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Identification and phytoremediation potential of spontaneous species in vineyard soils contaminated with copper

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ABSTRACT

Copper (Cu) contents in vineyard soils due to the application of cupric fungicides cause changes in the native covering flora. Under these conditions, the surviving individuals accumulate the metal in and decrease its availability in the soil, reducing the potential toxicity to grapevine. We have identified spontaneous plant species and their phytoremediation potential from vineyards of Isabella (*Vitis labrusca*) on two distinct soil types (Inceptisol and Entisol) contaminated with Cu. The results demonstrated that wild species displayed higher Cu contents in the roots than in the shoot, but had low bioaccumulation potential. During summer, the plants were unable to extract and stabilize the metal, although during the winter, *Lolium multiflorum*, *Cyperus compressus* and *Chrysanthemum leucanthemum* demonstrated phytostabilization potential. Among the investigated species, dry matter production and Cu accumulation by *Lolium multiflorum* indicated that the species is effective to decrease Cu availability in the soil.

KEYWORDS

Copper; cover crop; heavy metal; mitigation; Vitis sp

Introduction

The largest wine region in Brazil is Serra Gaúcha, in the State of Rio Grande do Sul. The humid climate in the region is highly favorable to fungal attack, thus the vineards are submitted to intensive applications of copper fungicides to prevent foliar diseases, such as mildew (*Plasmopora viticola*; Brunetto *et al.*, 2019). The continuous use of the fungicides increases plant contents of Cu, which accumulates in young roots responsible for water and nutrient absorption potentiating the toxicity to the grapevines (Brunetto *et al.* 2014; Miotto *et al.* 2014; Brunetto *et al.* 2019).

Heavy metal phytotoxicity encompasses a wide range of morphological, biochemical and physiological changes leading to a reduction in plant growth (De Conti *et al.* 2018, 2021), on an extent dependent on the concentration and chemical forms of the contaminants in the soil, associated to exposure time and constitutive and adaptive mechanisms of tolerance of the plants (Barceló and Poschenrieder 1992). Some plant species survive in heavy metal contaminated environments due to tolerance mechanisms (Silva *et al.* 2020), such as retention of the contaminants in the roots, compartmentalization in less susceptible cellular organelles such as the vacuoles, root exudation of the toxic compound and intracellular production of substances able to form stable complexes (Ferreira *et al.* 2014).

Spontaneous cover vegetation surviving in vineyard soils containing high levels of Cu may accumulate the metal and increase the levels of organic matter in the soil, thus reducing the bioavailability of the contaminant to grapevine (Silva *et al.* 2020). Therefore, a strategy to reduce Cu toxicity is seeding the spontaneous plants or maintaining them naturally in the vineyard to mitigate the phytotoxicity (Gardea-Torresdey *et al.* 2004; Ariyakanon and Winaipanich 2006; Nouri *et al.* 2009; Lorestani *et al.* 2011; Silva *et al.* 2020). This strategy can be performed by phytoremediation, using the plants to remove or reduce the toxicity of contaminant elements in soil (De Conti *et al.* 2018, 2019). Species with phytoremediation potential should be tolerant to contaminants and able to accumulate heavy metal in the biomass in order to contribute to reducing the availability of these elements in the soil. Ideally, phytoremediation species should also grow fast, produce high biomass and have low nutritional requirements (Ferreira *et al.* 2014; De Conti *et al.* 2019; Silva *et al.* 2020).

Plants potential to be effective phytoremediators involves bioconcentration and translocation factors, taking into account the metal concentration in the plant and soil, but also the relationship of the metal concentration in the shoots and roots (Yoon *et al.* 2006; Santos *et al.* 2010). Phytoremediation species were identified in several

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Table 1. Chemical attributes of the investigated Inceptisol and Entisol of the vineyards.

Soil Attributes[a]	Inceptisol	Entisol
Clay (g kg^{-1})	210	270
Organic matter (g kg^{-1})	46	23
pH_{H2O}	5.9	5.7
Al^{3+} ($cmol_c$ kg^{-1})	0.0	0.0
Available Cu (mg kg^{-1})	198.6	91.3
Available K (mg kg^{-1})	60	100
Available P (mg kg^{-1})	50.7	19.5
Exchangeable Ca ($mmol_c$ kg^{-1})	95.4	76.9
Exchangeable Mg ($mmol_c$ kg^{-1})	29.2	22.9
CEC $mmol_c$ kg^{-1}	154	127

Samples were taken from 0 to 20 cm in depth.
[a]P, K, Cu: extractor Mehlich-1; Ca2+, Mg2+, Al3+: extractor KCl 1 mol L^{-1}; CEC: cation exchange capacity in pH 7.0; Organic matter, by oxidation with $Na_2Cr_2O_7$ 2 mol L^{-1} + H_2SO_4 5 mol L-1; Clay: Pipette method

situations of contaminated soils (Yoon *et al.* 2006; Tangahu *et al.* 2011; Chirakkara and Reddy 2015), however the report of spontaneous species able to survive and accumulate heavy metal in Cu contaminated vineyard soils remains scarce.

The current work aimed to identify species from the spontaneous vegetation and their phytoremediation potential in vineyard soils contaminated with Cu.

Material and methods

Experiment description

The study was conducted from September 2012 to April 2013, in a vineyard in Bento Gonçalves, State of Rio Grande do Sul (RS), Brazil. The investigated vineyard consists of cultivar Isabella (*Vitis labrusca*) grafted on the rootstock Paulsen-1103, with 2.222 plants per hectare (3.0×1.5 m) conducted as pergola. At the sampling time, the vineyard was 40 years old with a history of annual applications of cupric fungicides. Approximately, the soil is mixed, with half of the area consisting of Entisol and the remaining of Inceptisol (NRCS-USDA 2014-Soil Survey Staff). The chemical attributes of the soils are shown in Table 1.

Collections, chemical analysis in soil and tissue and statistical analysis

Spontaneous vegetation was sampled from both soil types in September 2012 (end of winter) and March 2013 (end of summer). Plants were collect from the areas using a 0.50×0.50 m metal frame as guide, randomly launched onto the ground between the rows. At each season of the year and for each soil type, samples were taken from five locations in the vineyard.

The shoots and the roots were collected from all plants inside the sampling frame. Plant parts were segregated in the laboratory and the collected species identified according to Lorenzi 2006. Phytosociological variables were calculated, along with absolute frequency (AF), which corresponds to the absolute number of a given species, relative frequency (RF), which corresponds to the number of a given species in comparison to the total number of plants, dry matter of shoots (S) and roots (R), shoots dry matter per area (ADM), the ratio S/R and the contribution of each specie to total dry matter (CST). The similarity of species between both soil types was determined by Sorensen's similarity coefficient, as shown in Equation (1):

$$C = 2a/(2a + b + c) \quad (1)$$

where: a = number of common species; b and c = number of exclusive species from each soil type (Krebs 1999).

The shoots and roots were washed in distilled water or a solution containing 0.01 mol L^{-1} HCl, respectively, and immediately immersed in distilled water. Subsequently, plant parts were dried in an oven with forced air at 65 °C until constant mass. They were immediately weighed and ground in a Willey type mill. The samples were air-dried, sieved through a 2 mm mesh and stored for analyses.

Dried shoots and roots were submitted to nitric perchloric acid digestion. Copper contents in shoots [CuS] and roots [CuR] was made by atomic absorption spectrometry (AAS) according to Tedesco *et al.* 1995. Soil copper was extracted by Mehlich-1 extraction procedure and determined by AAS. The accumulation of Cu was calculated for the shoots (ACuS= [CuS] × MS_S1P/100) and roots (ACuR= [CuR] × DM_R1P/100), where [CuS] is the Cu concentration in the shoots, DM_S1P is shoots dry mass from one plant and in the, [CuR] is the concentration of Cu in the roots, DM_R1P is the roots dry mass from one plant. The ratios between the Cu contents of the shoots and the roots ([S]/[R]), accumulated Cu in shoots and roots (AS/AR) and dry matter of shoots and roots were also calculated. The exported Cu contents were estimated for each plant species (ESp), when cultivated alone in each soil type, and the total metal exported contents in the environment (TE). Copper translocation factor (TF_{Cu}) from the roots to the shoots and Cu bioconcentration factor for shoots (BCF_{CuS}) and roots (BCF_{CuR}) were also calculated (Equations 2, 3 and 4):

$$TF_{Cu} = [Cu]_S/[Cu]_R \quad (2)$$

$$BCF_{CuS} = [Cu]_S/[Cu]_{soil} \quad (3)$$

$$BCF_{CuR} = [Cu]_R/[Cu]_{soil} \quad (4)$$

where: $[Cu]_S$ is the concentration of Cu in the shoots of the plants, $[Cu]_{R,}$ in the roots, and$[Cu]_{soil}$ in the soil.

The means of the variables of species were compared by a non-parametric randomization test using the Euclidean Distance as a measure of similarity. Differences were significant when $p < 0.05$ using the MULTIV software (Pillar 2001).

Results

Identification and productive behavior of spontaneous vegetation

In collections carried out during summer ten spontaneous plant species were identified from Entisol and nine from Inceptisol vineyard. During winter, nine and ten plant species from Entisol and from Inceptsol, respectively (Table 2). The plant species observed and collected during summer were similar on both soil types, with Sorensen's similarity

Table 2. Absolute frequency (AF) and relative (RF) of each specie, shoots dry mass of each species (SDM) and total dry mass production of the plants per area (ADM), root dry mass (RDM), SDM/RDM ratio and contribution of each species to the total dry mass production (CSTDM) of the plant species collected in summer and winter, in a Inceptisol and Entisol planted with vines.

	Inceptsol						Entisols					
Species	AF (plants m^{-2})	RF (%)	SDM (g)	RDM (g $plant^{-1}$)	ADM (kg ha^{-1})	SDM/RDM	AF (plants m^{-2})	RF (%)	SDM (g)	RDM (g $plant^{-1}$)	ADM (kg ha^{-1})	SDM/RDM
	Summer Collect											
S. nodiflora	11.0 b	7.1	2.4 b	0.3 b	98.6 a	9.7 b	6.0 c	2.8	0.4 b	0.1 b	19.2 b	8.3 a
Setaria sp.	11.2 b	7.2	2.7 b	0.3 b	110.3 a	13.5 b	–		–	–	–	–
D. carota	–		–	–	–	–	5.6 c	2.6	0.8 b	0.2 b	34.9 b	4.7 b
I. cairica	4.0 c	2.6	0.7 b	0.3 b	30.5 b	4.3 b	9.6 c	4.4	1.8 b	0.2 b	72.9 a	9.6 a
S. rhombifolia	10.0 b	6.5	1.3 b	0.1 b	54.9 b	33.3 a	25.6 b	11.8	3.1 a	1.1 b	124.2 a	5.0 a
C. compressus	76.8 a	49.5	4.3 b	0.9a	173.1 a	4.2 b	100.8 a	46.6	7.2 a	2.5 b	290.3 a	2.8 b
E. heterophylla	4.0 c	2.6	0.6 c	0.08 c	24.1 b	7.6 b	4.0 c	1.8	0.1 c	0.1 b	3.2 c	3.2 b
R. obtusifolius	–		–	–	–	–	5.3 c	2.5	4.2 a	3.1a	169.4 a	1.3 b
C. leucanthemum	16.0 b	10.0	6.8 a	1.0 a	275.8 a	6.6 b	19.0 c	8.8	12.6 a	3.2 a	504.4 a	5.4 a
P. tomentosa	6.0 c	3.9	0.9 b	0.1 b	36.2 b	6.7 b	10.0 c	4.6	3.0 a	0.8 b	118.2 a	3.5 b
T. pratense	16.0 b	10.3	5.4 a	1.1 a	217.4 a	6.1 b	30.4 b	14.1	3.9 a	0.9 b	150.4 a	4.4 b
Total	**155.0**	**100**			**1020.9**		**216.3**	**100**			**1483.9**	
Sorensen's similarity coefficient	**0.86**											
	Winter Collect											
O. dillenii	7.2 c	2.5	1.8 c	0.1 b	74.1 b	11.6 ns	28.0 a	8.2	1.4 c	0.4 b	59.7 c	3.5 b
L. multiflorum	23.2 b	8.2	46.1 a	3.0 a	1847.7 a	15.5	25.6 a	7.5	81.6 a	7.9 a	3265.4 a	10.4 a
D. carota	4.0 c	1.4	7.5 b	1.3 a	303.0 b	24.8	7.2 b	2.1	6.3 b	1.1 b	255.6 b	12.3 a
V. sativa	10.4 c	3.6	5.3 c	0.2 b	215.6 b	39.9	–		–	–	–	–
S. rhombifolia	–		–	–	–	–	60.0 a	17.6	0.6 c	0.5 b	24.1 c	1.0 c
C. compressus	149.6 a	52.1	0.6 c	0.1 b	24.8 c	3.6	60.0a	17.6	0.2 c	0.1 c	8.6 c	2.5 b
R. obtusifolius	16.0 c	5.6	1.8 c	0.1 b	74.6 c	41.6	–		–	–	–	–
C. leucanthemum	24.0 b	8.2	2.0 c	0.4 b	80.8 c	5.8	127.2 a	37.3	6.3 b	2.6 a	254.0 b	2.7 b
S. oleraceus	5.6 c	1.9	12.8 b	1.8 a	512.2 b	7.3	–		–	–	–	–
P. tomentosa	40.0 b	13.9	6.6 b	0.8 b	264.4 b	22.1	24.8 a	7.3	4.3 b	0.9 b	172.7 b	7.6 a
T. campestre	–		–	–	–	–	4.0 b	1.2	1.3 c	0.2 c	53.6 c	6.3 a
T. pratense	7.2 c	2.5	11.0 b	1.2 a	440.6 b	10.3	4.0 b	1.2	6.3 b	0.9 b	152.5 b	3.9 b
Total	**287.2**	**100**			**3397.2**		**340.8**	**100**			**4246.2**	
Sorensen's similarity coefficient	**0.74**											

Same letters in the column do not differ statistically by the randomization test ($p > 0.05$); ns – not significant diferences.

Table 3. Cu content in the shoots (CuS), Cu accumulated in the shoots (ACuS) and Cu exported in the shoots of each species (CuESp), in plants collected in summer and winter, under a Inceptisol and Entisol, planted with vines.

	Inceptsol			Entisols		
Species	CuS (mg kg^{-1})	ACuS (ug $plant^{-1}$)	CuESp (g ha^{-1})	CuS (mg kg^{-1})	ACuS (ug $plant^{-1}$)	CuESp (g ha^{-1})
			Summer collect			
S. nodiflora	46.7 a	46.3 a	5.1 b	24.5 b	10.9 b	1.4 c
Setaria sp.	17.6 c	13.1 b	1.9 b	–	–	–
D. carota	–	–	–	12.3 c	8.1 b	0.7 c
I. cairica	18.6 c	14.2 b	0.7 b	22.6 b	13.8 b	2.1 c
S. rhombifolia	20.3 c	17.7 b	2.1 b	17.0 c	9.9 b	4.2 b
C. compressus	37.9 b	9.1 b	29.3 a	36.0 a	11.3 b	36.5 a
E. heterophylla	12.3 c	7.4 b	0.4 c	9.4 c	0.7 c	0.3 c
R. obtusifolius	–	–	–	15.4 c	49.5 a	0.8 c
C. leucanthemum	33.5b	69.5 a	5.3 b	25.7 b	171.3 a	4.9 b
P. tomentosa	57.7 a	32.7 a	3.3 b	28.7 b	45.2 a	2.9 b
T. pratense	32.5 b	46.5 a	5.1 b	30.8 b	26.4 a	9.1 b
Total			53.2			62.9
			Winter collect			
O. dillenii	42.2 a	27.5 b	3.0 b	43.4 b	9.2 c	12.1 b
L. multiflorum	11.1 b	92.4 a	2.3 c	14.8 c	251.2 a	3.8 c
D. carota	13.2 b	100.6 a	0.5 d	43.7 b	155.7 a	3.2 c
V. sativa	15.9 b	31.8 b	1.6 c	–	–	–
S. rhombifolia	–	–	–	34.3 b	1.5 c	21.1 a
C. compressus	46.5 a	0.7 c	67.5 a	69.0 a	0.9 c	42.4 a
R. obtusifolius	34.3 a	17.7 b	5.6 b	–	–	–
C. leucanthemum	26.2 b	14.5 b	6.3 b	52.8 a	8.1 c	69.2 a
S. oleraceus	18.8 b	177.8 a	1.0 c	–	–	–
P. tomentosa	28.4 b	17.2 b	11.3 b	28.6 c	21.3 c	7.1 c
T. campestre	–	–	–	10.6 c	14.1 c	0.4 d
T. pratense	16.2 b	95.7 a	1.1 c	20.4 c	82.3 b	0.8 d
Total			100.2			160.1

Same letters in the column do not differ statistically by the randomization test ($p > 0.05$).

coefficient of 0.86 (Table 2). The overall AF was 155 and 216.3 plants m^{-2} on Inceptisol and Entisol, respectively. The species with the highest RF on Inceptisol was *C. compressus*, with 48.5% soil coverage, followed by *T. pratense* and *C. leucanthemum*, with 10.3% and 10.0%, respectively. A high RF was also found for *C. compressus* on Entisol, with 46.0% coverage, followed by *T. pratense* and by *S. rhombifolia*, with 14.1 and 11.8%, respectively.

In the winter, covering crops were similar on both soil types, displaying a Sorensen's similarity coefficient of 0.74 (Table 2). *V. sativa*, *R. obtusifolius* and *S. oleraceus* were absent from Entisol. Besides, *S. rhombifolia* and *T. campestre* occurred exclusively in Entisol. AF was of 287.2 and 340.8 plants m^{-2} on Inceptisol and Entisol, respectively. The species with higher RF on Inceptisol was *C. compressus*, with 52.1% soil coverage, followed by *P. tomentosa* with 13.9%, *C. leucanthemum and L. multiflorum*, both with 8.2%. The species with the highest RF in Entisol was *C. leucanthemum*, with 37% soil coverage, followed by *C. compressus* and *S. rhombifolia*, with 17.6% each.

During the summer, ADM on Inceptisol was of 1020.9 kg ha^{-1} (Table 2). In the season *C. leucanthemum*, *T. pratense* and *C. compressus* achieved the highest production of dry matter, with 275.8, 217.4 and 173.1 kg ha^{-1}, respectively, corresponding to 27.3, 21.3, and 17.0%. Despite *C. compressus* presented an RF 5.7 times higher than that for *C. leucanthemum*. On Entisol, ADM was of 1483.9 kg ha^{-1}, whereas for *C. leucanthemum* was of 34.0%. However, the species contributed to the total dry matter, the highest RF was obtained for *C. compressus*, with 46.6% and AF, corresponding to 100 plants m^{-2}. Additionally, *S. rhombifolia*, that showed an RF of 11.8%, represented only 8.4% of ADM (Table 2).

The winter species growing on Inceptisol achieved a total ADM production of 3397.2 kg ha^{-1}. From the total, *L. multiflorum* produced 1847.7 kg ha^{-1}, which represents 41.8% of ADM. Although *C. compressus* had high AF and RF, when compared with other species, its SDM was lower, equivalent to 0.06 g. This behavior was opposite to that of *L. multiflorum*, which achieved production of SDM of 46.1 g and a frequency in the soil of 8.1%. The species *D. carota*, *T. pratense* and *S. oleraceus* reached equivalent yields of SDM corresponding to 7.5 g, 11.0 g and 12.8 g, respectively. However, these species were infrequency in the environment. On Entisol, the total ADM was of 4246.2 kg ha^{-1}, and 76.9% was produced by *L. multiflorum*. Although *C. leucanthemum* presented higher RF, corresponding to 127 plants per m^{-2}, *L. multiflorum*, was the only species with 7.5% of RF, producing 3265.4 kg ha^{-1} of ADM, while *C. leucanthemum* produced 254.0 kg ha^{-1} (Table 2). Furthermore, *S. rhombifolia* and *C. compressus*, both with 17.6% of RF, contributed with only 0.6 and 0.2% of ADM.

Summer species growing on Inceptisol gave rise to RDM values ranging from 1.1.0 g $plant^{-1}$ *(C. leucanthemum) and* 0.08 g per $plant^{-1}$ (*E. heterophylla*). On Entisol, *S. nodiflora* produced 0.16 g $plant^{-1}$ and *C. leucanthemum* 3.2 g $plant^{-1}$. Furthermore, winter plants from Inceptisol presented RDM values varying from 0.01 *(C. compressus*, *R. obtusifolius* and *O. dillenii*) to 3.0 g $plant^{-1}$ (*L. multiflorum*). On Entisol the smallest RDM was observed for *C. compressus* with 0.1 g $plant^{-1}$ and the largest, for *L. multiflorum* with 7.9 g $plant^{-1}$. In general, plants collected during winter showed

Table 4. Cu content in the roots (CuCR), Cu accumulated in the roots (ACuR), translocation factor (TF_{Cu}), bioconcentration factor in the shoots (BCF_{CuS}), bioconcentration factor in the roots (BCF_{CuR}), in different species of plants collected in summer and winter, in a Inceptisol and Entisol planted with vines.

	Inceptsol					Entisols				
Species	CuCR (mg kg^{-1})	ACuR (ug planta^{-1})	TF_{Cu}	BCFCuS	BCFCuR	CuCR (mg kg^{-1})	ACuR (ug planta^{-1})	TF_{Cu}	BCFCuS	BCFCuR
					Summer collect					
S. nodiflora	70.6 b	46.3 a	0.7 a	0.23 a	0.35 b	59.1 b	10.9 b	0.4 c	0.26 b	0.65 b
Setaria sp.	65.4 b	13.1 b	0.2 b	0.08 c	0.32 b	–	–	–	–	–
D. carota	–	–	–	–	–	19.0 c	8.1 b	0.6 a	0.13 c	0.21 c
I. cairica	26.6 c	14.2 b	0.7 a	0.09 c	0.13 c	41. b	13.8 b	0.5 b	0.24 b	0.46 b
S. rhombifolia	20.4 c	17.7 b	0.9 a	0.10 c	0.10 c	16.1 c	9.9 b	1.0 a	0.18 c	0.18 c
C. compressus	126.0 a	9.1 b	0.3 b	0.19 b	0.63 a	129.1 a	11.3 b	0.3 c	0.39 a	1.41 a
E. heterophylla	48.3 b	7.4 b	0.2 b	0.06 c	0.24 b	56.5 b	0.7 c	0.2 c	0.10 c	0.62 b
R. obtusifolius	–	–	–	–	–	10.6 c	49.5 a	1.4 a	0.16 c	0.12 c
C. leucanthemum	76.8 b	69.5 a	0.4 b	0.16 b	0.38 b	87.8 a	171.3 a	0.3 c	0.28 b	0.96 a
P. tomentosa	97.8 b	32.7 a	0.5 a	0.29 a	0.49 b	58.3 b	45.2 a	0.4 c	0.31 b	0.64 b
T. pratense	44.5 b	46.5 a	0.7 a	0.16 b	0.22 b	55.0 b	26.4 a	0.5 b	0.33 b	0.60 b
					Winter collect					
O. dillenii	49.9 b	27.5 b	0.87 b	0.21 a	0.25 b	41.7 c	9.2 c	1.0 b	0.47 b	0.45 c
L. multiflorum	187.5 a	92.4 a	0.05 e	0.05 b	0.94 a	274.3 a	251.2 a	0.05 d	0.16 c	3.0 a
D. carota	14.7 c	100.6 a	0.91 b	0.06 b	0.07 c	13.4 d	155.7 a	3.2 a	0.47 b	0.14 d
V. sativa	99.6 b	31.8 b	0.16 d	0.08 b	0.50 b	–	–	–	–	–
S. rhombifolia	–	–	–	–	–	31.3 c	1.5 c	1.1 b	0.37 b	0.34 c
C. compressus	229.5 a	0.7 c	0.20 d	0.23 a	1.15 a	69.0 c	0.9 c	1.0 b	0.75 a	0.75 c
R. obtusifolius	21.6 c	17.7 b	1.59 a	0.17 a	0.10 c	–	–	–	–	–
C. leucanthemum	134.1 a	14.5 b	0.22 d	0.13 b	0.67 a	266.3 a	8.1 c	0.1 c	0.57 a	2.91 a
S. oleraceus	59.5 b	177.8 a	0.32 c	0.09 b	0.30 b	–	–	–	–	–
P. tomentosa	117.9 a	17.2 b	0.24 c	0.14 b	0.59 a	95.8 b	21.3 c	0.3 c	0.31 c	1.04 b
T. campestre	–	–	–	–	–	49.2 c	14.1 c	0.2 c	0.11 c	0.53 c
T. pratense	47.4 b	95.7 a	0.35 c	0.08 b	0.23 b	52.0 c	82.3 b	0.3 c	0.22 c	0.56 c

Same letters in the column do not differ statistically by the randomization test ($p > 0.05$).

higher SDM/RDM ratios than plants collected during summer; but plants collected during winter growing on Inceptisol presented higher SDM/RDM mean.

Cu in shoots and roots

Summer spontaneous plants found in Inceptisol presented variations in CuS from 12.3 to 57.7 mg kg^{-1} (Table 3). The species presenting the highest CuESp *C. leucanthemum*, *T. pratense* and *C. compressus* showed ability to export 40 g Cu ha^{-1} (5.3 g + 5.1 g + 29.3 g) On the other soil, the species presenting the highest CuS were: *C. compressus*, *T. pratense*, *P. tomentosa* and *C. leucanthemum* (Table 3). However, the highest ACuS was observed in *C. leucanthemum*.

Among the collection held in winter on Inceptisol soil, *C. compressus* presented the highest CuS concentration, followed by *O. dillenii* and *R. obtusifolius* (Table 3). Individually, *S. oleraceus* presented higher ACuS, 177.8 ug plant^{-1}, followed by *D. carota* and *T. pratense*. The *C. compressus* exhibited a higher CuESp (67.5 g ha^{-1}), followed by *P. tomentosa* and *C. leucanthemum*. *C. compressus* the extraction of these three species is approximately 80.1 g, which corresponds to approximately 80% of the CuESp by plants on Inceptisol. On Entisol, the species that presented higher CuS were *C. compressus*, *C. leucanthemum*, *O. dillenii* and *D. carota*. However, the largest ACuS was observed in *L. multiflorum* (251.2 ug plant^{-1}), followed by *D. carota* and *T. pratense*. The *L. multiflorum* was highest potential for Cu accumulated in the shoots.

The values of CuCR and the ACuR in plant species collected during summer allowed to estimate the plants' ability to stabilize Cu stabilized as biomass, reducing Cu phytotoxicity to grapevine. On Inceptisol soil, CuCR ranged from 20.5 to 126.0 mg kg^{-1}, with the lowest content observed for *S. rhombifolia* and the highest for *C. compressus*. The ACuR ranged from 7.4 to 69.5 µg plant^{-1}, and the highest values were observed in *C. leucanthemum* (Table 4). On Entisol, CuCR ranged from 10.6 to 129.1 mg kg^{-1}, with highest contents for *C. compressus*. The lowest content was observed for *R. obtusifolius*. The ACuR ranged from 0.7 to 171.3 µg plant^{-1}, and the highest values were observed in *C. leucanthemum* and *R. obtusifolius* (Table 4).

Regarding species collected during the winter, values of CuCR and ACuR were intermediate (Table 4). On Inceptisol, the CuCR ranged from 14.7 to 229.5 mg kg^{-1}, where the lowest content was observed in *D. carota* and the largest in *C. compressus*. The values of ACuR ranged from 0.7 to 177.8 µg plant^{-1}, and the highest values were observed in *L. multiflorum* and *S. oleraceus*. On Entisol soil, CuCR ranged from 13.4 to 274.3 mg kg^{-1}, with highest content in *L. multiflorum* and *C. leucanthemum*. The lowest Cu content was observed in *D. carota*. The values of ACuR ranged from 0.9 to 251.2 µg plant^{-1}, and the highest values were observed in *L. multiflorum* (Table 4).

Discussion

Growth of species with potential for phytoremediation

From the total, *L. multiflorum* was the species with the highest yield, although *C. compressus* had high AF and RF, when compared with other species, its SDM was lower. The resulting index is likely to be due to species specific features, since it is classified to the genus *Cyperus* whose main characteristics are small height and variable growth periods throughout the lifecycle (Brighenti *et al.* 1997). This behavior was opposite to that of *L. multiflorum*, which achieved high production of SDM. The yield of *L. multiflorum* was the highest during the winter and the species was absent from both soils during the summer, since it is adapted to low temperatures it develops only in the winter, which explains its increased production of dry matter production in the period (Monteiro *et al.* 1996; Rodrigues *et al.* 2011).

The results observed in both seasons demonstrated that AF was higher for plants growing during winter, which can be partly attributed to the greater availability of environmental resources, but also because grapevines are dormant (Sozim *et al.* 2007), which increases the incidence of light on spontaneous species, fostering their growth. For some species, the presence of light is fundamental to trigger germination and the following developmental stages, also affecting/interfering with the dormancy of seeds in the soil (Benech-Arnold *et al.* 2000). Moreover, during the summer there was a reduction in growth and dry matter production of the spontaneous covering plants, due to the development and increased leaf are of the grapevine, leading to shading of the spontaneous vegetation (Carvalho 2014).

Additionally, the impaired growth and development of the plants can be attributed to higher Cu contents in both soils analyzed, reaching up to 198.6 mg kg^{-1} in Inceptisol and 91.3 mg kg^{-1} in Entisol. Natural Cu concentration in soils is usually of 13–24 mg kg^{-1} (Kabata-Pendias and Pendias 1986), although it can vary from 2 to 200 mg kg^{-1}, with an average of 30 mg kg^{-1} (Mortvedt 2000), depending on the soil type. Excessive Cu in the soil may cause morphological and physiological changes in plants, inhibiting nutrient absorptions, reducing the photosynthetic rate and growth, thus reflecting on dry matter production (Michaud *et al.* 2008; Toselli *et al.* 2009; Lequeux *et al.* 2010; Cambrollé *et al.* 2015). Moreover, taller plants tend to have higher dry matter production per plant and per area, which contributes to a higher accumulation of soil nutrients and Cu export potential by the plant (Brunetto *et al.* 2007; Melo *et al.* 2013). Therefore, species with high RF and with high dry matter production are desirable for Cu phytoextraction in contaminated soils.

Copper accumulation potential for phytoremediation

P. tomentosa and *C. compressus* had the highest Cu concentration. The species *C. leucanthemum* and *C. compressus* exhibited the highest CuESp. Interestingly, *C. leucanthemum* and *C. compressus* showed ability corresponding to 65 and 74% of the total exported by plants cohabiting the Inceptisol vineyard in summer and winter, respectively. The *C. compressus* was the species with the greatest potential of Cu translocation to shoots, presenting accumulation of 36.5 g ha^{-1}, followed by *T. pratense* in Entisols. The set of plants found on Entisol exported 62.9 mg ha^{-1}, with more than 58% being exported by *C. compressus* alone. These observations are probably due to its higher dry matter production leading to high absorption of the metal, in agreement with the results shown by Mota *et al.* 2013.

Our data indicates that on Entisol the *C. leucanthemum* was the species with the highest potential for Cu translocation to shoots, considering that more than 43% was exported by *C. leucanthemum* of a total exported of plants growing on Entisol of 160.0 mg ha^{-1}. This species possibly has mechanisms of tolerance to Cu and other heavy metals in the soil (Alvarenga *et al.* 2011; Soares *et al.* 2013), allowing this species to accumulate high contents of the metals in the shoots.

The values of CuCR and the ACuR in plant species collected during summer allowed to estimate the plants' ability to stabilize Cu stabilized as biomass, reducing Cu phytotoxicity to grapevine. *C. leucanthemum* and *R. obtusifolius* presented highest values of ACuR and these results demonstrate the importance of the yield of dry matter by the roots, as observed for *C. compressus* that has a high capacity to concentrate Cu in the roots, but low production of root dry matter, resulting in low Cu accumulation.

Regarding Cu concentration in the plants shoots and roots, most species presented CuS/CuCR (TF_{Cu}) ratios lower than 1.0 for both soils, indicating that the investigated plants concentrate more Cu in the roots that in the shoots and that the metal translocated from the root system to the shoots (Reilly and Reilly 1973). The relation CuS/CuCR for *D. carota* grown on Entisol during the winter was of 3.2, indicating that Cu concentration in the shoots was 326% higher than in the roots. *R. obtusifolius* grown on Entisol during summer and on Inceptisol during winter, accumulated 145% more Cu in the shoots than in roots.

About bioconcentration factors and translocation, most of plants germinated during the summer presented TF_{Cu} lower than 1.0, although *R. obtusifolius* had TF_{Cu}=1.4. Winter species with TF_{Cu} higher than 1.0 were *D. carota*, *C. compressus* and *R. obtusifolius*. None of the investigated species presented BCF_{CuS}, although *C. compressus, L. multiflorum, P. tomentosa* and *C. leucanthemum* had BCF_{CuR} higher than 1.0. Species with TF_{Cu} higher than 1.0 display phytoextraction potential, whereas those exhibiting bioaccumulation factor higher than 1.0 are potential phytostabilizers (Yoon *et al.* 2006; Branzini *et al.* 2012). Researches carried out by Alvarenga *et al.* 2011 showed that *L. multiflorum* has phytostabilization potential for heavy metals, such as Cu, Pb and Zn, in soils degraded by mining activities, developing no symptoms of nutritional deficiency or toxicity by metals.

Conclusions

The spontaneous species growing on vineyard soils contaminated with Cu presented high contents of the element in the shoots, but low bioaccumulation potential, indicating low potential to perform Cu phytoextraction.

During the summer, *R. obtusifolius* was present on Inceptisol and Entisol soils and presented phytoextraction capacity, whereas *C. compressus* presented phytostabilization capacity. Furthermore, the winter species *D. carota* exhibited phytoextraction potential and the species *L. multiflorum, R. obtusifolius* and *C. leucanthemum* demonstrated phytostabilization potential.

L. multiflorum was the sole investigated species presenting a dry matter production that may impact Cu availability in soil and, since significant part of its growth coincides with grapevine production and it displays tolerance to soil Cu, it can contribute to reduce the metal availability.

The tolerance mechanisms of the identified species need to be further investigated since under appropriate management they could, even if only transiently, reduce potential toxicity of Cu to grapevine.

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No potential conflict of interest was reported by the author(s).

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References

Alvarenga P, Fernandes RM, Varennes A, Vallini G, Duarte E, Cunha-Queda AC. 2011. Utilização de Lolium Perenne L. na fitoestabilização controlada de solos degradados por actividades mineiras. Rev Ciências Agrárias. 34:117–130.

Ariyakanon N, Winaipanich B. 2006. Phytoremediation of copper contaminated coil by *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var. radiata. J Sci Res Chula Univ. 31:49–56.

Barceló J, Poschenrieder CH. 1992. Respuestas de las plantas a la contaminación por metales pesados. Suelo y Planta. 2:345–361.

Benech-Arnold RL, Sánchez R, Forcella F, Kruk BC, Ghersa CM. 2000. Environmental control of dormancy in weed seed banks in soil. F Crop Res. 67(2):105–122. doi:10.1016/S0378-4290(00)00087-3.

Branzini A, González RS, Zubillaga M. 2012. Absorption and translocation of copper, zinc and chromium by Sesbania virgata. J Environ Manage. 102:50–54. doi:10.1016/j.jenvman.2012.01.033.

Brighenti AM, Silva JF, Sediyama T, Silveira JSB, Sediyama CS. 1997. Análise do crescimento da tiririca (*Cyperus Rotundus L.*). Rev Ceres. 44:97–110.

Brunetto G, Rosa DJ, Ambrosini VG, Heinzen J, Ferreira PAA, Ceretta CA, Soares CRFS, Melo GWB, Soriani HH, Nicoloso FT, *et al.* 2019. Use of phosphorus fertilization and mycorrhization as strategies for reducing copper toxicity in young grapevines. Sci Hortic. 248:176–183. doi:10.1016/j.scienta.2019.01.026.

Brunetto G, Melo GWB, Junior AS, Kaminski J, Ceretta CA. 2007. Taxa fotossintética e acúmulo de matéria seca e nutrientes em videiras jovens na Serra Gaúcha cultivadas em solos com excesso de cobre. Comun Técnico Embrapa Uva e Vinho. 80:8.

Brunetto G, Miotto A, Ceretta CA, Schmitt DE, Heinzen J, Moraes MP, Canton L, Tiecher TL, Comin JJ, Girotto E. 2014. Mobility of copper and zinc fractions in fungicide amended vineyard sandy soils. Arch Agron Soil Sci. 60(5):609–624. doi:10.1080/03650340.2013.826348.

Cambrollé J, García JL, Figueroa ME, Cantos M. 2015. Evaluating wild grapevine tolerance to copper toxicity. Chemosphere. 120:171–178. doi:10.1016/j.chemosphere.2014.06.044.

Carvalho LB. 2014. Monitoramento e manejo de plantas daninhas em videira de altitude. 1st ed. Santa Catarina: Lages.

Chirakkara R, Reddy K. 2015. Plant species identification for phytoremediation of mixed contaminated soils. J Hazard Toxic Radioact Waste. 19(4):1–10.

De Conti L, Ceretta CA, Melo GWB, Tiecher TL, Silva LOS, Garlet LP, Mimmo T, Cesco S, Brunetto G. 2019. Intercropping of young grapevines with native grasses for phytoremediation of Cu-contaminated soils. Chemosphere. 216:147–156. doi:10.1016/j.chemosphere.2018.10.134.

De Conti L, Marques ACR, Ceretta CA, Tarouco CP, Nicoloso FT, Ferreira PAA, Tiecher TL, Tassinari A, Bicalho da Silva IC, Brunetto G. 2021. Tolerance and phytoremediation potential of grass species native to South American grasslands to copper-contaminated soils. Int J Phytoremediation. 23(7):726–735. doi:10.1080/15226514.2020.1852528.

De Conti L, Melo GW, Ceretta CA, Tarouco CP, Marques ACR, Nicoloso FT, Tassinari A, Tiecher TL, Cesco S, Mimmo T, et al. 2018. Photosynthesis and growth of young grapevines intercropped with native grasses in soils contaminated with copper. Acta Hortic. 1235(1217):179–184. doi:10.17660/ActaHortic.2018.1217.23.

Ferreira PA, Brunetto G, Giachini AJ, Soares CRFS. 2014. Heavy metal uptake and the effect on plant growth: heavy metl remediation transport and accumulation in plants. In: Dharmendra Kumar Gupta, Chatterjee S, editors. 1st ed., Nova York: Nova, p. 258.

Gardea-Torresdey JLL, Peralta-Videa JR, Montes M, de la Rosa G, Corral-Diaz B. 2004. Bioaccumulation of cadmium, chromium and copper by Convolvulus arvensis L.: impact on plant growth and uptake of nutritional elements. Bioresour Technol. 92(3):229–235. doi:10.1016/j.biortech.2003.10.002.

Kabata-Pendias A, Pendias H. 1986. Trace elements in soils and plants. Florida: CRC Press.

Krebs CJ. 1999. Ecological methodology. 2nd ed. Menlo Park: Addison Wesley Educational Publishers.

Lequeux H, Hermans C, Lutts S, Verbruggen N. 2010. Response to copper excess in *Arabidopsis thaliana*: impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol Biochem. 48(8):673–682. doi:10.1016/j.plaphy.2010.05.005.

Lorenzi H. 2006. Manual de identificação e controle de plantas daninhas: plantio direto e convencional. 6th ed. Nova Odessa: Instituto Plantarum de Estudos da Flora.

Lorestani B, Cheraghi M, Yousefi N. 2011. Phytoremediation potential of native plants growing on a heavy metals contaminated soil of copper mine in Iran. World Acad Sci Eng Technol. 53:377–382.

Melo GW, Mezacasa J, Zalamena J, Oliveira PD, Freitas RF, Dal Magro R. 2013. A calagem pode mitigar os efeitos da fitotoxicidade do cobre em aveia (*Avena sativa*)? Comun Técnico Embrapa Uva e Vinho. 142:8.

Michaud AM, Chappellaz C, Hinsinger P. 2008. Copper phytotoxicity affects root elongation and iron nutrition in durum wheat (*Triticum turgidum* durum L.). Plant Soil. 310(1–2):151–165. doi:10.1007/s11104-008-9642-0.

Miotto A, Ceretta CA, Brunetto G, Nicoloso FT, Girotto E, Farias JG, Tiecher TL, De Conti L, Trentin G. 2014. Copper uptake, accumulation and physiological changes in adult grapevines in response to excess copper in soil. Plant Soil. 374(1-2):593–610. doi:10.1007/s11104-013-1886-7.

Monteiro ALG, Moraes A, Corrêa EAS. 1996. Forragicultura no Paraná. Londrina – PR: Comissão Paranaense de Avaliação de Forrageiras- CPAF.

Mortvedt JJ. 2000. Bioavailability of micronutrients. In: Sumner M, editor. Handbook of soil science. Boca Raton: CRC Press. p. 71–88.

Mota PRD, Fiorim ACR, Bôas RLV, Folegatti MV, Ludwig F, Silva M. E A d. 2013. Condutividade elétrica da solução nutritiva e acúmulo de macro e micronutrientes no cultivo de crisântem. Bragantia. 72(1):81–89. doi:10.1590/S0006-87052013005000015.

Nouri J, Khorasani N, Lorestani B, Karami M, Hassani a. H, Yousefi N. 2009. Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential. Environ Earth Sci. 59(2): 315–323. doi:10.1007/s12665-009-0028-2.

NRCS-USDA. 2014. Soil survey Staffv – Keys to Soil Taxonomy.Washington: Department of Agriculture.

Pillar VD. 2001. Multivariate exploratory analysis, randomization testing and bootstrap resampling. User's Guid. Porto Alegre: UFRGS.

Reilly A, Reilly C. 1973. Zinc, lead and copper tolerance in the grass stereochlaena cameronii (stapf) clayton. New Phytol. 72(5): 1041–1046. doi:10.1111/j.1469-8137.1973.tb02080.x.

Rodrigues DA, Avanza MFB, Dias LGGG. 2011. Sobressemeadura de aveia e azevém em pastagens tropicais no inverno. Rev Científica Eletrônicade Med Veterinária. 16:22.

Santos GD, Rodella AA, Abreu CD, Coscione AR. 2010. Vegetable species for phytoextraction of boron, copper, lead, manganese and zinc from contaminated soil. Sci Agric. 67(6):713–719. doi:10.1590/S0103-90162010000600014.

Silva ICB, Marques ACR, Quadros FF, Sans GA, Soares VM, De Conti L, Ceretta CA, Ferreira PAA, Toselli M, Brunetto G. 2020. Spatial variation of herbaceous cover species community in Cu-contaminated vineyards in Pampa biome. Environ Sci Pollut Res Int. 27(12):13348–13359. doi:10.1007/s11356-020-07851-z.

Soares R, Takeda IJM, Botari JC, Botari A. 2013. A fitorremediação como instrumento alternativo no saneamento ambienta. XIII Safety. Heal Environ World Congr. 13:448–452.

Sozim M, Ferreira FP, Ayub RA, Botelho RV. 2007. Época de poda e quebra de dormência em videiras cv. Niagara Rosada. Sem Ci Agr. 28(2):201–206. doi:10.5433/1679-0359.2007v28n2p201.

Tangahu BV, Sheikh Abdullah SR, Basri H, Idris M, Anuar N, Mukhlisin M. 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng. 939161:1–31.

Tedesco MJMJ, Gianello C, Bissani CA, Bohnen H, Volkweiss S. 1995. Análise de solo, plantas e outros materiais. 2nd ed. Porto Alegre: Universidade Federal do Rio Grande do Sul.

Toselli M, Baldi E, Marcolini G, Malaguti D, Quartieri M, Sorrenti G, Marangoni B. 2009. Response of potted grapevines to increasing soil copper concentration. Aust J Grape Wine Res. 15(1):85–92. doi:10.1111/j.1755-0238.2008.00040.x.

Yoon J, Cao X, Zhou Q, Ma LQ. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ. 368(2–3):456–464. doi:10.1016/j.scitotenv.2006.01.016.