



Review

Remediation of copper in vineyards – A mini review

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ARTICLE INFO

Article history:

Received 3 January 2012

Received in revised form

15 March 2012

Accepted 19 March 2012

Keywords:

Copper

Vineyard

Remediation

Phytoextraction

Microorganisms

ABSTRACT

Viticulturists use copper fungicide to combat Downy Mildew. Copper, a non-degradable heavy metal, can accumulate in soil or leach into water sources. Its accumulation in topsoil has impacted micro and macro organisms, spurring scientists to research *in situ* copper removal methods. Recent publications suggest that microorganism assisted phytoextraction, using plants and bacteria to actively extract copper, is most promising. As vineyards represent moderately polluted sites this technique has great potential. Active plant extraction and chelate assisted remediation extract too little copper or risk leaching, respectively. However, despite interesting pot experiment results using microorganism assisted phytoextraction, it remains a challenge to find plants that primarily accumulate copper in their shoots, a necessity in vineyards where whole plant removal would be time consuming and financially cumbersome. Vineyard remediation requires a holistic approach including sustainable soil management, proper plant selection, increasing biodiversity and microorganisms.

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1. Introduction

The goal of this review is to investigate and summarize the most important literature available on copper use and remediation in vineyards. The resulting document can be used to identify potential for improvement and opportunities for further research. Viticulture is an important and established crop on practically every continent. This review focuses on the influence of copper use in vineyards and the status and potential of remediating vineyards where copper accumulation is, or borders on, toxic. In viticulture, grapevines are plagued yearly by “Downy Mildew,” *Plasmopara viticola*, triggering the use of copper fungicide by farmers. Over time copper has accumulated in the topsoil, reaching toxic levels, causing plant stress and reducing soil fertility (McBride et al., 1981). Downy Mildew is problematic when weather is moist and wet. Under rainy or windy conditions, particles are expelled from the ground onto the vine canopy, where they subsequently contaminate open stomata (Salinari et al., 2006). Consequently, grape leaf gas exchange declines and plant stress increases during the ripening stage, reducing grape quality and grape sugar content (Jermiini et al., 2010b, 2010a).

Plants, soil, and their interface in this complex system will be discussed. To date reviews on heavy metal remediation have not focused on copper or vineyards specifically (Wenzel, 2009; Jadia

and Fulekar, 2009; Rajkumar et al., 2010; Baker et al., 1994; Kidd et al., 2009; McGrath et al., 2001). Two vineyard specific reviews have recently appeared, but neither addresses microbially assisted phytoextraction (Komárek et al., 2010; Pietrzak and Uren, 2011).

2. Copper

2.1. Copper as a Heavy Metal

Copper is a common metal pollutant (Maier et al., 2000). As an essential heavy metal it is by definition necessary for organism functions, but it is also potentially toxic. Uncontaminated soils generally have <20 mg Cu kg⁻¹ soil, but when copper is present in parent rock and natural minerals as much as 100 mg Cu kg⁻¹ is possible (McBride et al., 1981; Wightwick et al., 2006). Copper can be either immobile or mobile in the soil. Immobile copper is non-bioavailable and can be adsorbed or precipitated into the soil matrix. Copper is predominately cationic, naturally attracted to negatively charged clay minerals, anionic salts, organic matter, hydroxides, phosphorus, and sulfate and therefore producing complexes (Maier et al., 2000; Moolenaar, 1998). Although not bioavailable, newly formed complexes can prevent enzyme reactions or stimulate new reactions with enzyme–substrate complexes (Hinojosa et al., 2010). The optimal environment for sustaining immobility and preventing plant/organism uptake is with high cation exchange capacity (CEC), high pH, and high organic matter (OM) content (Maier et al., 2000). High CEC encourages metals to

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bind with soil aggregates, depending on the OM and clay content of the soil (Maier et al., 2000). High pH enhances the dissociation of organic acids and, therefore, the formation of complexes with metals, altering metal speciation and reducing bioavailability (Maier et al., 2000; Selim and Amacher, 2001).

When the opposite properties are observed, i.e. low pH, low CEC, and little organic matter, copper can be mobile and potentially bioavailable. Bioavailability is defined as the portion of a pollutant in the soil that is available for uptake by soil organisms and/or plants (Hinojosa et al., 2010). Dissolved organic carbon (DOC), or dissolved organic matter, also increase the potential mobility of copper, especially to groundwater and plant roots (Moolenaar, 1998). Low pH is often considered the most critical factor influencing the solubility of copper in soil solution (Maier et al., 2000; Hinojosa et al., 2010; Selim and Amacher, 2001; Nachtigall et al., 2007). Chemical speciation also plays a large role and toxicity decreases in this order: $\text{Cu}(\text{OH})^+/\text{Cu}(\text{OH})_2$, CuCO_3 , CuHCO_3 , $\text{Cu}(\text{CO}_3)_2^{2-}$, chloro-complexes (Komárek et al., 2010). Copper fractions do not correlate to total copper and should be measured separately (Maier et al., 2000; Ristic et al., 2006).

2.2. Copper in vineyards

The first commonly applied copper solution, “Bordeaux mixture” (CuSO_4), was used in France in 1885 (McBride et al., 1981). Pierre Marie Alexis Millardet, a French botanist, observed that copper sulfate, which had been effective for many years in preventing passersby from eating grapes along the road, was also efficient in combating Downy Mildew (McBride et al., 1981; Moolenaar, 1998). It is now known that Cu creates complexes in pathogens, destroying cell proteins and enzyme functions (Spencer-Phillips et al., 2002). Although the original application rate is not known, it can be speculated from recent restrictions that $>8 \text{ kg ha}^{-1} \text{ y}^{-1}$ was used. In much of the world CuSO_4 is no longer recommended for use as it is found to be too concentrated and toxic for workers and the environment. Instead, copper hydroxide ($\text{Cu}(\text{OH})_2$) and copper oxychloride ($\text{Cu}_2\text{Cl}_2(\text{OH})_4$), both less concentrated, are extensively sold and applied by grape cultivators. In 1995, copper solutions represented 20% of global fungicide sales (Spencer-Phillips et al., 2002).

Anthropogenic sources have increased the soil copper load. Almost all organic wineries use copper fungicides because it is currently the only organic option to fight fungi. Worldwide, 121 825 ha of organic grapes are cultivated, with Europe managing 100 000 ha (IFOAM, 2009). Despite strict certification, copper use has created a dispute over the sustainability of organic production (Russo, 2008; FAO, 2009). Its toxic characteristics have prompted governments to set copper limits. The Netherlands has banned copper use, while the European Union permits only $6 \text{ kg ha}^{-1} \text{ y}^{-1}$ in agriculture (Wightwick et al., 2008; European Commission, 2007). Some countries have no restrictions in place, such as Australia and the United States (Wightwick et al., 2008; R. Mazur, personal communication, August 26, 2011). Despite difficulties in calculating specific soil toxicity, as total concentrations rarely correlate to plant available concentrations, boundaries between nontoxic to toxic and toxic to critical total copper levels have been defined; in Australia and New Zealand $>60 \text{ mg Cu kg}^{-1}$ soil, in the Netherlands $>36 \text{ mg Cu kg}^{-1}$ and in the European Community 50 and $140 \text{ mg Cu kg}^{-1}$, limits for sewage sludge addition, are often used as indicators of toxicity (Maier et al., 2000; Pietrzak and McPhail, 2004; Council of the European Communities, 1986; BBodSchV, 1999). Due to the above stringent toxicity guidelines, most groups that set organic standards have copper limits below those required by their national governments; in Germany $3 \text{ kg ha}^{-1} \text{ y}^{-1}$, in Australia $8 \text{ kg ha}^{-1} \text{ y}^{-1}$, and in the United States it is prohibited

(BIOLAND, 2011; Wightwick et al., 2008; National Organic Program, 2000). This illustrates their awareness of the problem despite its widespread use. Although organic farmers are big users of copper fungicide, conventional farmers also use it, depending on the region; at rates of $1\text{--}2 \text{ kg ha}^{-1} \text{ y}^{-1}$ in Europe, $0\text{--}20 \text{ kg ha}^{-1} \text{ y}^{-1}$ in Australia and up to $65 \text{ kg ha}^{-1} \text{ y}^{-1}$ in Brazil, in order to supplement other chemicals (Nachtigall et al., 2007; Pietrzak and McPhail, 2004). Up to 90% of winegrowers in Australia apply Cu, contributing to the increase in copper levels in agricultural soils (Pietrzak and McPhail, 2004).

Fungicidal spraying contributes more copper to soils than any other agricultural activity (McBride et al., 1981). Copper is sprayed directly onto the vine canopy rather than over the entire vineyard area in a light mist using a radial blowing sprayer or, in steep regions, by hand. These methods, however, do not prevent the spray from drifting or falling onto the soil below. (Such machines are now available, but are expensive and are currently the exception in vineyards.) Therefore, containment of copper spray is low, resulting in non-point source pollution. This is problematic because copper cannot be degraded or destroyed (Maier et al., 2000). Although copper is found to be “one of the least mobile of the trace elements,” soluble fractions are always present (McBride et al., 1981). The total copper concentration is in fact distributed over all possible solid, liquid, and biotic forms and these have different effects on the environment (Maier et al., 2000). Also, copper applied anthropogenically is more potentially available to plants than naturally occurring copper (Fernández-Calviño et al., 2009a).

Historical and current applications have resulted in copper accumulation in the soil and total copper quantities have been measured in vineyards worldwide (see Table 1).

It has been suggested that areas with greater humidity and precipitation, such as Brazil or Champagne, France, exhibit higher copper concentrations than dry environments, due to higher copper use (Komárek et al., 2010; Fernández-Calviño et al., 2009b; Deluisa et al., 1996; Morgan and Taylor, 2004). However, even regions with low total copper levels can be labeled with either “warning” or “critical” alerts. These values, however, do not describe the distribution throughout the vineyard or their uptake by fauna or flora.

Copper accumulates in the topsoil and decreases with depth, eventually returning to natural levels between 40 and 60 cm (Ristic et al., 2006; Pietrzak and McPhail, 2004; Deluisa et al., 1996; Magalhaes et al., 1985; Besnard et al., 2001; Brun et al., 1998; Flores-Vélez et al., 1996; Rusjan et al., 2007; Komárek et al., 2008). Depth and quantity of copper accumulation do, however, increase in more tropical climates, such as Brazil (Mirlean et al., 2007). One study from Ristic et al. (2006) suggests that copper accumulation at depth decreases with increasing sand content, allowing copper to be more mobile and to leach out; however copper reduction at depth is seen in many soils with and without sand. Another characteristic of copper in vineyards is that it accumulates within rather than between the rows; copper is sprayed on the vines, under which the soil is left largely undisturbed (see Fig. 1A) (Wightwick et al., 2006; Pietrzak and McPhail, 2004; Komárek et al., 2008; Mirlean et al., 2007). Depending on the age of the vineyard and technical operations within the vineyard, i.e. removal of foliage or distribution of organic matter, the difference between copper quantities within and between the rows can be more or less significant (Mirlean et al., 2007). Plowing between rows is a common soil management technique in vineyards to reduce weed and plant growth under the vines. It is also thought to affect the distribution of copper in the vineyard. Generally, tilling can be as deep as 50–60 cm, resulting in lower copper concentrations, due to mixing, than in untilled areas (Ristic et al., 2006; Mirlean et al., 2007; Paoletti et al., 1998). Provenzano et al. (2010) observed that copper levels were the same

Table 1
Total copper and extractable copper values, including method, in vineyards around the world.

Location	Soil type	Total copper (mg kg ⁻¹)	Method for extractable copper fraction	Extractable copper fraction (mg kg ⁻¹)	Source
<i>Europe</i>					
Czech Republic	Various types	6	EDTA	11–81	(Komárek et al., 2008)
France & Mosel, Germany	—	60–360	Calcium chloride	0.43–0.70	(Fründ et al., 2007)
Burgundy, France	Calcaric cambisol and leptosol	57–332	—	—	(Parat et al., 2002)
Burgundy & Beaujolais, France	Rendosol and brunisol	17; 34	—	—	(Dousset et al., 2007)
Beaujolais, France	Acidic sandy	323	—	—	(Flores-Vélez et al., 1996)
Champagne, France	—	100–1500	—	—	(Besnard et al., 2001)
Hérault, S. France	Calcareous sandstone-slate and alluvial deposits	31–251	EDTA	2.3–95.1	(Brun et al., 1998)
			DTPA	1.5–82.1	
			Ammonium acetate	0.6–29.7	
			Calcium chloride	0.10–9.24	
Hérault, S. France	Calcareous sandstone-slate and alluvial deposits	20–251	EDTA	3.1–95.1	(Brun et al., 2001)
			DTPA	2.4–82.1	
			Ammonium acetate	1.6–29.7	
			Calcium chloride	0.10–9.24	
Reims, France	Calcaric cambisols	248–519	—	—	(Besnard et al., 2001)
Roujan, France	Various types	22–398	Calcium chloride	0–40	(Chaignon et al., 2003)
			EDTA	4–176	
Bari, Italy	Alkaline loam	93	DTPA	9.6	(Provenzano et al., 2010)
Piedmont, Italy	Acidic soils	251–372	KNO ₃ (Alva et al., 2000)	9–30	(Dell'Amico et al., 2008)
Torino, Italy	Dystric Eutrudept	478	KNO ₃	19	(Lagomarsino et al., 2010)
Italy	Various types	9–945	DTPA	0.5–418	(Deluisa et al., 1996)
			Calcium chloride	0–0.90	
Galicia, Spain	Sandy loam to loam granite and schist	41.5–583.1	Ammonium acetate	0.4–23.3	(Fernández-Calviño et al., 2008b)
			EDTA	6.3–212.6	
Galicia, Spain	Sandy loam to loam granite and schist	61–434	—	—	(Fernández-Calviño et al., 2008c)
Galicia, Spain	Sandy loam to loam granite and schist	25–272	Ammonium acetate	0.1–2.5	(Fernández-Calviño et al., 2008a)
			EDTA	1–141	
Rioja, Spain	Various types	22.5–66.8	—	—	(Herrero-Hernández et al., 2011)
NW Spain, N Portugal	Various types	25–666	Ammonium acetate	0.1–30.3	(Fernández-Calviño et al., 2009a)
NW Spain, N Portugal	Various types	100–268	Ammonium acetate	1–12	(Fernández-Calviño et al., 2010)
			DTPA	23–93	
			EDTA	31–98	
N Portugal	—	8–574	—	—	(Pessanha et al., 2010)
S Portugal	Calcareous loam	2	(Olsen, 1972)	14–46	(Magalhaes et al., 1985)
Serbia	Neutral soils	24–432	EDTA	5.1–92	(Ristic et al., 2006)
			Ammonium acetate	0–25	
Slovenia	Various types	87–120	Calcium chloride	0.04–0.06	(Rusjan et al., 2007)
Slovenia	Calcaric leptosol and regosol, anthrosol	65–99	—	—	(Rusjan et al., 2006)
<i>Asia</i>					
Taichung, Taiwan	Acidic sandy loam	9.1–100	—	—	(Lai et al., 2010)
<i>Oceania</i>					
Australia	Various types of acidic soil	22–228	MgCl ₂	0–4.3	(Pietrzak and McPhail, 2004)
Australia	Various types	6–223	—	—	(Wightwick et al., 2008)
Australia	Various types	1–223	Calcium chloride	0–0.94	(Wightwick et al., 2010)
Marlborough, New Zealand	Silt loam to clay loam	50–150	—	—	(Robinson et al., 2006)
New Zealand	Various types	1–259	—	—	(Morgan and Taylor, 2004)
<i>Africa</i>					
Stellenbosch, South Africa	—	10–20	—	—	(Eijsackers et al., 2005)
<i>Americas</i>					
Brazil	Acidic dystrophic lithic udorthent and humic dystrodept	1355–1381	DTPA	~375–550	(Nachtigall et al., 2007)
			Mehlich III	~850–1000	
			Calcium chloride	~1–15	
Brazil	Ferrasols, podzols, arenosols	36–3215	Calcium chloride	0.2–5	(Mirlean et al., 2007)
			Ammonium acetate	7.3–606	
			EDTA	19–1264	
New York, USA	Alton gravelly loam	87–142	—	—	(Taschenberg et al., 1961)

in the topsoil and at 40 cm depth when tillage was practiced. However, as most vineyards commonly utilize tillage as a practice, copper distribution in a completely untilled vineyard is yet to be investigated. Copper treated wooden posts, which support vine cordons, have also been found to leach copper into the surrounding soil, but untreated or lacquered wood posts can prevent it (Robinson et al., 2006). Knowledge of specific vineyard management and soil sample placement is important in vineyard research.

Some research indicates that as vineyards age they are subjected to long periods of Cu application, enhancing Cu accumulation (Morgan and Taylor, 2004; Rusjan et al., 2007; Fernández-Calviño et al., 2008a). Fernández-Calviño et al. (2008a) found that abandoned vineyards had the highest levels of total copper when compared to young and old vineyards. Pietrzak and McPhail (2004), however, did not see any difference between young and old vineyards in Australia with respect to total copper concentration, and

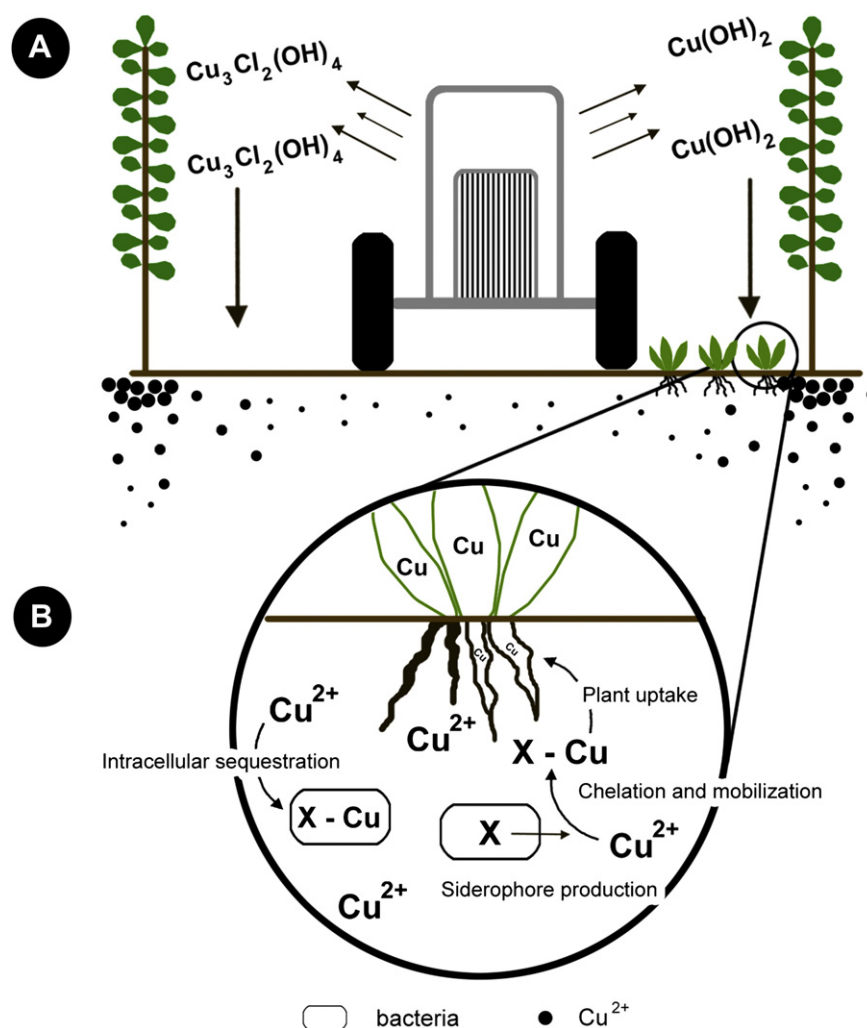


Fig. 1. A) Copper spraying and subsequent copper distribution in a vineyard. B) Microbially assisted phytoextraction, where bacteria use siderophores to chelate copper ions and enhance plant metal uptake.

found that vineyard age does not play a role in how much copper is used each year, suggesting that perhaps even less is applied in older vineyards, which produce fewer grapes. However, [Morgan and Taylor \(2004\)](#) suggest this result is due only to soil erosion from vineyard sites rather than from a true reduction in copper use or accumulation. CEC has also been shown to increase with vineyard age, but not with depth, which suggests that as a vineyard ages and more copper accumulates, it becomes less available ([Rusjan et al., 2007](#)).

In vineyards, most of the copper complexes with organic matter and clay soil fractions ([Besnard et al., 2001](#); [Flores-Vélez et al., 1996](#); [Parat et al., 2002](#); [Lagomarsino et al., 2010](#)). Copper is found in all soil fractions in different forms; its affinity for organic matter and clay particles has been verified. In this way copper indirectly prevents the complete decomposition and mineralization of organic matter ([McBride et al., 1981](#); [Moolenaar, 1998](#); [Parat et al., 2002](#)). It has also been observed that as copper concentrations increase, the fraction bound to organic matter also increases, illustrating a pattern similar to that of vineyard age and copper concentration ([Fernández-Calviño et al., 2008a](#)). Within the clay fraction copper demonstrated an affinity for Fe (oxyhydroxides) ([Morgan and Taylor, 2004](#); [Parat et al., 2002](#); [Lagomarsino et al., 2010](#)). [Morgan and Taylor \(2004\)](#) identified the largest copper fraction in vineyards as copper residuals, with organically bound copper closely followed by Fe bound copper. On the other hand, [Pietrzak and McPhail \(2004\)](#) measured greater fractions of active

copper fractions in the topsoil, where water soluble, sorbed and exchangeable fractions represented up to 50%, and organically bound forms only 25%. They also saw that sandy soils had less active and more organically bound copper. In general, however, analysis of organic matter and clay fractions gives a good estimate of where copper can be found.

Vineyard soil pH is generally neutral ([Wightwick et al., 2006](#); [Nachtigall et al., 2007](#)). Acidic pH between 4 and 6 can be found in specific regions, such as Spain and Brazil ([Fernández-Calviño et al., 2008a](#)). Under neutral pH copper is largely immobilized and therefore likely to accumulate in the root zone. Grapevines have deep rooting systems; so far, copper has not affected them ([Wightwick et al., 2008](#)). But topsoil could become toxic for cover crops and young, newly planted vines with shallow root systems. Toxicity depends on the form and concentration of copper as well as soil texture, and copper lowers soil pH, enhancing its solubility ([Hinojosa et al., 2010](#)). Copper can also influence the quantity of organic carbon in vineyard soil. When copper concentrations are high organic carbon also increases ([Besnard et al., 2001](#); [Flores-Vélez et al., 1996](#); [Parat et al., 2002](#); [Fründ et al., 2007](#)). [Fründ et al. \(2007\)](#) determined that copper levels $>180 \text{ mg Cu kg}^{-1}$ increase organic carbon and decrease microbial biomass. [Ristic et al. \(2006\)](#) found the same to be true for bioavailable copper.

Copper also affects sediments in watersheds. As vineyards are often planted on steep hillsides to increase the amount and

duration of sunshine, erosion is common, especially when cover crops are not planted between rows to stabilize the soil (Kosmas et al., 1997). Sediments in a Galician (Spain) river valley were found to have higher copper levels than nearby vineyard soils (422 mg kg^{-1} ; 204 mg kg^{-1} , respectively) (Fernández-Calviño et al., 2008b). The portion of potentially bioavailable copper was almost 50% of total copper measured and was bound mainly to organic matter in the sediments, as seen in soils (Fernández-Calviño et al., 2008b). However, in another river valley Fernández-Calviño et al. (2008c) found that copper levels did not exceed 209 mg kg^{-1} . Differences in the two studies may be due to their different locations and upstream land uses. Highest total copper observed in sediments was found downstream from an area with the largest percentage of vineyards (Fernández-Calviño et al., 2008c). As in vineyard soils, copper release from sediments increased in acidic environments. Water samples showed no correlation to copper in vineyards in Spain, but in Champagne, France basin water had $53.6 \mu\text{g Cu L}^{-1}$, higher than the LC_{50} for many aquatic species (Banas et al., 2010). The authors suggested storm water basins as a deterrent.

The effect of landscape or topography on copper levels is unclear. Rusjan et al. (2007) found that amongst plains, plateaus and terraces, copper levels were highest on terraces. Deluisa et al. (1996), in a comprehensive study of mountainous and plain regions in northern and southern Italy, found that the mountain zone had higher copper accumulation. They attributed this to differences in soil type and precipitation, the latter encouraging greater use of copper. However, in Australia Pietrzak and McPhail (2004) did not see a difference in copper application in Victoria (Australia) between humid regions and drier regions. They suggest, “slope, exposure, amount of sun received, surrounding vegetation, wind breakers” are factors in controlling mildew outbreaks. According to them the source of copper and organic matter content largely determine copper distribution. Other authors have suggested that erosion, leaching and plowing are the main determinants. Differences between findings may be due to vineyard age, but this information was not available.

2.3. Environmental impact of copper

Total copper has induced mal effects on organisms with as little as 55 mg kg^{-1} (Jänsch and Römbke, 2009). To better estimate potential complications bioavailable copper must also be analyzed. Measuring bioavailable copper directly is not possible; bioavailable fractions can be approximated by sequential or single-step extraction methods. Thus, fractions can only be labeled as exchangeable, soluble, complexed, etc. The exchangeable fraction is considered to be the most similar to bioavailable copper. Suggested extraction methods include ethylene diamine tetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), ammonium acetate, calcium chloride and ammonium nitrate (Komárek et al., 2008; VDLUFA, 2006; Marschner, 2003; Chaignon et al., 2003; Song et al., 2004; BBodSchV, 1999). DTPA was developed to chelate many heavy metals, not only copper, and could be an inconsistent indicator of the effect of Cu on yield (McBride et al., 1981). The EDTA Cu fraction tends to correlate with total Cu, not to plant available Cu, explaining why this extraction can lead to an overestimation of plant available Cu (Mirlean et al., 2007; Fernández-Calviño et al., 2008b; Song et al., 2004). CaCl_2 can predict copper levels in wild plants from acidic to neutral soils, but in alkaline soils it can underestimate copper (Brun et al., 1998; Komárek et al., 2008; Mirlean et al., 2007). The literature does not recommend a particular method, but governments often require specific analyses, such as ammonium nitrate in Germany. As the various methods may be extracting different parts of the same source, multiple analytical

methods in comparison with plant copper values may better clarify site-specific contamination.

On the macro-scale, plants and animals as well as ecosystem processes are impacted by copper pollution (McBride et al., 1981; Moolenaar, 1998). Plant toxicity reduces root growth, increases atrophy, and causes chlorosis (Moolenaar, 1998; Magalhaes et al., 1985). In vineyards plant toxicity depends on rooting depth. Grapevines root deeply within the soil in search of water. Therefore, vines do not often display toxicity, while grasses and shallow rooting plants do (Magalhaes et al., 1985). Copper accumulation can, however, affect the development of new vine shoots (Coïc and Coppent, 1989; Komárek et al., 2010; Romeu-Moreno and Mas, 1999). As plants age copper in vine leaves accumulates (Pessanha et al., 2010). Angelova et al. (1999) observed, however, that copper in grapevine leaves decreased substantially when copper spraying was halted and leaves were washed by precipitation before harvest. Pessanha et al. (2010) did not observe any significant increase in grape, wine, or must copper levels, despite an increase in leaves, a finding supported by Lai et al. (2010). However, Provenzano et al. (2010) measured increased copper levels in berries with copper applications in Italy, but no phytotoxicity in grape leaves was detected. They also observed higher accumulation in white chardonnay grapes (5 mg kg^{-1}) than in traditional red Italian varieties (1.7 mg kg^{-1}), but not at levels hazardous to human health. In their study copper concentration remained constant throughout the seasons, which contrasts with the leaf research of Angelova et al. (1999), perhaps due to differences in spraying frequency and duration. Angelova et al. also analyzed grape berries and found that vineyards in an industrial area had significantly higher copper levels than those in non-industrial areas, suggesting that atmospheric deposition was a significant contributor rather than uptake from the soil.

Brun et al. (1998) detected greater Cu in aboveground biomass of wild Asteraceae plants, known hyperaccumulators (Prasad and Freitas, 2003), than in Poaceae plants, which had high Cu in the root mass. Copper found in roots is mainly concentrated in the fine roots (Brun et al., 1998; Angelova et al., 1999). Brun et al. (2003) indicated that the Asteraceae species showed signs of chlorosis at copper levels equal to or greater than $200 \text{ mg Cu kg}^{-1}$, inducing delayed growth and low seed set. One species of Poaceae, *Dactylis glomerata*, however, was completely tolerant of copper. The effects of copper contaminated vineyard soils on other crop plants have provided a better understanding of copper distribution in plants. In general, neither maize nor tomatoes accumulated high levels of copper in their shoots (Chaignon et al., 2003; Brun et al., 2001). Brun et al. (2001) and Marschner (2003) suggest that the aerial parts of a plant do not indicate soil bioavailable Cu, but that the roots show the first signs of contamination. These results were seen in calcareous soils but not acidic soils where copper accumulation was much higher, suggesting that pH is a determinant in the speciation and activity of copper.

Earthworms play a key role in soil fertility, assisting in litter decomposition; they increase water-holding capacity with burrow tunnels and increase root penetration by loosening soil. While earthworms are also potential copper immobilizing agents, digesting copper and accumulating it in their tissue, they are also fatally affected by high copper quantities (McBride et al., 1981; Hinojosa et al., 2010; Udovic and Lestan, 2010). In 1984 copper chloride was seen to greatly suppress cocoon production with as little as 65 mg Cu kg^{-1} in sandy loam soils and $130 \text{ mg Cu kg}^{-1}$ in sandy soils (Ma, 1984). Paoletti et al. (1998) found that earthworm abundance, species number, and biomass were greatly reduced by both copper ($140\text{--}210 \text{ mg Cu kg}^{-1}$) and soil tillage in vineyards. Copper oxychloride has been shown in lab studies to decrease earthworm-burrowing rates and increase copper body content,

influencing growth and survival (Eijsackers et al., 2005). The authors saw mal effects with vineyard soils ($14.4 \text{ mg Cu kg}^{-1}$) and increasing in spiked soils ($55.4 \text{ mg Cu kg}^{-1}$). They believe this indicates a decrease in sustainable soil quality because only low numbers of big and old earthworms were observed (Eijsackers et al., 2005).

On a micro-scale copper “severely decreases the functional diversity of the soil microbial community and impairs specific pathways of nutrient cycling” (Kandeler et al., 1996). Microorganisms can interact with heavy metals in three ways: through sequestration and immobilization, enhancement of solubility, or potential harm due to toxicity (Maier et al., 2000). By crossing through the cell membrane, copper alters cell transcription, translation, and division, damaging cell DNA, denaturing proteins, and disturbing microbial biomass and communities (Maier et al., 2000).

Dell’Amico et al. (2008) found that, when comparing a high copper site (372 mg kg^{-1}) to a lower copper site (215 mg kg^{-1}) in acidic soils in Piedmont, Italy, the high copper site had higher exchangeable Cu and fewer fast growing, r-strategist bacteria, i.e. lower bacterial populations. Both sites contained copper tolerant bacteria, but the high copper site had more gram-negative bacteria, while the low copper site had more gram-positive bacteria. Bacteria have also been shown to increase community tolerance to copper (Díaz-Raviña et al., 2007). Known copper resistant bacteria are *Pseudomonas*, *Xantomonas* and *Escherichia coli* (Silver and Phung, 1996). *Pseudomonas* is the only bacteria that visually displays (by turning blue) copper storage in high copper environments (Silver and Phung, 1996).

Copper can affect soil microorganism enzyme activity, an aspect of biological health, cell membrane activity, and the processes of oxidation and reduction (Maier et al., 2000; Moolenaar, 1998; Hinojosa et al., 2010; White, 2009). It has been found to inhibit arylsulfatase, acid phosphatase, protease, urease, β -Glucosidase, and cellulase at varying levels of toxicity (Hinojosa et al., 2010). Phosphatase and arylsulfatase show the highest sensitivity to copper (Hinojosa et al., 2010). In vineyards, copper has been shown to reduce levels of dehydrogenase, β -glucosidase, and phosphatase, and to inconsistently affect urease, sometimes even increasing its activity at moderate copper levels (Fernández-Calviño et al., 2010). This suggests that copper decreases enzymes associated with the C and P cycles.

3. Remediation

3.1. Remediation strategies

Remediation efforts for heavy metals can be achieved in five ways: immobilization through pH alterations or OM addition, removal, sequestration, active mixing, and plant uptake or phytoextraction (Pietrzak and Uren, 2011; Maier et al., 2000).

Altering pH to immobilize copper, called phytostabilization, is often suggested in the literature (Kidd et al., 2009; Flores-Vélez et al., 1996; Robinson et al., 2006; Fernández-Calviño et al., 2008a). This method requires continuous addition of lime, however, and is only a temporary solution, as copper will increase with continued use. As for OM addition, Brandt et al. (2008) found that manure addition to copper laden soils reduces free Cu^{2+} ion activity, but increases copper bioavailability. Copper can actually form bioavailable complexes with dissolved organic matter (DOM) derived from manure. Nachtigall et al. (2007) studied whether or not poultry litter would reduce Cu availability; it did not. However, other studies have had contradictory results and recommended manure to reduce Cu mobility (Besnard et al., 2001; Flores-Vélez et al., 1996; Fernández-Calviño et al., 2008a; Lejon et al., 2008).

Second, removal through acid washing and incineration are not practical, financially or logistically, for large areas of land that continue to be utilized for crops. Third, sequestration through exopolymers has only been achieved under laboratory conditions and is, therefore, not viable for large tracts of land. Fourth, active mixing of top and deep soils to dilute Cu contamination destroys the soil structure and is also not possible directly within vinerows where Cu is highest.

Fifth, phytoextraction, uptake of soil heavy metals by plants, seems most feasible (Jadia and Fulekar, 2009). After sufficient growth and metal accumulation in roots and shoots, plants are harvested (Kidd et al., 2009). In general, phytoextraction is non-invasive; i.e. it retains soil fertility, is inexpensive, and utilizes the natural ecosystem to help control anthropogenic contamination. Phytoextraction is suggested for low to moderately contaminated environments, which vineyards often are (Jadia and Fulekar, 2009; Kidd et al., 2009). Vegetation cover also helps reduce erosion and maintain nutrients within the soil (Flores-Vélez et al., 1996). Morlat and Jacquet (2003) found that cover crops encourage vine roots to grow deeper by leaving less room for them in the upper soil and resulting in improved juice quality. However, cover crops are labor intensive to harvest. Three types of phytoextraction exist; no addition, chelate addition, and microorganism addition.

3.2. Plant selection

Plant selection is a key element of phytoextraction. Hyper-accumulator plants have the ability to take up more copper ($>20 \text{ mg kg}^{-1}$), particularly in their shoots (McGrath et al., 2001; Reuter and Robinson, 1997), than other plants and still remain physiologically unaffected by toxicity (Rajkumar et al., 2010; Kidd et al., 2009). They are technically defined as plants that can accumulate more than 0.1% copper in leaf dry matter (Baker et al., 1994). Hyperaccumulating plants have also been shown to support a higher fraction of metal resistant microorganisms in the rhizosphere when compared to non-accumulators (Kidd et al., 2009). Several hyperaccumulating species are found in the *Brassicaceae* family (Kidd et al., 2009). Unfortunately, hyperaccumulators often have low biomass and slow growth. Usually ruderal or rosette plants growing along the ground, they are not an efficient option in field-contaminated systems. High biomass crops that are not necessarily hyperaccumulators, but which take up high levels of trace metals, are now being researched for their potential (Kidd et al., 2009). There have also been suggestions that genes for metal hyperaccumulation should be transferred to more productive host plants (Baker et al., 1994), but this has not yet been pursued.

In a contaminated vineyard it is critical to choose plant types that accumulate large amounts of copper in their shoots, or aboveground biomass. In the narrow two-meter space between vinerows it is difficult to remove both roots and shoots without specialized, and currently non-existent, machinery. The second most important factor is to choose plants with relatively high biomass so that a greater amount of copper can be removed in fewer cuttings. Table 2 indicates plants tested for copper removal. Removal rates are not given, as there are no field trials.

Should these methods prove successful and sufficient copper is accumulated in the plants, what should then be done with the harvested biomass? Pyrolysis, anaerobic decomposition and burning of plant biomass after which copper is phytomined, are most often cited in the literature (Kidd et al., 2009).

3.3. Phytoextraction

Phytoextraction without addition requires hyperaccumulator plants (Poschenrieder et al., 2001). Physiologically, plants have the

Table 2
Plants selected for copper removal around world.

Location	Experiment type	Soil type and origin	Plant	Approach	Copper accumulated in shoots mg kg ⁻¹	Copper accumulated in roots mg kg ⁻¹	Source
<i>Europe</i>							
Hérault, S. France	Greenhouse pot experiment	Calcareous sandstone-slate and alluvial deposits	<i>Zea mays</i> cv. Gaucho	No addition	7.14–17.2	23.4–584	(Brun et al., 2001)
Hérault, S. France	Field	Calcareous sandstone-slate and alluvial deposits	Wild species	No addition; <i>Dactylis glomerata</i> <i>Poa annua</i> <i>Andryala integrifolia</i> <i>Hypochoeris radicata</i> <i>Senecio vulgaris</i> <i>Sanguisorba minor</i> <i>Rumex acetosella</i> <i>Allium polyanthum</i> <i>Rubia peregrina</i>	3.54–12.2 10.9–11.4 9.44–26.6 8.48–21.6 14.7–19.8 9.01–11.3 9.78–32.7 3.80–10.2 4.53–16.0	– – 4.56–18.00 7.40–16.5 14.9–28.3 9.81–13.1 – – –	(Brun et al., 1998)
Roujan, France	Pot experiment	Variable soil samples	<i>Lycopersicon esculentum</i> cv. St Pierre	No addition	4.1–13.9	19–189	(Chaignon et al., 2003)
Portugal	Greenhouse pot experiment	CuSO ₄ amended soil	<i>Brassica juncea</i>	Addition of microorganisms; <i>Achromobacter xylosoxidans</i>	~2–8	~30–180	(Ma et al., 2009)
Serbia	Outdoor pot experiment	Clay loam/Vineyard	<i>Brassica napus</i>	Addition of Chelators; EDDS EDTA	38.6–316.4 34.2–52.0	78.1–244.4 202.1–390.6	(Zeremski-Škorić et al., 2010)
Slovenia	Soil column experiment	Sandy loam/Vineyard	<i>Brassica rapa</i>	Addition of Chelators; Citric acid EDDS DTPA EDTA	11.28 37.81 24.28 21.71		(Kos and Leštan, 2004)
Spain	Soil and plant collection from 15 sites	Malachite outcrops	+32 species	No addition; <i>Sedum sediforme</i> <i>Hirschfeldia incana</i> <i>Hyparrhenia hirta</i> <i>Brachypodium retusum</i> <i>Echium vulgare</i> <i>Foeniculum vulgare</i> <i>Alyssum maritimum</i> <i>Psoralea bituminosa</i>	406 355 10 34 32 16 25 11	639 97 202 124 99 51 30 32	(Poschenrieder et al., 2001)
United Kingdom, Chile, China	Greenhouse pot experiment	Factory and mine tailings	<i>Silene vulgaris</i> <i>Elsholtzia splendens</i>	No addition; <i>Silene vulgaris</i> <i>Elsholtzia splendens</i>	0.77 ^a 0.60 ^a	0.75 ^a 0.74 ^a	(Song et al., 2004)
<i>South America</i>							
Brazil	Greenhouse pot experiment	Inceptisol and Mollisol/Vineyard	<i>Avena sativa</i>	Addition of microorganisms; <i>Pseudomonas putida</i> A1 <i>Stenotrophomonas maltophilia</i> A2 <i>Acinetobacter calcoeticus</i> A6	~1150–1200 mg kg ⁻¹ ~1100–1200 mg kg ⁻¹ ~1100–1300 mg kg ⁻¹		(Andreazza et al., 2010a)
<i>Asia</i>							
Zhejiang Province, China	Greenhouse pot experiment	Alluvial sandy loam/Agriculture	<i>Elsholtzia splendens</i>	Addition of Chelators; Glucose Citric acid	~85 ~75	~440 ~400	(Chen et al., 2006)
Zhejiang Province, China	Greenhouse pot experiment	Alluvial sandy loam/Agriculture	<i>Trifolium repens</i>	Addition of Chelators; Glucose Citric acid	~50 ~25	~320 ~360	(Chen et al., 2006)

^a Log transformed data.

ability to increase the solubility of metals, both through their roots and in the surrounding rhizosphere. Plant techniques to increase solubility include “acidification/alkalinisation, modification of soil redox potential, exudation of metal chelants and organic ligands” (Wenzel, 2009; Kidd et al., 2009). The amount and structure of root exudates (Quartacci et al., 2009) and the reducing capacity of roots play a large role (Kidd et al., 2009). Root exudates release metal chelating compounds to mobilize metals (McGrath et al., 2001). Vineyards represent a practical environment for this method, as copper is bioavailable in the root zone (Kidd et al., 2009). The role of microorganisms is difficult to assess because plants and microorganisms cooperate closely (Kidd et al., 2009).

Poschenrieder et al. (2001) found that the following species had high shoot/root ratios indicating copper resistance and high potential for phytoextraction: *Alyssum maritimum* (>1.5), *Hirschfeldia* (>6), *Reseda lutea* (>1.5), *Reseda phyteuma* (>1.5), *Ononis natrix* (>6), and *Spartium junceum* (>1.5). In particular, the authors suggest *R. phyteuma* for use in vineyards and orchards because it can grow in soils with as much as 950 mg kg⁻¹ copper while extracting as much as 51 mg Cu kg⁻¹ DM in its shoots (Poschenrieder et al., 2001). However, its low biomass limits this plant's efficiency.

Unfortunately, there is no published literature on natural phytoextraction in vineyards and the use of plants alone has not

resulted in efficient outcomes. Because successful phytoremediation depends on the accessibility of copper in the field (Chen et al., 2006), there are two possible methods for increasing copper availability; adding chelators or using microorganisms.

3.4. Chelate assisted phytoextraction

Chelate assisted phytoremediation consists of adding an acid amendment to the entire contaminated area, inducing an acidic environment in which the copper becomes more bioavailable. Examples are EDTA, citric acid, glucose, or DTPA (McGrath et al., 2001; Chen et al., 2006). Chen et al. (2006) conducted a laboratory experiment with contaminated agricultural soils. They found that both chelators used, citric acid and glucose, promoted the availability and uptake of copper, especially by a hyperaccumulator plant. They also found that the chelators did not negatively affect soil microorganisms, a potential problem due to leaching. Treatments with plants also had higher microbial diversity than those without plants (Chen et al., 2006).

Kos and Leštan (2004) investigated the ability of four chelators to assist plants in removing copper from contaminated vineyard soils. The authors found that all but citric acid increased copper mobility, although plant uptake did not increase in the same proportion. EDDS was found to be the most successful. However, this method was also found to induce copper leaching (Kos and Leštan, 2004). The authors suggested that horizontal permeable barriers be installed to decrease environmental degradation (Kos and Leštan, 2004). This would, however, be a costly investment requiring extensive soil upheaval in vineyards and is therefore not practical. Zereński-Skorić et al. (2010) also looked at vineyard soils to identify differences in EDTA and EDDS chelate assisted phytoextraction by *Brassica napus*. The authors found that EDTA was inefficient while EDDS had potential for copper extraction. However, large amounts of EDDS induced growth suppression in the plants.

In conclusion, EDDS assisted experiments in vineyard soils were most successful. However, unlike Chen et al. (2006), other authors have observed leaching (McGrath et al., 2001; Kos and Leštan, 2004; Zereński-Skorić et al., 2010). According to Wenzel (2009), due to the high cost of chelators and their unavoidable tendency to induce copper leaching it is not recommended in sites connected to groundwater. As groundwater is always present, chelate assisted phytoextraction is not suggested for vineyard sites.

3.5. Microbially assisted phytoextraction

Microorganisms aid in environmental processes such as mobility and availability of heavy metals in soil (Gadd, 2004). “Metal mobilization can arise from a variety of leaching mechanisms, complexation by metabolites and siderophores, and methylation” (Gadd, 2004). Microbially assisted phytoextraction is potentially a suitable tool for efficient remediation in vineyards. Bacteria that produce siderophores are exploited due to their capacity to mobilize metals, especially Fe, but also Cu, and to make metals more bioavailable for plants uptake (Wenzel, 2009; Rajkumar et al., 2010; Kidd et al., 2009; Gadd, 2004; Boukhalfa and Crumbliss, 2002). This method could be considered a “co-cropping component” (Wenzel, 2009) because the bacteria are added to the soil with the plants.

Metal resistant siderophore-producing bacteria are commonly found in areas of iron deficiency where their function is to make iron more accessible to plants (Van Loon, 2009). In heavy metal polluted environments the same bacteria can also chelate other non-ferrous metals, assisting plants in taking up more metal in a shorter period of time (see Fig. 1B) (Rajkumar et al., 2010). Over

500 siderophore compounds have been identified from bacteria occurring naturally in the rhizosphere (Boukhalfa and Crumbliss, 2002), which increases the likelihood of success because they should already be present in the soil. At least one study has shown that contaminated environments can even stimulate these bacteria, either because they are an integrated part of siderophore production or because pollution restricts Fe availability and pushes the bacteria into an iron deficient, and thus a siderophore necessary, environment (Rajkumar et al., 2010).

There are two plant strategies to accumulate metals; plants (dicots and monocots, except Poaceae) rely on reducing agents or acidification of the surrounding environment; or, plants (Poaceae) release siderophores (Rajkumar et al., 2010). Siderophores act as chelators and as solubilizing vehicles by taking metals into the bacterial cell membrane, reducing their charge and releasing them into the cell (Rajkumar et al., 2010). The siderophore can be destroyed or recycled (Rajkumar et al., 2010). In order to implement this method, bacteria resistant to toxins must be isolated and then inoculated into the particular environment in which they are resistant (Andreazza et al., 2010c).

Currently, only one study has attempted this method in a copper contaminated vineyard soil and to date only in the laboratory. “Three copper resistant bacterial isolates from oatmeal rhizosphere (*Pseudomonas putida* A1; *Stenotrophomonas maltophilia* A2 and *Acinetobacter calcoaceticus* A6) were used for the stimulation of copper phytoextraction” (Andreazza et al., 2010a). Copper uptake in the plant shoots was improved with the isolates (see Table 2 for figures). Overall, the bacteria assisted oats in taking up more copper than it naturally would. The authors suggest this method for the removal of copper from polluted sites (Andreazza et al., 2010a). Thereafter, they also isolated *Pseudomonas* sp. strain NA, a copper resistant bacteria, from a vineyard soil and investigated how it reduces copper from Cu(II) to Cu(I), which increases availability to ATPases and the cell (Andreazza et al., 2010b). It had greater than 50% efficiency in reducing Cu(II) using mainly a copper reductase enzyme and its efficiency increased with greater total copper levels. This suggests that siderophores and specifically the copper reductase enzyme boost copper bioavailability also to plants. As total copper increased and reduction percent increased, however, the bacterial biomass decreased. Therefore, more copper was reduced despite fewer bacteria. Copper reduction occurred between 20 °C and 35 °C with pH between 5 and 7, which suggests that reduction is possible over a range of temperatures if other factors, i.e. pH and pollutant concentration, are suitable (Andreazza et al., 2010c).

Other studies have suggested different enzymes or bacteria to enhance phytoextraction, such as histidine/proline, a Cu chelator in the xylem sap of *Brassica carinata* (Kidd et al., 2009). Plant growth promoting rhizobacteria (PGPR) have also been used to encourage plant growth, increasing metal uptake indirectly (Kidd et al., 2009; Ma et al., 2009). Uptake in roots or shoots remains unclear. These methods have not been tested in vineyards, but they provide interesting results and should be reviewed more closely as another method for combining bacteria and plants in copper removal.

A disadvantage of this method for wineries, as is the case for all phytoextraction techniques, is that it requires time to achieve Cu reduction in the soil and labor to remove the copper enriched plant mass (Wenzel, 2009). This is especially true if most of the copper is held in the roots. Also, since to date it has been demonstrated only in the laboratory, there remains the question of feasibility *in situ*.

4. Conclusions & outlook

Robinson et al. (2009) consider phytoextraction to be “unsuitable for most, if not all, sites due to low-extraction rates and problems caused by site heterogeneity, the limited rooting depth of

plants and the presence of contaminant mixtures.” The available literature does not wholly support this statement. First in vineyards copper contamination is low to moderate. Second copper resides in the topsoil, making it suitable for shallow depth remediation. Third vineyards represent a single contaminant site where only copper is found in high levels. Finally, site heterogeneity can be accommodated through improvements in methods and new ideas that could be integrated into vineyard systems over time. These include integrated pest management with increased biodiversity. Downy Mildew originates in the soil. Encouraging diverse plant life to create a barrier between the ground and the grapevines could further suppress fungal growth and also enhance dynamic microbial communities. When a complex system is diverse it adapts more easily to changes in toxicity, climate, soil type, etc. Focus on field experiments to define both true applicability and true resource competition in contaminated environments is also necessary (Wenzel, 2009). Pot experiments cannot replicate field environmental factors such as rooting density, compaction, and soil heterogeneity.

If no remediation techniques prove workable in vineyard ecosystems there are no options other than restricting copper to levels which can be removed through the afore-mentioned strategies or banning copper use completely. The former would require a much more precise application of copper. Precision application is already being utilized in certain parts of Europe and can be achieved with software programs indicating when to most effectively apply copper, including vitimeteo.at and agromefeo.de, the “Coptimizer,” the “Environmental Impact of an Organic Viticulture Indicator,” and others (Kuflik et al., 2009; Fragoulis et al., 2009; Pellegrini et al., 2010).

As alternative options for organic winegrowers are not yet effective (Heibertshausen et al., 2007) and the climate is changing, increasing potential for infection from plant pathogens (Salinari et al., 2006), a holistic remediation approach is necessary. Phytoremediation can be a sustainable technique creating zero waste, while improving soil quality (Haferburg and Kothe, 2010). Plant biodiversity, enhancement of microorganisms, specifically siderophore production, and reduced copper use, should be reinforced. Specifically, multidisciplinary relationships in the rhizosphere should be the focus of further research.

Acknowledgments

Thank you very much to the Faculty of Agriculture at the University of Hohenheim (Landesgraduiertenförderung) and the Carl Zeiss Stiftung for funding this work as a part of the Doctoral thesis by Kathleen Mackie. To our colleagues in the Soil Biology group at the University of Hohenheim and to Dr. Sabine Zikeli thank you for your attentive proofreading. Thank you, Manuel Hilscher, for bringing our illustration to life with your digital graphics skills and assistance. Finally, thank you to the anonymous reviewer who gave us very helpful comments during the revision process.

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