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Evaluation of two Brazilian indigenous plants for phytostabilization and phytoremediation of copper-contaminated soils

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(With 4 figures)

Abstract

Indigenous plants have been grown naturally and vigorously in copper contaminated soils. Thus, the aim of this study was to evaluate the phytoremediation ability of two indigenous plants naturally grown in two vineyard soils copper contaminated, and in a copper mining waste. However, it was evaluated the macro and micronutrient uptake and the potential of phytoremediation. So, a greenhouse study was carried out with *Bidens pilosa* and *Plantago lanceolata* in samples of vineyard soils (Inceptisol and Mollisol) copper contaminated, and in a copper mining waste. Plant growth, macro and micronutrient up take, tolerance index (TI), translocation factor (TF), metal extraction ratio (MER), bioaccumulation factor (BCF), plant effective number of the shoots (PENs), and plant effective number of the total plant (PENt) were analyzed. Both plants grown in vineyard soils showed high phytomass production and TI. *P. lanceolata* plants cultivated in the Inceptisol showed the highest copper concentrations in the shoots (142 mg kg^{-1}), roots (964 mg kg^{-1}) and entire plants (1,106 mg kg^{-1}). High levels of copper were phytoaccumulated from the Inceptisol by *B. pilosa* and *P. lanceolata* with 3,500 and 2,200 g ha^{-1} respectively. Both *B. pilosa* and *P. lanceolata* plants showed characteristics of high copper hyperaccumulator. Results showed that both species play an important role in the natural copper phytoaccumulation in both vineyard soils contaminated with copper, being important to its phytoremediation.

Keywords: Bidens pilosa, copper contaminated areas, copper phytoaccumulation, *Plantago lanceolata*.

Avaliação de duas plantas nativas brasileiras para fitoestabilização e fitorremediação de solos contaminados com cobre

Resumo

Plantas nativas crescem naturalmente e vigorosamente em solos contaminados com cobre. Assim, o objetivo deste estudo foi avaliar a capacidade de fitorremediação de duas plantas nativas, naturalmente encontradas em dois solos de vitivinicultura contaminados com cobre, e em rejeito de mineração de cobre. Foram avaliados os teores de macro e micronutrientes nos tecidos das plantas, e o potencial de fitorremediação. Assim, um estudo em casa de vegetação foi realizado com plantas de *Bidens pilosa* e *Plantago lanceolata*, com amostras de dois solos de vitivinicultura (Neossolos e Cambissolos) contaminados com cobre, e com rejeito de mineração de cobre. O crescimento das plantas, teores de macro e micronutrientes nos tecidos, índice de tolerância (TI), fator de translocação (TF), taxa de extração do metal (MER), fator de bioacumulação (BCF), número efetivo dos plantas da parte aérea (PENs) e número efetivo de plantas inteiras (PENt) foram analisados. Ambas as espécies cultivadas em solos vitivinicultura mostraram elevada produção de fitomassa e os TI. *P. lanceolata* cultivadas no Neossolo mostraram as concentrações de cobre mais elevados na parte aérea (142 mg kg^{-1}), nas raízes (964 mg kg^{-1}) e nas plantas inteiras (1.106 mg kg^{-1}). Altos níveis de cobre foram fitoacumulados pelas plantas *B. pilosa* e *P. lanceolata* com 3.500 e 2.200 g ha^{-1}, respectivamente, quando cultivadas em Neossolo. Ambas as espécies apresentaram características hiperacumuladoras de cobre. Os resultados mostraram que estas espécies desempenham um papel importante na fitoacumulação de cobre naturalmente em ambos os solos de vitivinicultura contaminados com cobre, sendo importantes para a fitorremediação.

Palavras-chave: Bidens pilosa, areas contaminadas com cobre, fitoacumulação de cobre, *Plantago lanceolata*.

1. Introduction

Copper contamination is an eminent problem often found in a wide range of soils, sediments and water courses (Lacerda et al., 2009; Andrade et al., 2010). Furthermore, in some cases as vineyards, copper is a fundamental agent used to control leave diseases, which it is commonly and constantly used to produce grapes and wines (Komárek et al., 2010). Also, copper mining waste sites are enormous areas with notable problems such as low nutrient content and high copper concentrations (Laybauer, 1998). However, polluted environments with heavy metals change the plants community during the time (Dazy et al., 2009). Indigenous or wild plants are located in heavy metal contaminated areas and their contribution to environment should be evaluated, once, these plants can uptake the heavy metals and then, mitigate the negative impact of the contamination of adjacent soils and water courses.

Moffat (1995) notified that researchers discovered natural and ornamental plants grown vigorously in metal contaminated areas; and they reported that phytoremediation would be much better and cost-effective using these plants than the use of conventional cleanup strategies. Native plants have been studied by their capacity to accumulate heavy metals in the shoots and roots by uptake from contaminated sites (Yoon et al., 2006) using their characteristics such as rusticity and adaptability.

Phytoremediation is the use of plants to reduce the concentrations or toxic effects of contaminants in the environments (Ali et al., 2013). Furthermore it has been used for cleaning up the environment with high success (Babu et al., 2013; Pandey, 2013). *Lonicera japonica,* an Asian native plant showed high accumulation and tolerance characteristics for cadmium (Cd), being a useful plant with potential in hyperaccumulating cadmium (Liu et al., 2009). The wild plant *Bidens tripartita*, other species of *B. pilosa* was found grown in a super-large antimony (Sb) deposit area with high concentrations of Sb in the roots (Qi et al., 2011). Also, *B. tripartite* has been studied to phytoremediation of Cd contaminated soils (Wei et al., 2010). Other wild plant, *Arthrocnemum macrostachyum* showed characteristics of Cd-hyperaccumulator (Redondo-Gómez et al., 2010); and *Spartina argentinensis*, an Argentine native plant showed high accumulation and tolerance characteristics for chromium (Redondo-Gómez et al., 2011). However, native plants with copper hyperaccumulation abilities are incipient and there is a gap of information that must be filled requiring more studies.

Many indigenous plants grown surrounding mining wastes showed high BCF with high heavy metal hyperaccumulation characteristics (González and González-Chávez, 2006), but *B. pilosa* and *P. lanceolata* plants were not found in these areas. In other study, *B. pilosa* was characterized as a potential Cd-hyperaccumulator plant with high potential for resistance, growth, BCF and TF for Cd (Sun et al., 2009). However, there is a paucity of studies with *B. pilosa* and *P. lanceolata* plants in copper contaminated sites, where both were found abundantly in vineyard soils and *P. lanceolata* was also found in copper mining waste area, both sites in Southern Brazil. Thus, it was evaluated the growth ability, macro and micronutrients uptake and different phytoremediation capability of *B. pilosa* and *P. lanceolata* plants in vineyard soils contaminated with copper and copper mining waste as a bioremediation tool to improve the soil quality.

2. Material and Methods

2.1. Soil and experiment characterization

A greenhouse experiment was carried out with topsoil samples (0-20 cm) taken from two 40 year old vineyard soils (Inceptisol and Mollisol) at Brazilian Agriculture Research Corporation research (EMBRAPA) farm located in Bento Gonçalves, RS, Southern Brazil. A native soil was sampled from a native forestry area located nearby the vineyards. The copper mining waste was sampled from a copper mine in Caçapava do Sul, RS, Southern Brazil. Copper mining waste, native soil, and the two types of copper contaminated soils were characterized to physical-chemical analysis (Table 1). All soil samples were air dried, ground and sieved (3 mm). It was not added any nutrient to treatments because of these indigenous plants are considering weeds to agricultural crops and consequently no nutrient recommendations is required.

Five replicates of 1 kg subsamples were placed in pots of 700 dm^3. Deionized water was then added to bring the soil moisture up to 80% field capacity water content and was maintained during the 64 and 85 days of plant growth for *B. pilosa* and *P. lanceolata* respectively. Four soil treatments were tested: Native soil (Control); Inceptisol; Mollisol; and copper mining waste (40% of Native soil and 60% of copper mining waste).

2.2. Plant growth for copper phytoremediation, harvest and analysis

Ten seeds of each species were seeded per pot. After 10 days of incubation, it was kept until the end of the study in each pot 4 and 3 plants of *B. pilosa* and *P. lanceolata* respectively. The pots were watered during the growth period to maintain soil water content near to 80% of field capacity. After the growth period, shoots were harvested and immediately measured for height and green mass. The height of *B. pilosa* plants was determined with respect to the main stem from the base to the tip of each plant and calculated the average. Shoots of both species were then oven-dried for 72 h at 60°C and the shoot dry weight was recorded. Green and dry roots biomass were also measured to compose the soil-root system. After green mass 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 dry matters were determined. Nitrogen was determined after digestion with concentrated sulfuric-peroxide by steam distillation, and quantification by titration. The macronutrients (P, K, Ca, Mg and S), copper and micronutrients (Zn, Mn, Na and Fe) were determined following digestion in concentrated nitric-perchloric acid by Inductively Coupled Plasma - Optical Emission Spectrometry.

Table 1. Chemical-physical* characteristics of soils: Native soil, Inceptisol and Mollisol, and copper mining waste.

Solo	pH	Carbon	Clay	Cu Extrac†	Cu Total	
	1:1	%		mg kg^{-1}		
Native soil	5.8	2.4	25	3.8 (1.0)	35	
Inceptisol	6.3	1.5	19	207 (171)	507	
Mollisol	6.0	1.4	29	142 (104)	281	
Waste	7.9	0.5	02	576 (479)	852	
	Ca	Mg	Mn	S	Zn	
	mg kg^{-1}					
Native soil	1860	150	59	6.3	8.1	
Inceptisol	2180	388	55	6.1	19.0	
Mollisol	1560	263	35	5.9	18.0	
Waste	4840	213	2	0.1	0.8	
	P (Extrac)	P (Total)	N (Extrac)		N (Total)	K
	mg kg^{-1}				%	mg kg^{-1}
			NH_4^+	NO_3^-		
Native soil	14	600	277	216	0.31	217
Inceptisol	28	700	12	11	0.20	142
Mollisol	27	900	13	10	0.18	167
Waste	32	700	1	2	0.01	32

*pH: pH in water ratio 1:1; Clay: dispersion with NaOH; Cu and Zn (extractable): extracted with 0.1 M HCl; Ca, Mg, Al and Mn (exchangeable): extracted with 1.0 M KCl; H+Al: titration; S: extracted with 500 M calcium fosfate; P and K (available): extracted with Mehlich-1; Cu and P (total): extracted with nitric-perchloric digestion. N (extractable): extracted with 1 M KCl; N (total): Kjeldhal. †Values in parenthesis are soil extractable Cu with 0.1 M HCl after liming.

2.3. Characterization of potential phytoremediation

The tolerance index (TI) was expressed on the basis of plant growth parameters including height, green and dry biomass of the roots and shoots (Wilkins, 1978). The translocation factor (TF) of the Cu, Zn, Na, Mn and Fe from the roots to shoots, and the bioconcentration factor (BCF) were calculated (Yoon et al., 2006; Shi and Cai, 2009). Also, the metal extraction ratio (MER) is defined as the ratio of metal accumulation in the shoots to that in soil (Mertens et al., 2005). The plant effective number of the shoots (PENs) and the plant effective number of the total plant (PENt) have been applied to evaluate the ability of remedying contaminated soil by a hyperaccumulator according with Sun et al. (2008).

2.4. Statistical analysis

The statistical design used was randomized complete block with five replicates. Statistical analysis was performed using ANOVA. When the significance difference was observed between treatments ($P \leq 0.05$), multiple comparisons were carried out using Tukey test.

3. Results and Discussion

3.1. Plant growth

Both *B. pilosa* and *P. lanceolata* grown vigorously in the both vineyard soils contaminated with copper; however, they poorly grown in the copper mining waste (Figures 1 and 2). *B. pilosa* plants showed high height after 64 days of growth in the both vineyard soils with height of 17 cm (Mollisol) and 10.4 cm (Inceptisol) (Figure 1). On the other hand, *B. pilosa* plants showed low growth in the copper mining waste with height of 4 cm (Figure 1).

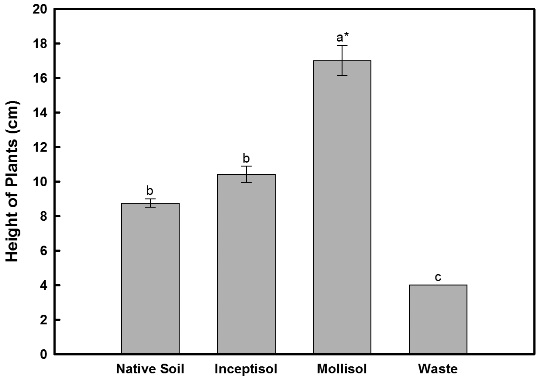

Figure 1. Plants height of *Bidens pilosa* after 64 days growing in different copper contaminated soils: Native soil (Control); Inceptisol; Mollisol and copper mining waste (Waste). *Different letters represent significant differences (P≤0.05) with Tukey test. Error bars are calculations of standard error.

B. pilosa plants cultivated in the Mollisol and Inceptisol showed the highest green mass production of the shoots (8.4 and 8.1 g pot^{-1}, respectively) and roots (14.6 and 9.3 g pot^{-1}, respectively) (Figure 2A), and dry mass of the shoots (1.6 and 1.3 g pot^{-1}, respectively) and roots (1.2 and 0.77 g pot^{-1}, respectively) (Figure 2B). Copper

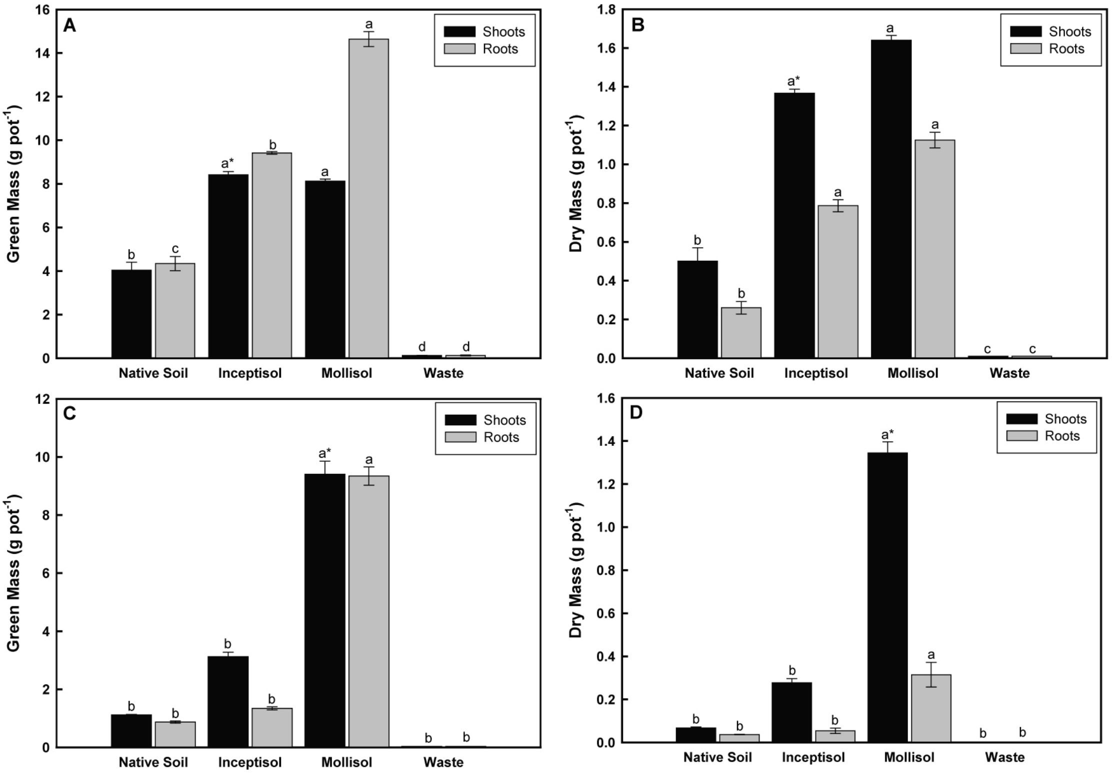

Figure 2. Green biomass (A) and dry biomass (B) production of the shoots and roots of *Bidens pilosa*; and green mass (C) and dry mass (D) production of the shoots and roots of *Plantago lanceolata* after 64 and 85 days of growth respectively in copper contaminated soils: Native soil (Control); Inceptisol; 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.

mining waste drastically affected both indigenous plants with green and dry mass production around 0.1 g pot^{-1}. *P. lanceolata* showed significantly high green and dry biomass production in the Mollisol followed by the Inceptisol treatment (Figure 2C, D). However, *P. lanceolata* showed high green mass production in both Mollisol and Inceptisol in the shoots (9.4 and 3.1 g pot^{-1}, respectively) and roots (9.3 and 1.3 g pot^{-1}, respectively) (Figure 2C), and also to dry mass of the shoots (1.3 and 0.27 g pot^{-1}, respectively) and roots (0.31 and 0.05 g pot^{-1}, respectively) (Figure 2D).

Heavy metals in toxic concentrations can interfere in the plant growth (Ke et al., 2007). Some indigenous plants (*Populus deltoids* and *P. nigra*) were negatively affected after exposure to toxic concentrations of cadmium (Wu et al., 2010). In other study, the *B. pilosa* dry weight was drastically reduced when Cd concentration was increased (Sun et al., 2009). Some authors proved that indigenous plants (*P. lanceolata* and *P. media*) with high nutrient demands showed high fitness as seeders respectable of nutrient availability after growth in negatively impacted areas (Latzel and Klimesova, 2009). However, the results in this study demonstrated a high potential of growth of both indigenous species (*B. pilosa* and *P. lanceolata*) in the both vineyard soils contaminated with copper, and low potential to growth in the copper mining waste.

Both *B. pilosa* and *P. lanceolata* showed high tolerance index (TI) values in the vineyard soils contaminated with copper; and copper mining waste showed high visual toxicity effects and promoted a very low tolerance by the both indigenous plants (Table 2). *B. pilosa* showed TI values between 2 to 4-folds higher than the control among the variables analyzed in the Mollisol and Inceptisol soils, showing that both vineyards in study can promote *B. pilosa* growth. Surprisingly, *P. lanceolata* plants showed the highest adaptability in both vineyard soils, especially in the Mollisol soil with TI values ranged from 839 to 23285% among the growth evaluations. It shows a high potential of both indigenous plants to growth in both vineyard soils contaminated with high copper concentrations.

In an ecological study, *B. pilosa* was classified as a copper tolerant plant with substantial growth in a high copper contaminated site (He et al., 2010). Other study with eight high potential bioenergy crops presented tolerance indexes in a range of TI of 13 and 111% (Shi and Cai, 2009). Compiling with the results obtained in this study, *B. pilosa* and *P. lanceolata* showed high tolerance to copper contaminated soils. It explains the potential to growth in the vineyard soils.

Table 2. Tolerance index (TI) of the height, green mass of the shoots and roots, and dry mass of the shoots and roots of *Bidens pilosa* and *Plantago lanceolata* plants after 64 and 85 days of growth respectively in three copper contaminated soils: Inceptisol, Mollisol and copper mining waste (waste).

Soils	***Bidens pilosa***				
		Green mass		**Dry mass**	
	Height	**Shoots**	**Roots**	**Shoots**	**Roots**
	%				
Inceptisol	119.14	208.46	273.33	216.97	302.56
Mollisol	194.37	201.24	328.00	337.33	432.69
Waste	45.71	3.16	2.00	2.88	3.85
Soils	***Plantago lanceolata***				
		Green mass		**Dry mass**	
	Height	**Shoots**	**Roots**	**Shoots**	**Roots**
	%				
Inceptisol	ND*	279.02	411.11	153.42	3476.19
Mollisol	ND	839.73	1992.59	1065.68	23285.71
Waste	ND	3.57	1.48	4.33	19.05

*ND not determined.

3.2. Macro and micronutrient uptake

Macro and micronutrient uptake and concentration in the shoots were affected in the both *B. pilosa* and *P. lanceolata* after growth for 64 and 85 days respectively in the copper contaminated soils (Table 3). *B. pilosa* plants cultivated in the Inceptisol did not show any significant depletion on all macro and micronutrient uptake in the shoots. On the other hand, *B. pilosa* cultivated in the other vineyard soil (Mollisol) showed significant low concentrations in the shoots for N (0.85 g kg^{-1}), K (1.6 g kg^{-1}), Ca (1.38 g kg^{-1}), Mg (0.57 g kg^{-1}), Zn (136 mg kg^{-1}) and Mn (85 mg kg^{-1}). *B. pilosa* cultivated in the copper mining waste treatment showed high depletion on K (1.45 g kg^{-1}), Ca (1.3 g kg^{-1}), Mg (0.47 g kg^{-1}), Zn (119 mg kg^{-1}), Fe (174 mg kg^{-1}) and Mn (61 mg kg^{-1}) uptake and concentration in the shoots.

P. lanceolata cultivated in the vineyard soils contaminated with copper showed no depletion in all macronutrients concentration in the shoots, on the contrary, some macronutrients showed the highest concentrations (Table 3). Due the low biomass production by *P. lanceolata*, it was not able to determine N and Na in all treatments; also, the amounts of macro and micronutrients in *P. lanceolata* grown in copper mining waste. Only micronutrients were affected after *P. lanceolata* cultivated in the Inceptisol and Mollisol for Fe (956 and 620 mg kg^{-1}, respectively) and Mn (197 and 106 mg kg^{-1}, respectively); and Zn (98 mg kg^{-1}) in the Mollisol treatment.

Macro and micronutrients accumulation in the roots were affected after both *B. pilosa* and *P. lanceolata* plants cultivated in the copper contaminated soils (Table 4). *B. pilosa* plants showed high nutrient accumulation when plants were cultivated in the Inceptisol such as P (0.15 g kg^{-1}), K (1.99 g kg^{-1}), Ca (0.51 g kg^{-1}), S (0.32 g kg^{-1}), Zn (381 mg kg^{-1}) and Na (2696 mg kg^{-1}). *B. pilosa* cultivated in the Mollisol showed low concentration of the most nutrients in the roots such as N (0.81 g kg^{-1}), K (1.54 g kg^{-1}), Ca (0.44 g kg^{-1}), S (0.2 g kg^{-1}), Zn (80 mg kg^{-1}) and Mn (44 mg kg^{-1}). *B. pilosa* did not show significant difference in P and Fe concentration in the roots after grown in copper contaminated soils. Macro and micronutrients uptake in the roots of *B. pilosa* and *P. lanceolata* cultivated in the copper mining waste also were not able to be determined due the insufficient biomass production to perform the analysis. *P. lanceolata* cultivated in the Inceptisol and Mollisol showed the same level of the macronutrients concentration in the roots for P (0.37 and 0.34 g kg^{-1}, respectively), K (2.60 and 2.27 g kg^{-1}, respectively), Ca (1.10 and 0.89 g kg^{-1}, respectively) and Mg (0.62 and 0.54 g kg^{-1}, respectively). Micronutrients evaluation in the roots of *P. lanceolata* cultivated in vineyard soils showed different trends and showed higher concentrations in the Inceptisol than the Mollisol for Zn (381 and 81 mg kg^{-1}, respectively) and Mn (257 and 91 mg kg^{-1}, respectively).

Generally, macro and micronutrients uptake and concentrations in the shoots and roots can be affected by heavy metal contamination in different soils (Ke et al., 2007). There is a paucity of information in the evaluation of the different effects on nutrient uptakes by indigenous plants, furthermore, it is incipient and requires more studies. However, it is notorious that both indigenous plants (*B. pilosa* and *P. lanceolata*) have high relationship in the nutrient cycling in the environment with high nutrient concentrations in the biomass; and these results can help in further studies and provide more substantial scientific information.

3.3. Copper phytoremediation

Copper concentration in the roots and total biomass were high when both *B. pilosa* and *P. lanceolata* were cultivated in the vineyard soils contaminated with copper (Figure 3). *B. pilosa* cultivated in the Inceptisol showed the highest copper concentration in the shoots, roots and entire plant with 36, 844, 880 mg kg^{-1} of copper, respectively; followed by plants cultivated in the Mollisol soil with

Table 3. Macro and micronutrients in dry mass of the shoots of the *Bidens pilosa* and *Plantago lanceolata* plants, after 64 and 85 days of growth respectively in different copper contaminated soils: Native soil (Control, no contaminated); Inceptisol; Mollisol and copper mining waste (waste).

	Bidens pilosa				
	N	**P**	**K**	**Ca**	**Mg**
	---------- **g kg^{-1}** ----------				
Native Soil	3.30±0.14a*	0.10±0.01a	2.88±0.14a	1.57±0.02ab	0.38±0.05b
Inceptisol	1.25±0.08c	0.15±0.01a	2.27±0.05a	1.76±0.09a	0.79±0.04a
Mollisol	0.85±0.02c	0.22±0.02a	1.60±0.05b	1.38±0.03b	0.57±0.00b
Waste	2.48±0.01b	0.27±0.01a	1.45±0.09b	1.30±0.05b	0.47±0.01b
CV(%)**	19.76	34.76	15.24	13.06	25.16
Soils	**S**	**Zn**	**Fe**	**Mn**	**Na**
	---- **g kg^{-1}** ----	---------- **mg kg^{-1}** ----------			
Native Soil	0.17±0.01a	80.05±1.36b	323.50±25.07a	303.73±5.54a	258.50±10.0a
Inceptisol	0.26±0.04a	385.10±23.7a	290.60±11.07a	307.83±8.62a	220.57±11.8a
Mollisol	0.09±0.00a	136.78±2.05b	226.45±17.19a	85.47±2.28b	161.70±8.79a
Waste	0.09±0.00a	119.40±5.09b	174.70±10.59b	61.17±3.75b	133.40±9.75a
CV(%)	58.45	48.73	35.65	36.40	30.74
	Plantago lanceolata				
Soils	**N**	**P**	**K**	**Ca**	**Mg**
	---------- **g kg^{-1}** ----------				
Native Soil	ND***	0.29±0.02b	5.45±0.18a	7.74±0.16a	0.42±0.01b
Inceptisol	ND	0.41±0.02a	4.61±0.14a	8.79±0.06a	0.99±0.04a
Mollisol	ND	0.37±0.01a	5.47±0.10a	7.12±0.19a	0.90±0.02a
Waste	ND	ND	ND	ND	ND
CV(%)	-	22.43	13.47	11.13	13.55
Soils	**S**	**Zn**	**Fe**	**Mn**	**Na**
	---- **g kg^{-1}** ----	---------- **mg kg^{-1}** ----------			
Native Soil	1.97±0.07a	232.2±4.70a	1473.0±67.8a	405.4±50.9a	ND
Inceptisol	1.49±0.07a	185.5±5.44a	959.3±16.6b	197.3±3.62b	ND
Mollisol	1.14±0.03a	99.8±1.14b	620.5±3.23b	106.8±1.82b	ND
Waste	ND	ND	ND	ND	ND
CV(%)	25.70	20.55	20.33	73.27	-

*Values are means ± standard error of the mean. Different letters in the column represent significant differences ($P \leq 0.05$). **CV is the coefficient of variation of the means. ***ND means not determined by low biomass production, being not enough to evaluations.

15, 395 and 410 mg kg^{-1} of copper in the shoots, roots and entire plant, respectively (Figure 3A). *P. lanceolata* showed the same behavior with higher levels of copper phytoaccumulation than *B. pilosa* plants, even in the native soil (Figure 3B). *P. lanceolata* plants cultivated in the Inceptisol and Mollisol showed high copper concentrations in the shoots (142 and 68 mg kg^{-1}, respectively), roots (964 and 452 mg kg^{-1}, respectively) and entire plant (1106 and 520 mg kg^{-1}, respectively) (Figure 3B).

The maximum copper concentration in the biomass of the indigenous plants grown in surrounding mining wastes was 110 mg kg^{-1} by *Stachys coccinea*, in the same study, other indigenous plants showed copper concentrations in a range between 10 and 35 mg kg^{-1} (González and González-Chávez, 2006). Other study with medicinal plants (*B. tripartita*, *Leonurus cardiaca*, *Marrubium vulgare*, *Melissa officinalis* and *Origanum heracleoticum*) showed copper concentration in plant parts in the following order: higher in the roots, than leaves, than flowers, than stems (Zheljazkov et al., 2008). Furthermore, *B. tripartite* another specie of *Bidens* showed the lower copper concentrations in the roots, however, wild plants demonstrated copper concentrations ranging from 20 to 40 mg kg^{-1} of dry mass of the roots, in the shoots showed a ranging between 40 and 60 mg kg^{-1} of dry mass, and in the whole plants showed a ranging between 60 and 110 mg kg^{-1} of dry mass. However, *Plantago* sp. grown such as wild vegetation in a pyrite mine located in the village of Aznalcóllar, Sevilla (Southern Spain) showed 22 mg kg^{-1} of copper in the phytomass (Del Río et al., 2002). This information demonstrates the high potential of both *B. pilosa* and *P. lanceolata* plants in copper phytoaccumulation, and the potential use of indigenous plants for phytoremediation.

Average values of phytomass production of the *B. pilosa* plants are between 3,000 and 6,000 kg ha^{-1} (Fleck et al., 2003). *P. major* can produce levels of 2,000 kg ha^{-1} of phytomass (Nascimento et al., 2007), once *P. lanceolata* can produce higher levels of phytomass than *P. major*.

Table 4. Macro and micronutrients in dry mass of the roots of the *Bidens pilosa* and *Plantago lanceolata* plants, after 64 and 85 days of growth respectively in different copper contaminated soils: Native soil (Control, no contaminated); Inceptisol; Mollisol and copper mining waste (waste).

Soils	***Bidens pilosa***				
	N	**P**	**K**	**Ca**	**Mg**
	---- g kg^{-1} ----				
Native Soil	2.52±0.07a*	0.09±0.00a	2.78±0.05a	0.67±0.04a	0.15±0.01c
Inceptisol	1.22±0.01b	0.15±0.01a	1.99±0.11b	0.51±0.02ab	0.31±0.02b
Mollisol	0.81±0.01c	0.13±0.01a	1.54±0.03c	0.44±0.01b	0.45±0.01a
Waste	ND**	ND	ND	ND	ND
CV(%)***	12.00	25.74	14.86	19.87	18.28
Soils	**S**	**Zn**	**Fe**	**Mn**	**Na**
	---- g kg^{-1} ----	---- mg kg^{-1} ----			
Native Soil	0.25±0.02ab	147.0±8.36b	1472.7±52.8a	256.8±17.6a	1631.0±126.2b
Inceptisol	0.32±0.02a	381.9±35.6a	1104.3±88.5a	150.3±7.14b	2696.2±146.0a
Mollisol	0.20±0.01b	80.5±2.36b	1037.7±45.6a	44.8±1.43b	1423.7±15.71b
Waste	ND	ND	ND	ND	ND
CV(%)	25.02	43.27	27.72	61.70	24.24

Soils	***Plantago lanceolata***				
	N	**P**	**K**	**Ca**	**Mg**
	---- g kg^{-1} ----				
Native Soil	ND	0.34±0.01a	6.52±0.18a	2.29±0.14a	0.47±0.01a
Inceptisol	ND	0.37±0.01a	2.60±0.16b	1.10±0.03b	0.62±0.03a
Mollisol	ND	0.34±0.02a	2.27±0.10b	0.89±0.04b	0.54±0.01a
Waste	ND	ND	ND	ND	ND
CV(%)	-	20.82	18.67	24.44	17.45
	S	**Zn**	**Fe**	**Mn**	**Na**
	---- g kg^{-1} ----	---- mg kg^{-1} ----			
Native Soil	0.72±0.02a	408.3±20.5b	5896.0±166.9a	607.7±29.3a	ND
Inceptisol	0.51±0.01b	1321.0±91.7a	3410.0±171.3a	257.3±14.8a	ND
Mollisol	0.24±0.01c	464.7±6.88b	1678.6±67.22b	91.4±1.80b	ND
Waste	ND	ND	ND	ND	ND
CV(%)	22.27	31.78	36.38	61.34	-

*Values are means ± standard error of the mean. Different letters in the column represent significant differences (P ≤ 0.05). **CV is the coefficient of variation of the means. ***ND means not determined by low biomass production, being not enough to evaluations.

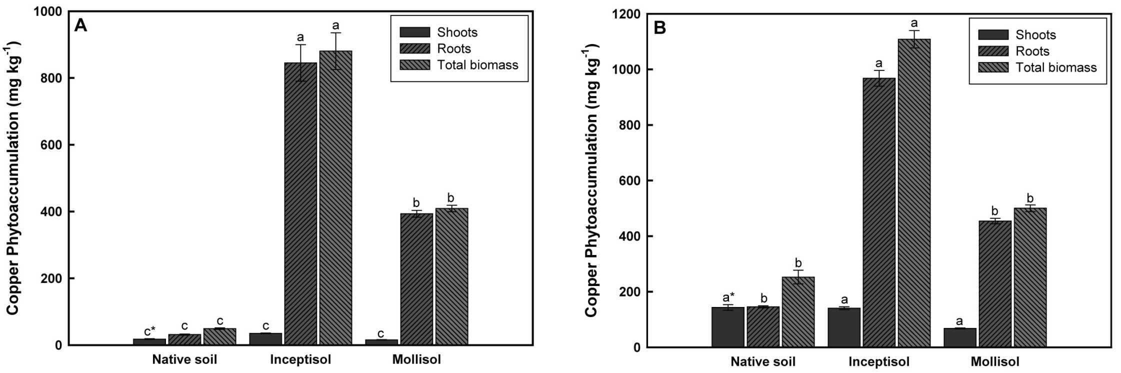

Figure 3. Copper concentrations in shoots, roots and entire plant in the biomass of *Bidens pilosa* (A) and *Plantago lanceolata* (B), after 64 and 85 days of growth respectively in different copper contaminated soils: Native soil (Control); Inceptisol and Mollisol. Error bars are calculations of standard error. *Different letters in the same color bars, represent significant differences (P≤0.05) with Tukey test.

Based on these references values, it was calculated the potential copper extraction by the indigenous plants assuming the phytomass production of 4,000 (*B. pilosa*) and 2,000 (*P. lanceolata*) g ha^{-1}. Both indigenous species showed high potential for copper phytoextraction in both vineyard soils contaminated with copper (Figure 4). High levels of copper can be phytoextracted by both *B. pilosa* and *P. lanceolata* in the Inceptisol with levels of more than 3,500 and 2,200 g ha^{-1} of copper phytoextracted respectively. Both plants cultivated in the Mollisol also showed high levels of copper phytoextraction, but significantly lower than the plants cultivated in the Inceptisol, with values of 1,600 and 1,000 g ha^{-1} for both *B. pilosa* and *P. lanceolata* respectively in the Mollisol. The estimated values of potential copper phytoextraction by indigenous plants show the importance of these plants mainly in copper phytostabilization of these areas. However, *B. tripartite* also showed potential for phytoremediation of Cd contaminated soils (Wei et al., 2010).

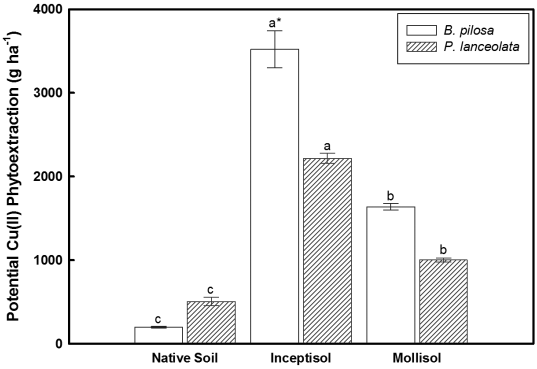

Figure 4. Potential of Cu(II) phytoremoval of *Bidens pilosa* and *Plantago lanceolata*, after 64 and 85 days of growth respectively in different copper contaminated soils: Native soil (Control); Inceptisol and Mollisol. Values are calculated in base on phytomass production of 4,000 kg ha^{-1} for *Bidens pilosa*, and 2,000 kg ha^{-1} for *Plantago lanceolata*. Error bars are calculations of standard error. *Different letters represent significant differences ($P \leq 0.05$) with Tukey test.

Both indigenous plants *B. pilosa* and *P. lanceolata* cultivated in copper contaminated soils showed low translocation factor (TF) with values of 0.04 and 0.15, respectively (Table 5). *B. pilosa* showed high bioaccumulation factor (BCF) grown in the Inceptisol and Mollisol with values of 4.08 and 2.77, respectively. Also, *P. lanceolata* plants showed high BCF for both vineyard soils with BCF values of 4.68 (Inceptisol) and 3.20 (Mollisol).

In one study with *P. major* and *B. alba*, it was demonstrated low TF values for copper with values of 0.43 and 0.8, respectively (Yoon et al., 2006). In other study, *B. pilosa* showed high TF with values higher than 2.4, when it was increased Cd concentration the TF values were decreased (Sun et al., 2009). Different plant stages also can interfere in the metal uptakes and transportation into the plants. It was demonstrated by Sun et al. (2009) which TF values for cadmium of *B. pilosa* at the flowering and mature stages were between 1.3-7.4 and 1.9-14.4, respectively.

Both *P. major* and *B. alba* plants cultivated in copper contaminated sites showed BCF values of 1.2 and 0.48, respectively (Yoon et al., 2006). *B. pilosa* plants showed high BCF to Cd with values between 1.2 and 5.6, depending of the physiologic stage of the plant and Cd concentration in the soil (Sun et al., 2009). BCF of wild plants growing on soil-slag mixtures surrounding slag heaps in Mexico showed different values in many species such as *Solanum elaeagnifolium* (4.6), *B. odorata* (1.6), *Asphodelus fistulosus*

Table 5. Translocation factor (TF) and bioaccumulation factor (BCF) for copper, copper extraction ratio (MER), plant effective number of shoots (PENs) and plant effective number of total plant (PENt) of *Bidens pilosa* and *Plantago lanceolata*, after 64 and 85 days of growth respectively in different copper contaminated soils: Native soil (Control); Inceptisol and Mollisol.

Soils	***Bidens pilosa***				
	TF	**BCF**	**MER**	**PENs**	**PENt**
			------- % -------	**--------------- plants[a] ---------------**	
Native Soil	0.57	8.27	14.82	446594	106642
Inceptisol	0.04	4.08	14.40	83001	2109
Mollisol	0.04	2.77	4.38	154344	3536
Waste	ND*	ND	ND	ND	ND
Soils	***Plantago lanceolata***				
	TF	**BCF**	**MER**	**PENs**	**PENt**
			------- % -------	**--------------- plants[b] ---------------**	
Native Soil	0.99	38.28	549.33	412957	190274
Inceptisol	0.15	4.68	65.74	102539	7844
Mollisol	0.15	3.20	21.70	43814	2985
Waste	ND	ND	ND	ND	ND

*ND means not determined by low biomass production, being not enough to evaluations. [a]Values are the average number of *Bidens pilosa* plants capable to remove 1 g of copper after 64 days of growth. [b]Values are the average number of *Plantago lanceolata* plants capable to remove 1 g of copper after 85 days of growth.

(0.2), *Schinus molle* (0.9), *Reseda luteola* (0.4) (González and González-Chávez, 2006). Other indigenous species such as *Acia raddiena* and *Avera javanica*, the BCFs were almost 0.2 and 0.23 respectively in plants grown in mine tailings (Rashed, 2010). However, it is notorious that the copper concentrations in these soils are lower than concentrations obtained in the vineyard soils studied, which increases the BCF values. However, the results of *B. pilosa* and *P. lanceolata* showed higher BCF to both *B. pilosa* and *P. lanceolata* in the vineyard soils compared with the most BCF values reported in the literature.

B. pilosa plants cultivated in the Inceptisol showed high metal extraction ratio (MER) with value of 14.40%, and it is compared to the native soil with MER of 14.82%. *B. pilosa* cultivated in the Mollisol also showed high MER index of 4.38%. Surprisingly the expectations, *P. lanceolata* cultivated in both vineyard soils showed the highest values of MER of 65.74% (Inceptisol) and 21.70% (Mollisol). MER index is related with the percentage of the copper that can be accumulated in the shoots to that in the soil (Mertens et al., 2005). It shows a high potential to extract the metals from soil, and it indicates these both indigenous species (*B. pilosa* and *P. lanceolata*) as copper hyperaccumulator plants.

The plant effective number of the shoots (PENs) necessary to extract 1 g of copper were high to *B. pilosa* cultivated in the Inceptisol (83,001 plants) and to *P. lanceolata* cultivated in the Mollisol (43,814 plants) (Table 4). Plant effective number of the total plant (PENt) showed the same behavior; however, the number of the plants was highly reduced to both *B. pilosa* and *P. lanceolata*. *B. pilosa* showed the PENt to Inceptisol and Mollisol soils of the 2,109 and 3,536 plants, and the *P. lanceolata* grown in the Inceptisol and Mollisol showed PENt of 7,844 and 2,985 plants, respectively. These PEN numbers were much higher than other plant such as *Piptatherum miliaceum* (Smilo grass) in edaphic Pb and Zn contaminated sites in short periods (Garcia et al., 2004). However, the *B. pilosa* and *P. lanceolata* species in this study were naturally found in the vineyard soils in high plant densities.

Indigenous plants with high hyperaccumulation capacity are especially common in the tropics and subtropics, apparently because the metal accumulation is a defense against plant-eating insects and microbial pathogens (Moffat, 1995). However, the results found in this study showed high capacity in growth, macro and micronutrient uptake, and copper phytoaccumulation in the biomass. Compiling all these characteristics of the both indigenous plants *B. pilosa* and *P. lanceolata*, it culminates in a high potential of these plants in copper phytoremediation and phytostabilization from copper contaminated soils.

4. Conclusions

The results presented in this study demonstrate that both indigenous plants *B. pilosa* and *P. lanceolata* are efficient tools for bioremediation of copper-contaminated sites such as vineyard soils. These plants demonstrate high growth potential with high tolerance to copper contaminated areas, acting as cover crops against the direct impact of the rainfall in the soil surface, reducing soil and water losses by surface runoff with consequently contamination of the adjacent environments. Furthermore, high macro and micronutrients were accumulated in the biomass, showing an important role in the nutrient cycling. However, high copper concentrations were extracted from soils and phytoaccumulated. Even these plants are considered weeds to agriculture, they are easily controlled and managed to do not affect negatively the vineyard production. Furthermore, both indigenous plants *B. pilosa* and *P. lanceolata* showed high potential to growth, phytostabilize and phytoremediate copper from both vineyard soils, being important candidates to phytoremediation of copper contaminated vineyards and to allow further alternative crops.

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