Norwegian University of Life Sciences
Faculty of Social Sciences
Department of International Environment and
Development Studies

Master Thesis 2015
30 credits

Copper Immobilization in Contaminated Vineyard Soils Using Biochar: An Investigation of the Effects of pH and DOC

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M.Sc. Thesis

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2015

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Declaration

I, Stephen Bell, declare that this thesis is a result of my research investigations and findings. Sources of information other than my own have been acknowledged and a reference list has been appended. This work has not been previously submitted to any other university for award of any type of academic degree.

Stephen Bell

December 2015

Acknowledgments

The completion of this work would not have been possible if not for the generous support and guidance I received from so many wonderful people. From my time in Austria, I must give enormous thanks to my co-supervisor Walter Wenzel of BOKU and my two AIT colleagues Gerhard Soja and Vladimír Fršták. All three gentlemen were unending in their encouragement, thoughtfulness, and daily assistance. I would like to thank Walter for sharing his passion for scientific research and encouraging me to take on new challenges. I am grateful for his openness and kindness as a supervisor, professor, and mentor. Likewise, I am very thankful to Gerhard for providing everything I needed to succeed during my time at AIT. He not only ensured I was fully supported as a student researcher, but he also showed true concern for my professional development. I am grateful for his genuine and invaluable counsel. Vladimír Fršták truly went above and beyond as my daily source of laboratory and chemistry wisdom. Enough positive things cannot be said as to his character as a researcher, supervisor, tutor, and friend. He took on extra work whenever I needed help. I would like to thank Vladimír for being a great tutor, and an even better friend. From my time in Norway, I would like to give thanks to my Noragric supervisor Elisabeth Molteberg for providing useful support and advice. Elisabeth was always the upmost professional and the ideal supervisor to help me succeed during my time abroad.

I received valuable assistance from several people during the experimental stages of my research. At AIT, I would like to thank Marion Graser, Christian Mayer, and Barbora Micháleková Richveisová for providing me with much needed technical support and for making my experience in the laboratory even more fun. At BOKU, I would like to thank Veronika Slavik for helping with my DOC measurements and to Julia von Chamier for performing the chemical characterization of our biochar.

Lastly, I would like to thank my family who have always supported me and gave me the opportunity to follow my passions all the way across the ocean.

Preface

The research presented here fulfils the thesis requirement for the Master of Science degree in International Environmental Studies offered by the Department of International Environment and Development Studies (Noragric) of the Norwegian University of Life Sciences (NMBU). Research was conducted during the spring and summer of 2015 and was performed in the laboratories of the University of Natural Resources and Life Sciences, Vienna (BOKU) and the Austrian Institute of Technology (AIT). Materials and support was provided through the "Copper stabilization in vineyard soils" (KUSTAW) joint-project administered by BOKU in partnership with AIT. Operating until January 2017, the KUSTAW project is aimed at developing methods for reducing the bioavailability of copper and improving soil fertility and soil life in the topsoil layers of vineyards. The application of organic soil additives, such as biochar, are explored for their copper sorption capacities and soil amelioration potential. The research presented here represents a small contribution to the wider KUSTAW research scope.

Abstract

Decades of copper-based fungicide application to vineyard soils has resulted in elevated soil-Cu concentrations observed in wine producing regions globally. While grapevines, and the wine itself, do not inherit abnormal levels of Cu, ecotoxicological concerns regarding soil microbiology, vegetation cover, and watershed contamination still remain. Biochar has been shown to have a high sorption capacity for cations, such as Cu^{2+}, and is therefore a potential soil amendment worth investigating. The purpose of this study is to determine the effect of biochar amendment on Cu mobility in contaminated vineyard soils at varying levels of pH and DOC content. The literature is inconclusive, and generally lacking, as to the effectiveness of wood-derived biochar on Cu immobilization, especially at lower amendment rates. It is hypothesized that the liming effect of biochar and the high amount of binding sites it contributes to a soil will result in increased Cu immobilization. Additionally, immobilization is expected to be positively correlated to pH and negatively correlated to DOC concentration in soil solution. Three Cu-contaminated Austrian vineyard soils were amended with 3% (w/w) biochar (woodchips, 480˚C) and compared physically and chemically to non-amended samples. Laboratory batch sorption and desorption experiments were conducted and supernatant Cu concentrations were measured using atomic absorption spectroscopy. For all samples, biochar increased soil pH, moisture content, and surface area while it decreased readily soluble Cu. Copper sorption kinetics of all amended and non-amended soils followed a characteristic two stage process of rapid initial sorption, comprising the majority of sorption capacity, followed by a stage of gradually increasing sorption until equilibrium was reached. Copper sorption was best defined by the Freundlich isotherm model. Biochar had a positive influence on Cu immobilization in the most acidic of the three soils (pH 5.8), which can be attributed to the liming effect. Sorption capacities of all samples were brought to comparable levels when reaction pH was controlled. The positive effect of biochar amendment on Cu immobilization was no longer apparent at the pH range investigated once the liming effect was nullified. DOC batch sorption experiment results indicated a positive correlation of DOC and Cu immobilization, which could be a result of humic acid adsorption to soil surfaces. The desorption capacities of biochar amended and non-amended soils were almost identical and very minimal, indicating no negative effect of biochar addition. Before complete endorsement can be made for this type of biochar for use in field trials and later vineyard remediation, further research is recommended involving lower reaction pH, more elaborate DOC experiments, and additional subsequent desorption steps.

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List of Symbols and Abbreviations

µL	Microlitres
µm	Micrometers
µS cm^{-1}	Microsiemens per centimeter
AAS	Atomic absorption spectrometer
Al	Aluminium
Ca	Calcium
CEC	Cation exchange capacity
Cl	Chlorine
Co	Cobalt
Cu	Copper
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DW	Deionized water
EC	Electrical conductivity
Eh	Reduction potential
Fe	Iron
g kg^{-1}	Grams per kilogram
h	Hour
ha	Hectare
IUPAC	International Union of Pure and Applied Chemistry
Mg	Magnesium
mg g^{-1}	Milligrams per gram
mg L^{-1}	Milligrams per litre
min	Minutes
Mn	Manganese
mol L^{-1}	Moles per litre
Ni	Nickel
OM	Organic matter
Pb	Lead
R^2	Coefficient of determination
rpm	Revolutions per minute
SD	Standard deviation
SOM	Soil organic matter
t	Tonne
w/w	Weight per weight
Zn	Zinc

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1. Introduction

As a major part of the terrestrial environment and the biosphere by extension, soils are integral to human civilization. Soils and soil biota transfer nutrients up the terrestrial trophic chain while providing a medium for biomass degradation and recycling. Aside from the obvious agricultural and economic benefits humans can reap from productive and healthy soils, they also provide several other functions. Soils can filter, buffer, immobilize, and transform the various elements of an ecosystem. In some cases, this refers to the presence of heavy metals which, although they are minor soil components, play an important role in soil fertility. The contamination of soils with inorganic pollutants, such as heavy metals like copper (Cu), is therefore an important topic in environmental studies.

The widespread use and adoption of copper-based fungicides in European and global viticulture have led to Cu-contaminated soils in many wine producing regions. One such fungicide, known as the Bordeaux mixture ($Ca(OH)_2 + CuSO_4$), has been used since the mid- to late-19th Century to combat vine diseases. Today, elevated soil-Cu levels have been measured in vineyards across the world prompting a wide range of studies addressing the various environmental concerns associated with this issue. Topics such as plant uptake, ecotoxicity, soil microbial impacts, soil-Cu spatial distribution, soil-Cu mobility, and overall wine industry risk assessment have been explored. However, research has been steadily moving from problem identification towards remediation strategies.

The need for heavy metal remediation in soils has led to several practical approaches that address different parameters of a study site. Relevant parameters include soil type, future intended land use, potential environmental impacts, and contaminant type, concentration, and bioavailability. One strategy receiving increasing global attention is the use of biochar as a soil amendment for the immobilization of various organic and inorganic contaminants. Biochar is the solid product of heating biomass (>300˚C) in the absence of oxygen, a process known as pyrolysis. The resulting char-like substance is a natural material that is relatively inexpensive and easy to produce, making it particularly attractive for landscape remediation strategies. Biochar has unique properties that either interact with soil contaminants directly or improve the natural capacity of soils to mitigate contaminant effects. Both of these pathways address Cu contamination in vineyard soils. The purpose of amending vineyard soils with biochar would therefore be to immobilize the soil-Cu present at elevated levels from repeated annual fungicide application.

Research on soil-Cu immobilization by biochar has been inconclusive and lacking due to the wide-range of experimental variables. Luckily, laboratory batch experiments are a quick and relatively efficient way to identify and reduce research gaps. The ideal follow-up to these kinds of experiments are large-scale trials in the field. The ultimate goal, often as it in environmental research, is to provide relevant data for policy makers and landowners to guide decision making, and in this case to improve remediation strategies.

The research presented here is an assessment of the Cu immobilization potential of one type of biochar amended to Cu-contaminated vineyard soils collected from three active vineyards in Austria. To fully understand the influence of biochar amendment, experiments were conducted with varying contact times, initial Cu concentrations, pH, and DOC levels. The batch sorption experiments were used to produce Cu sorption isotherms for data analysis. Additionally, desorption experiments were performed to determine the ability of the biochar amended soils to retain previously sorbed Cu.

Biochar amendment for the remediation of Cu-contaminated vineyards is an intriguing solution to an environmental issue that can be seen in every wine producing region of the world. But before biochar can be endorsed as a suitable remediation technique in this respect, proper research and evidence must be presented. This problem is complex and involves several topics. Understanding the global story of Cu-fungicides and vineyard soil contamination is necessary, but it is not the starting point. At the root of this environmental issue is the fundamental ability of soils to sorb (take), hold, and desorb (release) contaminants in nature, and Cu in particular.

2. Sorption Mechanisms in Soils

Soils are natural and very complex heterogeneous mediums with both organic and inorganic components that blend to create unique physical, chemical, and biological properties. Soils are not simply just an assortment of unconsolidated materials, and their properties cannot be predicted based alone on the combined properties of the components. Within every soil is a solid phase (mineral-organic matrix), liquid phase (soil solution), and gaseous phase (soil air), which all contribute in forming the overall soil properties (Kabata-Pendias, 2011). The ability of soils to sorb, or take, metal ions from the liquid phase to the solid phase has particular importance for issues relating to agriculture, soil pollution remediation, and waste management to name a few.

Sorption is a term used to explain the often unclear mechanism by which the loss of a metal ion occurs from a liquid to solid phase. Bradl (2004) describes three main sorption processes: adsorption (the two-dimensional accumulation of matter at the solid/water interface), surface precipitation (the development of a new solid phase in three-dimensions), and absorption (fixation of a metal species into the solid phase). When the exact mechanism is difficult to identify, sorption is the preferred term. The material that accumulates at a surface is called the sorbate, while the solid surface on which the sorbate accumulates is known as the sorbent. Sorption is a very important part of soil chemistry as it can determine the quantity, bioavailability, and mobility of plant nutrients, trace metals, pesticides, fungicides, and other organic chemicals.

Adsorption consists of two major types of interaction between the sorbate (heavy metal) and sorbent (soil components): specific and non-specific adsorption. Specific adsorption involves stronger, more selective, and less reversible chemical reactions (i.e. inner-sphere complexation), while non-specific adsorption involves weaker, less selective, and more reversible chemical reactions (i.e. outer-sphere complexation) (Bradl, 2004). Also known as chemisorption, specific adsorption is the binding of heavy metal ions to soil surfaces, including organic matter and soil minerals of varying charges. Ions in soil solution form stable complexes with surface functional groups, such as inorganic hydroxyl groups or organic functional groups, reacting with OH-groups which are negatively charged at high pH (Bradl, 2004). As Bradl (2004) explains, these inner-sphere reactions between a metal cation (Me), such as copper (Cu^{2+}), and a surface functional group (S) can be described by the following equation:

$$\text{S-OH} + \text{Me}^{2+} + \text{H}_2\text{0} \leftrightarrow \text{S-O-MeOH}_2^{+} + \text{H}^{+} \quad (1)$$

Soils contain various mixtures of hydrous oxide minerals and organic matter (OM) which provide the surface OH-groups that are able to take up metal ions and release their protons (H^+) to the soil solution. As pH is roughly a measure of the concentration and activity of hydrogen ions, specific adsorption reactions to soil surfaces and functional groups are a function of pH.

Non-specific adsorption, on the other hand, is an outer-sphere electrostatic interaction by which ions from the soil solution are attracted, exchanged, and bound to charged soil surfaces. Also known as ion exchange, cations and anions are swapped between binding sites at the soil surfaces and the diffuse ion swarm of the soil solution, depending on the strength of charge and other factors. These covalent bonds are weak, easily reversible, and can be broken and formed rapidly. A visual representation of some of the theorized metal ion interactions with a biochar particle surface, similar to soil surfaces, is provided in Figure 6. The main parameters that regulate the sorption and desorption processes of heavy metals in soils include pH, Eh, cation exchange capacity (CEC), silt and clay fractions, soil organic matter (SOM), oxides and hydroxides (mainly Fe, Mn, and Al), and microorganisms (Kabata-Pendias, 2011).

3. Copper Behaviour in Soils

Heavy metals are generally considered as a group of elements with specific metallic characteristics (e.g. density, conductivity, stability as cations) and atomic numbers over 20 (Raskin et al., 1994). While this definition is sometimes extended to include other elements, a key feature of heavy metals is their potential toxicity in natural systems at low concentrations. A few examples of heavy metals include copper (Cu), lead (Pb), iron (Fe), and nickel (Ni), cobalt (Co), zinc (Zn), and manganese (Mn). Copper, with atomic number 29, is a transitional metal and therefore has more than one oxidation state. Cuprous (Cu^{1+}) and cupric (Cu^{2+}) ions are commonly found in nature, however cuprous is unstable in solution and either forms cupric ions and Cu containing compounds or precipitates into solid copper (Oorts, 2013).

Soil-Cu is most often in the form of $Cu(H_2O)_6^{2+}$ ions adsorbed to clay minerals or co-precipitated on other mineral and organic soil components. However, soil pH can influence copper speciation greatly resulting in forms such as Cu^{1+}, Cu^{2+}, $Cu(Cl_2)^-$, $CuSO_4$, $Cu(OH)_2$, $CuCO_3$, and CuCl (Oorts, 2013). In most soils however, copper is found predominantly as Cu hydroxides and carbonates. Again depending on pH, copper can occur in soil solution as Cu^{2+}, $CuOH^+$, $Cu_2(OH)_2^{2+}$, $Cu(OH)_3^-$, $Cu(OH)_4^{2-}$, and $Cu(CO_3)_2^{2-}$ (Kabata-Pendias, 2011).

Generally speaking, soil-Cu concentration is linked to soil texture, with lower concentrations in light sandy soils and higher concentrations in loamy soils due to their higher capacity for heavy metal retention (Kabata-Pendias, 2011). The largest factors determining initial soil-Cu concentration however are parent material, soil formation process, and anthropogenic inputs from mining or agriculture for example. The parameters that can govern initial soil-Cu content in mineral soils are illustrated in Figure 1 as relative explanation index (REI) values calculated in Kabata-Pendias (2011). These values represent statistically significant relationships between various soil parameters and Cu, based on the correlation coefficient matrices calculated from several hundred soil samples.

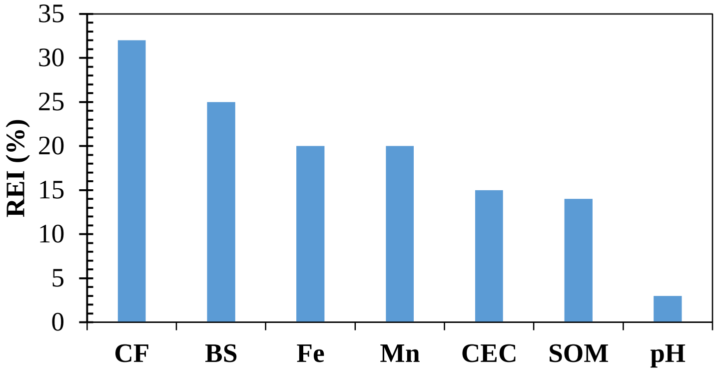


__Figure 1__ Relative explanation index (REI) of statistically significant relationships between Cu and various mineral soil parameters with 99% confidence. Soil parameters: CF (clay fraction), BS (base saturation), Fe (total iron content), Mn (total manganese content), CEC (cation exchange capacity), SOM (soil organic matter), pH (water pH from soil:water ratio 1:2.5) (Kabata-Pendias, 2011).

When interpreting such values, a distinction must be made between terms like initial soil-Cu (total) mentioned above, bioavailable soil-Cu, and readily soluble soil-Cu (leachable). In particular, the soil parameters featured here can have very different levels of significance in terms of soil-Cu mobility. The evolving factors that influence soil-Cu mobility and adsorption, as previously described above for heavy metals in general, include pH, oxidation and reduction potential, SOM, soil texture, soil mineral composition, temperature, soil hydrology, Cu type and speciation, heavy metal competition, and aging (Bradl, 2004, Kabata-Pendias, 2011). The behaviour, bioavailability, and toxicity of Cu are also determined to a larger extent by species (the molecular identity) than by total soil-Cu content.

Copper is well-known for its high affinity for soil organic matter (Temminghoff et al., 1997, Bradl, 2004). Cu^{2+} ions typically bind to inorganic and organic ligands when introduced in a soil, while binding to dissolved organic matter (DOM) in soil pore water (i.e. soil solution). Stable complexes form between Cu^{2+} ions and NH_2^-, SH^-, and OH^- groups in organic acids, keeping copper mobile in the soil solution (Oorts, 2013). In fact, in comparison with other trace metals as divalent ions, Cu^{2+} complexes with humic acids the strongest according to the following preference series: $Cu > Pb > Fe > Ni = Co = Zn > Mn = Ca$ (Adriano, 2001). Overall, humic and fulvic acids can be expected to form stable complexes with Cu in small concentrations leading to the conclusion that SOM plays an important role in modifying the interaction of Cu and inorganic soil components.

Copper ions interact with various soil components differently in terms of retention and release. Other than complexation with organic matter, Cu can be absorbed onto the surfaces of clays, Fe and Mn oxides, or exist in the lattice of primary silicate minerals or secondary minerals (e.g. carbonates, phosphates, sulphides) (Chaignon et al., 2003). Figure 2, which illustrates the adsorption of Cu to these soil components as a function of pH, shows not only the high Cu sorption preference for Mn oxide and soil organic matter but also the significance of pH on these interactions.

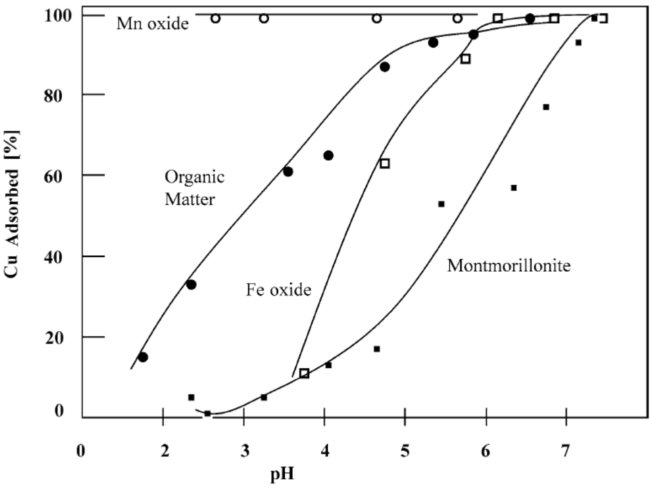


Figure 2 *Adsorption of Cu by different soil components as a function of pH (Bradl, 2004, redrawn after Adriano, 2001).*

The preferential order of Cu sorption affinity to these components from greatest to least are as follows: Mn-(hydr)oxides > SOM > Fe-(hydr)oxides > clay minerals (Bradl, 2004). However, soil organic matter (SOM) is the dominant factor in retaining soil-Cu as most dissolved Cu is complexed with DOM. In fact, several studies has found evidence of very high Cu-DOM complexation, often reaching 99% of total dissolved Cu in soil solution depending on pH (Sauve et al., 1997, Temminghoff et al., 1997, Ponizovsky et al., 2006).

Copper precipitation is limited under most soil conditions, leaving sorption processes as a more determining factor on Cu concentration in solution. Precipitation of Cu becomes relevant if there is a lack of ligands other than –OH for complexation, but it also depends on initial Cu concentration and pH. At or above pH 7-8 Cu solubility is very low, partly as a result of amorphous $Cu(OH)_2$ and tenorite (CuO) precipitation (Kabata-Pendias, 2011, Oorts, 2013). Indeed, sorption of divalent transition and heavy metal ions, such as Cu^{2+}, is very pH-dependent and is characterized by a narrow pH range called the sorption edge where sorption increases from very low amounts to almost 100% (Sparks, 2003). As pH increases, so do the negatively charged surface sites available for cations to interact with, which reduces cations in soil solution and therefore cation mobility and concentration. This increase in binding sites is a result of the reduced competition from H^+ ions. The opposite effect occurs as pH decreases with increased competition from the increased quantity of H^+ ions in solution. Most change in sorption or desorption occurs in the pH range of the sorption edge, which is in turn dependant on other variables such as the sorbent characteristics. The sorption edge of Cu onto a sediment composite (consisting essentially of Al-, Fe-, and Si-oxides) and onto humic acid (a form of dissolved organic carbon (DOC)) can be seen in Figure 3 and Figure 4 respectively. In both cases, adsorption nears 100% by pH 7.

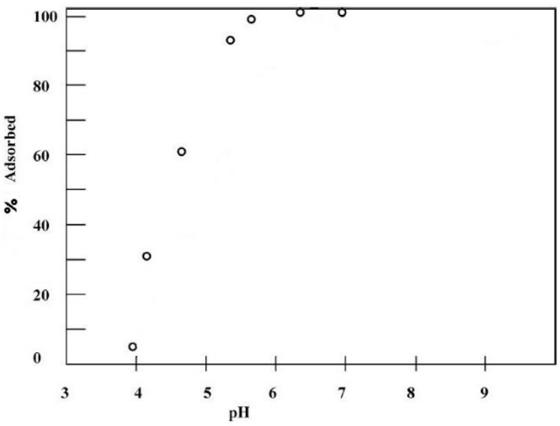


Figure 3 *Cu adsorption onto a sediment composite in 10^{-3} M $NaNO_3$ as a function of pH (Bradl, 2004, redrawn after (Apak, 2002)).*

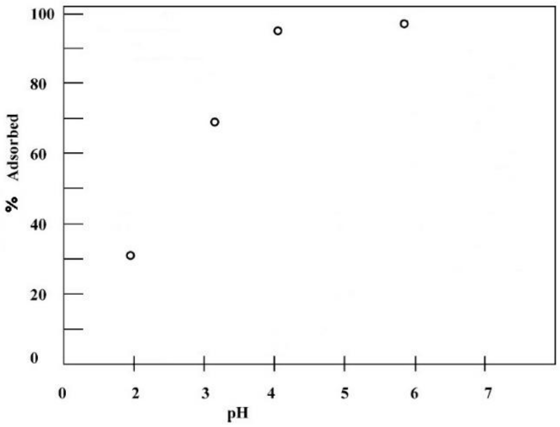


Figure 4 *Cu adsorption onto humic acid as a function of pH (Bradl, 2004, redrawn after (Kerndorff and Schnitzer, 1980)).*

This is also illustrated in Figure 2, where the Cu adsorption trend lines for each of the featured soil constituents converge to almost total adsorption again by pH 7. While heavy metals behave differently at different pH levels in terms of adsorption to sorbents like oxides or humic acid, most if not all follow the same basic trend of increasing adsorption with increasing pH. Similar to what can be seen in Figure 4, another study found increasing adsorption of Cu onto humic acid with increasing pH plateauing at nearly 100% at pH 7-9 but dropping sharply from pH 9-11 due to the dissolution of OM and subsequent soluble Cu-OM complexes (Jordão et al., 2001).

4. Copper Contamination in Vineyard Soils

Since the mid- to late-19th Century, application of copper-based fungicides was commonplace in European viticultural practices. In particular, the famous Bordeaux mixture ($Ca(OH)_2$ + $CuSO_4$) became widely adopted in France, and later elsewhere, to combat the spread of vine pathogenic fungi (Brun et al., 2001). Vine downy mildew (*Plamopara viticola*) is a prime example of an annual fugal disease that plagues grapevines. Others include grey mould (*Botrytis cinerea*) and back spot (*Diplocarpon rosae*). First discovered in Southwestern France in 1878, downy mildew quickly spread to other European vineyard regions leading to increased demand for fungicides such as the Bordeaux mixture (Brun et al., 2001). Other copper-based compounds used for similar purposes include $3Cu(OH)_2 \cdot CuCl_2$, $CuSO_4 \cdot 3Cu(OH)_2$, and Cu_2O (Komárek et al., 2010).

Best management practices in viticulture promote the regular application of fungicides during the entire growing season. In Australia, this is a common preventative management strategy regardless of whether fungal infection is present or not (McConnell et al., 2003). Today, European organic viticulture restricts the use of synthetic organic fungicides. However, some invaluable copper-based fungicides, ones that have not been deemed to toxic for vineyard workers by 21st Century standards, are allowed in minimized quantities (8 kg Cu ha^{-1} during first four years of vine cultivation, later capped at 6 kg Cu ha^{-1}) (EC, 2002). Depending on the plowing habits of individual famers, even an application rate at this level would result in 250-750 mg Cu kg^{-1} in topsoils after roughly 150 years of application (a time period since the introduction of the Bordeaux mixture for example), which is above the estimated EU predicted no effect concentration (PNEC) of soil-Cu (20-200 mg kg^{-1} depending on soil properties) (Ruyters et al., 2013). Typical application rates vary from 2 to 4 kg Cu ha^{-1} $year^{-1}$ (Komárek et al., 2010).

Copper from fungicides usually enters the soil matrix of vineyards due to wash-off from treated vine foliage during precipitation, but can also occur as a result of inaccurate spraying, spillage, or during natural leaf, vine, and other treated biomass accumulation onto the topsoil (Paradelo et al., 2008). In the IUPAC's database of pesticides and agrochemical properties, copper-based fungicides are compared to four other major classes of fungicides: phthalimide, dithiocarbamate, triazole, and strobilurin. In terms of environmental risk assessment, summarized in Table 1, copper-based fungicides are a less ideal choice than other synthetic organic fungicides.

Table 1 *Summary of the behaviour and toxicity of copper-based fungicides (IUPAC, 2010).*

Concern	Copper-based	Phthalimide	Dithiocarbamate	Triazole	Strobilurin
Mobility in soil	Low	Low	Low to medium	Low to medium	Low
Persistence in soils	High	Low	Low	Moderate to high	Low to moderate
Toxicity to earthworms	Low to moderate	Moderate	Low to moderate	Low to moderate	Low to moderate
Toxicity to aquatic invertebrates	High	Moderate	Low	Low	Low to moderate
Toxicity to fish	Moderate	Moderate	Low to moderate	Low	Low to moderate
Toxicity to aquatic primary producers	Moderate	Low to moderate	Low	Low	Low

As Wightwick et al. (2013b) suggests, due to the high persistence in soil of copper-based fungicides, switching to more use of strobilurins (e.g. trifloxystrobin), dithiocarbamates (e.g. mancozeb), and phthalimides (e.g. captan) should result in reduced risks to soil and aquatic organisms. Aside from vineyards, copper-based fungicides are also used on a wide variety of crops such as coffee, hops, apples, avocadoes, tomatoes, potatoes, and several other vegetables (Komárek et al., 2010).

Predictably, decades of repeated and steady application of such fungicides to grapevines in vineyards across Europe, Australia, and other global wine producing regions, have resulted in increased soil-Cu levels which raises several ecotoxicological concerns (Komárek et al., 2008, Komárek et al., 2010, Wightwick et al., 2010). Background soil-Cu levels in uncontaminated soils are mainly determined by parent material and usually range from 5 to 50 mg kg^{-1} dry weight in land considered arable, with some natural soils exhibiting concentrations above 100 mg kg^{-1} (Adriano, 2001, Oorts, 2013). However, the concentrations in fungicide treated wine producing regions have been commonly found to range between 200 to 500 mg Cu kg^{-1} (Brun et al., 2001). Concentrations reaching 1500 mg Cu kg^{-1} have been observed in France (Flores-Vélez et al., 1996) and some exceeding 3000 mg Cu kg^{-1} have been recorded in Brazilian vineyard soils (Mirlean et al., 2007). The soil-Cu concentrations cited here from various sources are expressed as milligrams of Cu per kilogram of dry soil (mg Cu kg^{-1}), usually according to aqua regia digestions (which give "near-total" values) or similar methods followed by inductively coupled plasma spectrometry (ICP) or atomic absorption spectrometry (AAS) analysis.

Concentrations of copper in vineyard soils observed at these high levels have warranted a wide range of studies exploring topics such as plant uptake (Brun et al., 1998, Brun et al., 2001, Chaignon et al., 2003, Toselli et al., 2009), general toxicity and soil microbial impacts (Komárek et al., 2010, Ruyters et al., 2013, Wightwick et al., 2013a), soil-Cu distribution and mobility (Fernández-Calviño et al., 2008, Mirlean et al., 2009, Fernández-Calviño et al., 2013), and overall wine industry risk assessment (Wightwick et al., 2013b). However, research focus has been steadily moving beyond problem identification and towards remediation techniques (Pietrzak and Uren, 2011, Mackie et al., 2012, Navel and Martins, 2014).

Due to the widespread adoption and use of copper-based fungicides in viticulture, the problem of soil contamination is not expected to subside. This becomes increasingly important as wine producing regions, which are exceptionally sensitive to climate, fluctuate geographically with global climate change. Trends already indicate a clear temperature increase, more so over land than water and with greater warming at high latitudes particularly in the Northern Hemisphere (IPCC, 2013). Recent studies project a decrease in viticulture suitability in traditional regions (e.g. Tuscany, Bordeaux, Rhône Valley) while an increase in suitable land is expected in the more northern regions of North America and Europe and regions of higher elevation (Hannah et al., 2013). Northern Europe, New Zealand, and Western North America can expect increases by 99, 168, and 231 percent respectively in land area suitable for viticulture from the current average (1961-2000) to predicted average (2041-2060) (Hannah et al., 2013).

New ecosystems and their soils will undoubtedly be introduced to the standard range of vineyard best management practices, including annual copper-based fungicide application. Contaminated lands converted from viticulture to other forms of agriculture, as a result of climate change or not, raise issues regarding crop health and even human health. In an assessment of the Australian viticulture industry, Wightwick et al. (2013b) found indications that copper-based fungicide application might have negative effects on vineyard lands that are transitioned to sustainable agriculture, but further research is required linking laboratory and field findings to determine real implications to crop health. Far from Australia, Komárek et al (2008) measured Cu concentrations of vineyard soils from small-scale wine producers in the Czech Republic. Most of the vineyard soils analyzed exceeded federally set limits for Cu in agricultural soils (60-100 mg kg^{-1} depending on soil classification, Ministry of the Environment of the Czech Republic) and the warning limits set by the EU (50 mg kg^{-1}). However, the highest Cu concentrations measured were actually in a non-active vineyard. The authors suggested that abandoned vineyard needed to be investigated for soil-Cu contamination before transitioning the land to other forms of agriculture.

In terms of vineyard hydrology and its relationship to local watersheds, soil-Cu contamination as a result of fungicide application is considered a form of non-point source pollution because it cannot be traced back to a single discrete source, such as a pipe. Instead, its origins are diffuse. The issue of Cu being transferred through vineyard soils via soil solution and ground water flow to nearby bodies of water is receiving increasing attention. A few studies have appeared in recent years specifically investigating pesticide and fungicide residue detection within vineyard watersheds following runoff events (Gregoire et al., 2010, Rabiet et al., 2010). Bereswill et al. (2012) assessed copper fungicide exposure in streams of the Palatinate wine region in south-west Germany. They not only found that Cu concentrations in stream waters and sediments were significantly correlated with fungicide application frequency and rate, but that they were also likely to cause ecotoxicological effects in the field. Copper entry into streams was due to surface runoff and the development of erosion rills on vineyard slopes.

While Cu is an essential trace metal for the human body, required for many enzymatic reactions, it does not decompose biologically and can therefore prove harmful when present in high doses (Hakeem et al., 2014). Even though privately connected copper piping is considered the main source of public drinking water contamination, it is possible that water from wells located near contaminated vineyards could contain elevated Cu levels as well (Komárek et al., 2010). Copper is absorbed primarily by the gastrointestinal tract, with some amounts passing to the liver. A study exploring the acute effects of Cu in drinking water on human health found that concentrations of 10 mg L^{-1} can modify the first phase of gastric emptying, resulting in nausea among adult test volunteers (Araya et al., 2003). Copper inhalation can also prove dangerous, especially for viticulture workers using copper-based fungicides. Santić et al. (2005) compared the risk of developing lung carcinoma by professional vineyard fungicide sprayers to a control group. The authors found that the yearly inhalation of particles from Bordeaux mixture fungicide sprays resulted in a mortality risk three times greater for vineyard sprayers.

For plants, Cu is an essential trace element as well, serving an important role in physiological processes such as photosynthesis and respiration, carbohydrate and nitrate metabolisms, water permeability, reproduction, and disease resistance (Kabata-Pendias, 2011). Copper deficiencies are therefore detrimental to the health of many plants and important Cu-sensitive crops such as wheat, oats, and sunflowers. However, elevated Cu concentrations can also be highly phytotoxic despite the general tolerance of most plants species. A summary by Kabata-Pendias (2011) on the negative effects of excessive Cu^{1+} and Cu^{2+} cations in plants highlights tissue damage, membrane permeability increase (resulting in nutrient leakage), inhibition of electron transport for photosynthesis, immobilization of Cu in cell walls, vacuoles, and protein complexes, and DNA damage (resulting in inhibited photosynthesis). Specific research on excess Cu in plants identifies Cu-chlorophyll complexes as potentially damaging to photosynthetic functions, while altered membrane permeability is caused by decreased root phospholipid (and associated compounds) levels (Chaffai et al., 2007, Zvezdanovic, 2007). As always, soil pH can be a very important factor in determining the bioavailability and toxicity of Cu to plants.

It is apparent that the remediation of Cu-contaminated vineyard soils is important for several reasons. As a heavy metal, Cu is non-biodegradable and can ultimately bioaccumulate in organisms if Cu containing compounds are taken up at a greater rate than they are metabolized or excreted. When considering the additional pressures presented by vineyard land use change, as a result of increasing climate change or other reasons, there is a clear temporal factor to this issue. Fortunately, soil remediation strategies and techniques are continuously being developed and improved to address important topics such as the need for long term effective Cu immobilization in vineyard soils.

5. Approaches to Soil Remediation

The issue of contaminated vineyard soils can benefit from decades of research on the remediation of soils impacted with Cu from mining, urbanization, waste management, and pesticide and fungicide use for other kinds of agriculture (Lado et al., 2008). Globally, the need for soil heavy metal remediation has led to several practical approaches. Each approach addresses different parameters of a contaminated site such as soil type, future intended land use, potential environmental impacts, and contaminant type, concentration, and bioavailability to name a few. However, due to the complex nature of soils and usually the presence of multiple active contaminants, remediation strategies can be difficult and expensive (Sparks, 1993). Often more than one strategy must be used to optimize the effects.

Soil remediation strategies can be *in situ* (i.e. on-site) or non-*in situ*. Non-*in situ* implies the removal of soil by excavation to be then treated on-site or elsewhere entirely. Some of these kinds of methods include soil spreading (excavated soils are treated and then dispersed), thermal treatment (high temperatures to break down contaminants), asphalt incorporation (immobilization in asphalt), solidification (encapsulating contaminants with additives and then landfilling), chemical extraction (cleaned with solvent/surfactant mixture), and simple excavation and landfilling (Sparks, 2003). It is generally understood that non-*in situ* methods are less idea than *in-situ* methods because they are costly, time consuming, much more invasive, and they raise concerns over contaminant exposure during excavation and transportation (Cui and Zhang, 2004). As well, non-*in situ* methods are not practical at a large scale or for sites with only slight contamination. *In situ* methods include soil amending, valorization (use of air flow and soil amendment, limited to volatile organic compound (VOC) materials), biodegradation (stimulated microbial activity), phytoremediation (use of plants), leaching (flow of water and surfactant to leach out contaminants), vitrification (immobilization through electric current), isolation (installation of subsurface barriers such as clay liners), and simple passive monitoring (Sparks, 2003).

The addition of soil amendments is a heavily studied *in situ* remediation strategy that aims to reduce contaminant mobility and bioavailability so waterways and organisms are unaffected. Organic materials are the most ideal choice of soil additives as they relatively cheap and easy to attain. They also require minimal pre-treatment and have minimal impacts because they are organic. Other benefits of using such additives is that there is often a surplus of organic residues, in agriculture for example, which can be put to convenient use. Amendments that are rich in carbon have been identified as suitable materials for contaminant immobilization and therefore risk reduction. In particular, recent research has concentrated heavily on biochar as the amendment of choice for organic and inorganic contamination management in soils (Beesley et al., 2010, Beesley et al., 2011, Zhang et al., 2013, Ahmad et al., 2014).

6. Biochar

Many definitions of biochar circulate between the academic, political, and public spheres due to its infancy as a scientific term. However, most definitions address the production conditions, source material, and its purpose as a soil amendment when describing biochar. In essence, biochar is the solid product of heating biomass (>300˚C) in the total or near-total absence of oxygen, a process called pyrolysis but known more simply as charring (Lehmann and Joseph, 2015). The best way to distinguish biochar from charcoal or other related carbon products is that it is designed and intended for environmental management, such as soil remediation and amelioration. The International Biochar Initiative (IBI), an organization formed in 2006 to promote biochar research, cooperation, and safe use for soil fertility and climate change mitigation, defines biochar as:

> A solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment. Biochar can be used as a product itself or as an ingredient within a blended product, with a range of applications as an agent for soil improvement, improved resource use efficiency, remediation and/or protection against a particular environmental pollution and as an avenue for greenhouse gas (GHG) mitigation. (2014)

While biochar is a relatively new subject of scientific inquiry and public interest, there is evidence that it has been used as a soil amendment for centuries by the ancient Amerindian populations of the Amazon basin. Amazonian Dark Earths, also known as the Terra Preta de Indio, are extensive carbon-rich patches of dark Amazon soil that contain biochar-like material. The plots are typically 40-60 cm deep and range in size from one to several hundred hectares, with the oldest sites dating back 2000 years (Mann, 2002). Since the realization that these soils were actually anthropogenic, theories have abounded crediting the development of advanced civilizations in the region to the use of these charred materials for soil improvement. Long lasting fertile soils in the Amazon are, in all senses of the term, an El Dorado. The exact intentions of the first biochar producers is still a matter of debate for some, but the fact remains that these carbon rich dark soils, with their sustained fertility, are a stark contrast to the typically acidic and agriculturally problematic rainforest soils.

The editors of the first comprehensive text on biochar knowledge, Professors Johannes Lehmann of Cornell University and Stephen Joseph of the University of New South Wales, believe that the recent increase in biochar research over the last two decades can be attributed to the discovery that the high organic carbon content and fertility of the Amazonian Dark Earths are linked to the biochar-like materials that they largely comprise of (Lehmann and Joseph, 2015). This can explain the widespread public interest and fascination about biochar. It represents one of the most popular sentiments about ancient cultures and their relationships with nature, namely the notion of indigenous wisdoms rediscovered. As Lehmann and Joseph also point out, biochar is not just another soil amendment like compost or manure aimed at improving soil properties. It is fundamentally more effective and longer lasting, due to its specific physical and chemical properties.

6.1. Physical and Chemical Properties

It must be acknowledged that describing biochar from a physical and chemical point of view, rather than from a production point a view, is much more difficult to do due to the wide range of possible feedstocks and pyrolysis conditions. However, generalities can be made from the most commonly studied types of biochar and these serve as the reference points for tweaking production processes to create the most fitting product for any giving purpose. It is also important to continue studying biochar properties and their behaviour in soils as research into environmental applications increase.

The pyrolysis process results in a several thousand fold increase in surface area as the feedstock becomes charred (Thies and Rillig, 2009). Porosity is a major feature of biochar that makes it attractive as a soil amendment. In particular, macropores in biochar particles serve to aerate soils, improve soil hydrology, provide channels for roots and root hair development, and provide soil microbial habitats (Lehmann and Joseph, 2015). Pore structures typically form in groups instead of along a uniform continuum and can range in diameter from tens of nanometers to up to hundreds of micrometers, depending on pyrolysis conditions and feedstock. Macropores can be found both within and on the surface of biochar particles and can be large in comparison to the smallest categories of soil particles: fine sand (0.1-0.25 mm), very fine sand (0.05-0.1 mm), silt (0.002-0.05 mm), and clay (less than 0.002 mm). Figure 5 illustrates the pore structure of a wood-derived biochar.

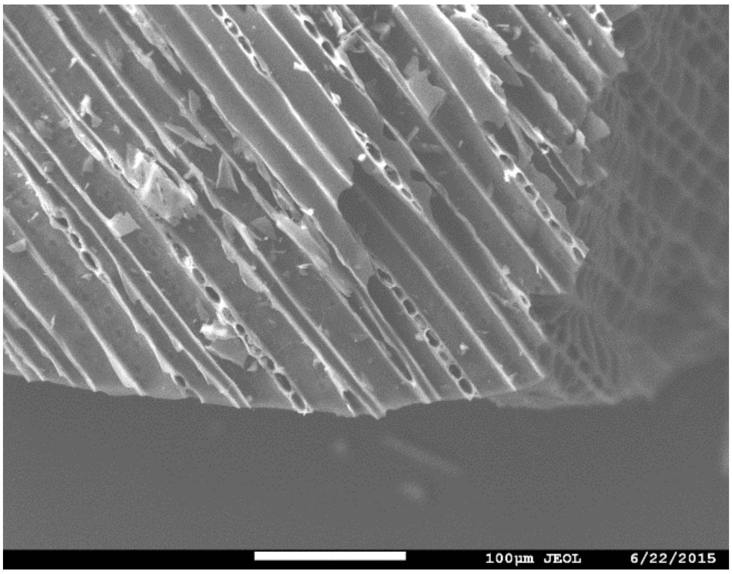


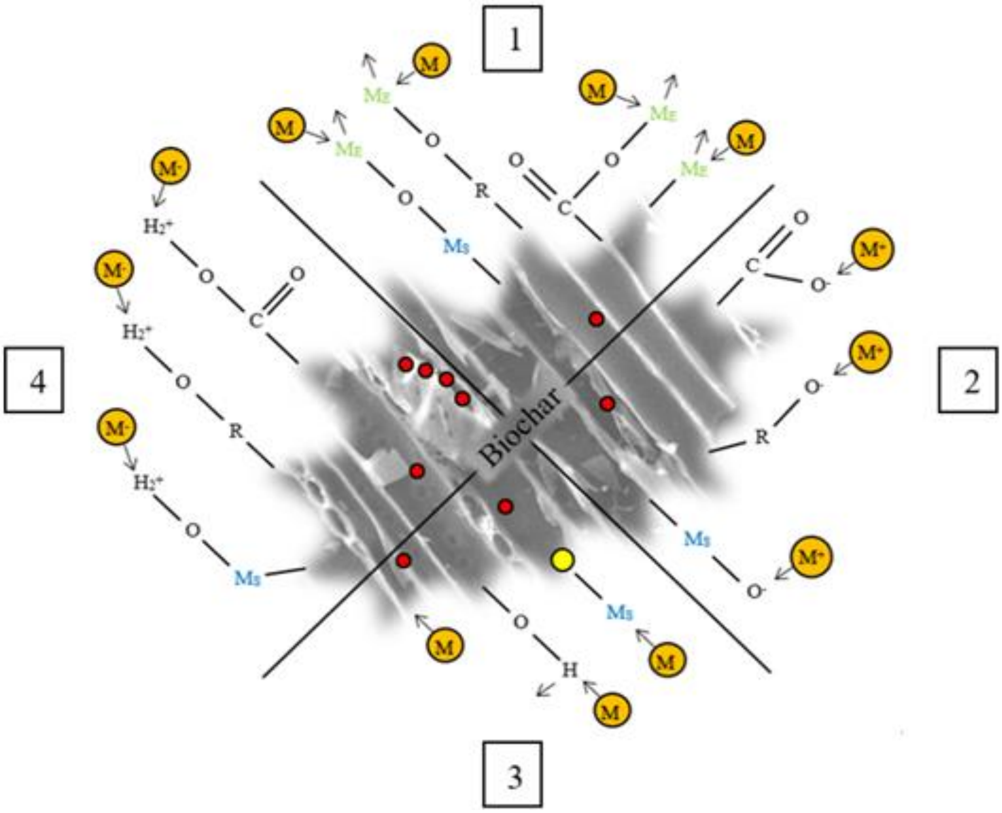
Figure 5 *Biochar (woodchips, 500˚C) particle magnified 250X with 100µm scale provided at the bottom. Macropores can be seen within the irregular patterning of the pyrolyzed wood fibres (photo: Libor Duriska, 2015).*

There is a relationship between pyrolysis temperature and several important biochar properties, aside from porosity. Mendez et al. (2013) examined sewage sludge and pruning waste-derived biochars and found that as treatment temperature increased from 400-600˚C so did pH, surface area, porosity, and total metal concentration for Cu, Ni, Zn, Cd, and Pb, while electrical conductivity (EC) and cation exchange capacity (CEC) decreased, especially closer to 600˚C. Biochar particle size distribution is also largely dependent on feedstock material and the pyrolysis conditions, with expected shrinkage and attrition to occur during heat treatment and post-treatment steps (Lehmann and Joseph, 2015).

Biochar is comprised mainly of stable aromatic forms of organic carbon and has the potential to function as a soil conditioner because of its high organic carbon content. These aromatic forms are in the shape of rings usually with six carbon (C) atoms, and without many oxygen (O) or hydrogen (O) atoms which are more common in organic matter (Lehmann and Joseph, 2015). Depending on feedstock, biochar has been found to contain upwards of 90% organic carbon (Chan and Xu, 2009). Soil organic matter (SOM), which can also be used as an effective soil amendment, releases immobilized contaminants as it decomposes (Hakeem et al., 2014). Biochar on the other hand, last much longer in soils due to being very chemically and biologically recalcitrant in nature and is likely to have more stable interactions with metal ions and retain them longer (Namgay et al., 2010, Cross and Sohi, 2011). Biochar pH is determined by feedstock and also pyrolysis temperature, due to the loss of acidic functional groups and the increase in ash content (Wu et al., 2012, Lehmann and Joseph, 2015). Generally, biochar has neutral to high pH and creates a liming effect in acidic soils, the extent to which depends on its acid neutralizing capacity.

6.2. Heavy Metal Sorption

The physical and chemical properties of biochar greatly influence its sorption capacity for both organic and inorganic contaminants. In general, biochar pyrolyzed at higher temperatures have increased porosity and surface area, which is better suited to organic contaminant sorption. Lower pyrolysis temperatures produce biochar with more O-containing functional groups and a higher cation exchange capacity, both advantageous for inorganic contaminant sorption (Ahmad et al., 2014). While surface area and porosity are relevant, the dominant mechanisms for inorganic contaminant and heavy metal sorption to biochar are ion-exchange, electrostatic attraction, and precipitation. This is where the liming effect of biochar becomes a major factor because higher pH increases the electrostatic attraction between soil and biochar particle surfaces and cations (Sposito, 1989). As shown in Figure 6, cations such as Cu^{2+} can bind to biochar via multiple pathways through various mechanism.



M_S	Metal attached to surface
M_E	Exchangeable metal ions
●	Physical adsorption
○	Ions in biochar
M	Metal ions (+/-)

Ion exchange	1
Cationic metal attraction	2
Precipitation	3
Anionic metal attraction	4

Figure 6 *Theorized mechanisms of biochar interactions with inorganic contaminants such as heavy metals (redrawn after Ahmad et al., 2014).*

Copper can be exchanged with readily leachable cations that are known to be present in biochar and certainly in soils (1), such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}). When these elements are exchanged with cations from the diffuse ion swarm, co-precipitation or inner-sphere complexation with complexed humic matter and mineral oxides in the biochar can occur (Zhang et al., 2013). Surface complexation of heavy metals with O-containing functional groups (2) and surface precipitation (3) are also possible.

In field conditions, it is difficult to predict all the parameters that could determine which mechanism of biochar sorption is dominant. Even when a biochar type is standardized and controlled before amendment in a contaminated soil, the cations already present in the soil and their concentrations will complicate remediation.

7. Biochar and Vineyard Soils

When considering economic factors, biochar has the same or greater sorption efficiency for some contaminants than other endorsed soil amendments such as activated carbon (Ahmad et al., 2014). This is because it can be produced in a more cost-effective way than activated carbon and it uses waste resources like agricultural residues. Unlike biochar, activated carbon is a material that has been treated in various ways, such as steam/chemical treatment at high temperatures, to increase surface area, adsorption capacity, and filtration ability (Lehmann and Joseph, 2015). According to McCarl et al (2009), a good estimation of the break-even price of biochar is around \$245 t^{-1} USD, approximately one sixth of the cost of commercially available activated carbon (~\$1500 t^{-1} USD). Additionally, biomass waste conversion to biochar is a convenient solution for safe and effective disposal of many materials. Even environmentally hazardous materials can be neutralized through conversion, such as the removal of active pathogens from solid wastes like animal litter or sewage sludge (Ahmad et al., 2014).

Biochar acquisition for vineyard remediation is a question of regional availability, considering biochar regional policy, costs of purchase, and product transportation. However, like many forms of agriculture, viticulture already has access to large amounts of potential feedstock, namely vine residues. A recent study of biochar production on Spanish vineyards revealed promising results regarding CO_2 sequestration. Using a mobile and self-sustaining pyrolysis reactor, authors Rosas et al (2015) were able to convert ripped vine wood to suitable biochar (550˚) at a production yield of 25-35% depending on pyrolysis conditions. The biochar met heavy metal content standards required in Spanish legislation for class B and C fertilizers and was below similar limits set by the International Biochar Initiative for Europe and internationally. The use of an on-site mobile reactor can also reduce costs and greenhouse gas emissions from biomass and biochar transportation, leading to a potential reduction of ~18 g of CO_{2eq} per bottle of wine (Rosas et al., 2015). The authors concluded that producing biochar in this manner and applying it to vineyard soils, as opposed to burning the excess biomass, can lead to significant reductions in the carbon footprint of global viticulture, resulting in a more socially and environmentally responsible industry.

Aside from use as a source carbon sequestration in viticulture practices, biochar is capable of also significantly improving vineyard hydrology and production. Genesio et al (2015) applied biochar (orchard pruning, 500˚C) to a non-irrigated vineyard in the Tuscany region of central Italy and found increased grape yield in amended plots without an impact on grape quality. The increase in yield was also inversely correlated with rainfall, supporting the use of biochar as an adaptation method to reduce water shortage impacts on production. While biochar is multi-faceted in its potential as a tool for environmental management, by improving crop production, regulating landscape hydrology, reducing carbon footprints, and even improving waste management, its interaction with soil contaminants is also notable.

The influence of biochar on soil pH is a major factor on its suitability as a remediation tool in contaminated vineyard soils. By creating a liming effect in soils, biochar can reduce Cu bioavailability. However, this is a reversible effect on pH, and therefore continuous land management is sometimes required to prevent the re-acidification of a soil and subsequent release of available Cu (Oorts, 2013). In a study of pH-dependent mobilization of cadmium, zinc, and lead, Houben et al (2013) found that biochar amended at 5% and 10% (w/w) rates reduced metal bioavailability. The authors concluded that biochar is a feasible *in situ* soil remediation technique for the immobilization of metals, but soil pH must be monitored to avoid unwanted re-acidification and release of contaminants.

Biochar is an interesting potential soil amendment for them remediation of various organic and inorganic contaminants introduced into natural ecosystems due to human activities. As a natural material that is relatively inexpensive and easy to produce, biochar is particularly attractive for sustainable landscape remediation, such as Cu-contaminated vineyards. Despite the increase in biochar focused research of late, many questions remain as to the effectiveness and suitability of each biochar type for the required task. The parameters surrounding a contaminated soil remediation strategy must be identified and supported with evidence before biochar policy recommendations can be made and final strategies implemented in the field by landowners. Laboratory based batch experiments are a common starting point when investigating a specific soil amendment type, such as a wood-derived biochar, and its capacity to influence a specific soil parameter, such as elevated soil-Cu. Further research naturally following in this progression would entail field studies with longer timespans to understand the impacts of aging as well as overall ecosystem implications. Relatively few studies have specifically explored biochar as a soil amendment for the remediation of Cu-contaminated vineyards soils. Therefore, an appropriate starting point for this topic is laboratory batch experiments, using common industry biochar and soil samples from active vineyards with varying levels of Cu contamination.

8. Research Objectives

The purpose of this study is to determine the effect of biochar amendment on Cu mobility in contaminated vineyard soils at varying levels of pH and DOC content. The literature is inconclusive, and generally lacking, as to the effectiveness of wood-derived biochar on Cu immobilization, especially at lower amendment rates (Beesley et al., 2010). Also, Cu in soils is known to have a strong affinity for organic matter complexation (Beesley and Dickinson, 2011) and becomes more mobile with decreasing pH (Temminghoff et al., 1997). It is hypothesized that the liming effect of biochar and the high amount of binding sites it contributes to a soil will result in increased Cu immobilization in amended soils compared to non-amended soils. The liming effect is expected to be the dominant short-term effect of biochar soil amendment on Cu sorption. Immobilization should be positively correlated to pH and negatively correlated to DOC concentration in soil solution.

Primary objectives are as follows:

- Determine Cu immobilization in biochar amended and non-amended soils through sorption and desorption batch experiments, exploring the importance of pH and DOC
- Determine the effect of contact time and initial concentration for Cu sorption in biochar amended and non-amended soils
- Investigate the influence of biochar amendment on soil solution pH
- Determine suitability of biochar for vineyard soil-Cu remediation at field-scale

9. Materials and Methods

9.1. Sample preparation

Three soils, named in this paper Rossatz (R), Harm (H), and Stroh (S) based on various identifiers, with elevated total Cu concentrations (201-342 mg kg^{-1}) were used for analysis. The soils were previously recovered from three sites in wine producing regions in the Austrian states of Lower Austria and Styria (Figure 7). Quartzite sand (QS) (0.1-0.3 mm, fire dried) was used as a control. All samples were sieved to less than 2 mm. Commercial woodchips biochar (BC) (Sohnenerde GmbH, Riedlingsdorf, Austria) pyrolyzed at 480˚C was sieved to 0.5-1 mm fraction size (Figure 8).

Figure 7 *Locations of Rossatz (R) and Harm (H) vineyards in Lower Austria, and Stroh (S) vineyard in Styria.*

The water holding capacities (WHC) of each of the three soils and the quartzite sand were determined following a basic sand-bath experiment and calculations. Dry weights were measured and moisture content calculated after oven drying samples and biochar at 105˚C for 24 h. Two pots were prepared with 100 g (dry weight) of each soil and quartzite sand. In one of the two pots for each sample, 3 g (dry weight) of biochar was added and mixed thoroughly to represent a 3% (w/w) amendment rate which corresponds to 90 t ha^{-1} incorporated at 0.2 m deep. The other pots without biochar represent non-amendment. With 100% WHC weight known from the sand-bath experiment, deionized water (DW) with a resistivity of 0.05 µS cm^{-1} (Synergy UV, Millipore) was added to all of the eight pots until 65% WHC weight was reached. The pots were then lightly covered with a thin film and then placed in a greenhouse for two weeks under typical greenhouse conditions for sample incubation. After incubation and later air drying, the samples were removed from the pots and sieved once more to 2 mm in preparation for experiments (Figure 9).

__Figure 8__ Woodchips biochar, pyrolysis at 480˚C.

__Figure 9__ Vineyard soils used for experiments with 3% (w/w) biochar amended samples directly below (from left to right: Sand, Rossatz, Harm, and Stroh).

9.2. Sample Physical and Chemical Characterization

9.2.1. Moisture Content

Samples were measured to 2 g and placed in an oven for 24 h at 105˚C. Moisture content was calculated following standard procedure.

9.2.2. pH

Samples were measured to 1 g and put in a centrifuge tube (50 mL, Nalgene) (Figure 10) along with 5 mL of 0.01 mol L^{-1} $CaCl_2$ solution (diluted with DW) and placed on a shaker for 24 h of end-over-end shaking at 150 rpm. All samples were prepared in triplicate. After shaking, sample tubes were centrifuged (5 min at 3500 rpm) and then the supernatant pH was measured using a benchtop pH meter (inoLab pH Level 2P, Weilheim, Germany).

__Figure 10__ Typical batch of samples in centrifuge tubes used for all experiments.

9.2.3. Electrical Conductivity

Samples were measured to 1 g and put in a centrifuge tube (50 mL, Nalgene) along with 10 mL of DW and placed on a shaker for 24 h of end-over-end shaking at 150 rpm. All samples were prepared in triplicate. After shaking, sample tubes were centrifuged (5 min at 3500 rpm) and then the supernatant electrical conductivity was measured using a benchtop conductivity meter (inoLab Cond Level 2P, Weilheim, Germany).

9.2.4. Specific Surface Area

Specific surface area, representing inner and external surface areas, was estimated using the methylene blue number method, closely resembling the method described by Nunes and Guerreiro (2011). Samples were measured to 0.5 g and put in a centrifuge tube (50 mL, Nalgene) along with 30 mL of a methylene blue solution (concentrations of 10, 25, 100, 250, and 500 mg L^{-1} diluted with DW) and placed on a shaker for 1 h of end-over-end shaking at 200 rpm. All samples were prepared in triplicate. After shaking, sample tubes were centrifuged (5 min at 3500 rpm) and then 1.5 mL of the supernatant was pipetted into a spectrophotometer crystal cuvette. Solutions were measured using a UV/VIS spectrophotometer (DU-640 Spectrophotometer, Beckman Coulter, U.S.A) at 630 nm. The equation used to calculate the amount of methylene blue adsorbed can be found in Nunes and Guerreiro (2011).

9.2.5. Readily soluble copper

Samples were measured to 0.5 g and put in a centrifuge tube (50 mL, Nalgene) along with 15 mL of DW and placed on a shaker for 24 h of end-over-end shaking at 150 rpm. All samples were prepared in triplicate. After shaking, sample tubes were centrifuged (5 min at 3500 rpm) and filtered through a 0.45 µm pore sized hydrophobic membrane filter to remove colloids from the solution. The supernatant was then measured for Cu concentration using an atomic absorption spectrometer (AAS) (AAnalyst 400, PerkinElmer) shown in Figures 11 and 12.

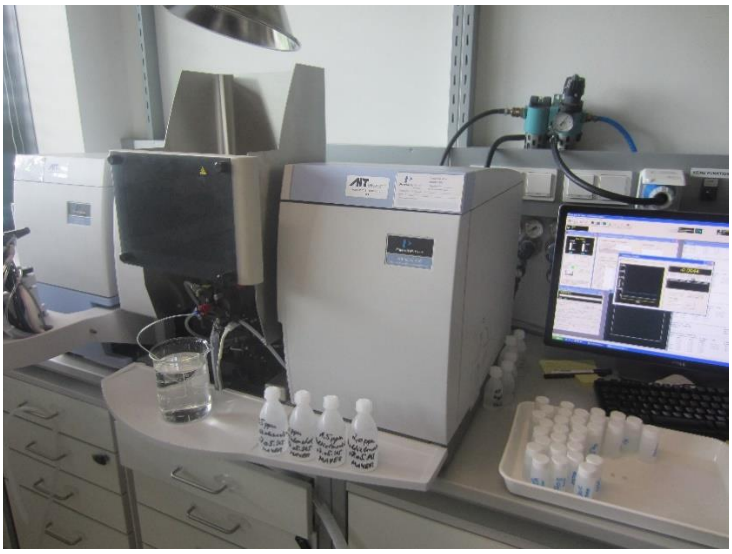


Figure 11 *AAS machine and computer. Flame atomization was used for all measurements and required manual sampling. Sample intake tube can be seen placed in the DW supply beaker during standby mode.*

9.3. Batch sorption experiments

Copper stock solutions were prepared at 50, 100, 150, 200, 250, 300, and 500 mg L^{-1} concentrations with copper (II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$, 97% assay, Sigma-Aldrich, Milwaukee, WI, USA) and DW. Soil samples were measured to 0.5 g and put in a centrifuge tube (50 mL, Nalgene) along with 15 mL of Cu stock solution of a specific concentration. Tubes were placed on a shaker for 24 h of end-over-end shaking at 150 rpm. All samples were prepared in triplicate. After shaking, sample tubes were centrifuged (5 min at 3500 rpm) and the equilibrium pH of the supernatant was measured using a benchtop pH meter (inoLab pH Level 2P, Weilheim, Germany). Samples were then immediately filtered through a 0.45 µm pore sized hydrophobic membrane filter to remove colloids from the solution and acidified with 50 µL of 65% HNO_3 (Suprapur, Merck, Darmstadt, Germany). Supernatant Cu concentrations were then measured using an atomic absorption spectrometer (AAnalyst 400, PerkinElmer) shown in Figures 11 and 12. Sample Cu sorption capacity was calculated with the following equation:

$$Q_{eq} = \frac{(C_0 - C_{eq}) \times V}{m} \tag{1}$$

where Q_{eq} is the Cu uptake (mg g^{-1}), C_0 is the initial liquid-phase concentration of Cu (mg L^{-1}), C_{eq} is the equilibrium liquid-phase concentration of Cu (mg L^{-1}), V is the volume (L) and m is the amount of soil sample (g).

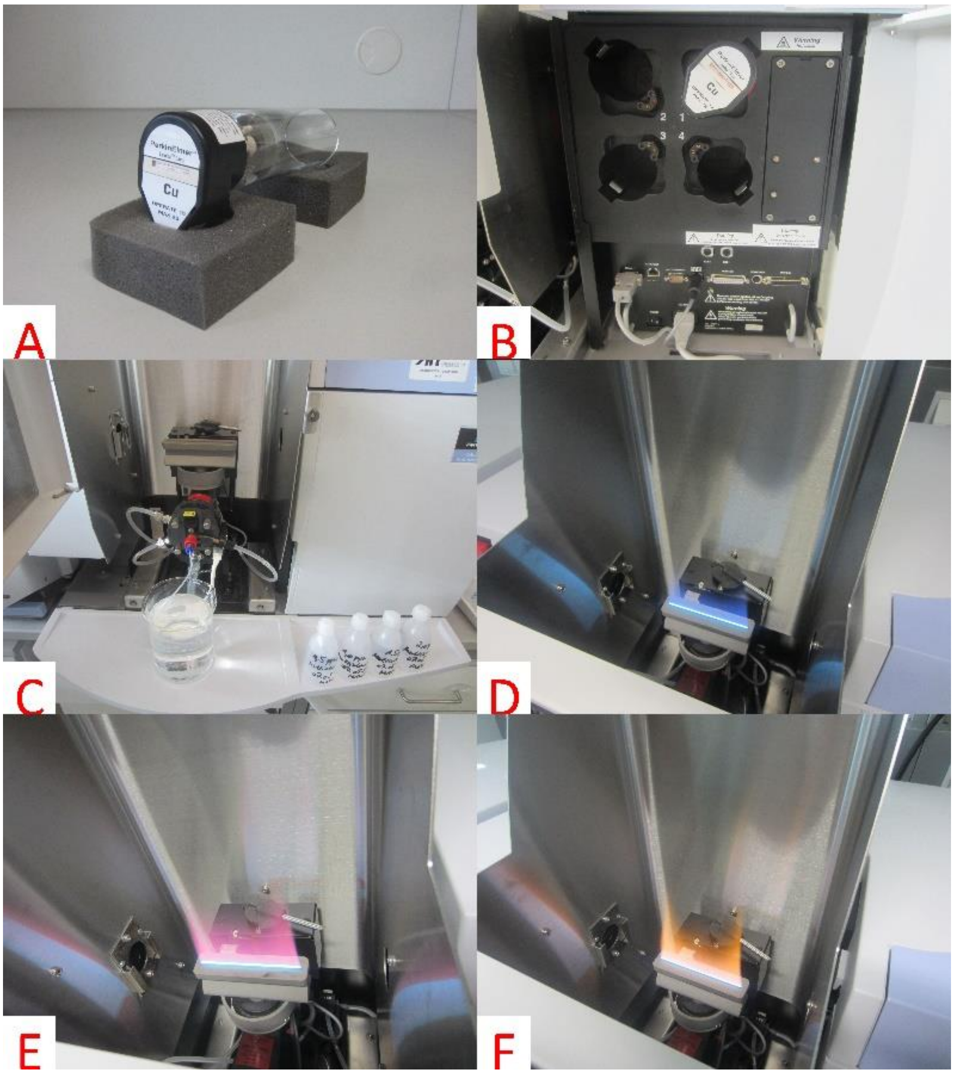


***Figure 12** AAS components (A: copper lamp, B: lamp installed in portal, C: flame nozzle before ignition with DW supply (beaker) and standard solutions for calibration tests (bottles), D: flame ignited in standby mode, E and F: Samples with different concentrations of Cu being measured).*

9.4. Adsorption models

In accordance with previous studies, equilibrium sorption data were analyzed using mathematical equations based on empirical adsorption models. The Langmuir, Freundlich, and Dubinin-Kaganer-Radushkevich (DKR) adsorption models were compared and adsorption isotherms were produced from the obtained data. Calculations were performed using nonlinear regression with the program MicroCal Origin 8.0 Professional (OriginLab Corporation, Northampton, MA, U.S.A).

The simplest and most commonly used of the three theoretical adsorption models, the Langmuir equation is as follows:

$$Q_{eq} = \frac{bQ_{max}C_{eq}}{1+bC_{eq}} \tag{2}$$

where Q_{eq} is the amount of sorbed Cu at equilibrium (mg g^{-1}), b is the isotherm coefficient characterizing biochar affinity to Cu ions in solution (L mg^{-1}), Q_{max} is the maximum Cu sorption capacity at saturated soil and biochar binding sites (mg g^{-1}), and C_{eq} represents the Cu equilibrium concentration in solution (mg L^{-1}).

The Freundlich adsorption model is considered more appropriate to describe adsorption processes on heterogeneous surfaces. It is determined according to the following equation:

$$Q_{eq} = KC_{eq}{}^{(1/n)} \tag{3}$$

where Q_{eq} is the amount of sorbed Cu at equilibrium (mg g^{-1}), K,n are the Freundlich empirical constants characterizing parameters and intensity of sorption processes (L g^{-1}), and C_{eq} is the Cu equilibrium concentration in solution (mg L^{-1}).

The DKR adsorption model is more linear in nature and is determined by the following equation:

$$lnQ_{eq} = lnQ_m - \beta_{\varepsilon}{}^2 \tag{4}$$

where Q_m is the monolayer maximum sorption capacity, β is the activity coefficient related to mean sorption energy, and ε is the Polanyi potential, which is equal to:

$$\varepsilon = RT ln(1 + \frac{1}{C_{eq}}) \quad (5)$$

where R is the gas constant (J mol^{-1} K^{-1}), T is temperature (K).

9.5. Sorption Kinetics

A kinetic study was performed using batch Cu sorption experiments with contact times of 10, 30, 60, 120, 240, 1440, and 2880 min. Soil samples were measured to 0.5 g and put in a centrifuge tube (50 mL, Nalgene) along with 15 mL of 300 mg Cu L^{-1} stock solution prepared with copper (II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$, 97% assay, Sigma-Aldrich, Milwaukee, WI, U.S.A) and DW. Tubes were placed on a shaker for the specified period of time of end-over-end shaking at 150 rpm. All samples were prepared in triplicate. After shaking, sample tubes were centrifuged (3500 rpm) and the equilibrium pH of the supernatant was measured using a benchtop pH meter (inoLab pH Level 2P, Weilheim, Germany). Samples were immediately filtered through a 0.45 µm pore sized hydrophobic membrane filter to remove colloids from the solution and then acidified with 50 µL of 65% HNO_3 (Suprapur, Merck, Darmstadt, Germany). Supernatant Cu concentrations were then measured using an atomic absorption spectrometer (AAnalyst 400, PerkinElmer) shown in Figures 11 and 12. The models used for fitting kinetic data are pseudo-first order (Lagergren equation) and pseudo-second order. The pseudo-first order equation can be defined as follows:

$$\frac{dQ_t}{dt} = k_1\left(Q_{eq} - Q_t\right) \quad (6)$$

where Q_t is the amount of Cu sorbed at time t (mg g^{-1}), Q_{eq} is the amount of Cu sorbed at equilibrium (mg g^{-1}), and k_1 is the rate constant of the pseudo-first order process (min^{-1}).

The pseudo-second order equation can be defined as follows:

$$\frac{dQ_t}{dt} = k_2(Q_{eq} - Q_t)^2 \quad (7)$$

where Q_t is the amount of Cu sorbed at time t (mg g^{-1}), Q_{eq} is the amount of Cu sorbed at equilibrium (mg g^{-1}), and k_2 is the rate constant of the pseudo-second order process (g mg^{-1} min^{-1}).

9.6. pH study

To investigate the influence of the reaction pH, a potassium phosphate pH buffer (KH_2PO_4 and K_2HPO_4) was used. Stock solutions (1 mol L^{-1}) of both phosphates were prepared and then combined at known amounts (see Appendix A) to create buffers for pH 6, 6.5, 7, 7.5, and 8. Each buffer solution was then diluted with DW to various concentrations, which were measured for pH periodically (inoLab pH Level 2P, Weilheim, Germany). It was determined that a 0.01 mol L^{-1} concentration was the most stable and was to be used for all subsequent experiments. Soil samples were measured to 0.5 g and put in a centrifuge tube (50 mL, Nalgene) along with 14 mL of pH buffer solution. Samples were then spiked with 1 mL of a 4.5 g Cu L^{-1} stock solution, prepared with copper (II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$, 97% assay, Sigma-Aldrich, Milwaukee, WI, USA) and DW, to create a 300 mg Cu L^{-1} solution in the reactor tubes. Tubes were placed on a shaker for 24 h of end-over-end shaking at 150 rpm. All samples were prepared in triplicate. After shaking, sample tubes were centrifuged (5 min at 3500 rpm) and the equilibrium pH of the supernatant was measured using a benchtop pH meter. Samples were immediately filtered through a 0.45 µm pore sized hydrophobic membrane filter to remove colloids from the solution and then acidified with 50 µL of 65% HNO_3 (Suprapur, Merck, Darmstadt, Germany). Supernatant Cu concentrations were then measured using an atomic absorption spectrometer (AAnalyst 400, PerkinElmer) shown in Figures 11 and 12.

9.7. DOC study

A forest floor litter mixture served as the source of humic and fulvic acid extractions for the dissolved organic carbon (DOC) used in this study. Here, DOC is considered the carbon portion of the dissolved organic matter (DOM), but the distinction is unimportant under the parameters of this study. The forest litter (sieved to <2 mm) was shaken end-over-end for 24 h at laboratory temperature with DW mixed at a 1:10 (w/w) ratio. The resulting slurry was then filtered through standard paper filters. The filtered slurry was then poured into a 20 cm long dialysis membrane, which was clipped shut at both ends and placed into a large glass beaker filled with DW. A peristatic pump provided fresh DW water to the bottom of the beaker to create a slow overflow current at the top, ensuring the membrane would always be in contact with unsaturated water (Figure 13). The dialysis membrane allowed salts and low molecular weight organics to be flushed out, while higher molecular weight organics, such as humic and fulvic acids, remained inside the membrane walls. The electrical conductivity of the external dialysis solution (mixture of DW and exuded solution) in the beaker was measured several times a day until a decrease to around 3 µS cm^{-1} was reached. The forest litter extract samples were then measured for non-purgeable DOC (vario TOC cube, Elementar Analysensysteme, Hanau, Germany). Soil samples were measured to 0.5 g and put in a centrifuge tube (50 mL, Nalgene) along with 14 mL of forest litter extract DOC dilutions of varying concentrations prepared with DW. Samples were then spiked with 1 mL of a 4.5 g Cu L^{-1} stock solution, prepared with copper (II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$, 97% assay, Sigma-Aldrich, Milwaukee, WI, USA) and DW, to create a 300 mg Cu L^{-1} solution in the reactor tubes. Tubes were placed on a shaker for 24 h of end-over-end shaking at 150 rpm. All samples were prepared in triplicate. After shaking, sample tubes were centrifuged (5 min at 3500 rpm) and the equilibrium pH of the supernatant was measured using a benchtop pH meter (inoLab pH Level 2P, Weilheim, Germany). Samples were immediately filtered through a 0.45 µm pore sized hydrophobic membrane filter to remove colloids from the solution and then acidified with 50 µL of 65% HNO_3 (Suprapur, Merck, Darmstadt, Germany). Supernatant Cu concentrations were then measured using an atomic absorption spectrometer (AAnalyst 400, PerkinElmer) shown in Figures 11 and 12.

***Figure 13** Dialysis phase of the DOC extraction process. A peristatic pump provides a steady flow of fresh DW across a dialysis membrane used to filter forest litter extracts contained inside (dark liquid). Samples of the yellowish solution outside of the membrane were tested for electrical conductivity until stabilizing around 3µS cm^{-1}.*

9.8. Desorption study

Copper desorption was measured once at pH 6, 7, and 8, using previously prepared phosphate pH buffer solutions (see pH study methods). Soil samples were measured to 0.5 g and put in a centrifuge tube (50 mL, Nalgene) along with 14 mL of pH buffer solution. Samples were then spiked with 1 mL of a 4.5 g Cu L^{-1} stock solution, prepared with copper (II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$, 97% assay, Sigma-Aldrich, Milwaukee, WI, USA) and DW, to create a 300 mg Cu L^{-1} solution in the reactor tubes. Tubes were placed on a shaker for 24 h of end-over-end shaking at 150 rpm. All samples were prepared in triplicate. After shaking, sample tubes were centrifuged (5 min at 3500 rpm) and the equilibrium pH of the supernatant was measured using a benchtop pH meter (inoLab pH Level 2P, Weilheim, Germany). Samples were immediately filtered through a 0.45 µm pore sized hydrophobic membrane filter to remove colloids from the solution and then acidified with 50 µL of 65% HNO_3 (Suprapur, Merck, Darmstadt, Germany). Supernatant Cu concentrations were then measured using an atomic absorption spectrometer (AAnalyst 400, PerkinElmer) shown in Figures 11 and 12. The remaining centrifuge tubes with soil samples were then placed in an oven at 60˚C for 24 h and then weighed again to determine loss of mass during sorption batch experiment. All tubes were then filled with 15 mL of the original pH buffer solution and placed on a shaker for 24 h of end-over-end shaking at 150 rpm. As before, samples were centrifuged and the supernatants were measured for pH, filtered, acidified, and measured for Cu concentration.

10. Results and Discussion

10.1. Soil and Biochar Physical and Chemical Characteristics

Biochar (woodchips, 480˚C) had predictable effects on the moisture content, pH, and surface area of the amended soils in relation to non-amended soils. Table 2 provides the basic soil characteristics investigated. A pH increase was observed in every soil indicating a liming effect from the 3% (w/w) biochar amendment rate. This is an effect well documented in the literature and has been attributed to the dissolution of the alkaline substances in biochar (Novak et al., 2009, Van Zwieten et al., 2010, Hass et al., 2012, Houben et al., 2013). During pyrolysis, base cations such as Ca^{2+}, Mg^{2+}, K^+, and Na^+ in feedstock biomass are transformed into oxides, hydroxides, and carbonates like ash which contribute to its alkali nature and liming potential (Yuan et al., 2011). Biochar increased soil solution pH in the sand standard by 0.2, in Rossatz soil by 0.09, Harm soil by 0.18, and Stroh soil by 0.22. Although a small increase, this was enough to have some influence on the sorption capacity of each soil, which will be explored later. A similar study on heavy metal contaminated soils used biochar (willow stems, 400˚C) amendment rates of 1% and 2% (w/w) and also found a small but noticeable increase in pH of 0.07 (Trakal et al., 2011). In comparison, another study amending soils at 10% (w/w) with biochar (woodchips, 450˚C) found an increase in pH of 1.1 in a an moderately acidic soil (pH 5.9), and 0.2 in a moderately alkaline soil (pH 8.1) (Rees et al., 2014).

***Table 2** Basic soil physical and chemical characteristics with and without 3% (w/w) biochar amendment.*

Soil	Moisture Content (%)	EC (µS cm^{-1})	pH	Specific Surface Area (m^2 g^{-1})
Sand	0.25	43.05	6.91	-
Sand + 3% BC	0.5	60.95	7.11	-
Rossatz	0.76	133.65	7.09	61.28
Rossatz + 3% BC	1.01	119.3	7.18	67.56
Harm	3.09	192.7	7.08	52.9
Harm + 3% BC	3.09	172.1	7.26	60.10
Stroh	3.36	135.75	5.84	98.43
Stroh + 3% BC	3.36	132.45	6.06	103.88

The most acidic of the soils, Stroh (pH 5.84), also responded to biochar amendment with the highest increase in pH. It is apparent that the extent of the liming effect of biochar in this study is a factor of the amendment rate and initial soil pH. Biochar pH has been shown to become more alkaline with higher pyrolysis temperature and is undoubtedly a determining factor as well (Yuan et al., 2011, Méndez et al., 2013).

Biochar amendment had no effect on moisture content for Harm or Stroh soil, but resulted in an increase of 0.25% for both Sand and Rossatz soil, which has the largest proportion of sand (64.2%) among the three soils (Table 3). Biochar is expected to have a greater positive influence on water retention in coarse-textured soils or soils with large amounts of macropores which are both characteristic of sandy soils (Glaser et al., 2002). Electrical conductivity was reduced in all three soils with the addition of biochar, dropping 14.35 µS cm^{-1} for Rossatz, 20.6 µs cm^{-1} for Harm, and 3.3 µs cm^{-1} for Stroh soil. Sand increased by 17.9 µS cm^{-1}. Due to the high porosity of biochar, soils that have been amended can be expected to increase in surface area, as was the case here. Biochar amended Rossatz soil increased in specific surface area by 6.28 m^2 g^{-1}, Harm by 7.2 m^2 g^{-1}, and Stroh by 5.45 m^2 g^{-1}. The smaller effect on Stroh soil could be attributed to its higher humus content (Table 3). Soil organic matter (SOM) like humus is naturally high in surface area and so the replacement of SOM-rich soil volume with biochar would therefore result in a smaller increase in surface area. Specific surface area of SOM is considered to range between 550 to 850 m^2 g^{-1} (Sparks, 2003). Humus is synonymous with SOM and can be defined as the "total of the organic compounds in soil exclusive of undecayed plant and animal tissues, their "partial decomposition" products, and the soil biomass" (Sparks, 2003). Humus content for Rossatz, Harm, and Stroh are in the expected range for surface horizon mineral soils (0.5-5% by weight) (Sparks, 2003).

The results of the sand samples with and without biochar amendment for specific surface area were confounding as a control in comparison to trends with the soil samples. This called into question the inherent quality of the sand for further laboratory use, especially as a control. Later results confirmed the unsuitability of the sand for sorption experiments as a control due to impurities. Sand sorption and desorption results have therefore been omitted from this paper and in discussion.

***Table 3** Soil texture and organic matter (AGES).*

Soil	TOC (%)	Humus (%)	Sand (%)	Silt (%)	Clay (%)
Rossatz	0.88	1.5	64.2	25	10.8
Harm	1.62	2.8	19.5	57.3	23.1
Stroh	2.73	4.7	44.6	34.1	21.3

According data provided by the Austrian Agency for Health and Food Safety (AGES), the three soils had Cu contamination levels commonly found in vineyards with a history of copper-based fungicide application (200-500 mg kg^{-1} according to Brun et al., 2001). Table 4 provides Cu concentration after both ethylenediaminetetraacetic acid (EDTA) and aqua regia extractions. EDTA provides a measure of potentially available Cu ions, whereas aqua regia provides a measure of residual or non-bioavailable Cu and is therefore more relevant for a study such as this. The historical and current fungicide application rates of these three vineyards is unknown. However, assuming similar application rates, the importance of soil pH on Cu concentration is apparent. As the most acidic soil, Stroh had the least total Cu content which could be a result of a history of increased leaching and Cu mobility. Adsorption and heavy metal retention typically becomes more difficult at lower more acidic pH. More extensive chemical information and metal concentrations for Rossatz, Harm, and Stroh, in addition to Cu, are provided in Appendix B.

***Table 4** Soil copper concentration (AGES).*

Soil	Cu - EDTA (mg kg^{-1})	Cu - Aqua regia (mg kg^{-1})
Rossatz	251.3	341.7
Harm	207	293.1
Stroh	97.3	201.4

As demonstrated by the observed liming effect, the biochar was strongly alkaline with a pH of 8.5 (Table 5). In a review of several studies using biochars from multiple feedstocks and pyrolysis conditions, average biochar pH was 8.1 (Chan and Xu, 2009). Copper content was very low in comparison to a similar product used in a study by Rees et al (2014). While having somewhat comparable pH (9.2), total carbon (75.21%), and total nitrogen (0.26%), the woodchips biochar (450˚C) used in their study contained 9.8 mg kg^{-1} of Cu. This goes to show once again that feedstock and pyrolysis conditions can have significant impacts on biochar properties and create different end products. For another example, Houben et al (2013) used a commercially produced biochar from a miscanthus straw feedstock pyrolyzed at 600˚C for a similar investigation of heavy metal immobilization. While heavy metal content was negligibly low in comparison to their soil samples, the biochar had a pH of 10.24, total carbon of 53.5%, and total nitrogen of 0.31%.

Table 5 *Woodchips biochar chemical characteristics.*

Woodchips biochar	
Copper (mg kg^{-1})	0.161
pH	8.5
Total Carbon (% dry mass)	79.19
Nitrogen (% dry mass)	0.44

Biochar amendment also resulted in a greater Cu retention, albeit in very low quantities (Figure 14). Batch experiments revealed that biochar reduced Cu solubility by 48.3% (2.22 µg g^{-1}) for Rossatz, 36.8% (1.52 µg g^{-1}) for Harm, and 4.5% (0.1 µg g^{-1}) for Stroh soil. However, these values are averages and subject to standard deviation which diminishes the effect of biochar amendment for Stroh soil. For Rossatz and Harm soils, the reduction in Cu solubility due to biochar amendment is likely a combined result of increased pH, which reduces Cu^{2+} mobility due to reduced competition with H^+ ions for sorption sites, and increased surface area which also increases potential binding sites. Any solubilized Cu ions are more likely to be readsorbed in amended Rossatz and Harm soils to biochar and soil particles, reducing the amount of leachable Cu. For Stroh, the replacement of soil volume with biochar and the consequent liming effect had less of an impact on Cu solubility. It is possible that a history of extensive leaching in Stroh soil due to its acidic soil pH has removed most of the weak outer-sphere Cu complexes (i.e. cation exchange processes), leaving only strong inner-sphere Cu complexes (i.e. chemisorption). This could partly explain both the lower overall amounts of readily soluble Cu in Stroh and the limited effect of biochar amendment.

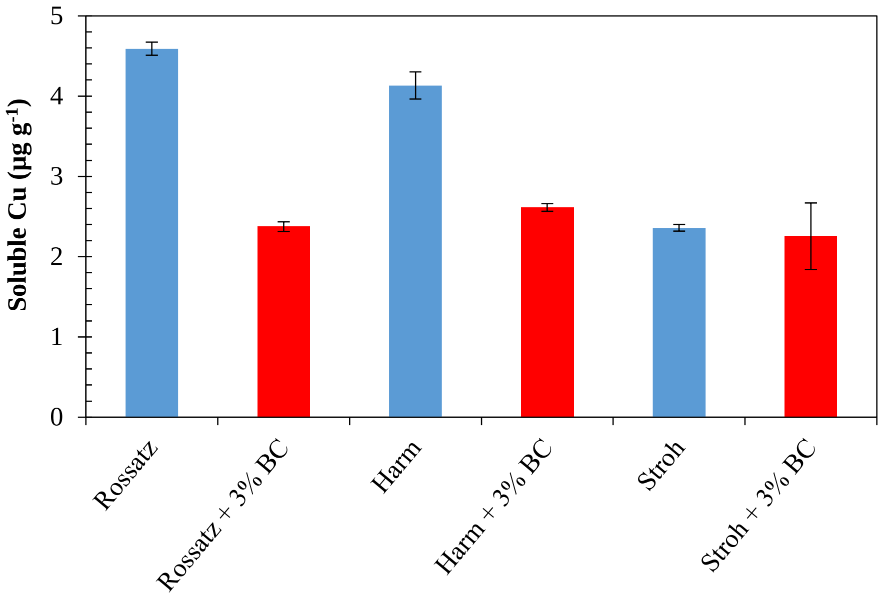


Figure 14 *Readily soluble Cu batch experiment with standard deviation bars.*

10.2. Effect of Contact Time

The kinetic process of Cu sorption for amended and non-amended samples was characterized by two stages (Figure 15). Rapid sorption occurred in all samples within the first 60 min of contact time. Following this initial stage, Cu sorption continued to increase slowly until reaching equilibrium at 1440 min of contact time for Stroh samples, and between 1440 and 2880 min for Rossatz and Harm samples. The intensity of the first stage can be attributed to the inherent abundancy of sorption sites for a specific sample. The second stage is a much slower sorption process and contributes much less immobilization of Cu^{2+} cations. It can be surmised that Cu uptake (Q_{eq}) was not significant beyond 1440 min. Therefore, an equilibrium time of 1440 min (24 h) is suitable for sorption experiments with these samples.

Rossatz and Harm had negligible differences between amended and non-amended samples. While the general trend indicated more sorption in amended soils, a few samples actually showed an opposite effect of biochar amendment for Rossatz and Harm. Clearly shown at 240 min for Rossatz, the non-amended soil immobilized slightly more Cu than the amended soil. In addition to this discrepancy and the overall negligible positive and negative differences, a few of the differences were within the standard deviation of both amended and non-amended soils. This further challenges any possible positive effect of biochar for Rossatz and Harm.

However, the effect of biochar was visible in Stroh samples. At each contact period, Stroh soil with biochar amendment had a higher sorption capacity than non-amended soil. The increased sorption of amended Stroh samples can be attributed primarily to pH. As the most acidic of the soils, Stroh benefited from the liming effect of biochar which has been shown to increase heavy metal sorption (Novak et al., 2009, Hass et al., 2012, Houben et al., 2013). Stroh had the highest increase in pH due to biochar addition, from pH 5.84 to pH 6.06. As well, increases in pH from lower starting points can result in a larger improvement in sorption, illustrated by the trend lines of Cu sorption by soil components in Figure 2. Copper sorption slows at higher pH due to total ion immobilization. The extent of the effect of biochar on Cu sorption, whether positive for Stroh or negligible for Rossatz and Harm, also appeared to be irrespective of contact time. There was no noticeable increase or decrease in biochar attributed adsorption at different contact times.

In order to fully understand the sorption kinetics, data was analysed using kinetic models with pseudo-first order and pseudo-second order equations as described in Frišták et al. (2015). These equations were used to determine the equilibrium Cu sorption (Q_{eq}), rate constants (k), and the coefficient of determination (R^2) for the soil samples and were obtained with non-linear regression analysis. Using the R^2 values, the efficiency and suitability of each model can be evaluated (Table 6). In comparison, it is clearly apparent that Cu sorption kinetics for each sample is described with more significance by the pseudo-second order equation than the pseudo-first order equations. The rate constants are important for determining the residence time required for completing adsorption reactions which has very useful applications in terms of environmental management. They are defined as the change in concentration of a reactant per unit time. Kinetic models are not concerned with the many factors that influence adsorption capacity, such as the initial sorbate concentration, reaction temperature, solution pH, sorbate particle size and amount, and solute type. Instead, they are only concerned with the effect of observable parameters on reaction rate. While both equations compare solution concentrations to the adsorption capacity of solids to describe adsorption rate, pseudo-second order has more commonly been successfully applied to describe the adsorption of metal ions from aqueous solution (Qiu et al., 2009). The pseudo-second order rate equation is able to "describe chemisorption involving valency forces through the sharing or exchange of electrons between the adsorbent and adsorbate as covalent forces, and ion exchange" (Ho, 2006).

Table 6 *Pseudo-first and pseudo-second order rate constants for the sorption process of Cu by Rossatz, Harm, and Stroh soils with and without biochar amendment.*

	Pseudo-first order rate constants			**Pseudo-second order rate constants**		
Sample	**Q_{eq} (mg g^{-1})**	**k_1 (min^{-1})**	**R^2**	**Q_{eq} (mg g^{-1})**	**k_2 (g mg^{-1} min^{-1})**	**R^2**
Rossatz	3.289	0.154	0.920	3.451	0.068	0.955
Rossatz + 3% BC	3.318	0.178	0.896	3.478	0.077	0.928
Harm	5.745	0.187	0.917	5.987	0.051	0.945
Harm +3% BC	5.787	0.157	0.914	6.070	0.040	0.948
Stroh	4.079	0.190	0.966	4.202	0.094	0.981
Stroh +3% BC	4.282	0.206	0.964	4.399	0.103	0.972

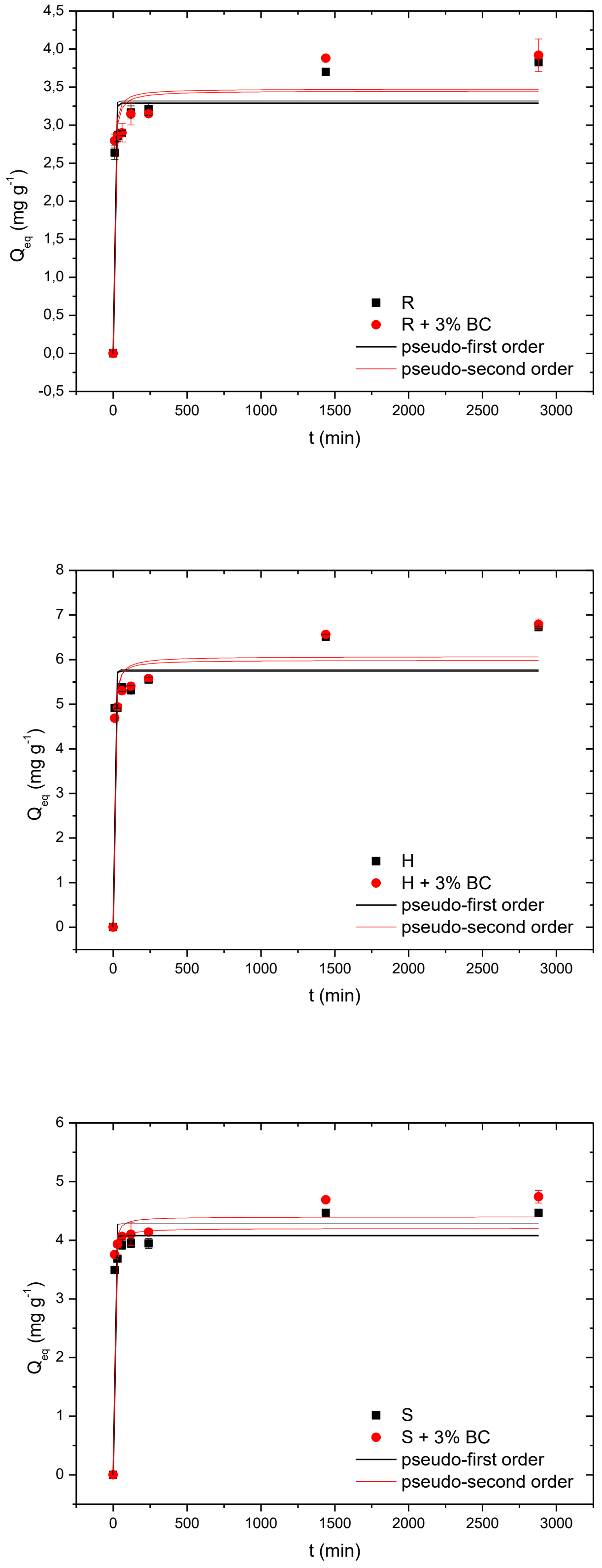


Figure 15 *Effect of contact time on Cu sorption capacity of Rossatz (R), Harm (H), and Stroh (S) soils with and without 3% (w/w) biochar amendment.*

10.3. Effect of Initial Cu Concentration

Based on the findings of the kinetic experiment, it was determined that the amended and non-amended samples reached Cu sorption equilibrium within 24 h. Sorption isotherms were produced comparing the Cu sorption capacity of each amended and non-amended soil to Cu concentration in solution (Figure 16). Immediately, a characteristic "L" curve is visible for each isotherm. The sorption behaviour of each sample was appropriate to be described by Langmuir, Freundlich, and DKR adsorption models. The equations for each model were used to determine sorption constants and correlation coefficients shown in Table 7 with standard deviations. Based on R^2 values, it is clearly evident that the best fitting model for every sample is the Freundlich model. Although both Langmuir and Freundlich equations are widely used in soil sorption studies, Freundlich is often the best simple equation (Barrow, 2008). Langmuir assumes that ions do not affect the surface of the sorbent except from occupying sorption sites, which is untrue as they affect surface charge and pH. Langmuir also requires that the sorption reaction occurs with a uniform homogenous surface. This is unrealistic for soils because they are such heterogeneous materials. Freundlich assumes a heterogeneous surface and so it is no surprise that it predicts sorption behaviour for metal ions in soils better. Copper sorption has been known to follow both Freundlich and Langmuir models (Bradl, 2004). The gradual continuous increase in sorption capacity characterizing the Freundlich isotherm model shape, and differentiating it from the Langmuir model, is because of the consideration of surface precipitation after surface complex formation has reached capacity. This difference between the Freundlich and Langmuir models can be clearly seen in the coloured trend lines of Figure 16.

As in the kinetic study, biochar amendment had the greatest effect on Stroh soil. This effect also became more pronounced at higher initial Cu concentrations in soil solution. Once again, the addition of biochar to Harm samples had negligible effects. Biochar in Rossatz soil appeared to have a slight positive effect on Cu sorption, however standard deviation diminishes the extent of its effect. Despite sharing similar pH and surface area, Harm had a much higher sorption capacity than Rossatz. At a Cu solution concentration of approximately 300 mg L^{-1}, non-amended Harm (pH 7.08) had a sorption capacity 6.51 mg g^{-1} whereas non-amended Rossatz (pH 7.09) had a sorption capacity of 3.83 mg g^{-1}. This difference can be attributed to the higher silt (57.3%) and clay (23.1%) fractions of Harm. Rossatz, on the other hand, is 64.2% sand. Harm also has 2.8% humus content compared to Rossatz with 1.5%. Non-amended Stroh (pH 5.84) soil had a sorption capacity of 4.47 mg Cu g^{-1}, at an approximate reaction solution concentration of 300 mg Cu L^{-1}. While being the most acidic of the soils, Stroh can claim a higher sorption capacity than Rossatz due to its much higher humus content of 4.7% and higher silt and clay fractions (Table 3). In a similar study, Uchimiya et al. (2011a) used one clay-rich, alkaline soil, called San Joaquin, and one sandy acidic soil, called Norfolk. Similar to alkaline Harm, San Joaquin had a high natural heavy metal sorption capacity while Norfolk did not. However, upon amendment with a broiler litter biochar, Norfolk soil had greater enhancement of Cu sorption capacity than San Joaquin soil. These findings mirror what Stroh has exhibited here, and once again illustrate the importance of the liming effect of biochar. The importance of clay contents for heavy metal retention in soils is also supported by the high natural capacities of San Joaquin and Harm for Cu sorption.

Table 7 *Langmuir, Freundlich and DKR equilibrium parameters (± SD) for Cu sorption of Rossatz (R), Harm (H), and Stroh (S) soils with and without 3% (w/w) biochar amendment. Obtained by non-linear regression analysis.*

Soil	**Model**	Q_{max} (mg g^{-1})	*b* (L mg^{-1})	*K* (L g^{-1})	*1/n*	Q_m (mg g^{-1})	β (mol^2 J^{-2})	R^2
Rossatz	Langmuir	3.56±0.24	0.59±0.06	-	-	-	-	0.890
	Freundlich	-	-	1.33±0.09	0.20±0.02	-	-	0.967
	DKR	-	-	-	-	2.74±0.24	1.639*10^{-7}	0.727
Rossatz + 3% biochar	Langmuir	3.14±0.23	0.57±0.02	-	-	-	-	0.870
	Freundlich	-	-	1.27±0.11	0.22±0.02	-	-	0.99
	DKR	-	-	-	-	2.77±0.23	1.303*10^{-7}	0.750
Harm	Langmuir	6.24 0.42	0.84±0.03	-	-	-	-	0.916
	Freundlich	-	-	2.53±0.22	0.21±0.02	-	-	0.941
	DKR	-	-	-	-	5.37±0.51	1.11*10^{-7}	0.776
Harm + 3% biochar	Langmuir	6.21±0.42	0.85±0.25	-	-	-	-	0.916
	Freundlich	-	-	2.54±0.20	0.22±0.02	-	-	0.942
	DKR	-	-	-	-	5.36±0.51	1.12*10^{-7}	0.773
Stroh	Langmuir	4.18±0.33	0.14±0.04	-	-	-	-	0.908
	Freundlich	-	-	1.30±0.11	0.23±0.02	-	-	0.957
	DKR	-	-	-	-	3.24±0.36	1.189*10^{-6}	0.673
Stroh + 3% biochar	Langmuir	4.33±0.37	0.19±0.01	-	-	-	-	0.891
	Freundlich	-	-	1.46±0.11	0.22±0.02	-	-	0.957
	DKR	-	-	-	-	3.39±0.39	5.553*10^{-7}	0.670

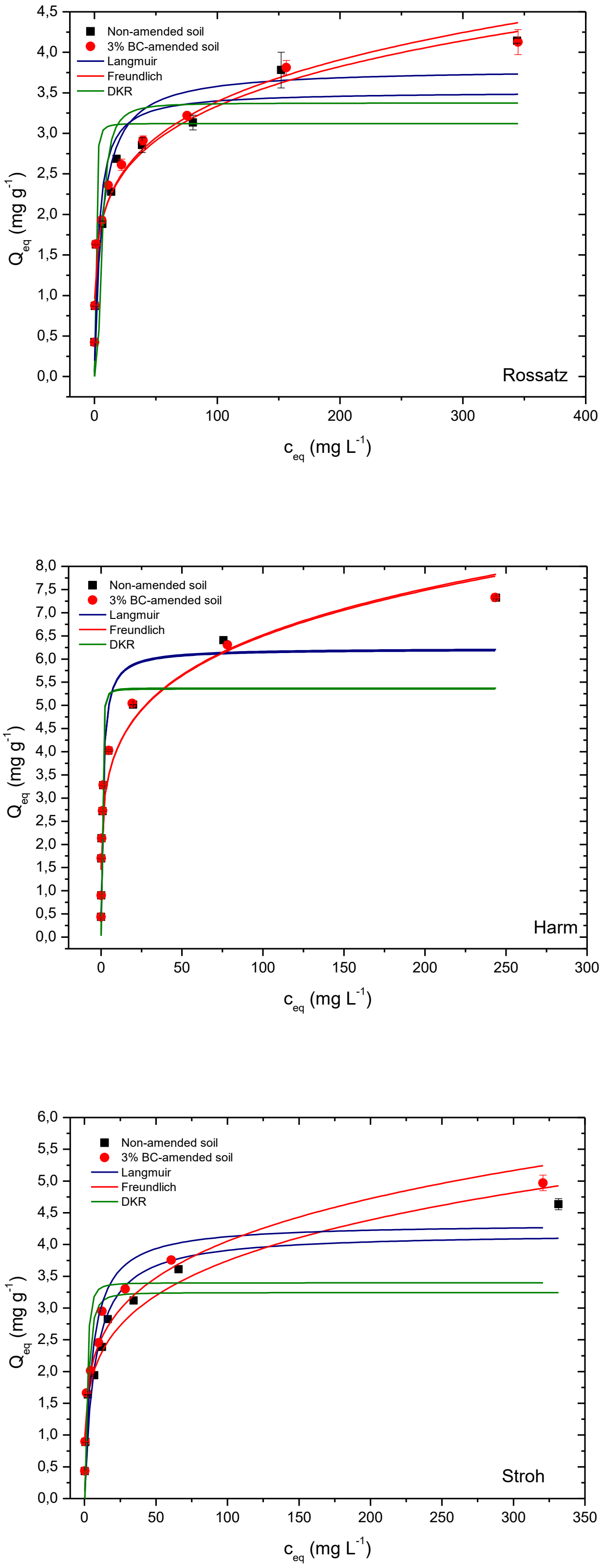


Figure 16 *Copper sorption isotherms of Rossatz, Harm, and Stroh soils with and without 3% (w/w) biochar (BC) amendment using Langmuir, Freundlich, and DKR equilibrium parameters (±SD).*

10.4. pH study

To better understand biochar influence on Cu sorption capacity, the liming effect should be isolated and removed. Controlling for pH can provide more insight into the other mechanisms biochar offers for Cu immobilization. To achieve this, a pH buffer was used at pH 6, 6.5, 7, 7.5, and 8. The potassium phosphate buffer stabilized the pH of all samples during sorption reactions. Aside from biochar addition, added Cu in soil solution has been known to reduce pH, with higher reductions at higher added Cu concentrations (Yu et al., 2002, Uchimiya et al., 2011a).

The Cu sorption results of amended and non-amended soils after 24 h of contact time with 300 mg Cu L^{-1} at the buffered pH levels are shown in Figure 17. The initial impression for all soil samples is the once again that biochar addition had a negligible effect on the sorption capacity for Cu^{2+} cations. In the case of Rossatz, there are even indications of reduced Cu immobilization due to biochar amendment at pH 7, 7.5, and 8. What is more important however is that the added Cu immobilization of biochar in Stroh samples, witnessed during the kinetic study and during the study with varying initial Cu concentrations, was largely nullified once the liming effect was removed due to the pH buffering system. This indicates that the dominant positive short-term effect of biochar addition is the liming effect. The slight variations between biochar amended and non-amended soils are also deemed negligible due to the highly heterogeneous nature of soils, the small quantities of samples used in experiments (0.5 g), and human error in measure, handling, and analysis of samples.

Sorption capacity increased for all soils and reached similar ranges (8.7-9 mg g^{-1}) due to pH buffering. This large increase is expected considering the higher available negative sorption sites as pH increases. The pH buffering system ensured higher reaction pH than the previous sorption experiments while also reducing the acidifying effect that occurs from Cu addition to soil solution (in this case 300 mg L^{-1}). There is a very slight increasing trend in sorption as pH increases for Rossatz and Harm. The overall flat trend for all samples of all soils is attributed to the sorption edge of Cu in soils which peaks and plateaus by pH 6-7 (Figures 2, 3, and 4). This supports the conclusion that the pH buffering system selected (pH 6-8) was limited in range and not very suitable and for the interpretation of Cu^{2+} cation sorption in these soils.

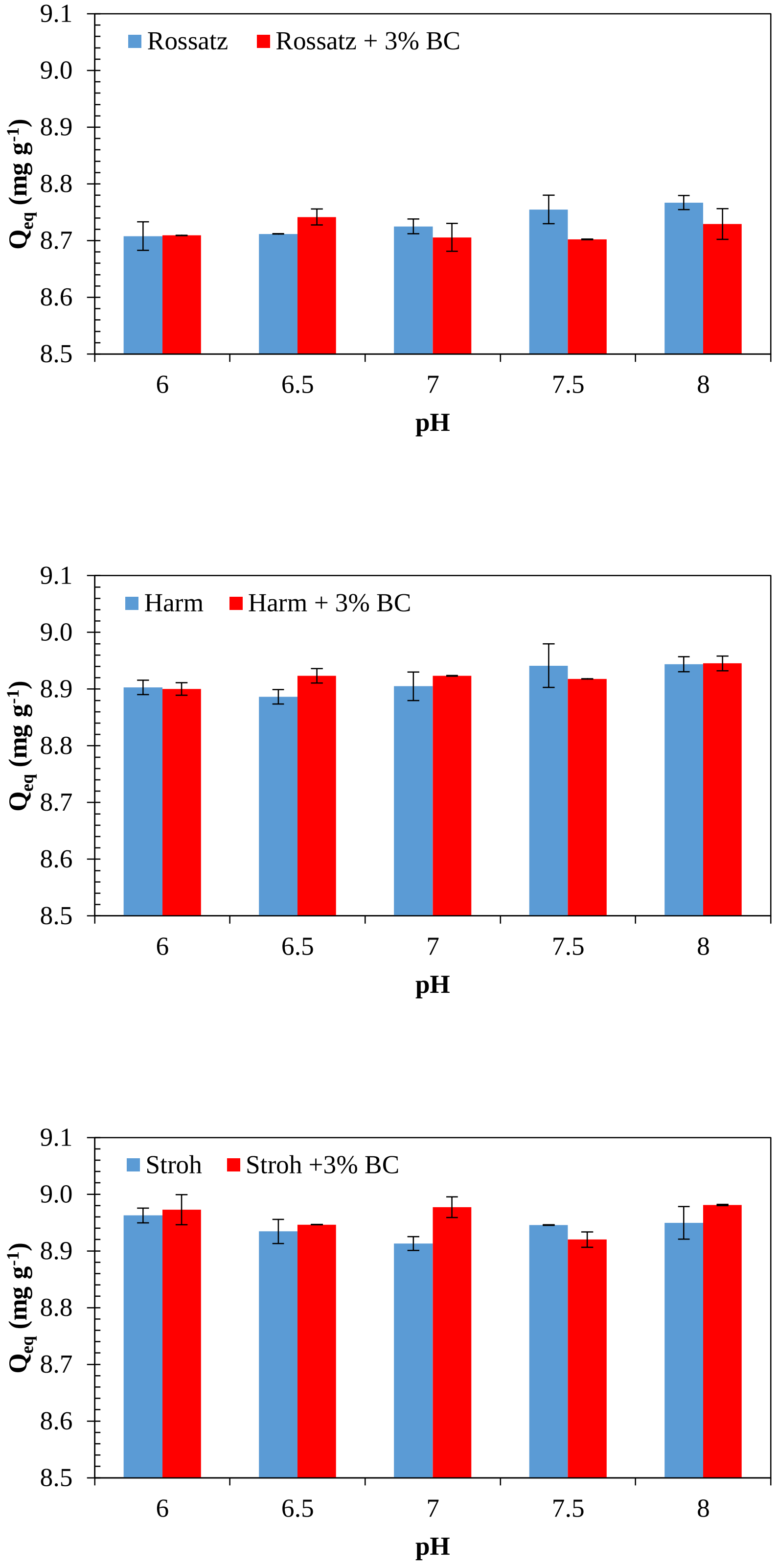


Figure 17 *Effect of pH on Cu sorption by Rossatz, Harm, and Stroh soils with and without 3% (w/w) biochar with standard deviation bars.*

10.5. DOC study

The dissolved organic carbon (DOC) added for experiments was derived from extracted humic and fulvic acids from a sample of forest floor litter. It is widely known that Cu has a strong affinity for DOC-complexation (Sauve et al., 1997, Temminghoff et al., 1997, Ponizovsky et al., 2006). It has been suggested that increases in DOC associated with soil amendments are responsible for increased Cu mobilization (Beesley et al., 2010). While DOC was measured and predictably increased with increasing concentration of the humic extract, the results indicated an inverse relationship of DOC and Cu mobilization. Instead, the sorption characteristics of the extracted acids themselves seemed to dominate the mobility of Cu in these samples. The DOC stock solutions and the sample supernatant solutions were also measured for Ca^{2+} and Mg^{2+} to determine if there was any easily observable evidence of competitive cation sorption with Cu^{2+}. Concentrations of both cations indicated no observable loss or gain in solution (see Appendix C).

Copper immobilization was noticeably improved in both biochar amended and non-amended samples of Harm and Stroh with the addition of 65 mg DOC L^{-1} compared to adsorption without added DOC, whereas Rossatz only showed a slight improvement for biochar amended samples. Copper sorption of non-amended Harm increased 0.45 mg g^{-1} and amended Harm increased 0.71 mg g^{-1}, while non-amended Stroh increased 0.82 mg g^{-1} and amended Stroh increased 0.98 mg g^{-1}. The greater increase in sorption by biochar amended soil compared to non-amended soil was also seen in Rossatz with the introduction of DOC.

Overall sorption capacities of the three soils with and without biochar increased along with increasing DOC in a linear fashion (Figure 18). Biochar amended Rossatz soil showed greater Cu adsorption capacity compared to non-amended soil as a result of introduced DOC in solution. Without added DOC, biochar had a negligible effect on Cu sorption in Rossatz soil. Rossatz has the most sand content and the least humus content of the three soils, meaning biochar amendment could greatly improve DOC adsorption and therefore the associated DOC-Cu complex adsorption. The enhanced Cu adsorption by biochar amended Rossatz was maintained at every level of DOC addition. As mentioned, all Harm and Stroh samples experienced a clear hike in overall Cu sorption capacity with the addition of DOC. The positive effect of biochar on Cu sorption in Stroh was slightly improved with the addition of DOC and maintained at all subsequent DOC concentrations. Biochar in Harm had a negligible effect, as previously mentioned, and the addition of DOC did not seem to noticeably change this other than the enhancement that occurred after initial DOC addition. If anything, added DOC equalized the sorption capacities of biochar amended and non-amended Harm further, especially at higher DOC concentration.

The overall trend of increasing Cu immobilization with increasing DOC concentration can be explained due to adsorption of humic acids to soil surfaces, assuming Cu^{2+} cations complexed extensively to DOC in the humic extract. Temminghoff et al (1997) found that DOC absorption increased with decreasing pH in a Cu contaminated sandy soil. At pH 3.9 all humic acids had been adsorbed or coagulated in the soil column, while only 40-50% was adsorbed at pH 6.6. Lower pH results in more positively charged metal (hydr)oxides that attract negatively charged DOC. The pH drop in Rossatz, Harm, and Stroh soil samples that occurred with increasing DOC addition could indicate increased humic acid sorption (see Appendix D). Rossatz amended and non-amended soil decreased in pH by 0.21 and 0.23 respectively from the initial pH at 65 mg DOC L^{-1} to the final pH at 251 mg DOC L^{-1}. Amended and non-amended Harm decreased in pH to a lesser amount, by 0.04 and 0.03 respectively, while Stroh decreased by 0.20 and 0.16 respectively. The original introduction of DOC (65 mg L^{-1}) and Cu spiking (300 mg L^{-1}) resulted in a decrease in pH in amended and non-amended Rossatz by 2.12 and 2.17 respectively, in Harm by 1.7 and 1.9, and in Stroh by 1.45 and 1.57. Despite the consistent decreases in pH which should increase Cu mobility, Cu sorption capacities increased in nearly all samples. In fact, pH was measured in the range of 4.2-5.3 in all soil samples with and without biochar, classifying the soil pH of the samples in the range of “very strong acid” to “strong acid” (USDA, 1993). Jordão et al (2001) concluded that, assuming similarities between commercial humic acid and those that are naturally derived, humic acid is capable of immobilizing high concentrations of Cu^{2+} cations in soils at pH values typical of acid soils.

Coagulation of dissolved organic matter into (semi) solid organic matter has been linked previously to increases in soil binding capacity (Temminghoff et al., 1997). A study by Komy et al (2014) determined humic acids did indeed enhance the rate and amount of Cu^{2+} cation adsorption to soil minerals (kaolinite and hematite). While their findings also indicated increasing adsorption with increasing pH, this could be due to the preparation of soil materials with humic acid before Cu addition. The authors aimed to create stable humic acid and soil mineral complexes and even washed the samples to remove free humic acid from solution. Their findings showing a positive effect of increasing pH could be attributed to reduced competition for H^+ ions for binding sites. Whereas, the negative effect of increasing pH in the results shown here is more likely because the Cu was added at the same time which resulted in pre DOC-Cu complexation. The complexed DOC-Cu were then bound to soil materials according to humic acid adsorption characteristics which increases with decrease pH (Temminghoff et al., 1997). Further research is required to determine the differences in humic acid addition to soils before or during Cu addition to soil solution.

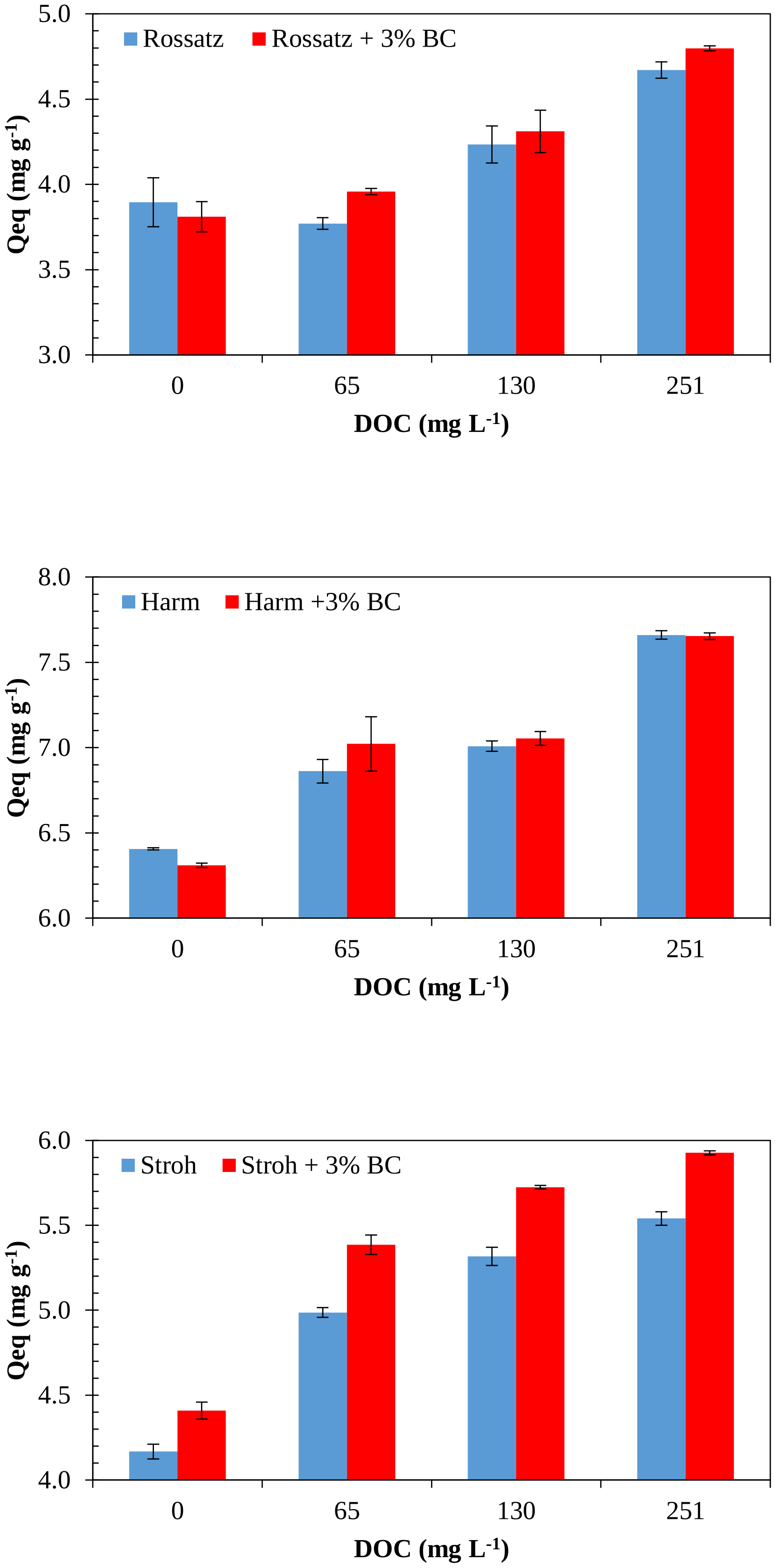


Figure 18 *Effect of DOC on Cu sorption by Rossatz, Harm, and Stroh soils with and without 3% (w/w) biochar with standard deviation bars.*

10.6. Desorption

Copper is known to form strong inner-sphere complexes with soil and biochar surface functional groups which are less reversible than cation exchange or non-specific adsorption (Bradl, 2004). These irreversible bindings also occur rapidly, as shown in Figure 15. Uchimiya et al (2011b) noted the fast kinetics of Cu sorption when observing negligible changes in Cu concentration between 24 h and 6-week equilibration periods. Additionally, the authors found significant hysteresis during desorption experiments after equilibrium indicating the irreversible nature of Cu biding to soil components.

Copper desorption for Rossatz, Harm, and Stroh soils with and without biochar amendment was measured in a sorption-desorption batch experiment using potassium phosphate pH buffer solutions at pH 6, 7, and 8. The percent of Cu desorbed from the samples after only one desorption step are shown in Table 8. For all samples, desorption was negligibly low with less than 1% of previously adsorbed Cu being released at all pH levels. A decreasing desorption trend is apparent as pH increases for all samples. While this is to be expected, it is also insignificant due to such small differences in values. As mentioned before, heavy metal cations like Cu^{2+} are less soluble as pH rises (to an extent) and are therefore less likely to be released and be mobile at pH 8 than at pH 6 (Sparks, 2003).

The negligibly low Cu desorption that occurred during the batch experiments was also observed by Uchimiya et al (2011b). The vast majority of Cu desorption in their experiments occurred only after a third desorption step and after using an acetate buffer (pH 4.9). The first two desorption steps using synthetic rainwater produced negligible Cu desorption. The biochar (broiler litter, 700˚C) used by the authors was also amended at a much higher rate of 20% (w/w) than the rate employed here.

The desorption capacities of biochar amended and non-amended soils are almost identical indicating no negative effect of biochar amendment. Considering the very small desorption (<1%) of Cu by the soils without biochar, a positive effect would be unexpected. Instead, the concern is whether biochar amendment is likely to increase desorption. The data indicates that biochar does not increase the natural desorption capacitates of the soils.

Table 8 *Copper desorption after one desorption step using pH buffer solutions.*

Soil	pH	Cu Sorption (mg g^{-1})	Cu Desorption (mg g^{-1})	Cu Desorption (%)
Rossatz	6	8.74	0.05	0.54
	7	8.70	0.04	0.47
	8	8.72	0.03	0.32
Rossatz + 3% BC	6	8.74	0.05	0.54
	7	8.74	0.04	0.46
	8	8.72	0.03	0.30
Harm	6	9.00	0.04	0.48
	7	8.95	0.04	0.46
	8	8.95	0.03	0.29
Harm + 3% BC	6	8.94	0.04	0.49
	7	8.90	0.04	0.45
	8	8.92	0.03	0.29
Stroh	6	8.92	0.05	0.61
	7	8.96	0.05	0.53
	8	8.99	0.04	0.47
Stroh + 3% BC	6	8.95	0.05	0.60
	7	8.97	0.05	0.51
	8	8.97	0.03	0.37

11. Conclusions

Biochar amendment for Cu-contaminated vineyard soils is an interesting soil remediation strategy that deserves thorough research. However, many questions remain as to the suitability of a specific biochar for a specific task. Differences in pyrolysis conditions and feedstocks can create very different end products that might address or worsen the issue at hand. Additionally, soils are extremely heterogeneous materials which make results difficult to predict and replicate. One simple and relatively quick way to begin to address these questions is the use of laboratory batch experiments.

The addition of woodchips biochar (pyrolysis at 480˚C) to the Cu-contaminated vineyard soils examined here resulted in several predictable affects. Firstly, biochar increased pH in all samples. This liming effect of biochar has been observed often in studies (Novak et al., 2009, Hass et al., 2012, Houben et al., 2013). Biochar also increased moisture content for Rossatz soil, which has the highest sand content of the three soils. Biochar is known to improve water retention in sandy, coarse-textured soils which have large amounts of macropores (Glaser et al., 2002). Likewise, biochar increased surface area for all soils, but had a larger influence on Rossatz due to the replacement of low surface area sand particles. Copper solubility was reduced in biochar amended Rossatz and Harm soils, most likely due to increased pH and binding sites. Based on the chemical and physical changes brought on by 3% (w/w) biochar amendment, it was concluded that this biochar improved soil parameters and did not exacerbate the problem of Cu contamination in these soils based on the investigations performed, warranting further experiments.

Copper sorption kinetics of all amended and non-amended soils followed a characteristic two stage process. The initial rapid sorption within 60 min was followed by a gradually slowing increasing stage until equilibrium was reached at 1440 min of contact time for Stroh samples, and between 1440 and 2880 min for Rossatz and Harm samples. The kinetic process for all samples was more accurately described by the pseudo-second order equation than by the pseudo-first order equation (Lagergren equation). There was no noticeable increase or decrease in biochar attributed adsorption at different contact times.

Copper sorption was best defined by the Freundlich sorption isotherm model. This model considers surface precipitation after surface complexation has reached capacity to describe the second stage of gradually increasing sorption with increasing initial Cu concentration which was seen in the results for all samples. The positive influence of biochar on Cu immobilization can be attributed to the liming effect, most easily seen with Stroh soil.

Sorption capacities of all samples were brought to comparable levels when reaction pH was controlled. Additionally, the positive effect of biochar amendment on Cu immobilization in Stroh soil was no longer apparent once the liming effect was removed. It can be surmised that the dominant positive short-term effect of biochar is related to its influence on soil pH. As well, the lack of change between sorption capacities at each pH used for analysis (pH 6, 7, and 8) supports the conclusion that the pH buffering system selected was not very suitable for interpretation of Cu sorption in these soils.

While DOC was measured and predictably increased with increasing concentration of the humic extract, the results indicated an inverse relationship of DOC and Cu mobilization. Instead, the sorption characteristics of the extracted humic acids themselves seemed to dominate the mobility of Cu in these samples. The overall trend of increasing Cu adsorption with increasing DOC addition to solution can be explained due to the adsorption of the humic acids to soil surfaces, assuming Cu^{2+} cations complexed extensively to DOC in the humic and fulvic acid extracts. Further research is required to determine the differences between humic acid addition to soils before and during Cu addition to soil solution.

Biochar had no negative effect on Cu immobilization during subsequent desorption. The desorption capacities of biochar amended and non-amended soils were almost identical and very minimal (<1%). A positive effect would be unexpected due to the small desorption already present, so the concern instead is whether biochar amendment is likely to increase desorption. The data indicates that this biochar type does not increase the natural desorption capacitates of the soils under the limited parameters of the experiment performed. Further consecutive desorption steps at lower pH are recommended for a better understanding of the true potential of this biochar for long-term irreversible Cu immobilization.

Before complete endorsement can be made for this type of biochar for use in Cu-contaminated vineyard soils, further laboratory research is recommended involving lower reaction pH, more elaborate DOC experiments, and additional subsequent desorption steps. The natural follow-up to these experiments would be field studies. Large-scale field trials are important not only for extending our knowledge base of biochar as a soil amendment, but for guiding remediation strategies so that they may be more effective and long-lasting.

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Appendix A: Potassium phosphate pH buffer preparation

Preparation of 0.1 M potassium phosphate pH buffer

pH*	**Volume of 1 M K_2HPO_4 (ml)**	**Volume of 1 M KH_2PO_4 (ml)**
5.8	8.5	91.5
6.0	13.2	86.8
6.2	19.2	80.8
6.4	27.8	72.2
6.6	38.1	61.9
6.8	49.7	50.3
7.0	61.5	38.5
7.2	71.7	28.3
7.4	80.2	19.8
7.6	86.6	13.4
7.8	90.8	9.2
8.0	94.0	6.0

Dilute combined amounts of 1 M stock solution to 1 L with distilled H_2O

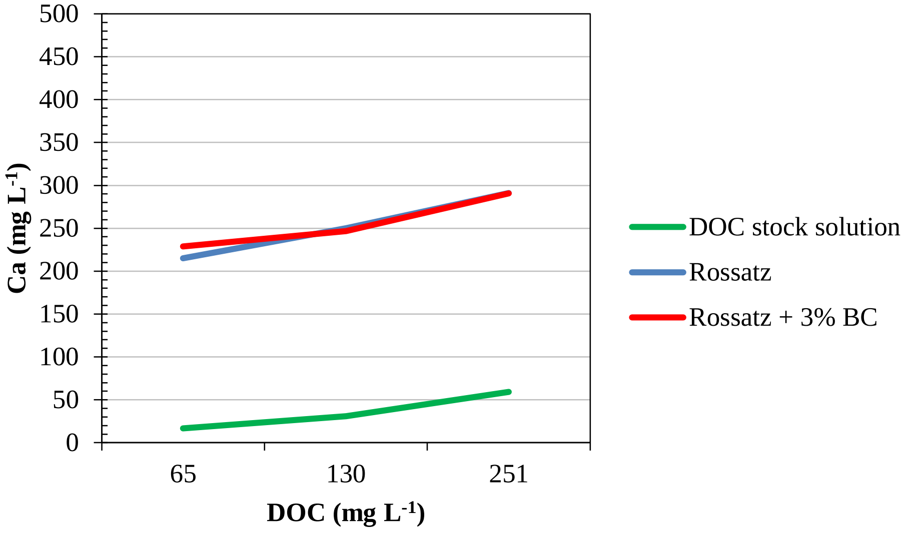
*****at 25˚C

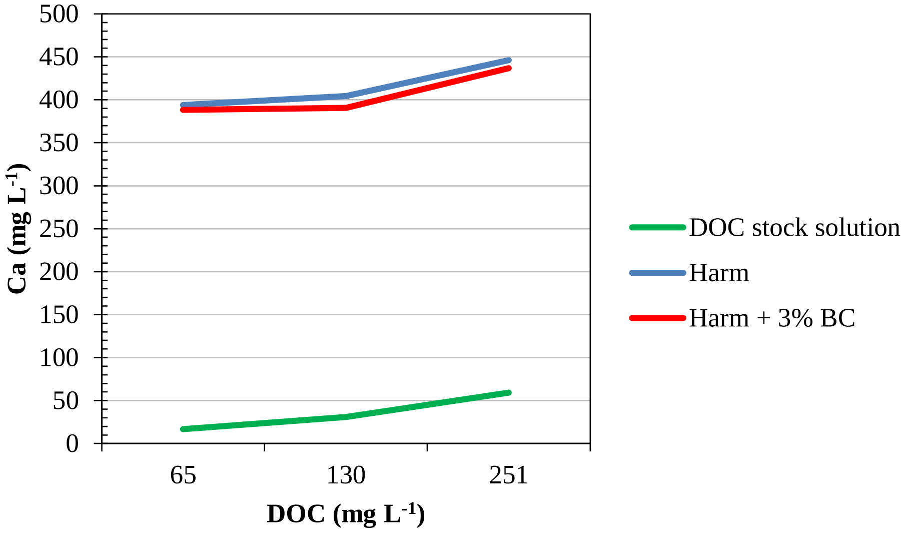
Appendix B: Soil elemental and chemical composition

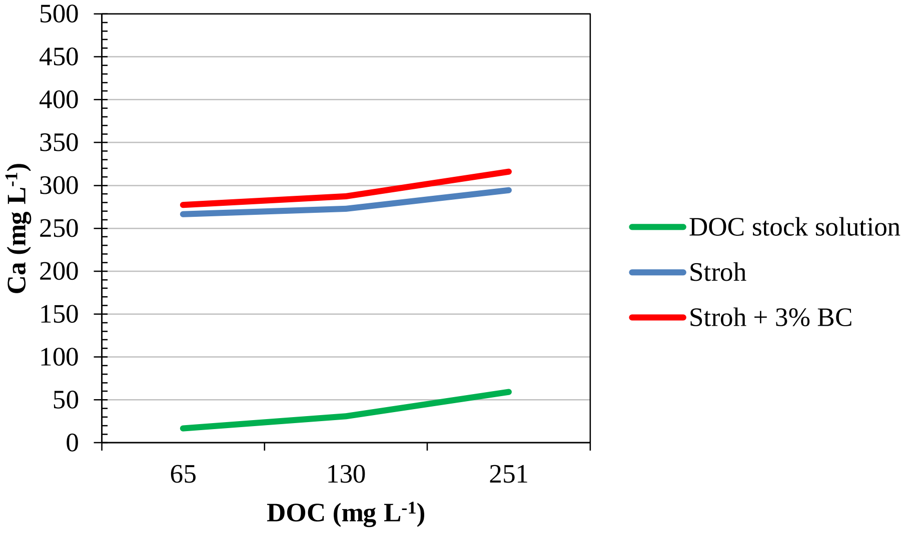
Element	Rossatz	Harm	Stroh
Arsenic Aqua regia (mg kg^{-1})	4.8	10	3.2
Cadmium Aqua regia (mg kg^{-1})	0.2	0.3	0.1
Chromium Aqua regia (mg kg^{-1})	28.1	31	38
Cobalt Aqua regia (mg kg^{-1})	6.7	8.5	13
Copper EDTA (mg kg^{-1})	251.3	207	97.3
Copper Aqua regia (mg kg^{-1})	341.7	293.1	201.4
Iron EDTA (mg kg^{-1})	134	175	432
Lead Aqua regia (mg kg^{-1})	17.5	17	20.4
Manganese EDTA (mg kg^{-1})	335	484	291
Molybdenum Aqua regia (mg kg^{-1})	0.2	0.3	0.3
Nickle Aqua regia (mg kg^{-1})	39.1	27.2	33.9
Phosphate (mg $100g^{-1}$)	65.9	97.1	13.6
Phosphorus (mg kg^{-1})	287	424	59
Potassium (mg kg^{-1})	225	548	257
Vanadium Aqua regia (mg kg^{-1})	28.5	40.3	59.5
Zinc EDTA (mg kg^{-1})	12.1	19.4	9.4
Zinc Aqua regia (mg kg^{-1})	48.9	75.5	91.6

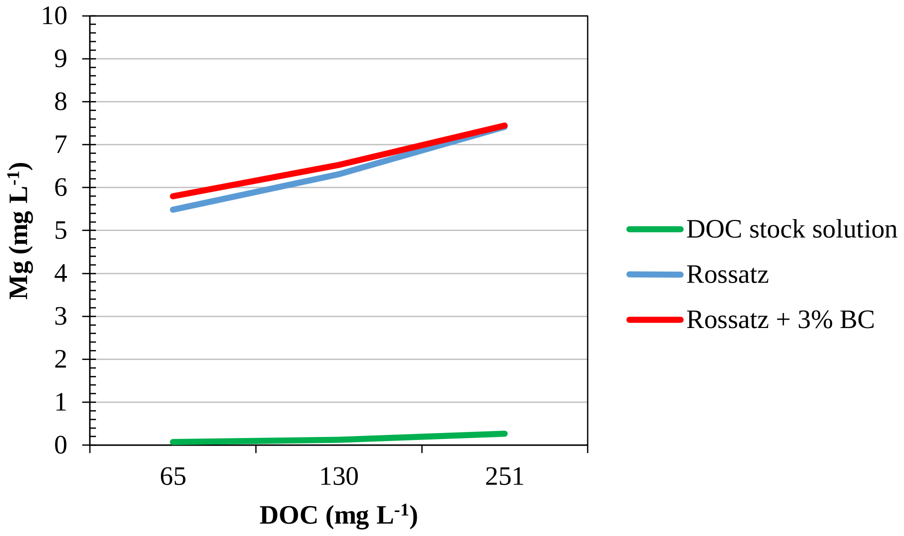
Source: Austrian Agency for Health and Food Safety (AGES)

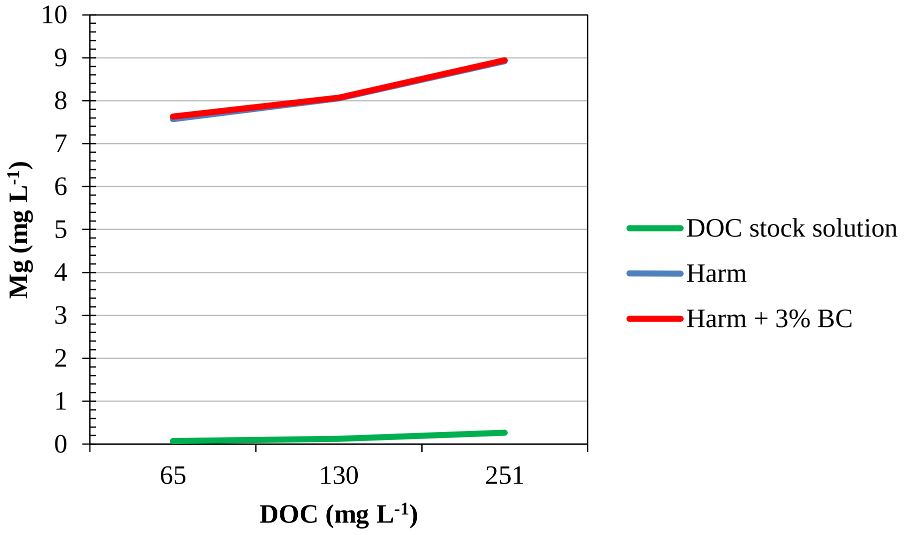
Appendix C: Ca and Mg cation concentrations during DOC study

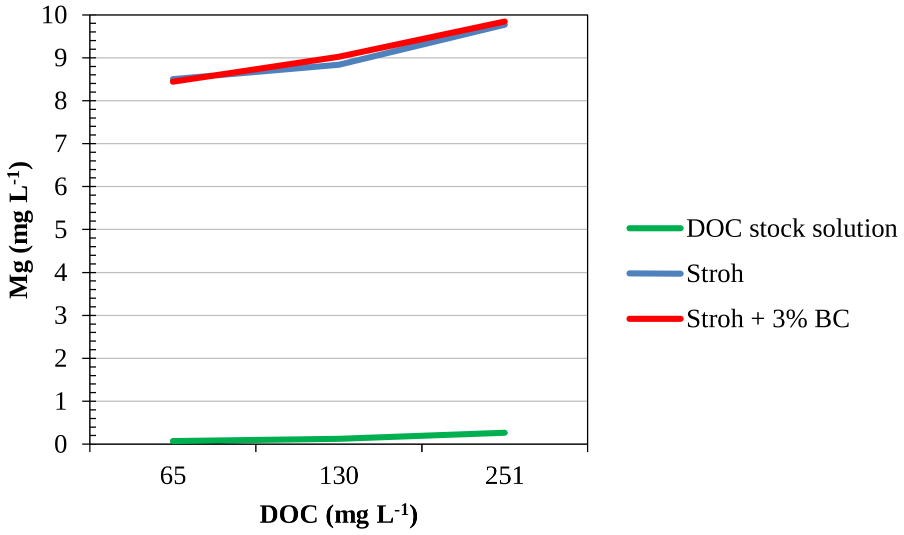




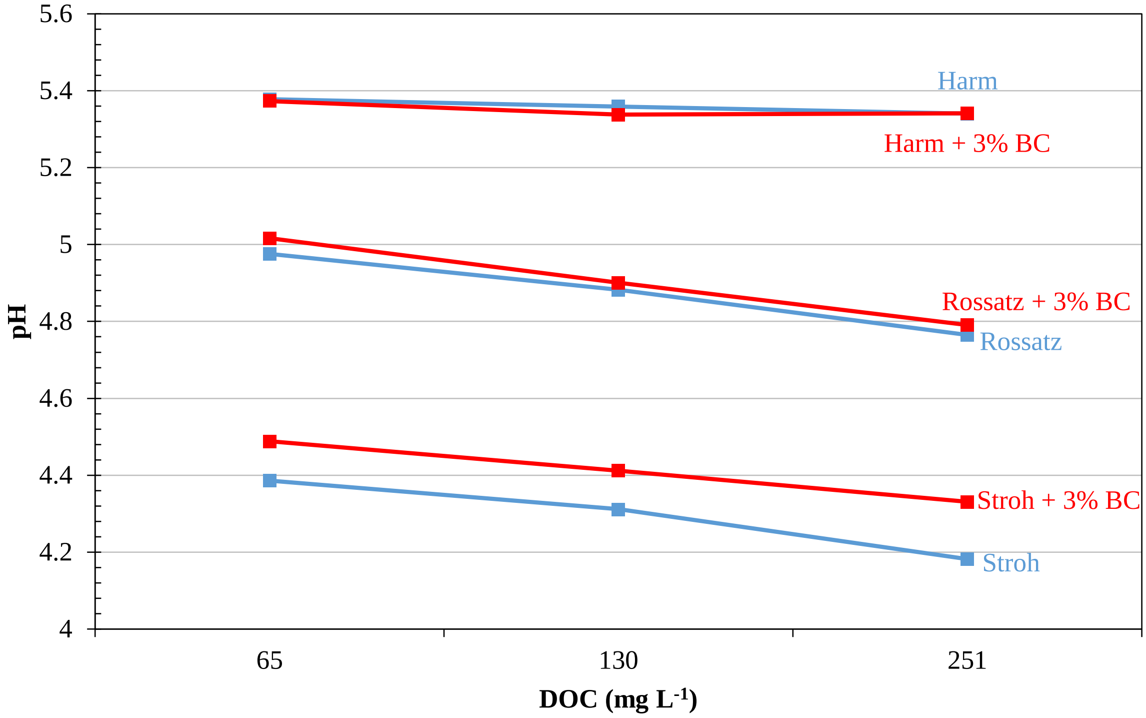



Mg (mg L^{-1})
10
9
8
7
6
5
4
3
2
1
0
65
130
251
DOC (mg L^{-1})
DOC stock solution
Rossatz
Rossatz + 3% BC


Mg (mg L^{-1})
10
9
8
7
6
5
4
3
2
1
0
65
130
251
DOC (mg L^{-1})
DOC stock solution
Harm
Harm + 3% BC


Mg (mg L^{-1})
10
9
8
7
6
5
4
3
2
1
0
65
130
251
DOC (mg L^{-1})
DOC stock solution
Stroh
Stroh + 3% BC

Appendix D: Change in pH during DOC study



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