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Identifying candidates for the phytoremediation of copper in viticultural soils: A systematic review

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ABSTRACT

For many years, copper-based fungicides have been used in viticulture and have contributed to increasing concentrations in soils. Today, it is not uncommon to find vineyard soils with total copper topsoil concentrations above 100 mg kg^{-1}, which may have consequences for both the environment and human health. Phytoremediation, the use of plants to remove heavy metals from soils, is a promising and environmentally-friendly method to extract copper from soils. The objectives of this study were to review and synthesise the current knowledge on copper phytoremediation in vineyard soils and identify future applications. A systematic literature search in Web of Science was conducted on 19 July 2022 and resulted in twenty-seven papers meeting the inclusion criteria. Approximately one third of the papers were from Brazil and most of the experiments had been carried out in pots. In some studies, the addition of bacteria or chelators was also evaluated. Some species, such as *Plantago lanceolata* L. or *Ricinus communis* L., can accumulate copper in their tissues at concentrations above 1000 mg kg^{-1}. Addition of bacteria and chelators to the soil can also increase the copper uptake capacity by plants. However, most of the species evaluated accumulate copper in the roots, rather than in the shoots, thus limiting the implementation of this method in practice. Further studies are thus needed to find other hyperaccumulator plants. Future research should focus primarily on the ability of plants to accumulate copper in their aerial parts, their ability to transfer copper from roots to shoots, and their biomass production under high soil copper concentrations. Longer-term experiments and more *in situ* testing are also needed to evaluate the potential for development and use of copper phytoremediation in vineyards. To conclude, species of the Poaceae and Lamiaceae families are the most promising so far for phytoremediation. Identifying plants able to translocate copper from the roots to the aerial parts will be an important factor in the success of this method.

1. Introduction

In 2020, the world's vineyards covered 7.3 million hectares, all production types combined (OIV, 2021). This area has been stable overall in recent years, although with heterogeneous development between countries (OIV, 2021). The European Union has nearly half of the world's vineyards with 3.3 million hectares, mainly in Spain (961 million ha), France (797 million ha) and Italy (719 million ha) (OIV, 2021). China has the third largest vineyard area in the world with 785 million (OIV, 2021). Like other crops, grapevine is susceptible to numerous pests and diseases, including downy mildew caused by *Plasmopora viticola* and for which the first means of control dates back to the 19th century (Komárek et al., 2010). In 1882, Pierre Marie Alexis Millardet discovered by accident the fungicidal action of copper sulphate combined with lime ($CuSO_4$–$5H_2O$ + $Ca(OH)_2$) against downy mildew, while, in 1885, Bordeaux mixture, one of the best known fungicides to date, was introduced (Millardet, 1933; Russell, 2005). Since then, the use of copper (Cu) as a fungicide in viticulture has continued to increase and new formulations have been developed to control various fungi (Klittich, 2008).

Given widespread use of copper in viticulture, it is not uncommon to find total copper topsoil concentrations above 100 mg kg^{-1} (Deluisa et al., 1996; Vavoulidou et al., 2005; Wightwick et al., 2008; Fernández-Calviño et al., 2009), whereas this value is generally not exceeded in uncultivated land (Pietrzak and McPhail, 2004; Ruyters et al., 2013). Indeed, there was one case of total concentration above 3000 mg kg^{-1} in the soil of a 100-year-old vineyard (Mirlean et al., 2007). Climate and location play an important role in the use of Cu-based fungicides. In New Zealand, the median copper application is 1.2 kg ha^{-1} $year^{-1}$ (Morgan and Taylor, 2004), in Italy it is 7.4 kg ha^{-1}

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year^{-1} (Kovačič et al., 2013) while in Brazil it is 30 kg ha^{-1} year^{-1} (Da Rosa Couto et al., 2015). The distribution of copper in viticultural soils varies greatly from region to region but also within a vineyard itself, between the vine row and the inter-row (Mackie et al., 2013; Ballabio et al., 2018). This is due to its low mobility and its high ability to associate or adsorb to soil particles (Cesco et al., 2021). The bioavailability of copper is strongly related to soil properties, such as pH (most important factor), organic matter content, clay content, phosphate content, and cation exchange capacity (CEC) (Zaidi et al., 2003; Pinto et al., 2014). In addition, the biological activity of the soil as well as environmental conditions, such as moisture, temperature, or oxidation state, can influence its mobility.

Although copper is a plant micronutrient, the accumulation of this heavy metal in viticultural soils directly impacts plants by reducing soil fertility and productivity (Mäder et al., 2002; Hendgen et al., 2018). In addition, it also impacts living soil organisms (Flemming and Trevors, 1989). Copper toxicity in grapevine is expressed by a decrease and modification of the root system, a decrease in dry matter production, a modification of plant nutrient uptake (mainly P and K), leaf chloroses and a decrease in chlorophyll (Ambrosini et al., 2018; Cesco et al., 2021). In a recent meta-analysis, Karimi et al. (2021) shows that repeated high inputs of copper-based fungicides decreased soil microbial activity by 30%, collembola and enchytraeid reproduction by 50%, and earthworm biomass by 15%. In addition, young earthworm development and cocoon production were greatly reduced even at soil copper concentrations slightly below 9 mg kg^{-1}, while a concentration of 16 mg kg^{-1} decreased reproduction and greatly impacted populations (Helling et al., 2000).

To decontaminate soils polluted with heavy metals, chemical, physicochemical, and biological methods exist (Sikdar and Kundu, 2018). The mobility or toxicity of heavy metals can, for example, be limited by using oxidizing, reducing or neutralizing chemicals. Electronic treatments, such as the use of reactive permeable barriers or filtration and adsorption methods are all possible physicochemical means. Biological treatments rely on the use of living organisms such as fungi, bacteria or plants. Traditional methods, such as excavation and landfilling, do not provide adequate solutions for such operations and are neither economically viable nor environmentally feasible on a large scale (Garbisu and Alkorta, 2001; Fan et al., 2012). In contrast, phytoremediation, a biological method based on the use of plants, is a potentially cost-effective and promising approach. Several phytoremediation techniques exist, including phytoextraction, which uses plants that accumulate heavy metals in their tissue. The aerial parts can then be harvested and exported for safe treatment by drying, ashing or composting. Some plants are defined as "hyperaccumulators" and are those that are capable of accumulating copper to a concentration higher than 1000 μg g^{-1} (ppm) of dry matter (Baker and Brooks, 1989). Hyperaccumulator plants are also characterized by a ratio of heavy metal concentration between shoots and roots >1 (Baker et al., 1999). They thus accumulate more heavy metals in the aerial parts than in the root system. Phytoremediation has the advantages of (1) being able to be performed *in situ*, (2) being inexpensive, (3) being ecologically beneficial, and (4) being socially accepted (Bouhadi et al., 2021). Its main disadvantages are (1) the time required to obtain results and (2) the intrinsic characteristics of hyperaccumulator plants (e.g., often accumulate a single specific element, grow slowly and/or produce little biomass).

Phytoremediation is a potential technology for *in situ* removal of accumulated copper from vineyard soils. Yet few studies have been conducted to date and many of those are not *in situ*. Furthermore, few have been multilocational. Despite widespread use of copper and risks of copper toxicity in vineyards, an economically important agricultural system, there has been no recent or systematic synthesis of existing studies nor identification of precise research gaps. The aim of this study was to systematically review the existing literature on what options there are for phytoremediation of copper in contaminated vineyard soils. A previous review on the possibilities of copper remediation in vineyards was done in 2012 but in a narrative format (Mackie et al., 2012). The objectives of this study were to identify where studies have been done, describing them, compiling a list of candidate species for phytoremediation of copper in vineyard soils and estimating their extraction potential. We also aimed to identify knowledge gaps and suggest possibilities for future development of this method to guide further field trials and implementation of phytoremediation in practice.

2. Methods

2.1. Data inclusion criteria

A literature search was conducted in Web of Science on 19 July 2022 using the following keyword strings in the topic field: (viticulture OR vineyard* OR wine-growing OR "vitis vinifera" OR "grape production*" OR "grapevine production*" OR grape* OR grapevine*) AND (bioremediation OR phytoremediation OR phytoextraction OR phytostabilization OR phytoaccumulation OR remediation OR "biological method*" OR "copper-accumulating plant*" OR hyperaccumulate* OR bioadsorbent* OR bio-adsorbent*) AND (copper OR Cu OR heavy-metal* OR "toxic element*" OR "copper pollution" OR "copper availability" OR "copper-contaminated site*" OR "copper phytoextraction"). The search was conducted using only English terms yet no restrictions on language or document type were made. No time restrictions were applied. The initial search resulted in 130 publications.

2.2. Data exclusion criteria

The articles were then evaluated by analyzing them using the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) method (Moher et al., 2009) (Fig. 1). A "backward snowball" (Wohlin, 2014) was also done by checking the reference sources of the captured papers, resulting in eight additional publications. Twenty-seven publications were therefore used in the systematic review.

2.3. Data extraction

The following data were extracted: (1) location; (2) soil texture; (3) concentration of copper in study soils; (4) study design (experimental or observational); (5) types of experiment conducted; (6) plant species studied; (7) plant families studied; (8) treatments evaluated (factors, control, number); (9) duration of study; (10) response variables.

The response variables of importance that were compiled from the studies were (i) plant aerial copper concentration; (ii) plant root copper concentration; (iii) total plant copper concentration; (iv) plant aerial dry mass; (v) plant root dry mass; (vi) total plant dry mass; (vii) plant height; (viii) translocation factor; (ix) estimated copper phytoextraction potential.

2.4. Additional data synthesis and analysis

When the texture of the studied soils was not mentioned in the papers, but the clay, silt and sand contents were available, it was characterized using the online "Soil Texture Calculator tool" (USDA, 2022). For some studies, response variable data, such as total copper concentrations or total plant dry masses, were calculated according to the results reported in the articles. When not reported, the translocation factor, i.e. the ability of a plant to translocate heavy metals from the roots to the aerial parts (Nirola et al., 2015), was calculated by dividing the copper concentration of the aerial plant parts by the copper concentration of the roots.

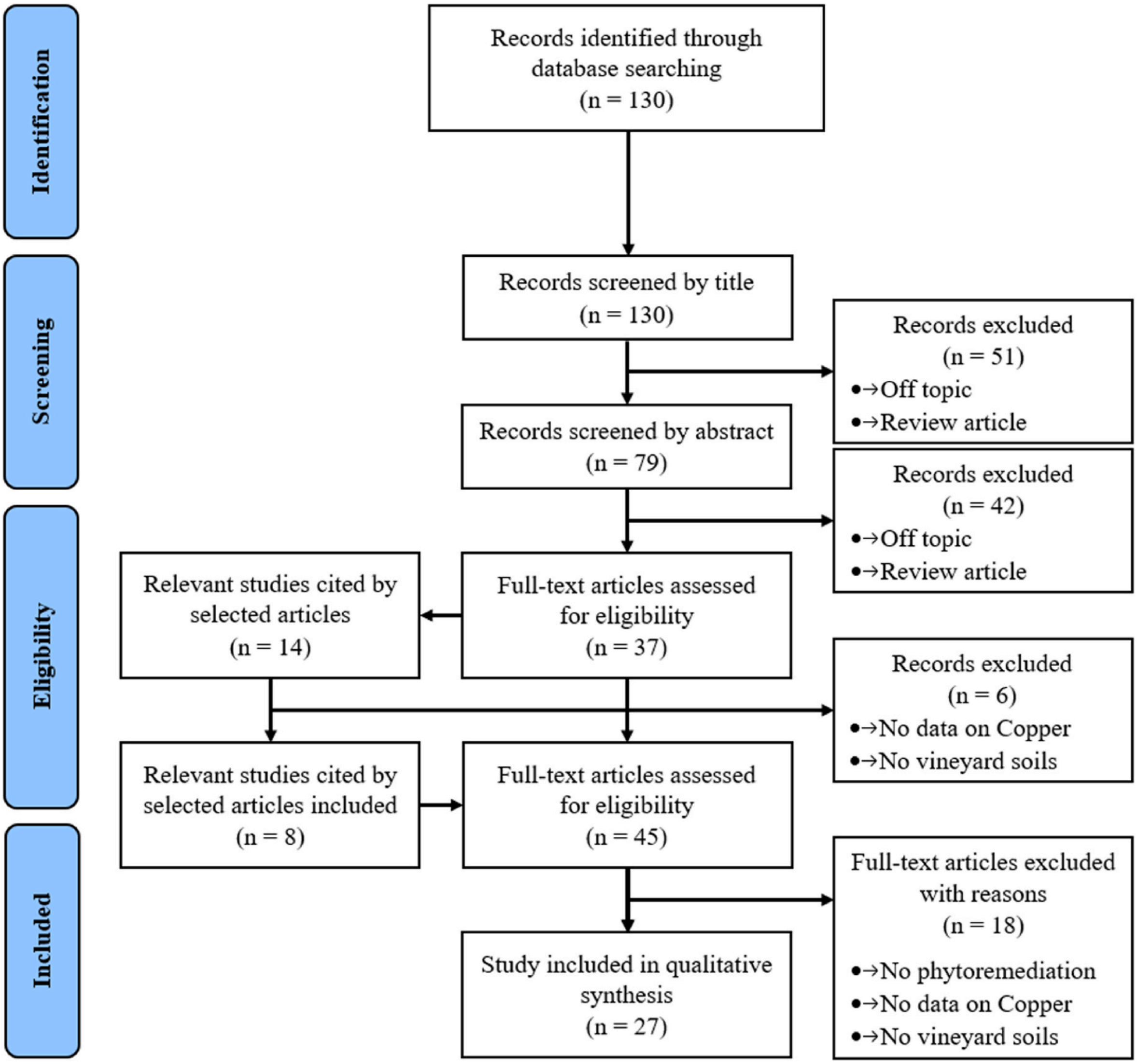

Fig. 1. PRISMA flow diagram.

3. Results and discussion

3.1. Categorisation and description of the general framework of studies

Of the 27 studies, nine were from Brazil. Seventeen were conducted in Europe, including eight in France and two each in Switzerland, Italy, and Spain. Tunisia was the only African country where an experiment was performed (Fig. 2a). All studies in South America were conducted in Brazil, despite being only the third largest wine producer on the continent, far behind Argentina and Chile (OIV, 2021).

Almost three quarters of the studies conducted (n = 19) used phytoremediation alone. Remaining studies assessed if the addition of bacteria (Andreazza et al., 2010; Fatnassi et al., 2015; Randriamamonjy et al., 2021; D'Incau et al., 2022), chelators (Kos and Leštan, 2004; Zeremski-Škorić et al., 2010), bioadsorbents (Fernández-Calviño et al., 2017), or immobilizing amendments (Shaheen et al., 2015) complemented phytoremediation (Fig. 2b). No study was found on the addition of fungi despite this method being promising for phytoremediation of heavy metals (Meier et al., 2012).

Of the twenty-seven papers found, 15% (n = 4) were observational studies of spontaneous vineyard flora and potentially copper-accumulating species (Fig. 2c). Approximately two-thirds of the studies (n = 19) were conducted *ex situ* with vineyard soil, whereas only four experiments were conducted directly in vineyards. *Ex-situ* studies were mostly conducted in pots in a greenhouse (n = 12), a growth chamber (n = 2) or an outdoor growing chamber (n = 1). All soil textures except "sandy clay", "silt" and "silty clay" were represented in the papers. The most represented soil textural class was loam, followed by sandy clay loam, silt loam, sandy loam and clay loam.

Concerning trial duration, (i.e., excluding observational *in situ* studies), 83% (n = 19) of the experiments lasted less than three months, of which fourteen lasted between one and two months. One research was conducted in pots in the greenhouse for four months (Malagoli et al., 2014) and two *in situ* experiments continued for up to one year (Mackie et al., 2014, 2015). Two papers did not mention experimental duration because these consisted of analyzing durum wheat (*Triticum turgidum durum* L.) plants sown in former vineyard soils (Michaud et al., 2007) or ryegrass (*Lolium perenne* L) plants growing in vineyard inter-rows (Duplay et al., 2014). Most trials were thus conducted over a short experimental period and on young seedlings.

Among the factors evaluated, the copper concentration of aerial plant parts was always analysed, whereas the copper concentration of roots was examined in 81% (n = 22) of the papers (Fig. 3). Total plant copper concentrations and translocation factors were mentioned or could be calculated for twenty-three (85%) and twenty-two papers (81%) respectively. The phytoextraction potential of copper per hectare

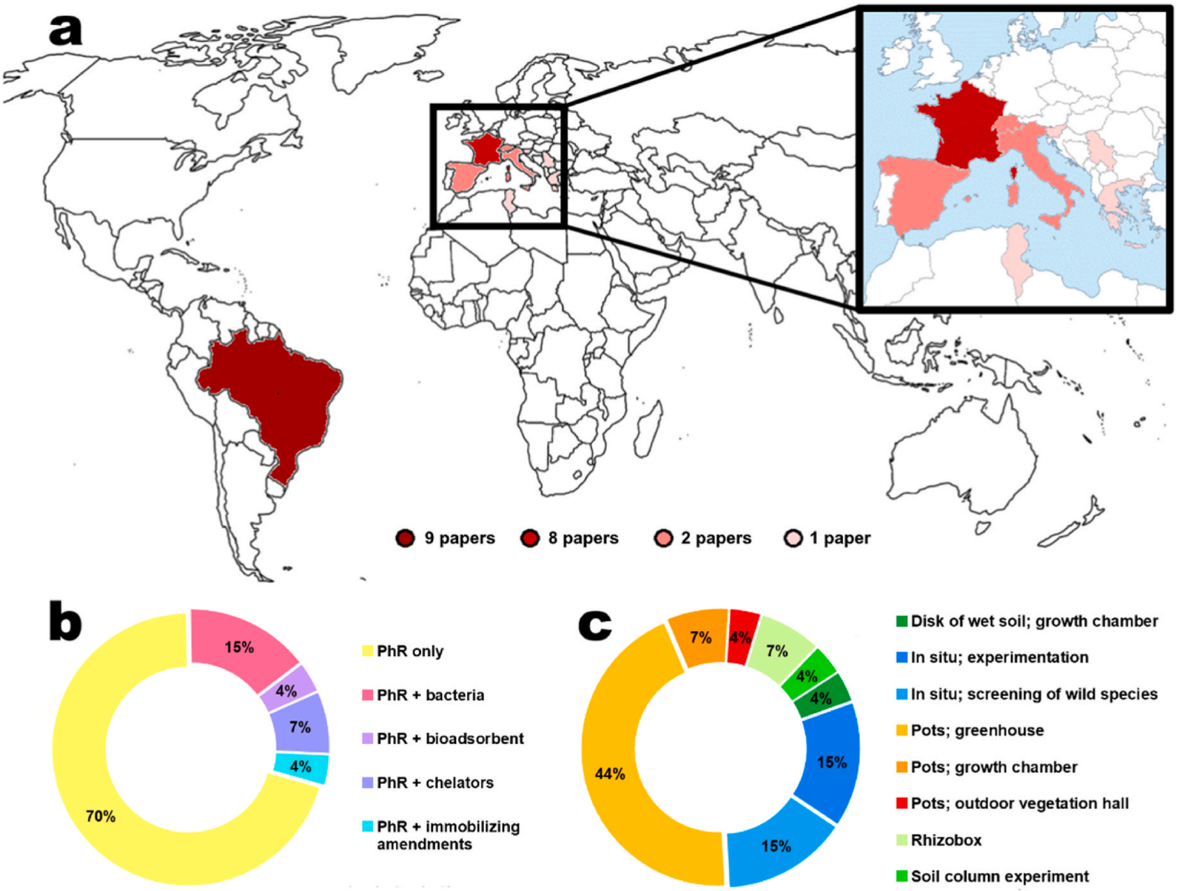

Fig. 2. Characteristics of the 27 reviewed studies on copper phytoremediation in contaminated vineyard soils. **a** Location of studies by country. **b** Method of phytoremediation (PhR) performed. **c** Type of experiment/study performed (n = 27; [1] – [27], for codes, see Appendix 1).

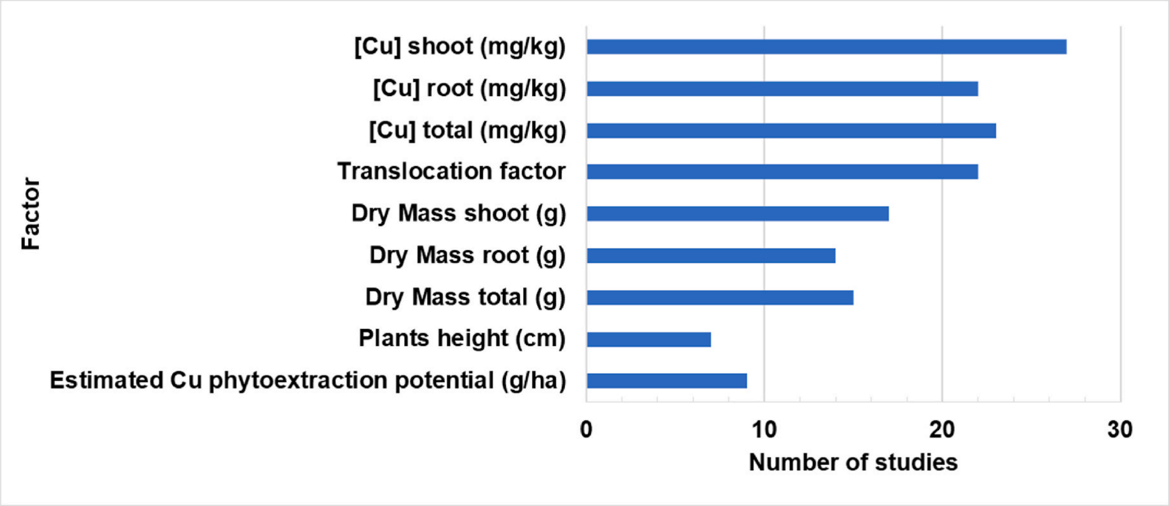

Fig. 3. Number of studies grouped by type of factor assessed or by data available to calculate them in the articles (n = 27; [1] - [27]). The translocation factor is the ratio of copper concentration between aerial parts and roots.

was estimated in only nine studies (33%).

3.2. *Phytoremediation of vineyard soils*

3.2.1. *Copper levels in vineyard soils studied*

Due to the properties of copper in soils (low mobility, altered bioavailability depending on soil properties, etc.) and the various current extraction methods, there are some limitations on comparability. Copper concentrations usually decrease with depth (Deluisa et al., 1996). In analyses of different soils taken from a depth between 0 and 25 cm and using EDTA as the extraction method, many vineyards had an available copper concentration below 75 mg kg^{-1} (Fig. 4). Sixteen soils with copper concentration above 100 mg kg^{-1} were also surveyed. Total pseudo and total copper concentrations obtained with Aqua regia and

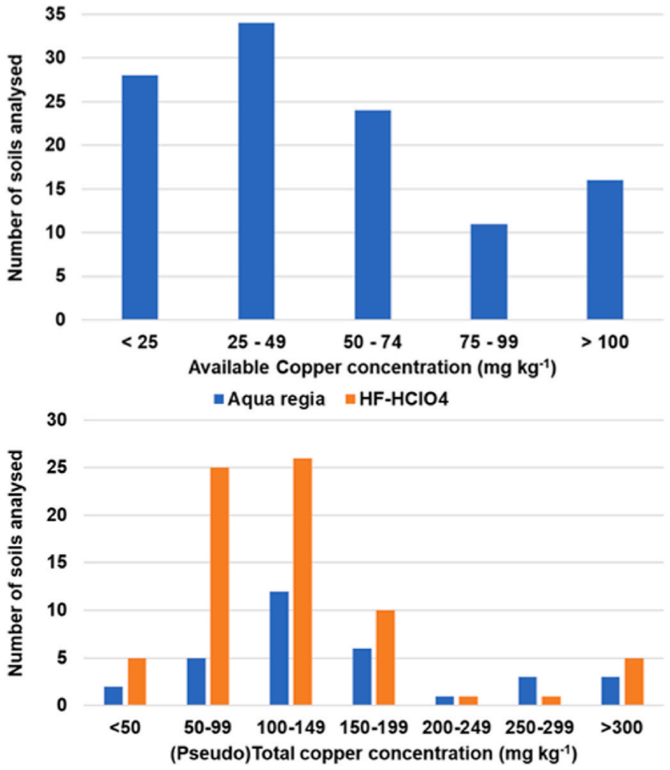

Fig. 4. Distributions of available (extractive = EDTA), pseudo-total (extractive = Aqua regia: 1 part HNO_3/3 parts HCl) and total (extractive = $HF–HClO_4$) Copper concentrations in various 0–25 cm high soil analyses (n = 11; [6] – [12], [16], [21], [23], [26]).

$HF-HCLO_4$ as extractives were generally between 50 and 200 mg kg^{-1}, respectively. Concentrations higher than 300 mg kg^{-1} have also been found, while an exceptional total copper concentration of 1030 mg kg^{-1} was found from a vineyard soil in the department of Hérault, France (Michaud et al., 2007).

Chaignon et al. (2003) analysed several vineyard soils in the Occitanie region of southern France and characterized the copper concentrations at different depths. In general, it was found that concentrations decreased with depth, following a similar trend to results obtained by Deluisa et al. (1996). For five analysed soils, Chaignon et al. (2003) compared copper concentrations (both available and total) between 0-2 cm and 2–15 cm depths. They found that available copper concentrations ranged between 22 and 163 mg kg^{-1} at 0–2 cm depth, yet ranged between 4 and 61 mg kg^{-1} at 2–15 cm depth (Chaignon et al., 2003). Total copper concentration showed the same tendency, ranging from 75 - 346 mg kg^{-1} at 0–2 cm depth versus from 22 to 146 mg kg^{-1} at 2–15 cm depth. Generally, sampling points with the highest surface concentrations also had the highest copper levels at depth. For two of the five sampling points analysed, the copper concentrations between 0 and 2 cm depth were slightly lower than those between 2 and 15 cm (Chaignon et al., 2003). This can easily be explained by runoff and leaching. Given the concentration in the topsoil, special attention should be paid to the root system architecture of the plants. It seems judicious to select species that develop a strongly branched root system on the surface to absorb maximal copper.

In vineyards, soil copper concentrations greater than ten times those in soil of adjacent areas have been found (Brun et al., 2001; Krishnamurti et al., 2007; Andreazza et al., 2010; Malagoli et al., 2014; Girotto et al., 2016). This clearly demonstrates the unintended impacts of using copper-based plant protection products.

3.2.2. *In situ phytoremediation – screening of wild species*

More than thirty-nine wild plant species were collected from vineyards in France, Spain and Brazil and analysed to quantify copper concentrations in plants and find candidates for phytoremediation (Brun et al., 1998; Campillo-Cora et al., 2019; Da Silva et al., 2020; Melo et al., 2021) (Table 1). In general, plant total copper concentrations did not exceed 100 mg kg^{-1} dry mass. In a study conducted by Melo et al. (2021), five species had higher copper accumulation capacities: *Cyperus compressus* L. (105.0–276.0 mg kg^{-1}), *Chrysanthemum leucanthemum* L. (103.0–319.1 mg kg^{-1}), *Lolium multiflorum* Lam (198.6–289.1 mg kg^{-1}), *Paulownia tomentosa* Premna (126.2–175.6 mg kg^{-1}) and *Vicia sativa* L. (115.5 mg kg^{-1}). Nevertheless, they cannot be defined as hyperaccumulator plants according to the criteria defined by Baker and Brooks (1989). Moreover, these species accumulated copper mainly in the root system and not in the aerial parts. All species tested, except *Ageratum conyzoides* L., accumulated less than 100 mg kg^{-1} copper in aerial parts and translocation factors varied greatly between species but also between individuals of the same species. It should be noted that the species *Allium polyanthum* Schult. & Schult.f., *Andryala integrifolia* L., *Conyza albida* Willd. ex Spreng, *D. carota*, *Rumex acetosella* L., *Rumex induratus* Boiss. & Reut. and *Phytolacca americana* L. had translocation factors close to or greater than 2. In a study by Campillo-Cora et al. (2019), an exceptional translocation factor of 5.9 was calculated for *Chenopodium album* Bosc ex Moq. All these species, particularly the latter one, could be candidates for copper phytoremediation in contaminated vineyard soils.

3.2.3. *Phytoremediation – experiments*

Among the plants used in the experiments, 38% (n = 14) were Poaceae (Fig. 5). A quarter of species used (n = 9) were Fabaceae, four from the Brassicaceae and the Asteraceae. Of the forty-five plant families with currently known heavy metal hyperaccumulating plants (Bouhadi et al., 2021), Brassicaceae, Fabaceae, and Poaceae were well represented in the experiments.

Of the thirty-six species used in the experiments, three can be considered as hyperaccumulators (Table 2). These were *Avena sativa* L. (Poaceae; oats), *Plantago lanceolata* L. (Plantaginaceae; ribwort plantain) and *Ricinus communis* L. (Euphorbiaceae; castor bean), and they accumulated concentrations of more than 1000 mg kg^{-1} of copper in their tissues. These plants stored nearly all of the copper in their root systems but had significant phytoextraction potential per hectare. Andreazza et al. (2015b) calculated that *P. lanceolata* could extract up to 2.2 kg of copper per hectare from the soil over a growing season, while *R. communis* could extract from 3.1 kg ha^{-1} to 5.9 kg ha^{-1} (Andreazza et al., 2013). Although the total copper concentrations of these two species were similar, their phytoremediation potential differed. This can easily be explained by their respective development and the biomass produced over a year, greater for castor bean, which is also tolerant of high heavy metal concentrations (Wang et al., 2016). Nevertheless, the higher biomass production of castor bean could also be a disadvantage as it is a strong competitor for water and nutrients, it may limit access to the crop due to its fast growth and given that it is tall, it may shade the vine. On the other hand, *P. lanceolata* is a shorter perennial plant, which is less competitive with the crop. Two other plants, *Arachis pintoi* (Fabaceae) and *Bidens pilosa* L. (Asteraceae) also had high phytoextraction potentials, up to 2.5 kg ha^{-1} and 3.5 kg ha^{-1}, respectively (Andreazza et al., 2011; Andreazza et al., 2015a).

A study by Chaignon et al. (2002) highlighted that wheat grown under iron deficient conditions in copper-contaminated vineyard soils took up more than 10-fold more Cu compared with a control, probably due to the plant releasing siderophores which increased availability. In another study, adding chelators to soils greatly increased copper concentrations in aerial parts of oilseed rape (*Brassica napus* L.) and resulted in translocation factors between 1.44 and 4.05 (Zeremski-Škorić et al., 2010). Kos and Leštan (2004) also observed an increase in copper concentration in aerial parts of *Brassica rapa* var. *Pekinensis* (Lour.) Hanelt

Table 1

Main results obtained in articles that have made observations of spontaneous flora in vineyards. When several data were available for the same species in a paper, the minimum and maximum obtained for each factor are mentioned. (n = 4).

Plant species	[Cu] total (mg kg^{-1})	[Cu] shoot (mg kg^{-1})	[Cu] root (mg kg^{-1})	Dry Mass total (g)	Dry Mass shoot (g)	Dry Mass root (g)	Translocation factor	Source
Allium polyanthum	15.7–16.1	3.8–10.2	5.5–12.3				0.3–1.9	[7]
Andryala integrifolia	14.0–57.4	9.4–26.6	4.6–37.4				0.5–2.1	
Dactylis glomerata		3.5–12.2						
Hypochoeris radicata	15.9–37.8	8.5–21.6	7.4–16.5				0.9–1.3	
Poa annua		10.9–11.4						
Rubia peregrina	17.0–87.0	4.5–22.6	9.2–71.0				0.2–0.9	
Rumex acetosella	15.1–49.7	9.8–32.7	5.3–17.0				1.8–1.9	
Sanguisorba minor	21.1–22.1	9.0–11.3	9.8–13.1				0.7–1.2	
Senecio vulgaris	29.6–48.1	14.7–19.8	14.9–28.3				0.7–1.0	
Chenopodium album	52.0	45.3	6.7				5.9	[8]
Conyza albida	19.9–72.3	9.8–44.1	10.1–28.2				1.4–2.3	
Digitaria sanguinalis	58.4–108.8	6.6–36.8	49.6–73.3				0.4–0.7	
Phytolacca americana	41.0–81.7	4.9–62.5	19.2–36.1				1.8–2.9	
Picris hieracioides	46.1–133.5	12.3–62.5	30.4–71.0				0.2–1.5	
Rumex induratus	20.3	13.5	6.8				2.0	
Setaria viridis	39.3	12.3	27.0				0.5	
Tolpis barbata	57.1	36.8	20.3				0.7	
Ageratum conyzoides		22.7–126.7						[11]
Cynodon dactylon		32.7–49.0						
Desmodium barbatum		24.6–42.7						
Paspalum notatum		34.1–84.3						
Paspalum plicatulum		51.2–117.6						
Richardia brasiliensis		30.9–63.2						
Cypressus compressus	105.0–276.0	36.0–69.0	69.0–229.5	0.3–9.7	0.2–7.2	0.1–2.5	0.2–1.1	[22]
Chrysanthemum leucanthemum	103.0–319.1	25.7–52.8	76.8–266.3	2.4–15.8	2.0–12.6	0.4–3.2	0.1–0.4	
D. carota	25.7–62.7	12.3–43.7	13.4–19.0	1.0–8.8	0.8–7.5	0.2–1.3	0.6–3.2	
E. heterophylla	60.6–65.9	9.4–12.3	48.3–56.5	0.2–0.7	0.1–0.6	0.1	0.2	
I. cairica	45.2–63.6	18.6–22.6	26.6–41.0	1.0–2.0	0.7–1.8	0.2–0.3	0.5–0.7	
Lolium multiflorum	198.6–289.1	11.1–14.8	187.5–274.3	49.1–89.5	46.1–81.6	3.0–7.9	0.1	
O. dillenii	85.1–92.1	42.2–43.4	41.7–49.9	1.8–1.9	1.4–1.8	0.1–0.4	0.9–1.0	
P. tomentosa	126.2–175.6	28.4–57.7	58.3–117.9	1.0–7.4	0.9–6.6	0.1–0.9	0.2–0.5	
R. obtusifolius	26.0–59.9	15.4–34.3	10.6–21.6	1.9–7.3	1.8–4.2	0.1–3.1	1.4–1.6	
S. nodiflora	83.6–117.3	24.5–46.7	59.1–70.6	0.5–2.7	0.4–2.4	0.1–0.3	0.4–0.7	
S. oleraceus	78.3	18.8	59.5	14.6	12.8	1.8	0.3	
S. rhombifolia	33.1–65.7	17.0–34.3	16.1–31.1	1.1–4.2	1.3	0.1	0.9	
Setaria sp.	83.0	17.6	65.4	3.0	2.7	0.3	0.2	
T. campestre	59.8	10.6	49.2	1.5	1.3	0.2	0.2	
T. pratense	60.7–85.8	16.2–32.5	44.5–55.0	4.8–12.2	3.9–11.0	0.9–1.2	0.3–0.7	
V. sativa	115.5	15.9	99.6	5.5	5.3	0.2	0.2	

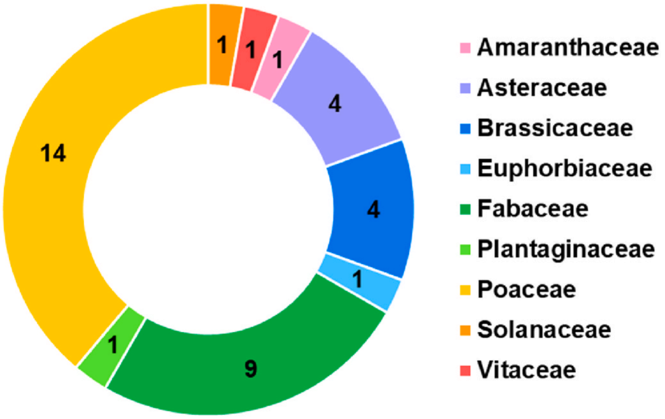

Fig. 5. Plant families of species used in the experiments (n = 22; [1] – [6], [9], [10], [12] – [21], [23] – [26]).

when EDTA, DTPA or EDDS were added to the soil. Among plant families, the Poaceae appear to be the most effective at accumulating copper in tissues with several species showing maximum total concentrations above 500 mg kg^{-1} (Brun et al., 2001; Michaud et al., 2007; Malagoli et al., 2014; Girotto et al., 2016). In a study conducted by Andreazza et al. (2011), the addition of bacteria as a supplement to phytoremediation boosts the copper extraction potential of *A. sativa* plants, increasing the concentrations of the heavy metal in the aerial parts and the total biomass produced to a lesser extent.

3.3. Study limitations and future research

Phytoremediation is a promising technique to extract copper from vineyard soils accumulated from years of fungicide application. Research on copper hyperaccumulating plants for viticulture is in its early stages, but some promising species are emerging, such as *P. lanceolata* and *R. communis*. However, many factors need to be considered when implementing this method. First, the selected plants should not unduly compete with the crop for water, nutrients and light. Special attention must be paid to the use of the space available for the hyperaccumulating plants and that used by the vine. They should not interfere with the maintenance and harvesting of the grapes, nor should they host or favour crop pests or diseases. For example, it might be possible to intercrop *P. lanceolata* both in the interrow space and between plants within the row as this species has a relatively low aerial development. On the other hand, it would not be possible to intercrop *R. communis* within the vine row given its tall stem and as it would shade the vine, unless harvested at a young stage. Combinations of different species might increase total uptake but, in this case, plants with complementary morphologies (pivotal and superficial root systems, covering and erect plants, etc.) should be selected. Finally, studies are currently focused on the search for hyperaccumulator species but not on the

Table 2

Main results obtained in the pot experiments. When several data were available for the same species in a paper, the minimum and maximum obtained for each factor are mentioned. (n = 23)

Key: EDTA: ethylenediamine tetraacetic acid; EDDS: Ethylenediamine disuccinic acid; DTPA: diethylenetriamine-pentaacetate.

Plant species	Additives/Complements	[Cu] total (mg kg^{-1})	[Cu] shoot (mg kg^{-1})	[Cu] root (mg kg^{-1})	Dry Mass total (g)	Dry Mass shoot (g)	Dry Mass root (g)	Translocation factor	Source
Avena sativa			12.6						[20]
Brassica napus			3.6						
Chenopodium spp.			9.0						
Secale cereale			4.5						
Trifolium incarnatum			14.3						
Vicia villosa			8.4						
Bidens pilosa		410–880.	15–36.	395–844.	2.07–2.80	1.30–1.60	0.77–1.20	0.04	[2]
Plantago lanceolata		520–1106.	68–142.	452–964.	0.32–1.61	0.27–1.30	0.05–0.31	0.15	
Festuca rubra cv. Merlin		533–899.	32–59.	501–847.		1.65–5.67		0.06–0.09	[21]
Sinapis alba		216–484.	17–41.	193–443.		0.76–3.32		0.07–0.10	
Species mix	–		19.58–38.33						[19]
	8 t ha^{-1} biochar		18.33–44.17						
	55 t ha^{-1} compost		22.08–32.50						
	63 t ha^{-1} biochar (8 t ha^{-1}) + compost (55 t ha^{-1})		21.67–40.00						
Arachis pintoi		527–827	27–52	475–800.	1.27–2.80	1–2	0.27–0.80	0.04–0.11	[4]
Avena sativa	–	1379–1549	55–62	1324–1487	0.30–0.82	0.23–0.52	0.07–0.30	0.04	[5]
	Pseudomonas putida	1197–1285	127–175	1070–1110	0.49–0.70	0.33–0.46	0.16–0.24	0.12–0.16	
	Stenotrophomonas maltophilia	1132–1186	65–110	1022–1121	0.38–0.80	0.24–0.52	0.14–0.28	0.06–0.11	
	Acinetobacter calcoaceticus	1132–1340	55–90	1077–1250	0.50–0.75	0.30–0.46	0.20–0.29	0.05–0.07	
Avena strigosa cv. UPF Moreninha		37.9–766.6	13.7–38.3	24.2–738.9	0.06–0.20	0.30–0.16	0.02–0.04	0.03–0.57	[16]
Brassica napus	–	237.2	16.6	220.6		40.48		0.08	[27]
	2 mmol kg^{-1} EDDS	283.0	38.6	244.4		38.87		0.16	
	4 mmol kg^{-1} EDDS	222.7	131.5	91.2		25.32		1.44	
	8 mmol kg^{-1} EDDS	394.5	316.4	78.1		14.35		4.05	
	2 mmol kg^{-1} EDTA	236.3	34.2	202.1		40.81		0.17	
	4 mmol kg^{-1} EDTA	339.3	51.5	287.8		31.45		0.18	
	8 mmol kg^{-1} EDTA	442.6	52.0	390.6		32.10		0.13	
	2 mmol kg^{-1} EDTA + 2 mmol kg^{-1} EDDS	276.6	40.	236.6		40.48		0.17	
	4 mmol kg^{-1} EDTA + 4 mmol kg^{-1} EDDS	439.3	295.6	143.7		16.94		2.06	
Brassica rapa var. *Pekinensis* (Nagaoka F1)	–		11.28			5.41			[17]
	5 mmol/kg citrate		11.23			5.88			
	5 mmol/kg EDTA		21.71			5.21			
	5 mmol/kg DTPA		24.28			5.33			
	5 mmol/kg EDDS		37.81			4.87			
Helianthus annus		53.25–78.94	17.45–40.60	34.46–38.34	1.01–7.17	0.90–6.24	0.11–0.93	0.51–1.06	[1]
Helianthus annus cv. Velox	–	55.25	11.4	43.85	0.81	0.48	0.33	0.26	[24]
	Pseudomonas fluorescens ATCC 13525	95.70	13.2	82.50	1.07	0.76	0.31	0.16	
Helianthus annus cv. Velox	–	87.19	13.3	73.89	0.62	0.31	0.30	0.18	[13]
	Pseudomonas putida ATCC 8209	135.60	15.6	120.00	0.98	0.51	0.47	0.13	
Lolium perenne		0.06–0.08	0.2–0.3	0.4–0.6				0.39–0.79	[12]
Lolium perenne	Crushed mussel shell 0 g kg^{-1}	300	50	250	0.07	0.04	0.03	0.20	[15]
	6 g kg^{-1}	121	22	99	0.13	0.08	0.05	0.22	
	24 g kg^{-1}	89	19	70	0.13	0.07	0.06	0.27	
	48 g kg^{-1}	110	29	81	0.07	0.04	0.03	0.36	
Lycopersicon esculentum cv. St Pierre		23.1–196.8	4.1–13.9	19–189.				0.04–0.42	[10]
Ricinus communis		588 – 1143	7–12	581–1131	4.65–5.26	2.70–2.90	1.95–2.36	0.01	[3]
Triticum aestivum		59.10–234.85	9.10–12.12	50–222.73				0.05–0.18	[18]
Triticum aestivum cv. Aroona	No stress	73.68	5.26	68.42	2.3	1.9	0.4	0.08	[9]
	Stress application (no Zn for 1 week)	71.05	5.26	65.79	2.2	1.7	0.5	0.08	
	Stress application (no Fe for 1 week)	168.42	15.79	152.63	1.6	1.1	0.5	0.10	
	No stress	26.31	7.89	18.42	1.6	1.1	0.5	0.43	

(*continued on next page*)

Table 2 (*continued*)

Plant species	Additives/Complements	[Cu] total (mg kg^{-1})	[Cu] shoot (mg kg^{-1})	[Cu] root (mg kg^{-1})	Dry Mass total (g)	Dry Mass shoot (g)	Dry Mass root (g)	Translocation factor	Source
Triticum aestivum cv. Songlen	Stress application (no Zn for 1 week)	44.73	5.26	39.47	1.7	1.2	0.5	0.13	
	Stress application (no Fe for 1 week)	275.	17.11	257.89	1.0	0.7	0.3	0.07	
Triticum turgidum durum		19 – 744	6–39	11–705				0.05–0.88	[23]
Vicia faba cv. Bachaar	–	179.5	46.9	132.6	16.43			0.37	[14]
	Mixture of *Rhizobium sp.* CCNWSX0481. *R. leguminosarum* cv. *viciae*. *Enterobacter cloacae* and *Pseudomonas sp.* 2(2010)	111.5	28.9	82.6	21.43			0.26	
Zea mays			11.03–42.07		6.53–16.15	2.95–10.57	3.58–5.58		[26]
Zea mays	–	53.5	11.8	41.7	2.8	1.6	1.2	0.28	[25]
	2.5% Zeolite	43.3	12.2	31.1	2.4	1.5	0.9	0.39	
	2.5% Al-oxide	36.5	10.4	26.1	2.1	1.2	0.9	0.40	
	2.5% Mn-oxide	38.8	10.5	28.3	2.3	1.3	1.0	0.37	
	2.5% phosphate rock	44.	10.	34.	2.6	1.4	1.2	0.29	
	2.5% activated charcoal	29.2	7.3	21.9	2.5	1.4	1.1	0.33	
	2.5% compost from olive oil processing wastes	37.	8.8	28.2	2.0	1.1	0.9	0.31	
	2.5% commercial peat soil material	35.5	9.8	25.7	2.5	1.4	1.1	0.38	
Zea mays cv. Gaucho		31.66–594.80	7.21–17.20	23.4 – 584.	0.69–1.78	0.49–1.40	0.16–0.38	0.02–0.35	[6]

treatment and disposal of such plants after their export from the plot. Yet this is an essential consideration as to not just relocate the problem elsewhere and must not be neglected.

Other limitations of phytoremediation include the time required to treat soils, which can be long and would need to be done repeatedly over several years. The copper must be in a form bioavailable to plants, and the effectiveness of the method depends on the location. In addition, the soil volume treated is limited to the rooting depths of the plants, soils must be moderately contaminated with copper to allow plant survival, and areas must be relatively large for the method to be applied effectively.

The phytoremediation trials that have been conducted to date using vineyard soil as a growing medium are of short duration, with most lasting less than three months. More and longer *in situ* trials are needed that focus on biomass production under field conditions and not just on the translocation factors. Promising preliminary results have already been obtained adding bacteria, fungi and/or chelators as a complement to plants so such interactions should be further investigated.

More in-depth studies over more than one season should be carried out with promising species such as *A. sativa*, *B. pilosa*, *B. napus*, *C. album* and *P. lanceolata* to evaluate their potential for copper phytoextraction per hectare through the export of the aboveground biomass currently seem to be the most promising for the implementation of copper phytoremediation in vineyards. Finally, fast-growing species, particularly Euphorbiaceae, Asteraceae and Lamiaceae, which are under-researched, should be evaluated.

4. Conclusion

The accumulation of copper in vineyard soils, predominantly due to the repeated use of copper fungicides, requires remediation in some fields. Phytoremediation is a promising technique for the future. Plants such as *P. lanceolata* or *R. communis* have a high potential to extract copper which would be accentuated by adding micro-organisms. Few other species have demonstrated high tissue copper accumulation capacities when grown in contaminated vineyard soils. Plants belonging to the Poaceae and Lamiaceae families are candidates, yet few have been evaluated, to date. The key factors for successful phytoremediation in practice are the ability to concentrate copper in their aerial parts, to translocate copper from roots to shoots and to produce high biomass during their life cycle. Therefore, more focus on these aspects is needed in future experiments.

Phytoremediation does have important limitations such as the long timeframe, that it can only be used in soils with a moderate level of soil contamination to allow plant survival or the surface area of the treatment that is limited to the plant roots. Furthermore, the effectiveness of the method is highly dependent on the species selected and the edaphic and climatic conditions. Further laboratory experiments and *in situ* research are needed to find new copper hyperaccumulating species. Subsequently, trials should be conducted in vineyards and over longer periods of time to also take into account interactions with the crop and to evaluate the management of these species in practice. Finally, consideration should also be given to the management of copper-enriched plants when they are exported from the plots and their *ad hoc* treatment.

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