important tool in vineyards (Ambrosini et al., 2015).

Our results suggest that it is not only the amendments themselves that may reduce metal toxicity (e.g. by providing new sorption surfaces), but also the effect on soil properties like pH associated with the application of alkaline biochars and lime. This is supported by the weak but significant relationship between CaCl₂-extractable Cu and pH in both the historically contaminated and freshly spiked soils, as demonstrated in **Table 6**. The interaction between DOC and Cu is less clear. Increasing DOC may cause the mobilization of Cu due to the increase in soluble Cu-organic complexes and causing their leaching (Beesley and Dickinson, 2010; Uchimiya et al., 2011, Ahmad et al., 2013). However, in this particular study, there appeared to be no relationship between Cu and DOC, as demonstrated by the R values in **Table 6**. A study conducted by Mackie et al., (2015) reached a similar conclusion, in which biochar, compost and biochar–compost did not immobilize exchangeable soil Cu content in a vineyard. Instead, they found that use of cover crops significantly reduced the amount of exchangeable soil Cu within

two growing seasons.

As the soluble Cu concentrations were not consistently reduced with the amendments, the first hypothesis is rejected.

Table 7: Relationship between CaCl₂ – extractable Cu with pH and DOC for freshly spiked soils; R for a linear relationship

Freshly Spiked	
CaCl ₂ Cu : pH	R=.246, p=0.000
CaCl ₂ Cu : DOC	R=.008, p=0.340

Table 8: Relationship between CaCl₂ – extractable Cu with pH and DOC for historically contaminated soils; R for a linear relationship

Historically Contaminated	
CaCl ₂ Cu : pH	R= 0.178, p=0.002
CaCl ₂ Cu : DOC	R= 0.0004, p=0.859

5.3 Evaluation of biochar and Cu aging effects during incubation

The short-term effects of biochar as a soil conditioner have been well-documented, including increasing soil carbon stocks, increasing pH, and improving microbial populations (Ahmad et al., 2013; Ippolito et al., 2012; Verheijen et al., 2009). There is also evidence to suggest that biochar is an effective tool in immobilizing metals such as Cu, although the duration of these effects and the mechanisms behind them are still not yet well understood (Uchimiya et al., 2011; Beesley et al., 2011; Park et al., 2011). Whether immobilization is the result of the interaction with the surface area of the biochar, or due to changes in soil conditions such as pH and DOC, will help determine if biochars are appropriate for long-term Cu sequestration (Li et al., 2015; Houben et al., 2013; Borchard et al., 2012; Bolan et al., 2014). The short-term consequences may be due to the ‘priming effect’ of biochar, i.e. changes in the mineralization of soil organic matter due to the addition of new substrates. It is suggested that the addition of biochar may initially increase mineralization, but that this decreases over time and there is a shift from a positive to a negative priming effect, decreasing the rate of mineralization (Singh and Cowie, 2014; Wagner and Kaupenjohann, 2015; Zimmerman et al., 2011; Ameuer et al., 2018). This mineralization is mirrored by the decomposition of biochar, which is initially rapid after

application, but changes to a slow and partial decomposition in the following years (Kuzyakov et al., 2009). However, even this priming effect is uncertain as some authors suggest that there may only be a slight negative effect, or even no priming effect at all (Wang et al., 2016; Verheijen et al., 2009).

In the historically contaminated soils from both Rossatz and St. Stefan, the six-week incubation had a higher pH than in the three-year incubation, although this difference was only significant for some of the amendments, and may be due to a transient priming effect (Wagner and Kaupenjohann, 2015). In contrast, the DOC levels in the three-year incubation were significantly higher than in the six-week incubation for nearly all amendments in both sites, and parallels findings by Li et al. (2015) that demonstrated increasing DOC over time. It has been hypothesized that this increase is the result of the dissolution of the biochar organic carbon (Beesley et al., 2010). With a decrease in pH and an increase in DOC in the three-year incubation, it would be assumed that this would correlate with an increase in soluble Cu (Li et al., 2015; Uchimiya et al., 2011; Chaignon et al., 2003; Komarek et al., 2010; Mackie et al., 2015; Temminghof et al., 1997). Instead, there was a significant decrease in CaCl₂-extractable Cu in the three-year incubation as compared to the six-week incubation. In the analysis of Cu²⁺ for both sites, there was no significant difference between the two incubation periods, although this could be the result of the large standard deviation values in the six-week incubation.

While it is not possible to state with certainty the reason for this apparent contradiction in soil conditions and soluble Cu, it may be the result of the aging of the biochar. One defining feature of biochar is its stability and slow degradation, leading to its extended presence in the soil, which has important implications for carbon sequestration and soil conditioning, as well as the long-term immobilization of metals (Wang et al., 2016; Zimmerman, 2010; Li et al., 2015; Ahmad et al., 2013; Bolan et al., 2013). As the biochar is aged, it undergoes oxidation through both biotic and abiotic processes, although only the exterior surface may be affected (Zimmerman, 2010; Cheng et al., 2006). This results in the formation of more oxygen containing surface functional groups, increasing the CEC and Cu sorption (Uchimiya et al., 2011; Li et al., 2015; Borchard et al., 2012). As hydrophobic biochar is aged, the oxidization of the surface may lead to more hydrophilic reactions, further influencing the Cu sorption capability (Joseph et al., 2010). The observed decrease in CaCl₂-extractable Cu perhaps can then be attributed to the increased oxidation of the biochar as the result of aging, despite the contradicting soil conditions.

However, in the freshly spiked soils, the opposite trend is presented, based on the comparison between the six-week incubation and the 3 yr + Cu spike. In St. Stefan especially, there was a significant increase in CaCl₂-extractable Cu in the three-year incubation as compared to the six-week incubation, a result that was less pronounced in Rossatz. This mirrors the findings by Li et al. (2015), in which the addition of biochar did initially decrease the availability of Cu, but which subsequently increased in the following years. However, this increase in Cu corresponded to an increase in DOC concentration, which was not observed in our study. Instead, there was a significant decrease in DOC for nearly all amendments in the three-year incubation in St. Stefan, with less pronounced results in Rossatz. However, the increase in CaCl₂-extractable Cu was coupled with an observable, although not necessarily significant, decrease in pH. Although the variation may not be statistically significant, it may suggest that even minor reversions to the baseline pH may lead to remobilization of Cu (Bolan et al, 2014.; Houben et al., 2013). The trend was repeated for Cu²⁺, in which the 3 yr+Cu spike had increased amounts compared to the six-week incubation. This has important implications for the long-term immobilization of Cu. It seems to suggest that the pH is an important controlling factor in determining Cu availability in freshly spiked soils. Although the pH change was not statistically significant, it appears to override the pure aging effect, that is the oxidation, of biochar. Why this occurred in freshly spiked soils and not in historically contaminated soils is discussed in the next section.

Another factor to consider is the aging of the Cu as well. Although most metals do not undergo microbial or chemical degradation, aging may have a significant effect on Cu fraction. The exchangeable Cu, including the water and acid soluble fraction, is incorporated into fractions that can be oxidized, and is readily mobile and bioavailable. The residual fraction becomes inactive after incorporation into the crystalline lattice of the soil. Fast processes, such as precipitation or occlusion into organic matter, may decrease the soluble Cu concentration rapidly after initial application. Slower reactions, such as diffusion of cations into micropores in the soil occur over a longer time period, and may further deplete bioavailable Cu over the longer timespan (Anxiang et al., 2008; Ma et al., 2006a; Ma et al., 2006b; Okonokhua, 2014). In a study presented by Houben et al. (2013), the bioavailability of metals decreased gradually with time when the soils were amended with biochar, perhaps due to diffusion into the micropores of the biochar. This aging of the Cu may explain the significant decreases demonstrated in the Cu

spike+ 3yr incubation as compared to the 3yr +Cu spike incubation. These decreases occurred in both the CaCl₂-extractable Cu as well as the Cu²⁺ for the two locations, and were not accompanied by significant pH change. The soil of St. Stefan showed a significant increase in DOC for the Cu spike+3yr incubation, which is contradictory to the decrease in Cu and suggests that other aging-related changes that favor Cu immobilization were more influential.

This follows the reasoning of hypothesis ii), in which the longer-term incubations had a more marked decrease in Cu. The exception to this is the 3 yr+Cu spike incubation, as this had higher concentrations than in the six-week incubation. Hence, these mixed results only partly support this hypothesis.

5.4 Effectiveness of amendments in historically contaminated vs. freshly spiked soils

Copper may be less mobile in historically contaminated soils than in freshly spiked soils due to the increase in equilibration time associated with historically contaminated soils (Lock and Janssen, 2002; Smolders et al., 2012; Oorts et al., 2006; Ma et al., 2006a). The more mobile Cu in freshly spiked soils has not yet been complexed, leading to an increased probability of leaching (Oorts, et al., 2006; Ma et al., 2006a). Leaching is responsible for the removal of not only excess Cu from the soil, but also other salts and protons in the soil solution that may affect Cu bioavailability (Okonokhua, 2014). The difference in the age of Cu in the soil also has important implications for understanding the toxicity of Cu as laboratory results may not always accurately reflect field conditions (Okonokhua, 2014; Lock and Janssen, 2002; Oorts et al., 2006). The influence of Cu aging in the soil may affect the effectiveness of the applied biochar amendments due to the difference in bioavailability of the Cu. It was hypothesized in this study that the amendments will have a more pronounced effect in the freshly contaminated soils than in the historically contaminated; however, due to the contradictions within the two sites, the results only partly support this hypothesis.

There is not a strong distinction in the comparison of the freshly spiked and historically contaminated soils of Rossatz. In the six-week incubation of the historically contaminated soils, only two amendments, K and K+CA, appeared to have a significant influence on the amount of CaCl₂- extractable Cu, and in fact, resulted in an increase in Cu. This may be attributed to the significant increase in DOC that accompanied both amendments (Uchimaya et al., 2011; Beesley

and Dickinson, 2010; Wagner and Kaupenjohann, 2015; Park et al., 2011). However, in the comparison of the freshly spiked soils, no amendment significantly changed the amount of CaCl_2 -extractable Cu as compared to the control, although some increases could be observed. As in the historically contaminated soils, the amendments K and K+CA once again demonstrated a significant increase in DOC, but this time without the accompanying increase in Cu. There was a significant increase in pH for K+CA, which may have counteracted the increase in DOC, as increasing pH is associated with reduced Cu solubility (Uchimiya et al., 2010; Karami et al., 2011; Houben et al., 2013).

A similar trend was repeated in the 3 yr+Cu spike incubations for Rossatz as well. In the historically contaminated soils, K, as in the six-week incubation, had a significant increase in CaCl_2 -extractable Cu. However, there was also a significant decrease in the wBC amendment. The increase in CaCl_2 -Cu in K corresponded to an increase in DOC, whereas the decrease demonstrated in wBC followed a decrease in DOC. This follows the findings that increasing DOC concentration has an adverse effect on Cu immobilization (Beesley and Dickinson, 2010; Wagner and Kaupenjohann, 2015; Park et al., 2011). For the freshly contaminated soils, the same trend is repeated as in the six-week incubation, with no amendment significantly decreasing the amount of CaCl_2 -Cu. Unlike in the six-week incubation, there was no significant changes in pH or DOC. This lack of strong distinction between the historically and freshly contaminated soils of Rossatz makes it difficult to determine the influence of Cu aging in soil. In the analysis of Cu^{2+} , no amendment appeared to have a significant effect, regardless of Cu aging in the soil or length of incubation.

For the soil of St. Stefan, the amendments appeared to have a stronger influence than for the soil of Rossatz. In the six-week incubation of the historically contaminated soils, only two amendments had significant decreases in CaCl_2 -Cu, wBC and L. The decrease in CaCl_2 -Cu in L was accompanied by an increase in pH. While there was no increase in pH for wBC, wBC was the only amendment to not have a significant increase in DOC. However, for Cu^{2+} , nearly all amendments, with the exception of wBC, had significant decreases. In the six-week incubation of the freshly contaminated soil, however, all amendments demonstrated a significant decrease in CaCl_2 -Cu and Cu^{2+} , although there were no significant changes in pH or DOC. This seems to imply that the aging of Cu in the soil did have an influence. It has been suggested that the bioavailability of Cu may decrease with increasing equilibration time, and that Cu toxicity is

higher in freshly spiked soils as compared to historically contaminated ones (Oorts et al., 2004; Ruyters et al., 2013).

In the 3 yr+Cu spike incubations, however, the opposite trend is presented. In the historically contaminated soils, all amendments had significantly decreased amounts of CaCl₂-Cu and Cu²⁺. However, in the freshly contaminated soils, only the K and L amendments had significant decreases both in CaCl₂-Cu and Cu²⁺, although they also paradoxically reported a significant increase in DOC. The decrease in CaCl₂-Cu in L corresponds to an increase in pH that is not present for wBC. This contradicts the findings by Lock and Janssen (2002), who found that extracted copper concentrations were significantly higher in freshly spiked soils, possibly due to the decrease in pH that occurred after spiking. While it is not possible to state with certainty the reason for this apparent contradiction for the soil of St. Stefan, perhaps it may also be attributed to the aging of biochar or the metal and in a three-year incubation period, the aging of the biochar had a greater effect. It may be that the labile Cu fractions decreased with increasing incubation time, but that the residual Cu and the Cu bound to the crystalline structure within the soil became more mobile after time (Arias-Estevez et al., 2007; Ruyters et al., 2013; Smolders et al., 2012). However, due to the contradictions within the two sites, the hypothesis that the amendments will have a stronger influence on the freshly contaminated soils is not fully supported.

5.5 Influence of soil properties

The fractionation of Cu, and as a result, its bioavailability, is strongly influenced by soil properties, such as pH (Li et al., 2015; Uchimiya et al., 2012; Chaignon et al., 2003; Ippolito et al., 2012). With increasing pH, increasing negative charges and the precipitation of hydroxides, carbonates and phosphates could lead to a decrease in metal adsorption (Rieuwerts et al., 1998; Ippolito et al., 2012). This was demonstrated for the soil from St. Stefan, as the lime amendment consistently had the largest decrease in CaCl₂-extractable Cu and Cu²⁺. The addition of lime could also lead to the precipitation of Cu-hydroxides or carbonates, further increasing the decrease of Cu (Brunetto et al., 2006). However, there may also be unintended consequences to the lime application, such as the interaction with elements such as phosphorus, which would have negative consequences on plant uptake of nutrients (Brunetto et al., 2006; Pietrzak and Uren,

2011). At neutral pH, however, immobilization through the complexation with organic matter may play an important role (Chaignon et al., 2003; Ruyters et al., 2013). As most biochars are naturally alkaline, their application often induces a liming effect on the soils. In a study by Soja et al., (2017), the biochar amendment treatments had a stronger effect in the acidic soil as in the neutral soil, a result that is mirrored in this study.

DOC also plays an important role in governing Cu mobility. On the one hand, Cu in vineyard soils may be strongly bound to soil organic matter (Strawn and Baker, 2008), but on the other hand, Cu may also be mobilized with increasing DOC concentration (Beesley and Dickinson, 2010; Wagner and Kaupenjohann, 2015; Park et al., 2011). It is suggested that biochars have a more stable binding effect of metals than compost, as compost has higher DOC concentrations. This was also reflected in this study, in which compost often resulted in increased CaCl_2 Cu values. However, compost can lead to an increase in soil fertility and plant growth, which could prove useful in other methods of removing copper from the soil, such as in phytoremediation (Beesley et al., 2013, Karami et al, 2011).

There is also evidence to suggest that the composition of the soil may also have an effect. In a study conducted by Uchimiya et al. (2011), Cu retention was compared between a clay-rich, alkaline soil and an eroded, acidic sandy loam soil. The clay-rich soil had a significant heavy metal sorption capacity, whereas the sandy loam had a lower capacity. This was attributed to the influence of the clay minerals, which had a higher CEC. In the present study, the soil from Rossatz was calcareous and had lower clay and organic matter contents, but a higher sand content as compared to the soil from St. Stefan. The amendments had limited impact on the reduction of either CaCl_2 Cu or Cu^{2+} for the soil from Rossatz, while they had much greater influence on Cu reduction in the soil from St. Stefan. However, whether this is due to either the composition or the pH of the soils, it is not possible to state, and more research is required in this direction.

6. Summary and Conclusions

This study was a continuation of the assessment of various organic amendments in the reduction of Cu in freshly spiked and historically contaminated vineyards. The purpose was to determine the long-term effects of the biochar amendments and to determine the effect of both

biochar aging and soil aging on Cu immobilization. The results demonstrated the following:

1. The amendments had varied results on Cu reduction. The compost amendment even increased the amount of soluble Cu in the soil, perhaps as a result of increasing DOC. The amendment with the greatest reduction was the lime amendment in the acidic soil, which consistently reduced both CaCl₂-extractable Cu and Cu²⁺.
2. In the historically-contaminated soils, the long-term incubation resulted in significantly reduced soluble Cu as compared to the short-term. This corresponds to the literature which suggests that while initial application is followed by quick complexation, this continues through time, although it may slow.
3. In the freshly spiked soils, the influence of incubation appeared to be site-dependent. For example, for the calcareous soil from Rossatz, there was a significant reduction in soluble Cu between the short-term incubation, and the Cu spike before the long-term incubation. This suggests the amendments have ability to further immobilize Cu over the incubation period. However, in the three-year incubation followed by the Cu spike, the resulting high concentrations suggest the biochar amendments are not as effective at immobilizing freshly added Cu. For the soil from St. Stefan, there appeared to be no significant difference between the short-term incubation and the Cu spike before the long-term incubation. However, as for the soil from Rossatz, there was a significant increase in the long-term incubation before the Cu spike.
4. There were significant differences between the two soils, suggesting the influence of soil properties and their interaction with the amendments. For example, the more acidic soil of St. Stefan exhibited a stronger response to the amendments when compared to the calcareous soil from Rossatz. This suggests that the amendments' liming effect, which is more pronounced in acidic soils, was an important factor for Cu immobilization in this study. Understanding the influence of soil properties highlights the need to select a biochar most appropriate for the soil in order to have the greatest impact possible on reducing bioavailable Cu.

As Cu fungicides are currently the most effective tool in fighting disease in viticulture, it is unlikely that our reliance on them will decrease in the future. Instead, focus should be given on remediation, in order to preserve soil health and functionality. This requires more research in the long-term effects of biochar amendments, especially on their reduction of heavy metals. The various interactions with the amendments and the soil properties can be complex and requires a better understanding of the involved processes. One limitation to this study was determining exactly how great the impact to the Cu^{2+} concentration was. Due to the high standard deviation present in most of the calculations, it is not possible to draw a definite conclusion, and instead only assumptions could be made. This could be improved upon in future studies, as understanding the impact on Cu^{2+} is necessary for determining Cu toxicity.

It is evident that for the most effective reduction, particular consideration must be given when choosing a biochar. This is especially important for the biochars that have been modified with activation processes as this increases the cost. Continued research into Cu remediation strategies, especially their long-term effects, is necessary for ensuring the continued health of viticulture around the world.

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10. Appendix

Identification of the Samples

a.) Böden/Soil

Code	Beschreibung/ Description
R	Rossatz
S	St. Stefan

b.) Varianten/Amendments

Code	Varianten Beschreibung/ Amendment Description
N	Kontrolle (ohne Kalk)/ Control
K	Kompost (40 t TM/ha)/ Compost
KH25	Kompost + Holz-BC (25:75)/ Compost + WoodBC
H	Holz-BC (100%)/ Wood BC
KZS	Kompost + ZS-aktivierte Holz-BC (25:75)/ Compost + Citric Acid activated WoodBC
KOH	Kompost + H ₂ O ₂ -oxidierte Holz-BC (25:75)/ Compost + H ₂ O ₂ oxidized WoodBC
Kalk	Kontrolle mit Kalk (nur für saure Böden)/ Lime (only for acidic soils)

c.) Incubation Treatment

6 week Incubation	
1	no Cu spike
2	Cu spike

3 year Incubation	
a	3 year incubation then Cu spike
b	3 year incubation; no Cu added
c	Cu spike then 3 year incubation

d.) Probencode Beispiele/ Sample Code Example

Code	Beschreibung/Description
R/KH75/3b	Rossatz/Variante 5 (Kompost+Holz-BC 75:25)/Wiederholung 3/ 3 year incubation; no Cu added
2/S/N/1	6 week incubation with Cu spike /Stefan/Kontrolle/Wiederholung 1

Table 9(a-d): How the samples are identified based on the site, soil characteristics, amendments, and incubation treatment applied

Measured and Calculated Values for Short-term Incubation

Sample	Total Cu (mg/L)	DOC (mg/L)	pH (0.01 M CaCl ₂)	Total CaCl ₂ -extracted Cu (ug/kg)	Cu ²⁺ (ug/kg)	Cu ²⁺ Concentration (mol/L)	% Cu ²⁺ of total CaCl ₂ - Cu
1/R/N/1	0.013	2.459	6.65	127.160	4.484	7.056E-09	3.53
1/R/N/2	0.010	3.064	6.63	101.874	1.727	2.718E-09	1.693
1/R/N/3	0.010	2.675	6.68	96.487	1.783	2.806E-09	1.857
1/R/K/1	0.021	5.784	6.6	210.922	2.380	3.745E-09	1.128
1/R/K/2	0.019	6.172	6.74	192.982	1.098	1.728E-09	0.569
1/R/K/3	0.021	5.956	6.65	207.191	1.701	2.677E-09	850
1/R/KH25/1	0.012	3.021	6.319	123.646	5.842	9.193E-09	0.711
1/R/KH25/2	0.012	3.582	6.763	122.852	1.273	2.004E-09	1.035
1/R/KH25/3	0.012	3.107	6.835	121.178	1.385	2.179E-09	1.145
1/R/KOH/1	0.013	3.668	6.71	133.477	1.700	2.675E-09	1.278
1/R/KOH/2	0.012	3.280	6.47	119.144	3.197	5.031E-09	2.687
1/R/KOH/3	0.012	3.323	6.7	117.982	1.659	2.610E-09	1.406
1/R/KZS/1	0.015	5.481	7.05	150.801	0.257	4.049E-10	0.186
1/R/KZS/2	0.014	4.359	7.025	135.111	0.415	6.530E-10	0.307
1/R/KZS/3	0.014	4.920	6.94	135.802	0.418	6.582E-10	0.308
1/R/H/1	0.010	2.546	6.37	96.942	4.387	6.903E-09	4.522
1/R/H/2	0.009	2.589	6.66	92.979	1.886	2.968E-09	2.028
1/R/H/3	0.009	2.502	6.81	86.010	1.092	1.719E-09	1.27
1/S/N/1	0.010	3.841	6.097	98.941	3.473	5.466E-09	3.508
1/S/N/2	0.010	3.495	6.19	95.563	3.262	5.134E-09	3.398
1/S/N/3	0.009	3.798	5.884	92.932	4.671	7.351E-09	5.023
1/S/K/1	0.010	6.431	6.276	103.248	0.822	1.293E-09	0.798
1/S/K/2	0.010	6.388	6.386	100.827	0.596	9.380E-10	0.59
1/S/K/3	0.012	6.992	6.508	120.486	0.513	8.075E-10	0.428
1/S/KH25/1	0.009	4.316	6.101	86.788	2.046	3.220E-09	2.352

1/S/KH25/2	0.008	4.532	6.214	84.668	1.356	2.134E-09	1.595
1/S/KH25/3	0.008	4.186	6.113	82.510	1.920	3.021E-09	2.313
1/S/KOH/1	0.008	4.359	6.113	80.451	1.625	2.557E-09	2.031
1/S/KOH/2	0.009	4.359	6.053	88.977	2.329	3.665E-09	2.617
1/S/KOH/3	0.009	4.704	6.388	86.392	0.828	1.302E-09	0.962
1/S/KZS/1	0.010	5.352	6.252	97.395	1.152	1.812E-09	1.187
1/S/KZS/2	0.010	5.222	6.268	102.857	1.340	2.109E-09	1.301
1/S/KZS/3	0.010	5.136	6.256	103.933	1.463	2.303E-09	1.407
1/S/H/1	0.007	3.495	6.034	72.785	2.505	3.942E-09	3.431
1/S/H/2	0.007	3.495	5.98	74.306	2.869	4.515E-09	3.877
1/S/H/3	0.008	3.452	5.985	83.465	3.066	4.825E-09	4.459
1/S/Kalk/1	0.006	4.704	6.924	64.624	0.074	1.170E-10	0.114
1/S/Kalk/2	0.007	4.575	6.717	69.993	0.201	3.158E-10	0.287
1/S/Kalk/3	0.007	4.920	6.888	67.752	0.085	1.338E-10	0.125
2/R/N/1	0.015	2.705	6.46	150.216	8.232	1.296E-08	5.48
2/R/N/2	0.021	2.274	6.44	214.132	24.810	3.904E-08	11.586
2/R/N/3	0.042	2.187	6.48	421.393	89.460	1.408E-07	21.23
2/R/K/1	0.051	6.202	6.74	505.952	11.042	1.738E-08	2.182
2/R/K/2	0.068	5.425	6.68	679.471	33.635	5.293E-08	4.951
2/R/K/3	0.043	6.850	6.48	426.663	14.720	2.316E-08	3.45
2/R/KH25/1	0.035	3.353	6.49	345.195	42.394	6.671E-08	8.597
2/R/KH25/2	0.022	3.008	6.51	221.113	13.779	2.168E-08	6.231
2/R/KH25/3	0.024	2.705	6.59	235.180	16.495	2.596E-08	7.014
2/R/KOH/1	0.026	3.051	6.58	255.191	15.792	2.485E-08	6.189
2/R/KOH/2	0.015	3.223	6.48	145.330	5.050	7.947E-09	3.482
2/R/KOH/3	0.022	3.137	6.54	223.946	12.226	1.924E-08	5.458
2/R/KZS/1	0.025	3.698	6.7	252.980	7.810	1.229E-08	3.089
2/R/KZS/2		5.037	6.79				
2/R/KZS/3	0.022	4.864	6.88	216.140	1.687	2.654E-09	0.781
2/R/H/1	0.024	2.619	6.58	236.067	18.076	2.845E-08	7.659
2/R/H/2	0.041	2.230	6.466	410.519	84.707	1.333E-07	20.611
2/R/H/3	0.068	2.446	6.54	682.646	168.734	2.655E-07	24.705
2/S/N/1	0.112	4.691	5.903	1115.860	318.493	5.012E-07	28.538
2/S/N/2	0.124	5.382	5.935	1243.506	314.934	4.956E-07	25.316
2/S/N/3	0.085	5.339	6.148	854.255	131.184	2.064E-07	15.361
2/S/K/1	0.039	6.807	6.276	385.970	13.747	2.163E-08	3.561
2/S/K/2	0.027	7.411	6.429	266.175	3.584	5.640E-09	1.345
2/S/K/3	0.068	7.368	6.16	675.007	46.500	7.318E-08	6.889
2/S/KH25/1	0.017	4.950	6.177	168.941	5.491	8.641E-09	3.249
2/S/KH25/2	0.034	5.468	6.685	340.122	6.663	1.049E-08	1.959

2/S/KH25/3	0.031	5.598	6.142	308.239	16.238	2.555E-08	5.272
2/S/KOH/1	0.039	5.080	6.013	388.867	38.189	6.010E-08	9.817
2/S/KOH/2	0.051	5.123	6.114	506.268	54.233	8.535E-08	10.718
2/S/KOH/3	0.077	5.684	6.115	767.333	101.032	1.590E-07	13.172
2/S/KZS/1	0.045	6.505	6.302	453.725	20.328	3.199E-08	4.477
2/S/KZS/2	0.015	6.116	6.156	145.851	2.669	4.201E-09	1.828
2/S/KZS/3	0.021	5.986	6.126	214.936	6.866	1.080E-08	3.193
2/S/H/1	0.062	4.519	6.07	622.339	103.764	1.633E-07	16.682
2/S/H/2	0.023	6.505	6.255	234.336	5.319	8.370E-09	2.273
2/S/H/3	0.016	4.346	6.464	160.723	3.456	5.439E-09	2.147
2/S/Kalk/1	0.007	5.727	6.931	66.022	0.045	7.110E-11	0.068
2/S/Kalk/2	0.006	8.318	6.712	59.728	0.030	4.799E-11	0.051
2/S/Kalk/3	0.005	5.943	6.901	52.626	0.026	4.086E-11	0.049

Table 10: The short-term incubation (6 weeks) values for both historically contaminated and freshly spiked soils in Rossatz and St. Stefan. The measured values are Total Cu, DOC, pH, and total CaCl₂-extracted Cu. The calculated values, using *Visual MINTEQ 3.1*, are Cu²⁺, the Cu²⁺ concentration, and the % Cu²⁺ in the CaCl₂-extracted Cu.

Measured and Calculated Values for Long-term Incubation

Sample	Total Cu (mg/L)	DOC (mg/L)	pH (0.01 M CaCl ₂)	Total CaCl ₂ -extracted Cu (ug/kg)	Cu ²⁺ (ug/kg)	Cu ²⁺ Concentration (mol/L)	% Cu ²⁺ of total CaCl ₂ -Cu
R/N/1a	0.032	2.347	5.87	323.644	97.791	1.54E-07	30.181
R/N/2a	0.041	2.520	5.79	407.832	138.454	2.18E-07	33.934
R/N/3a	0.036	2.477	6.48	359.231	55.279	8.70E-08	15.399
R/N/1b	0.009	5.887	5.83	90.538	2.055	3.23E-09	2.259
R/N/2b	0.010	5.844	5.88	96.366	2.116	3.33E-09	2.204
R/N/3b	0.009	5.887	6.48	91.590	0.444	6.99E-10	0.483
R/N/1c	0.014	2.218	6.91	135.581	3.299	5.19E-09	2.426
R/N/2c	0.014	2.304	6.47	138.917	9.418	1.48E-08	6.775
R/N/3c	0.014	2.174	6.2	138.899	16.900	2.66E-08	12.158
R/K/1a	0.046	3.642	6.48	459.563	46.705	7.35E-08	10.154
R/K/2a	0.045	4.074	6.62	445.598	27.226	4.28E-08	6.105
R/K/3a	0.040	4.074	6.56	402.869	24.651	3.88E-08	6.117
R/K/1b	0.014	7.787	6.42	136.155	0.688	1.08E-09	0.506
R/K/2b	0.013	7.139	6.23	131.996	1.280	2.01E-09	0.97
R/K/3b	0.013	7.139	6.46	129.984	0.678	1.07E-09	0.522
R/K/1c	0.019	4.376	6.42	189.905	5.478	8.62E-09	2.883

R/K/2c	0.115	4.549	6.53	1146.710	181.576	2.86E-07	15.831
R/K/3c	0.023	4.247	6.5	229.106	7.399	1.16E-08	3.231
R/KH25/1a	0.054	2.779	6.42	544.841	109.445	1.72E-07	20.082
R/KH25/2a	0.047	2.736	6.55	469.125	71.019	1.12E-07	15.144
R/KH25/3a	0.034	2.779	6.46	343.231	43.140	6.79E-08	12.578
R/KH25/1b	0.010	5.758	6.47	95.608	0.533	8.39E-10	0.556
R/KH25/2b	0.009	5.887	6.37	93.650	0.636	1.00E-09	0.677
R/KH25/3b	0.009	5.628	6.41	91.895	0.602	9.48E-10	0.55
R/KH25/1c	0.015	2.606	6.26	146.427	12.255	1.93E-08	8.394
R/KH25/2c	0.014	2.520	6.51	141.517	7.572	1.19E-08	5.332
R/KH25/3c	0.014	2.563	6.52	136.611	6.613	1.04E-08	4.827
R/KOH/1a	0.035	2.692	6.47	348.966	46.353	7.29E-08	13.282
R/KOH/2a	0.040	2.520	6.61	397.971	53.742	8.46E-08	13.504
R/KOH/3a	0.035	2.477	5.91	349.102	100.225	1.58E-07	28.717
R/KOH/1b	0.009	5.499	6.36	89.441	0.674	1.06E-09	0.757
R/KOH/2b	0.009	5.542	6.66	89.867	0.281	4.42E-10	0.312
R/KOH/3b	0.009	5.628	6.58	89.361	0.338	5.33E-10	0.38
R/KOH/1c	0.013	2.649	6.5	133.151	6.045	9.51E-09	4.545
R/KOH/2c	0.014	2.261	6.46	139.255	9.975	1.57E-08	7.176
R/KOH/3c	0.012	2.390	6.51	122.329	6.062	9.54E-09	4.968
R/KZS/1a	0.032	2.736	6.57	324.044	32.886	5.18E-08	10.15
R/KZS/2a	0.035	2.822	6.41	348.442	46.723	7.35E-08	13.426
R/KZS/3a	0.032	2.649	6.547	323.287	36.036	5.67E-08	11.157
R/KZS/1b	0.010	6.060	6.19	103.650	1.186	1.87E-09	1.141
R/KZS/2b	0.010	6.406	6.403	99.187	0.539	8.48E-10	0.545
R/KZS/3b	0.010	6.060	6.2	95.469	0.944	1.49E-09	0.994
R/KZS/1c	0.012	3.426	6.439	118.327	3.069	4.83E-09	2.601
R/KZS/2c	0.012	2.952	6.02	117.404	9.418	1.48E-08	8.049
R/KZS/3c	0.013	2.995	6.33	127.478	6.118	9.63E-09	4.817
R/H/1a	0.036	1.915	6.43	364.716	87.967	1.38E-07	24.101
R/H/2a	0.033	6.146	6.26	328.147	12.344	1.94E-08	3.763
R/H/3a	0.027	2.045	6.26	266.714	56.951	8.96E-08	21.329
R/H/1b	0.007	5.628	6.3	72.888	8.778	1.38E-08	3.287
R/H/2b	0.007	4.938	6.28	74.287	0.695	1.09E-09	0.939
R/H/3b	0.007	4.549	6.08	73.247	1.305	2.05E-09	1.788
R/H/1c	0.011	2.433	6.21	108.162	8.248	1.30E-08	7.636
R/H/2c	0.011	2.390	6.305	110.427	7.427	1.17E-08	6.751
R/H/3c	0.009	2.261	5.93	92.355		1.70E-04	11.769
S/N/1a	0.126	5.197	6.045	1256.677	303.521	4.78E-07	24.146

S/N/2a	0.122	5.326	5.84	1222.707	336.063	5.29E-07	27.478
S/N/3a	N/A		N/A				
S/N/1b	0.011	5.067	5.89	108.975	3.627	5.71E-09	3.327
S/N/2b	0.012	5.628	6.135	118.666	2.134	3.36E-09	1.793
S/N/3b	0.011	5.499	5.81	110.176	4.913	7.73E-09	3.311
S/N/1c	0.066	5.542	5.56	663.857	149.568	2.35E-07	22.525
S/N/2c	0.050	5.887	5.614	496.538	78.975	1.24E-07	15.89
S/N/3c	0.036	5.715	5.78	358.423	36.901	5.81E-08	10.307
S/K/1a	0.050	6.017	5.99	501.514	24.754	3.90E-08	6.914
S/K/2a	0.071	5.758	6.01	707.455	96.958	1.53E-07	13.714
S/K/3a	0.064	5.931	6.266	635.748	52.290	8.23E-08	8.221
S/K/1b	0.008	7.183	6.18	84.379	0.511	8.05E-10	0.609
S/K/2b	0.009	6.837	6.194	86.191	0.582	9.16E-10	0.677
S/K/3b	0.008	7.010	6.307	81.170	0.355	5.59E-10	0.439
S/K/1c	0.021	5.887	6.19	209.748	5.947	9.36E-09	2.832
S/K/2c	0.024	6.449	6.48	241.020	4.715	7.42E-09	1.956
S/K/3c	0.019	6.362	6.08	190.596	5.147	8.10E-09	2.695
S/KH25/1a	0.092	4.679	6.056	922.132	202.025	3.18E-07	21.911
S/KH25/2a	N/A		N/A				
S/KH25/3a	0.107	4.419	5.96	1074.268	305.885	4.81E-07	28.481
S/KH25/1b	0.007	5.456	6.252	74.124	0.595	9.36E-10	0.804
S/KH25/2b	0.007	5.628	5.84	73.810	1.427	2.25E-09	1.929
S/KH25/3b	0.007	5.326	5.864	72.551	1.482	2.33E-09	2.03
S/KH25/1c	0.036	5.024	6.07	363.578	31.423	4.95E-08	8.632
S/KH25/2c	0.061	4.765	5.87	611.554	116.696	1.84E-07	19.067
S/KH25/3c	0.082	4.592	6.053	818.781	168.994	2.66E-07	20.634
S/KOH/1a	0.123	4.419	6.01	1229.297	365.758	5.76E-07	29.762
S/KOH/2a	0.100	4.635	5.973	995.166	251.846	3.96E-07	25.311
S/KOH/3a	0.112	4.635	5.97	1120.851	307.849	4.84E-07	27.462
S/KOH/1b	0.008	5.456	6.24	81.824	0.776	1.22E-09	0.946
S/KOH/2b	0.008	5.283	6.19	77.400	0.818	1.29E-09	1.063
S/KOH/3b	0.008	5.499	6.091	79.368	1.003	1.58E-09	1.27
S/KOH/1c	0.051	5.197	6.02	509.137	60.956	9.59E-08	11.975
S/KOH/2c	0.035	8.176	6.038	350.285	12.005	1.89E-08	3.43
S/KOH/3c	0.037	7.312	5.93	371.415	20.285	3.19E-08	5.467
S/KZS/1a	0.114	4.592	5.967	1137.899	4.181	6.58E-09	3.674
S/KZS/2a	0.076	4.549	5.76	763.175	200.253	3.15E-07	26.245
S/KZS/3a	0.080	4.463	5.81	802.744	213.324	3.36E-07	26.565
S/KZS/1b	0.007	5.240	5.78	73.128	1.812	2.85E-09	2.482
S/KZS/2b	0.007	5.240	5.951	73.330	1.278	2.01E-09	1.751

S/KZS/3b	0.007	4.938	5.79	71.663	1.949	3.07E-09	2.707
S/KZS/1c	0.036	7.355	5.916	364.506	19.854	3.12E-08	5.439
S/KZS/2c	0.031	7.528	5.65	313.115	21.207	3.34E-08	6.775
S/KZS/3c	0.032	7.442	6.118	315.967	10.108	1.59E-08	3.198
S/H/1a	0.167	4.290	5.79	1666.968	695.956	1.10E-06	41.75
S/H/2a	0.053	4.419	6.005	534.304	87.776	1.38E-07	16.437
S/H/3a	0.111	4.074	6	1105.899	340.327	5.36E-07	30.77
S/H/1b	0.007	5.369	5.75	73.498	1.828	2.88E-09	2.504
S/H/2b	0.006	4.635	6.232	64.263	0.650	1.02E-09	1.015
S/H/3b	0.006	4.376	5.98	63.473	1.280	2.01E-09	2.031
S/H/1c	0.041	6.880	5.91	414.950	29.343	4.62E-08	7.07
S/H/2c	0.041	6.708	6.109	407.476	21.485	3.38E-08	5.279
S/H/3c	0.030	6.665	6.14	303.240	11.114	1.75E-08	3.668
S/Kalk/1a	0.012	6.146	6.756	116.680	0.305	4.80E-10	0.26
S/Kalk/2a	0.010	5.758	6.57	102.824	0.471	7.41E-10	0.457
S/Kalk/3a	0.009	5.974	6.27	92.427	0.761	1.20E-09	0.828
S/Kalk/1b	0.005	7.183	6.18	50.475	0.154	2.43E-10	0.308
S/Kalk/2b	0.005	6.924	6.548	47.312	0.047	7.34E-11	0.099
S/Kalk/3b	0.005	7.053	6.46	49.716	0.070	1.09E-10	0.139
S/Kalk/1c	0.006	9.255	6.718	55.811	0.019	2.99E-11	0.034
S/Kalk/2c	0.005	8.392	6.53	54.996	0.045	7.15E-11	0.083
S/Kalk/3c	0.006	9.687	6.07	57.815	0.147	2.31E-10	0.253

Table 11: The long-term incubation (3 years) values for both historically contaminated and freshly spiked soils in Rossatz and St. Stefan. The measured values are Total Cu, DOC, pH, and total CaCl₂-extracted Cu. The calculated values, using *Visual MINTEQ 3.1*, are Cu²⁺, the Cu²⁺ concentration, and the % Cu²⁺ in the CaCl₂-extracted Cu.

Historically Contaminated CaCl₂-Cu (µg kg⁻¹) Values

	Rossatz			St. Stefan	
	6 weeks	3 years		6 weeks	3 years
CO	108.5 (±16.4)	93.5 (±2.6)	CO	96.0 (±3.0)	112.6 (±5.3)
K	203.7 (±9.5)	134 (±3.1)	K	108.0 (±10.4)	83.9 (±2.5)
wBC	92.0 (±5.5)	73.5 (±0.6)	wBC	76.7 (±5.5)	67.1 (±5.6)
K+wBC	122.6 (±1.3)	95 (±2.0)	K+wBC	85.0 (2.0)	73.5 (±0.8)
K+H₂O₂	123.5 (±8.6)	89.5 (±0.6)	K+H₂O₂	85.0 (±4.6)	79.5 (2.2)
K+CA	140.6 (±8.9)	101.5 (±4.5)	K+CA	101.3 (±3.8)	72.7 (±0.9)
			L	67.7 (±2.5)	49.2 (±1.7)

Table 12: Average values of the CaCl₂-extractable Cu (µg kg⁻¹) for each amendment for the historically contaminated soils. ± values indicate standard deviation, n=3.

Historically Contaminated Cu²⁺ (µg kg⁻¹) Values

	Rossatz			St. Stefan	
	6 weeks	3 years		6 weeks	3 years
CO	2.7 (±1.6)	1.5 (±0.9)	CO	3.8 (±0.8)	3.1 (±0.9)
K	1.7 (±0.6)	0.9 (±0.3)	K	0.6 (±0.2)	0.5 (±0.1)
wBC	2.5 (±1.7)	1.5 (±0.9)	wBC	2.8 (±0.3)	1.3 (±0.6)
K+wBC	2.8 (±2.6)	0.6 (±0.1)	K+wBC	1.8 (±0.3)	1.2 (±0.5)
K+H₂O₂	2.2 (±0.9)	0.4 (±0.2)	K+H₂O₂	1.6 (±0.8)	0.9 (±0.1)
K+CA	0.4 (± 0.1)	0.9 (±0.3)	K+CA	1.3 (0.2)	1.7 (±0.4)
			L	0.1 (±0.1)	0.1 (±0.1)

Table 13: Average values of the Cu²⁺ (µg kg⁻¹) for each amendment for the historically contaminated soils. ± values indicate standard deviation, n=3.

Freshly Spiked CaCl₂-Cu (µg kg⁻¹) Values

Rossatz			
	6 weeks	3yr + Cu	Cu + 3yr
CO	261.9 (±141.8)	363.6 (±42.3)	137.8 (±1.9)
K	537.4 (±129.3)	436.0 (±29.5)	139.7 (±122.5)
wBC	443.1 (±225.1)	319.9 (±49.5)	103.6 (±9.8)
K+wBC	267.2 (±67.9)	452.4 (±101.8)	141.5 (±4.9)
K+H₂O₂	208.2 (±56.6)	365.3 (±28.3)	131.6 (±8.6)
K+CA	156.4 (±136.7)	331.9 (±14.3)	121.1 (±5.6)

St. Stefan			
	6 weeks	3yr + Cu	Cu + 3yr
CO	1071.2 (±198.4)	1239.7 (±24.0)	506.3 (±152.9)
K	442.4 (±210.2)	614.9 (±104.5)	213.8 (±25.5)
wBC	339.1 (±248.0)	1386.4 (±396.7)	375.2 (±62.5)
K+wBC	272.4 (±91.0)	998.2 (±107.6)	598.0 (±227.9)
K+H₂O₂	554.2 (±193.7)	1115.1 (±117.2)	410.3 (±86.3)
K+CA	271.5 (±161.5)	901.3 (±205.9)	331.2 (±28.9)
L	59.5 (±6.7)	104.0 (±12.2)	56.3 (±1.5)

Table 14: Average values of the CaCl₂-extractable Cu (µg kg⁻¹) for each amendment for the freshly contaminated soils. ± values indicate standard deviation, n=3.

Freshly Spiked Cu²⁺ (µg kg⁻¹) Values

Rossatz

	6 weeks	3yr + Cu	Cu + 3yr
CO	40.8 (±42.9)	97.2 (±41.6)	9.9 (±6.8)
K	19.8 (±12.1)	32.9 (±12.1)	6.4 (±1.0)
wBC	114.6 (±75.5)	72.5 (±15.5)	8.8 (±1.8)
K+wBC	24.2 (±15.8)	74.5 (±33.3)	8.8 (±3.0)
K+H₂O₂	13.0 (±1.8)	66.8 (±29.2)	7.4 (±2.3)
K+CA	4.7 (±4.3)	38.5 (±7.3)	6.2 (±3.2)

St. Stefan

	6 weeks	3yr + Cu	Cu + 3yr
CO	317.7 (±35.7)	319.8 (±16.3)	88.5 (±56.9)
K	21. (±22.4)	61.3 (±32.1)	5.3 (±0.6)
wBC	15.4 (±19.1)	518.1 (±177.8)	20.6 (±9.1)
K+wBC	9.5 (±5.9)	254.0 (±51.9)	142.8 (±26.1)
K+H₂O₂	64.5 (±32.7)	308.5 (±57.0)	31.1 (±26.2)
K+CA	10.0 (±9.2)	206.8 (±6.5)	17.1 (±6.1)
L	0.03 (±0.01)	0.5 (±0.2)	0.07 (±0.1)

Table 15: Average values of the Cu²⁺ (µg kg⁻¹) for each amendment for the freshly spiked soils. ± values indicate standard deviation, n=3.