

Potential Phytoextraction and Phytostabilization of Perennial Peanut on Copper-Contaminated Vineyard Soils and Copper Mining Waste

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Received: 13 December 2010 / Accepted: 17 January 2011 /
Published online: 1 February 2011
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Abstract This study sought to evaluate the potential of perennial peanut (*Arachis pintoi*) for copper phytoremediation in vineyard soils (Inceptisol and Mollisol) contaminated with copper and copper mining waste. Our results showed high phytomass production of perennial peanut in both vineyard soils. Macronutrient uptakes were not negatively affected by perennial peanut cultivated in all contaminated soils. Plants cultivated in Mollisol showed high copper concentrations in the roots and shoots of 475 and 52 mg kg⁻¹, respectively. Perennial peanut plants showed low translocation factor values for Cu, although these plants showed high bioaccumulation factor (BCF) for both vineyard soils, Inceptisol and Mollisol, with BCF values of 3.83 and 3.24, respectively, being characterized as a copper hyperaccumulator plant in these soils. Copper phytoextraction from Inceptisol soil was the highest for both roots and entire plant biomass, with more than 800 mg kg⁻¹ of copper in whole plant. The highest potential copper phytoextraction by perennial peanut was in Inceptisol soil with copper removal of 2,500 g ha⁻¹. Also, perennial peanut showed high potential for copper phytoremoval in copper mining waste and Mollisol with 1,700 and 1,500 g of copper per hectare, respectively. In addition, perennial peanuts characterized high potential for phytoextraction and phytostabilization of copper in vineyard soils and copper mining waste.

Keywords *Arachis pintoi* · Copper-contaminated sites · Phytoremediation · Phytostabilization · Copper phytoextraction

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Introduction

Copper is an essential micronutrient for living organisms, but at high exposure levels, it becomes a serious health problem. Human activities such as horticulture, industrial production, and mining operations often pollute the environment. In vineyards, copper content sprays are widely used to control parasitic leaf diseases [1]. Copper mining operations contaminate the environment and produce high quantities of copper mining waste with high copper concentrations [2]. Furthermore, these environments promote copper contaminations of adjacent areas, and it is necessary to apply efficient techniques for remediation and/or stabilization of copper contamination to protect soil and water quality.

Phytoremediation is a cost-efficient process for the removal of heavy metals from contaminated soil using plants as a means to transfer these metals in vegetal biomass [3] whose usefulness has already been demonstrated [4–16]. Besides, the interaction of phytoextraction and phytostabilization in vineyards and copper mining areas can be an efficient alternative for bioremediation, reducing the negative environmental impact.

Phytostabilization is the immobilization of a contaminant in the soil through absorption and accumulation by roots, adsorption onto roots, or precipitation in the plant root zone [3]. However, phytostabilization is the use of whole plants and/or plant roots to prevent contaminant migration through the wind and the water erosion, leaching, and soil dispersion [3]. However, this technique has been used in a wide range of contaminated sites such as mine soils [17, 18], and soils contaminated with heavy metals [19, 20]. There are some characteristics required for phytostabilization such as high stability of the contaminants in the roots and high biomass production. The use of this technique consequently will reduce the metal transport in the environment through surface runoff or wind-blown dust [3] due the covering of the soil surface. Also, perennial plants have a slight advantage in these required characteristics because it keeps growing in the field over time enhancing soil surface protection against the soil erosion factors.

Perennial peanut is an extensive crop used to perennial pasture for feeding animals [21–23], and it can increase the nutrient into soils such as green manure [24]. Also, this plant has a high biomass production with addition of high levels of nitrogen into the soil system [23] and biological nitrogen fixation [25]. Through all these characteristics, perennial peanut can be used as a cover crop, and also to protect the soil surface against the runoff and erosion, promoting to nutrients build up and recycling in vineyard soils. These properties of the perennial peanuts make it an attractive option for phytoremediation of vineyard soils contaminated with copper, especially due the lack of studies addressing the perennial peanut and heavy metal phytoextraction and its contribution in the bioremediation sites, which could be a win-win scenario, merging cover crop benefits and protection of the soil and water quality. Thus, this work sought to evaluate the potential of perennial peanut plants to copper phytoextraction and phytostabilization in two vineyard soils contaminated with copper and copper mining waste by the evaluation of some characteristics such as plant growth, nutrient uptake, and copper phytoremediation properties.

Material and Methods

Soil Characterization

A greenhouse experiment was carried out with samples of the topsoil (0–20 cm) layer from two copper-contaminated soils, and a copper mining waste from Southern Brazil. Two of the most used soils in vineyards (Inceptisol and Mollisol) were collected from vineyards at

Brazilian Agriculture Research Corporation farm located in Bento Gonçalves, RS, Brazil ($29^{\circ}09' 53.92''$ S and $51^{\circ}31'39.40''$ W). A native soil from a forestry area located near to the vineyards was used as a control. The copper mining waste was collected from a mine in Caçapava do Sul, RS, Brazil ($30^{\circ}29'43.48''$ S and $53^{\circ}32'37.87''$ W). All samples were air-dried, ground, and sieved (3 mm) prior to physical-chemical analysis, well characterized by Andreazza et al. [26]. Soil analysis showed that native soil, Inceptisol, Mollisol, and copper mining waste had extractable copper concentrations of 3.8, 207, 142, and 576 mg kg^{-1} , respectively. Based on contrasting differences in the soil copper content, we tested four treatments: native soil (control), Inceptisol, Mollisol, and copper mining waste. The copper mining waste was mixed with the native soil, being 40% of native soil and 60% of copper mining waste.

Five subsamples of 1 kg of each treatment were placed into 0.7-L plastic pots. Deionized water was then added to bring the soil moisture to 80% of water holding, and it was kept in this moisture by 30 days to stabilization; the same moisture was kept over the experimental period. Based on the nutrient recommendations to crops of Southern Brazil, soil samples (1 kg) were amended with nutrient solution consisting of $4.5 \text{ mg B (H}_3\text{BO}_3)$ and $25 \text{ mg S ((NH}_4)_2\text{SO}_4$) and mixed. Other nutrients were classified as “adequate” and were not supplied. Soil pH was set up to 6.5 using CaCO_3 .

Plant Growth and Copper Phytoaccumulation Analysis

One plant per pot of perennial peanut (*Arachis pintoi* Krapov and Gregory) to evaluate copper phytoaccumulation was used. Nitrogen was not added to plants. The pots were watered during the growth period to maintain soil water content near to 80% of field capacity. After 47 days of growth, shoots were harvested and immediately measured for wet mass and dry mass production. Shoots were then oven-dried for 72 h at 60°C and weighed. The soil-root system was measured in the wet mass and dry mass. After wet mass was measured, each plant root was separated by washing with deionized water, oven-dried for 72 h at 60°C , and weighed for further analysis.

Nutrient concentration in the roots and shoots was determined [27]. Nitrogen was determined after digestion with concentrated sulfuric–peroxide by steam distillation, and quantification by titration. The macronutrients (P, K, Ca, Mg, and S) and micronutrients (Cu, Zn, Mn, and Fe) were determined following digestion in concentrated nitric–perchloric acid by inductively coupled plasma–optical emission spectrometry.

The translocation factor (TF) of copper, zinc, iron, and manganese from root to shoot and bioaccumulation factor (BCF) of copper were calculated as follows [28, 29]:

$$\text{TF} = [\text{Cu}]_{\text{shoot}} / [\text{Cu}]_{\text{root}}$$

where $[\text{Cu}]_{\text{shoot}}$ is the concentration of copper, zinc, iron, and manganese in the shoots, and $[\text{Cu}]_{\text{root}}$ is the concentration of copper in the roots.

$$\text{BCF} = [\text{Cu}]_{\text{shoot and root}} / [\text{Cu}]_{\text{soil}}$$

where $[\text{Cu}]_{\text{shoot and root}}$ is the concentration of copper in the shoots plus roots, and the $[\text{Cu}]_{\text{soil}}$ is the concentration of copper in the soil.

Statistical Analysis

Descriptive statistics (means, standard error, and standard deviations ($N-1$)) were performed. Analysis of variance (ANOVA) was used to test differences in treatment

effects. The means of the treatments were evaluated by Tukey test with 5% of error probability.

Results

Plant Growth

Perennial peanut plants cultivated in the soils contaminated with copper showed high of both wet and dry mass production (Fig. 1). The perennial peanut cultivated in the Mollisol showed the highest wet biomass production of both shoots and roots with 9 and 4 g per plant, respectively (Fig. 1a). Also, it was observed that the dry matter production of perennial peanut in the shoots and roots (2 and 0.8 g per plant, respectively) was high in the Mollisol (Fig. 1b). Plants cultivated in the Inceptisol and in the copper mining waste showed high dry mass production in the shoots (1.0 and 0.3 g per plant, respectively) and roots (0.27 and 0.2 g per plant, respectively; Fig. 1b). However, the shoots and roots dry mass production of plants cultivated in both Inceptisol and copper mining waste were on average twofold lower than the results obtained in the Mollisol.

Macro- and Micronutrients Uptake

Perennial peanut plants cultivated in the Inceptisol contaminated with copper showed higher macronutrients concentration in the shoots for N 2.35, P 0.27, K 3.69, Mg 0.88, and S 0.61 g kg⁻¹ than all the other treatments (Table 1). Plants cultivated in both vineyard soils contaminated with copper (Inceptisol and Mollisol) showed the highest P, K, and Mg concentrations in the shoots. Perennial peanut cultivated in the copper mining waste only showed the highest concentration of Ca and S in the shoots with 4.54 and 0.53 g kg⁻¹, respectively. Furthermore, the native soil only showed the nitrogen concentration higher than other treatments with 2.44 g kg⁻¹ in the shoots biomass, probably due the highest soil organic matter content which contributes to more nitrogen released by the decomposition, increasing the soil nitrogen availability to plants. On the other hand, the determination of the macronutrients in the roots after 47 days of growth did not show significant differences among the treatments in the vineyard soils

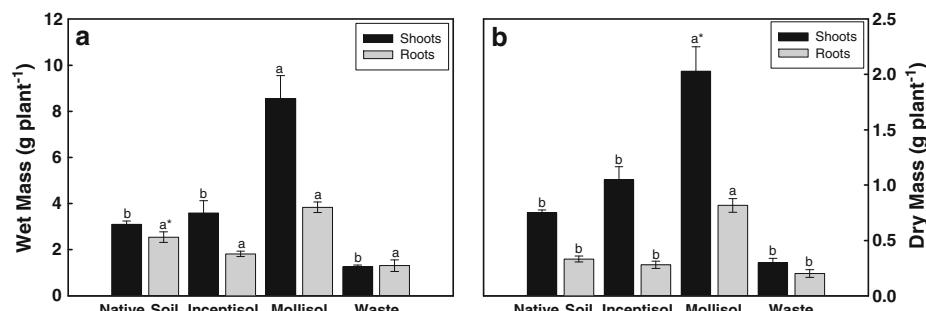


Fig. 1 Wet mass (a) and dry mass (b) of the shoots and roots of perennial peanut plants after growing in copper-contaminated soils: native soil (Control), Inceptisol, Mollisol, and copper mining waste (Waste).

*Different letters in the same color bar represent significant differences ($P \leq 0.05$) with Tukey test. Error bars are calculations of standard error

Table 1 Macronutrients in dry mass of the shoots of the perennial peanut plants, after growing in different copper-contaminated soils: native soil (control, not contaminated), Inceptisol, Mollisol, and copper mining waste (waste)

	N (g kg ⁻¹)	P (g kg ⁻¹)	K (g kg ⁻¹)
Native soil	2.44±0.06a	0.07±0.00b	1.91±0.02b
Inceptisol	2.35±0.04ab	0.27±0.01a	3.69±0.01a
Mollisol	1.96±0.10bc	0.27±0.01a	3.95±0.16a
Waste	1.91±0.09c	0.16±0.01a	2.38±0.26b
CV (%)	11.16	14.44	9.18
	Ca (g kg ⁻¹)	Mg (g kg ⁻¹)	S (g kg ⁻¹)
Native soil	1.47±0.03c	0.34±0.01b	0.23±0.01c
Inceptisol	2.76±0.03b	0.88±0.01a	0.61±0.01a
Mollisol	3.10±0.13b	0.90±0.03a	0.36±0.02b
Waste	4.54±0.14a	0.76±0.00a	0.53±0.04a
CV (%)	11.23	13.44	7.24

CV coefficient of variation of the means

Values are means ± standard error of the mean. Different letters in the column represent significant differences ($P\leq 0.05$) with Tukey test

contaminated with copper and in the copper mining waste compared to native soil (without copper contamination; Table 2).

The micronutrient uptake by perennial peanut was affected according to the soil type and soil copper content (Table 3). Zinc and Mn were found in the highest concentration in the shoots and roots of perennial peanut plants cultivated in the Inceptisol. The Zn concentration in the shoots of perennial peanuts ranged from 303 to 320 mg kg⁻¹ (Mollisol and Inceptisol, respectively) which were the highest value obtained. The same tendency

Table 2 Macronutrients in dry mass in the roots of the perennial peanut plants, after growing in different copper-contaminated soils: native soil (control, not contaminated), Inceptisol, Mollisol, and copper mining waste (waste)

	N (g kg ⁻¹)	P (g kg ⁻¹)	K (g kg ⁻¹)
Native soil	3.19±0.07a	0.11±0.01a	2.08±0.30a
Inceptisol	2.55±0.14b	0.22±0.02a	3.29±0.21a
Mollisol	2.04±0.07b	0.25±0.01a	2.76±0.12a
Waste	2.25±0.05b	0.13±0.02a	2.55±0.76a
CV (%)	11.95	30.47	32.01
	Ca (g kg ⁻¹)	Mg (g kg ⁻¹)	S (g kg ⁻¹)
Native soil	0.88±0.15a	0.37±0.05a	0.51±0.09a
Inceptisol	1.31±0.05a	0.93±0.04a	0.63±0.01a
Mollisol	1.43±0.10a	0.69±0.02a	0.44±0.02a
Waste	1.29±0.27a	0.55±0.16a	0.46±0.01a
CV (%)	30.15	44.22	36.85

CV coefficient of variation of the means

Values are means ± standard error of the mean. Different letters in the column represent significant differences ($P\leq 0.05$) with Tukey test

Table 3 Micronutrients in dry mass in the shoots and roots of the perennial peanut plants, after growing in different copper-contaminated soils: native soil (control, not contaminated), Inceptisol, Mollisol, and copper mining waste (waste), and translocation factor

	Shoots		
	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)
Native soil	54.02±1.79b*	149.97±12.39b	161.13±4.23b
Inceptisol	320.50±1.09a	366.90±10.25b	397.20±0.02a
Mollisol	303.85±1.53a	492.50±29.13b	122.50±0.19b
Waste	65.43±10.8b	1025.23±169.5a	139.03±4.08b
CV (%)	10.46	36.08	17.28
	Roots		
	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)
Native soil	63.66±9.38a	516.50±66.56b	136.12±27.50a
Inceptisol	295.23±40.9a	1925.00±239.0a	306.12±57.90a
Mollisol	216.07±35.2a	2215.67±229.5a	216.33±32.90a
Waste	64.52±10.6a	2621.00±67.41a	53.57±4.52a
CV (%)	70.10	71.87	87.46
	Translocation factor ^a		
	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)
Native soil	0.85	0.29	1.18
Inceptisol	1.09	0.19	1.30
Mollisol	1.41	0.22	0.57
Waste	1.01	0.39	2.60

CV coefficient of variation of the means

Values are means ± standard error of the mean. Different letters in the column represent significant differences ($P\leq 0.05$) with Tukey test

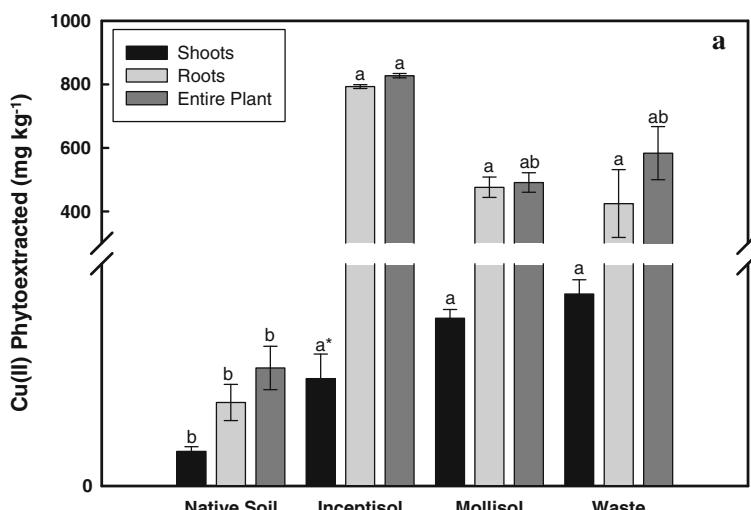
^a Metal concentration ratio of plant shoots to roots

was observed for the Zn concentration in the roots of perennial peanuts which the Zn concentration was more than fourfold in both Mollisol and Inceptisol than the other treatments (Table 3). Contrasting results were observed to Fe concentration in the shoots and roots of the perennial peanuts. The Fe concentration in the shoots of perennial peanut cultivated in the copper mining waste was more than twofold than the Fe concentration in the shoots of perennial peanut cultivated in the others soils studied (Table 3). However, the Fe concentration in the roots of perennial peanut were similar among the copper-contaminated soils, which were more than 3.5-fold than the Fe concentration in the roots of the plants grown in the native soil (Table 3). The concentration of Mn in the shoots of perennial peanut plants cultivated in the Inceptisol was the highest among the treatments (397 mg kg⁻¹). Although we observed the highest value of Mn concentration in the roots of the plants grown in the Inceptisol, the Mn concentration in the roots was not statistically significant among the treatments (Table 3). In general, our results indicate high TF in the perennial peanuts for Zn and Mn (1.01 and 2.6, respectively) in the copper mining waste. Also, the perennial peanuts showed high TF for Zn in the Inceptisol and in the Mollisol with TF values of 1.09 and 1.41, respectively. Perennial peanut plants showed low TF for Fe in all treatments with values ranged from 0.19 to 0.39. For Mn, high values of TF were observed in native soil and Inceptisol (1.18 and 1.30, respectively).

Copper Phytoextraction and Phytostabilization

Copper concentrations in the shoots and roots of perennial peanut cultivated in the copper contaminated soils were higher than the control treatment (Fig. 2a). Although the copper concentration in the shoots and roots in the peanut plant slightly differed among copper-contaminated treatments, perennial peanut plants cultivated in the Inceptisol showed the highest copper accumulation in the roots and entire biomass, with more than 800 mg kg⁻¹ of copper bioaccumulated in whole plant. Plants cultivated in the Mollisol showed copper accumulation in the roots of 475 and 52 mg kg⁻¹ in the shoots (Fig. 2a). It promoted a low TF value (0.05; Fig. 2b).

Perennial peanut plants cultivated in the copper mining waste showed the highest copper concentration in the shoots (60 mg kg⁻¹), and the highest TF value (0.14) among the treatments contaminated with copper (Fig. 2). Plants cultivated in the Inceptisol showed the lowest TF value (0.04; Fig. 2b). However, perennial peanut plants showed high copper BCF in the natural soils (BCF of 6.91), demonstrating the high potential to copper bioaccumulation. Also, perennial peanut plants cultivated in the vineyard soils showed high potential for copper bioaccumulation in both Inceptisol and Mollisol, with BCF values of



b	Bioaccumulation Factor (BCF)**	Translocation Factor (TF)***
Native Soil	6.91	0.41
Inceptisol	3.83	0.04
Mollisol	3.24	0.11
Waste	0.74	0.14

Fig. 2 Copper concentrations in shoots, roots, and total copper bioaccumulated by perennial peanut plants in different copper-contaminated soils: native soil (*Control*), Inceptisol, Mollisol, and copper mining waste (*Waste*; a). b Bioaccumulation and translocation factors. Error bars are calculations of standard error. *Different letters in the same color bars represent significant differences ($P \leq 0.05$) with Tukey test. **BCF metal concentration ratio of plants roots to soil. ***TF metal concentration ratio of plant shoots to roots

3.83 and 3.24, respectively (Fig. 2b). However, the copper translocation from roots to shoots was not high; on the other hand, the high copper accumulation was observed in the roots both in vineyard soils and in the copper mining waste, demonstrating a high potential of perennial peanut for phytostabilization of copper in these conditions.

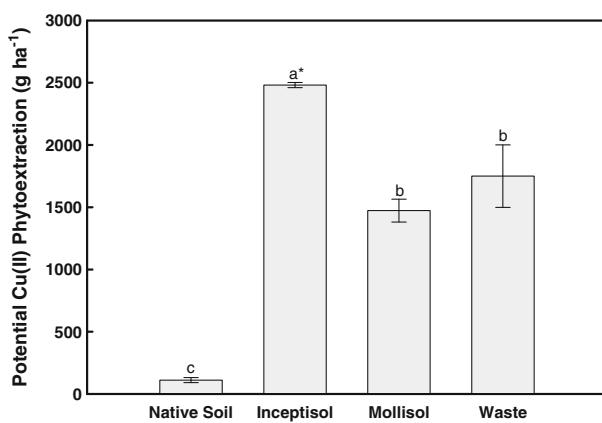
Perennial peanuts showed high potential to copper phytoextraction from vineyard soils contaminated with copper and copper mining waste (Fig. 3). The highest potential of copper phytoextraction by perennial peanut in our greenhouse experiment was in Inceptisol with copper removal about $2,500 \text{ g ha}^{-1}$. Also, the perennial peanuts cultivated in the copper mining waste and in the Mollisol showed high potential to copper phytoremoval with 1,700 and 1,500 g of copper per hectare, respectively. These results indicate the potential use of perennial peanut to copper removal in areas with high copper content that are limiting the proper crop growth and development.

Discussion

Copper-contaminated areas such as vineyards and copper mining waste are considerate problematic sites needing remediation to allow the establishment of alternative crops. Thus, we tested the potential of perennial peanut such as phytoremediation alternative to use in the copper-contaminated sites, due its high phytomass production. Perennial peanut plants showed high biomass production in all soils contaminated with copper, mainly in both vineyard soils. However, in a similar pot experiment developed to evaluate the peanut (*Arachis hypogaea*) tolerance in a cadmium (Cd)-contaminated soil and its potential use to Cd remediation, peanut showed both shoot and root biomass production of 0.37 and 0.14 g, respectively, in a soil with 200 mg kg^{-1} of Cd after 28 days of growth [29]. It shows that *A. pintoi* showed high biomass production compared to *A. hypogaea* in the contaminated sites with heavy metals. Oatmeal plants (*Avena sativa*) is a crop widely used as a cover crop in the vineyard areas contaminated with copper in Southern Brazil, and this species showed low biomass production in the Inceptisol and Mollisol [26] compared with perennial peanuts. These results showed a high potential use of perennial peanuts to bioremediation of copper-contaminated sites such as vineyard soils and copper mining waste.

Heavy metals can disturb the uptake and movement of other nutrients in the plants, and consequently, heavy metals can promote toxic effects on mineral nutrition and metabolism

Fig. 3 Potential of Cu(II) phytoremoval of perennial peanut plants in different copper-contaminated soils: native soil (*Control*), Inceptisol, Mollisol, and copper mining waste (*Waste*). Values as calculated in base on biomass production at $3,000 \text{ kg ha}^{-1}$. Error bars are calculations of standard error. *Different letters represent significant differences ($P \leq 0.05$) with Tukey test



of plants [30]. In general, this study did not demonstrate negative effect in the macronutrient uptake and concentrations in the roots and shoots. However, zinc concentrations in the roots and shoots increased when perennial peanuts were cultivated in both Inceptisol and Mollisol (vineyard soils). On the other hand, the effect on nutrient uptake by *Rumex japonicus* was observed to P and Fe which decreased due the soil copper contamination, while Ca concentrations in the shoots increased [9]. In addition, the high macro- and micronutrient uptake by perennial peanut plants cultivated in the copper-contaminated soils showed an important role of this plant in the nutrient cycling and resistance to copper toxicity. It can be important to promote the plant growth of other plants, where also it can help to phytostabilize and/or phytoextract copper within consortium in these different situations.

Plants ability in uptake and accumulate metals from soils can be estimated using the BCF, which is defined as the ratio of metal concentration in the roots to that in soil; the ability to translocate metals from the roots to the shoots is measured using the TF, which is defined as the ratio of metal concentration in the shoots to that in the roots [28]. TF is considered high if the value is equal or higher than 1.0, showing a high potential of nutrient or metal transfer from roots to shoots [28]. Our results demonstrate high capacity of perennial peanut plants in translocate and accumulate Zn and Mn in the shoots, but low capacity for copper.

Plants and soils have been monitored in contaminated sites with toxic levels of heavy metals [31]. Furthermore, in the same study, it was observed that the *Acia raddiana* and *Avena javanica* plants grown in these areas increased heavy metals concentrations in both shoots and roots, but they did not show high bioaccumulation factor indices for copper (0.1 and 0.15, respectively). However, our results showed higher bioaccumulation factor for *A. pintoi* cultivated in both vineyard soils (Inceptisol and Mollisol, with BCF of 3.83 and 3.24, respectively) and copper mining waste (0.74) than Rashed [31] study. These results showed a high potential use of perennial peanut plants for copper phytoremediation in copper-contaminated sites.

Perennial peanuts are widely cultivated for feed animals as pasture. Furthermore, this kind of pastures can produce high biomass quantities ranging from 2,000 to 5,000 kg ha⁻¹ of dry mass [21]. To calculate the potential of copper phytoextraction, we adopted the mean value of biomass production of 3,000 kg ha⁻¹. Thus, it was possible to estimate the amounts of copper phytoextracted by the perennial peanuts from these soils. Perennial peanuts were able to phytoextract copper concentrations in the vineyard soils and copper mining waste with amounts higher than other species. Evaluation on copper uptake by a hyperaccumulator plant (*Elsholtzia splendens*) in a polluted soil showed copper concentrations in the shoots of 10 mg kg⁻¹ under greenhouse conditions (pot); however, root copper concentrations were up to 1,700 mg kg⁻¹ in the pot experiment and 1,260 mg kg⁻¹ in the field experiment [5].

McCutcheon and Schnoor [3] classify copper uptake by plants into three different categories: resistant, accumulator, and hyperaccumulator plants. Our study showed that perennial peanut grown in copper-contaminated soil was an accumulator plant with copper phytoextraction ranged from 400 to 800 mg kg⁻¹ in entire plant (Fig. 2). However, in other research [32], it was observed that plants that showed BCF higher than 1.0 were considered hyperaccumulator plants. Thereby, most of the copper absorbed by plants was mainly found in the roots, and in our results, it showed a low TF by *A. pintoi* plants from roots to shoots. Our values of TF for copper were lower than *Cyperus eculentus*, *Gentiana pennelliana*, and *Stenotaphrum secundatum* with values of 2.8, 0.56, and 0.77, respectively [28]. However, the same study showed TF values for copper lower than our results with TF of 0.13 to

Phyla nodiflora. It shows a potential of perennial peanuts for copper phytostabilization in the vineyards soils and copper mining waste if the entire plant will not be harvested once perennial peanut has high BCF with high growth in both vineyard soils contaminated with copper.

Some plants with high biomass production, such as *Brassica rapa*, *Cannabis sativa*, *Helianthus annuus*, and *Zea mays*, showed copper concentrations in the shoots ranged from 2.7 to 15 mg kg⁻¹, with the lowest copper concentration observed in *Z. mays*, while *C. sativa* showed the highest copper concentration in the shoots [33]. In our study, perennial peanut plants extracted more than 50 and 60 mg of copper per kilogram of shoot biomass after 47 days of growth in the Mollisol soil and in the copper mining waste, respectively. Thus, these copper concentrations might not cause healthy problem to feed swine with the shoots biomass of perennial peanuts grown in the copper-contaminated sites. Some studies showed that plant biomass with copper content that ranged from 18 to 217 mg kg⁻¹ are often used to feed swine [34]. However, the copper translocation to edible parts in the plants is uncommon [35].

In summary, perennial peanut (*A. pintoi*) plants showed high potential for copper removal from the vineyard soils contaminated with copper and copper mining waste. This species showed high biomass production in the vineyard soils and in the copper mining waste; biomass production can be compared to native soil under study. Macronutrient uptake by perennial peanut plants was not negatively affected after cultivation in the copper-contaminated soils. Thereby, this species can be an important candidate to use copper phytoextraction and copper phytostabilization in the vineyard soils contaminated with copper and copper mining waste.

Acknowledgments This project was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

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