International Journal of Phytoremediation

ISSN: 1522-6514 (Print) 1549-7879 (Online) Journal homepage: http://www.tandfonline.com/loi/bijp20

Arbuscular mycorrhizal fungi in the growth and extraction of trace elements by Chrysopogon zizanioides (vetiver) in a substrate containing coal mine wastes

Edenilson Meyer, Diana Marcela Morales Londoño, Rafael Dutra de Armas, Admir José Giachini, Márcio José Rossi, Shantau Camargo Gomes Stoffel & Cláudio Roberto Fonsêca Sousa Soares

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION
2017, VOL. 19, NO. 2, 113–120
http://dx.doi.org/10.1080/15226514.2016.1207596

Taylor & Francis
Taylor & Francis Group

Arbuscular mycorrhizal fungi in the growth and extraction of trace elements by *Chrysopogon zizanioides* (vetiver) in a substrate containing coal mine wastes

Edenilson Meyer, Diana Marcela Morales Londoño, Rafael Dutra de Armas, Admir José Giachini, Márcio José Rossi, Shantau Camargo Gomes Stoffel, and Cláudio Roberto Fonsêca Sousa Soares

Biological Sciences Center, Microbiology, Immunology and Parasitology Department, Federal University of Santa Catarina (UFSC), Florianópolis, Santa Catarina, Brazil

ABSTRACT

Vetiver *(Chrysopogon zizanioides)* is a fast-growing, high biomass producing plant employed for environmental rehabilitation. The study evaluated the effects of arbuscular mycorrhizal fungi (AMF) on the growth and trace element phytoextracting capabilities of vetiver in a substrate containing coalmine wastes in Southern Brazil. AMF included *Acaulospora colombiana, Acaulospora morrowiae, Acaulospora scrobiculata, Dentiscutata heterogama, Gigaspora margarita*, and *Rhizophagus clarus*. Among those, *A. colombiana, G. margarita,* and *R. clarus* promoted higher growth. AMF stimulated average increments in the accumulated P of 82% (roots), 194% (shoots first harvest—90 days) and 300% (shoots second harvest—165 days) and affected the phytoextraction of trace elements by vetiver, with larger concentrations in the roots. Plants inoculated with *A. colombiana, A. morrowiae,* and *A. scrobiculata*, in addition to the control, presented the highest levels of Cu and Zn in the roots. Overall, *G. margarita* stimulated the highest production of biomass, and, therefore, showed the most significant levels of trace elements in the plants. This work shows the benefits of certain AMF (especially *A. morrowiae*, *G. margarita*, and *R. clarus*) for the production of biomass and P uptake by vetiver, demonstrating the potential of those species for the rehabilitation of coal-mine-degraded soils.

KEYWORDS
Heavy metals; phytoremediation; revegetation; soil contamination

Introduction

Mineral coal extraction has a great impact on the Brazilian economy. A vast amount of this commodity is destined to energy production and for the production of glass and ceramics. From the reserves found in Brazil, 89.25% are in the state of Rio Grande do Sul, 10.41% in Santa Catarina, 0.32% in Paraná, and 0.02 in the state of São Paulo (ANEEL 2008). Coal extraction in Brazil occurs either in mines or in open pits by soil layer inversions. These procedures often cause environmental impacts due to ashes and improper waste disposal, as well as by the exposition of pyrite (FeS_2) (Sanchez and Formoso 1990). In addition, open pits generally cause soil to lose its structure. Oxidation of wastes left unattended and with no proper soil casting causes serious damages to the ecosystems by the formation of acid mine drainage that affect underground as well as aboveground waters. Furthermore, the highly acidic environment from the oxidation of pyrite may cause the dissolution of aluminosilicate minerals, increasing the bioavailability and, consequently, the toxicity of trace elements in the soil (Barnhisel *et al.* 1982; Sharpley *et al.* 2003).

A few procedures are available to reduce the problems caused by the coal extraction activities—one of them is phytoremediation. One good example of plant species appropriate for the rehabilitation of coal extraction areas is vetiver (*Chrysopogon zizanioides*). This species is suitable for phytoextraction (Roongtanakiat and Sanoh 2011), phytostabilization (Meeinkuirt *et al.* 2013), or rhizofiltration. It tolerates adverse climatic and edaphic environments, aside from stabilizing the chemical and physical conditions of the surrounding soil due to the large, fasciculated, and deep root system. Furthermore, it may act as a natural biological barrier to trace elements dissolved in the soil (Truong 2000).

Another distinguishing characteristic of the species is its ability to associate with arbuscular mycorrhizal fungi (AMF), increasing the plant's absorption of nutrients and water (Newsham et al. 1995). AMF maybe responsible for up to 80% of all P, 60% of all Cu, 25% of all N and Zn, and 10% of all K absorbed by plants (Marschner and Dell 1994). These microorganisms also contribute considerably to soil restoration (Bedini *et al.* 2009) due to hyphal extensions (Jastrow *et al.* 1998) and by the exudation of glomalins (Leake *et al.* 2004), which not only contribute to soil aggregation but also increase the soil total organic carbon (Lovelock *et al.* 2004).

AMF have demonstrated importance in the process of revegetation of degraded soils, by stimulating, among other things, growth of plants in environments polluted with trace elements (Soares and Carneiro 2010; Nogueira and Soares 2010). Environmental rehabilitation by the fungi may happen by direct and indirect mechanisms, including AMF retention capacity (direct) and the contribution to the production of plant biomass (indirect). Therefore, AMF increase the

CONTACT Cláudio Roberto Fonsêca Sousa Soares crfsoares@gmail.com Biological Sciences Center, Microbiology, Immunology and Parasitology Department, Federal University of Santa Catarina (UFSC), Florianópolis, Santa Catarina, 88040-900, Brazil.

phytoextraction capabilities of the associated plant species (Silva *et al.* 2006; Rangel *et al.* 2014).

In that respect, the aim of this study was to evaluate the effects of AMF in the growth and extraction of trace elements by *Chrysopogon zizanioides* (vetiver) in a substrate contaminated with wastes from the mineral coal mining region of Criciúma, Southern Brazil.

Materials and methods

Soil sampling

Soil containing coalmine wastes, now on denominated substrate, was collected in the upper layer (0–20 cm) near Criciúma (28°44′18.40″S 49°24′42.62″W), in the state of Santa Catarina, Southern Brazil, whose chemical characteristics are shown in table 1 (Tedesco 1995). Cadmium (Cd), Pb, and Zn levels were above the tolerance limits preconized by governmental environment agencies (CETESB 2014), which are 1.3, 72, and 86 mg kg^{-1}, respectively. The above-described conditions characterize the substrate as highly acidic, with high levels of trace elements and low fertility. The substrate was placed in 300-cm^3 pots and sterilized at 121°C for 2 hours for the removal of autochthonous AMF propagules.

Seedling preparation and AMF inoculation

Seedlings of vetiver purchased from a commercial company (Deflor Bioengenharia) were propagated in a greenhouse ($\bar{x}$ temperature 23.4°C) in a sterile substrate composed of sand and vermiculite (1:1) v/v). Before transplanting, seedlings were standardized to 10-cm height and the roots washed in tap water to remove the remaining substrate and elements that could potentially interfere with the experiment.

The experiment followed a completely randomized design with four replications and seven treatments: six treatments inoculated with the AMF *Acaulospora colombiana, Acaulospora morrowiae, Acaulospora scrobiculata, Dentiscutata heterogama, Gigaspora margarita*, and *Rhizophagus clarus* isolated from the area described above, and a non-inoculated control (NI). AMF selection was based on the benefit for the growth of plants in contaminated soils, as described by Silva *et al.* (2006). Inoculation was done using a suspension of 50 spores per pot. Spores came from pure cultures of *Brachiaria decumbens* grown in the greenhouse. Spores extraction followed the wet sieving and decanting method described by Gerdemann and Nicolson (1963).

Table 1. Chemical characterization of substrate containing coal mine wastes.

Chemical attributes	
pH_{H2O}	3.8
Organic matter, g kg^{-1}	86
Al^{3+}, $cmol_c$ dm^{-3}	6.9
Ca^{2+}, $cmol_c$ dm^{-3}	1.9
Mg^{2+}, $cmol_c$ dm^{-3}	3.1
$P_{Mehlich-1}$, mg dm^{-3}	0.84
Trace elements*, **mg kg^{-1}**	
Cadmium	17.3
Lead	125
Zinc	422
Arsenic	8.60
Chromium	11.3
Copper	17.7

*3051B method

Experimental evaluation

Plants were periodically watered and fortnightly supplied with a low P (15 mg dm^{-3}) Hoagland and Arnon nutritional solution (Hoagland and Arnon 1950). The first evaluation was performed when the plants were three months old. Plant́s shoots were cut 5 cm from the soil, and height and dry biomass were determined. The experiment was carried out for 165 days and aside from the aboveground biomass, the second harvest also focused on root biomass, percent of mycorrhizal colonization, number of AMF spores, levels and accumulation of P, as well as the determination of the levels of trace elements (Cr, Pb, Cu, Zn, As, and Cd) in the plant's shoots and roots.

For the evaluation of the mycorrhizal colonization, 1 g of roots was treated according to Phillips and Hayman (1970) protocol, and the percentage was determined by the grid intersection method proposed by Giovannetti and Mosse (1980). Spores were extracted from 50 cm^3 of soil following the previously described methodology. They were collected on a filter paper in a grid pattern, washed with distilled water for an even spreading over the entire grid, and counted under a stereoscopic microscope.

Roots and shoots were dried at 60°C for the determination of dry biomass. Dried biomass was ground and digested (nitro-perchloric digestion) following Zasoski and Burau (1977) for the determination of P according to Murphy and Riley (1962). The determination of trace elements was by inductively coupled plasma mass spectrometry (ICP-AMS), on a Perkin-Elmer Optima 7000DV model. Accumulated P and trace element phytoextraction were determined by multiplying the levels of each element by the respective dry mass of shoots and roots.

Statistical analyses

Data were tested using a two-way analysis of variance (ANOVA) performed using Sisvar (Ferreira 1998), followed by a Scott–Knott test to separate means ($p < 0.05$). Graphs were obtained on SigmaPlot 12 software (Systat Corp.).

Results

Percentage of colonization and spore counting

The highest mycorrhizal colonization percentage was observed for *R. clarus* (28.6%), significantly differing from other treatments, followed by *A. colombiana* (7.2%) and *G. margarita* (6.4%). Average colonization for *A. scrobiculata, A. morrowiae*, and *D. heterogama* was 0.8% for the three fungi (Figure 1A).

As for spore counting, plants inoculated with *R. clarus* had, in average, 384 spores per 50 cm^{-3}. Average counting for the remaining treatments was approximately 8.4 spores per 50 cm^{-3} (Figure 1B).

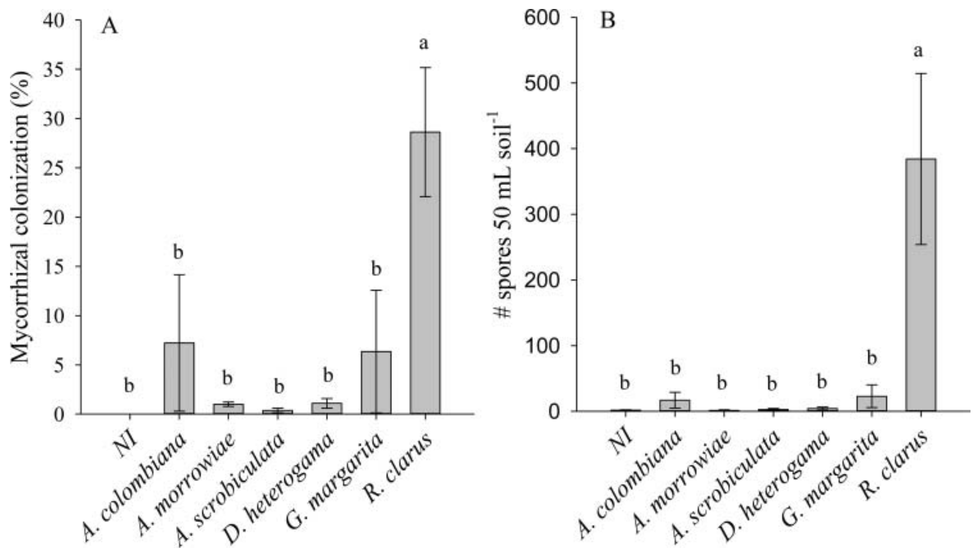

Figure 1. Mycorrhizal determination in plants of vetiver (*Chrysopogon zizanioides*) grown in a greenhouse for 165 days in a substrate containing coalmine wastes. (a) Percentage of colonization. (b) Number of arbuscular mycorrhizal spores. NI = Non-inoculated. Different letters are significantly different (Scott–Knott test, $p < 0.05$). Vertical bars represent ± mean standard error (n = 4 replicates).

Plant height and dry mass of shoots and roots

Fungal inoculation did not affect the height of the plants, showing average values between 41 and 45 cm for the first and second harvest, respectively (data not shown). Regardless of AMF inoculation, total shoot dry mass was 90% higher in the second compared to the first harvest. For both harvests, inoculations with *G. margarita* and *A. colombiana* promoted average increases in plant growth of 116% and 62%, respectively (Figures 2A and 2B). On the other hand, AMF inoculation had no effect over the production of roots (Figure 2C).

Level and accumulation of P in the shoots and roots of vetiver

For all treatments, the levels of P in the plant́s shoots were in average 65% higher in the first compared to the second harvest. For both harvests, the levels of P in the shoots were always higher for the inoculated treatments, with the exception of *A. scrobiculata* (Figure 3A and 3B). For the first harvest, inoculation with *R. clarus* showed increments of 229% in the contents of P in the shoots, and 88% in the contents of P in the roots (Figure 3C). AMF inoculation increased the average levels of accumulated P by 82% (roots second harvest), 194% (shoots first harvest), and 300% (shoots second harvest) (Figure 3D, 3E, and 3F).

Concentration of trace elements in the shoots and roots of vetiver

The inoculation with AMF significantly affected the levels of Cr, Cu, Pb, and Zn in the shoots and roots of vetiver (Table 2). Overall, the roots presented higher levels of trace elements than the shoots for both harvests. Among the elements analyzed, Cr and Zn had the highest levels in the shoots followed by Cu and Pb. The effects of AMF inoculation in the levels of trace elements varied according to each harvest for both the shoots

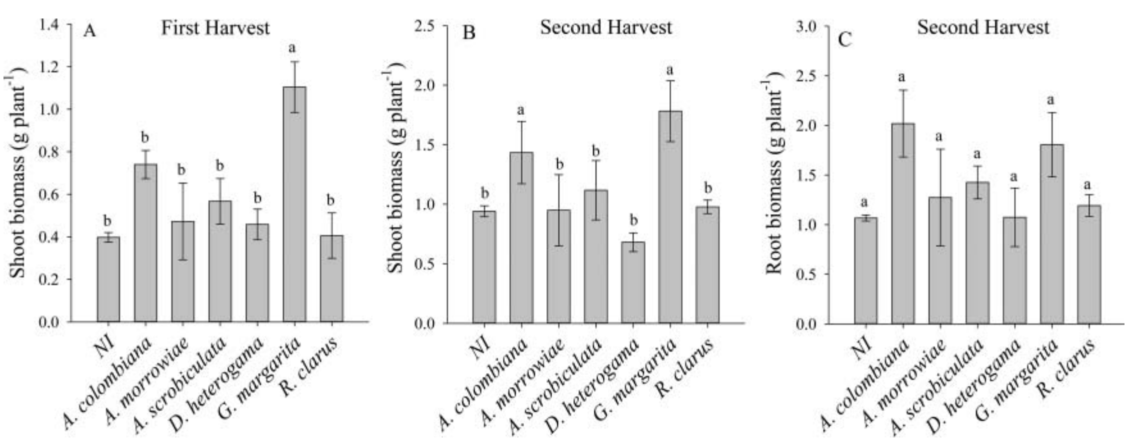

Figure 2. Evaluation of the growth of vetiver (*Chrysopogon zizanioides*) inoculated with AMF in a substrate containing coalmine wastes after 90 (first harvest) and 165 days (second harvest) of cultivation. Dry biomass of shoots after 90 days (a) and 165 days (b). Dry biomass of roots after 165 days (C). NI = Non-inoculated. Different letters are significantly different (Scott–Knott test, $p < 0.05$). Vertical bars represent ± mean standard error (n = 4 replicates).

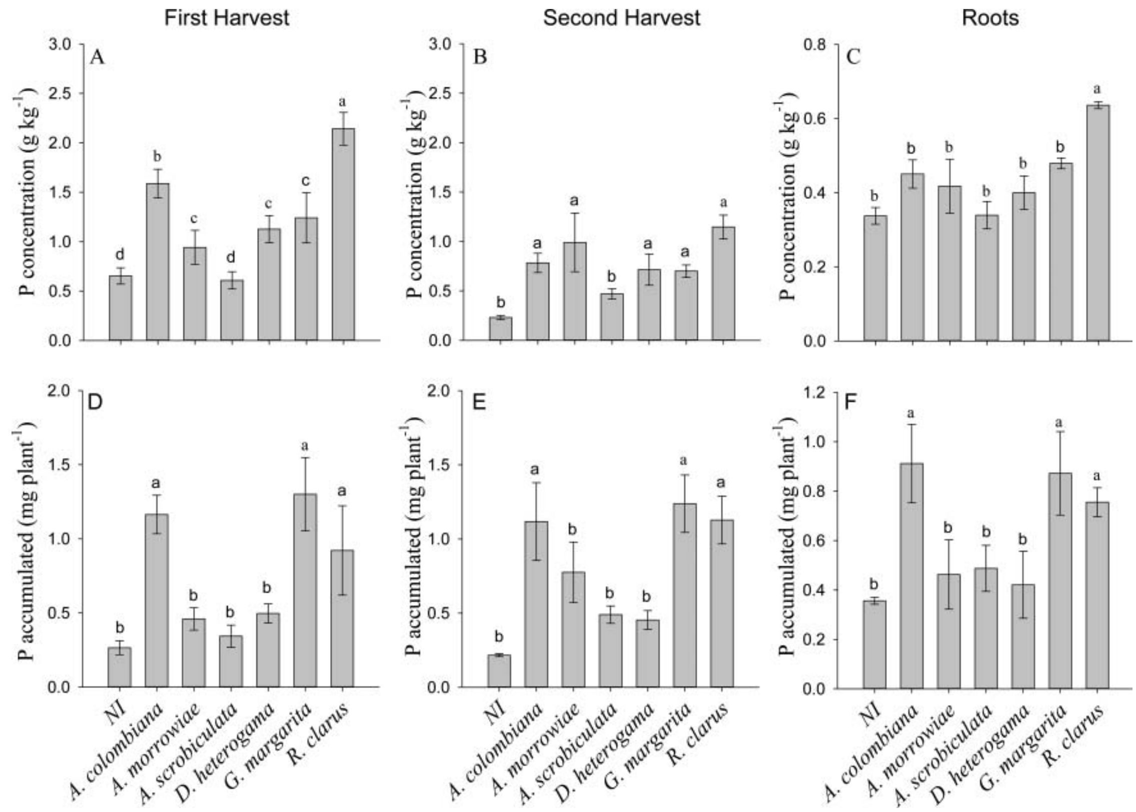

Figure 3. Concentration and accumulation of P in the shoots and roots of vetiver (*Chrysopogon zizanioides*) evaluated after 90 and 165 days of cultivation in a substrate containing coalmine wastes. Shoot P concentration and accumulation after 90 days (A and D, respectively) and 165 days (B and E, respectively). Root P concentration (C) and accumulation (F) after 165 days. NI = Non-inoculated. Different letters are significantly different (Scott–Knott test, $p < 0.05$). Vertical bars represent ± mean standard error (n = 4 replicates).

and the roots. Some fungi increased, while others decreased, the levels of trace elements in the plants. *A. morrowiae* and *A. scrobiculata* increased the levels of Cr for the first harvest. For the second harvest, plants inoculated with *A. morrowiae, R. clarus*, and *D. heterogama* presented the lowest accumulated levels of Cr. *R. clarus* presented the highest accumulated levels of Pb for the first harvest. The same fungi, in addition to *D. heterogama*, had the highest accumulation of Pb for the second harvest. Plants inoculated with *A. colombiana, A. morrowiae*, and *A. scrobiculata*, in addition to the control, presented the highest levels of Cu and Zn in the roots. On the other hand, plants inoculated with *G. margarita* had 35% less Zn in the roots when compared to the control (Table 2).

Accumulation of trace elements in the shoots of vetiver

The accumulation of trace elements in the shoots varied according to the AMF inoculated, showing different phytoextracting profiles for each harvest (Figure 4). In general, the second harvest showed higher accumulation levels of trace elements, with the exception of Pb. Among the fungi, *G. margarita* promoted significant increments in the accumulation of Cr, Pb, Cu, and Zn in the first harvest (Figure 4A, 4C, 4E, and 4G). This is due mainly to the contribution this isolate has on biomass productivity. For the second harvest, *G. margarita* promoted increments in the order of 26%, 28%, 1839%, and 250% in the accumulated quantities of Cr, Cu, Pb, and Zn,

Table 2. Levels of trace elements in the shoots and roots of vetiver (*Chrysopogon zizanioides*) inoculated with AMF cultivated in a substrate containing coal mine wastes.

	Trace element levels in shoots								Trace element levels in roots			
	First harvest				Second harvest							
	mg kg^{-1}											
Treatments	Cr	Pb	Cu	Zn	Cr	Pb	Cu	Zn	Cr	Pb	Cu	Zn
NI[/1]	308 *b*	39 *b*	129 *a*	204 *b*	509 *a*	1.3 *d*	151 *a*	292 *a*	384 *b*	65 *a*	284 *a*	589 *a*
A. scrobiculata	458 *a*	49 *b*	113 *a*	143 *b*	452 *a*	n.d[/2]	121 *b*	460 *a*	689 *a*	103 *a*	234 *a*	640 *a*
A. morrowiae	438 *a*	32 *b*	182 *a*	181 *b*	230 *c*	32 *b*	112 *b*	427 *a*	605 *a*	82 *a*	208 *b*	752 *a*
G. margarita	338 *b*	61 *b*	104 *a*	348 *a*	401 *b*	14 *c*	102 *b*	514 *a*	434 *b*	72 *a*	150 *b*	382 *b*
R. clarus	253 *c*	100 *a*	122 *a*	110 *b*	234 *c*	49 *a*	156 *a*	454 *a*	492 *b*	147 *a*	243 *a*	621 *a*
D. heterogama	219 *c*	25 *b*	123 *a*	162 *b*	314 *c*	56 *a*	114 *b*	327 *a*	612 *a*	73 *a*	186 *b*	504 *b*
A. colombiana	297 *b*	48 *b*	113 *a*	198 *b*	368 *b*	35 *b*	133 *a*	408 *a*	346 *b*	79 *a*	192 *b*	576 *a*

[/1]Non-inoculated.
[/2]Not detected.
*Different letters in columns are significantly different (Scott–Knott test, $p < 0.05$).

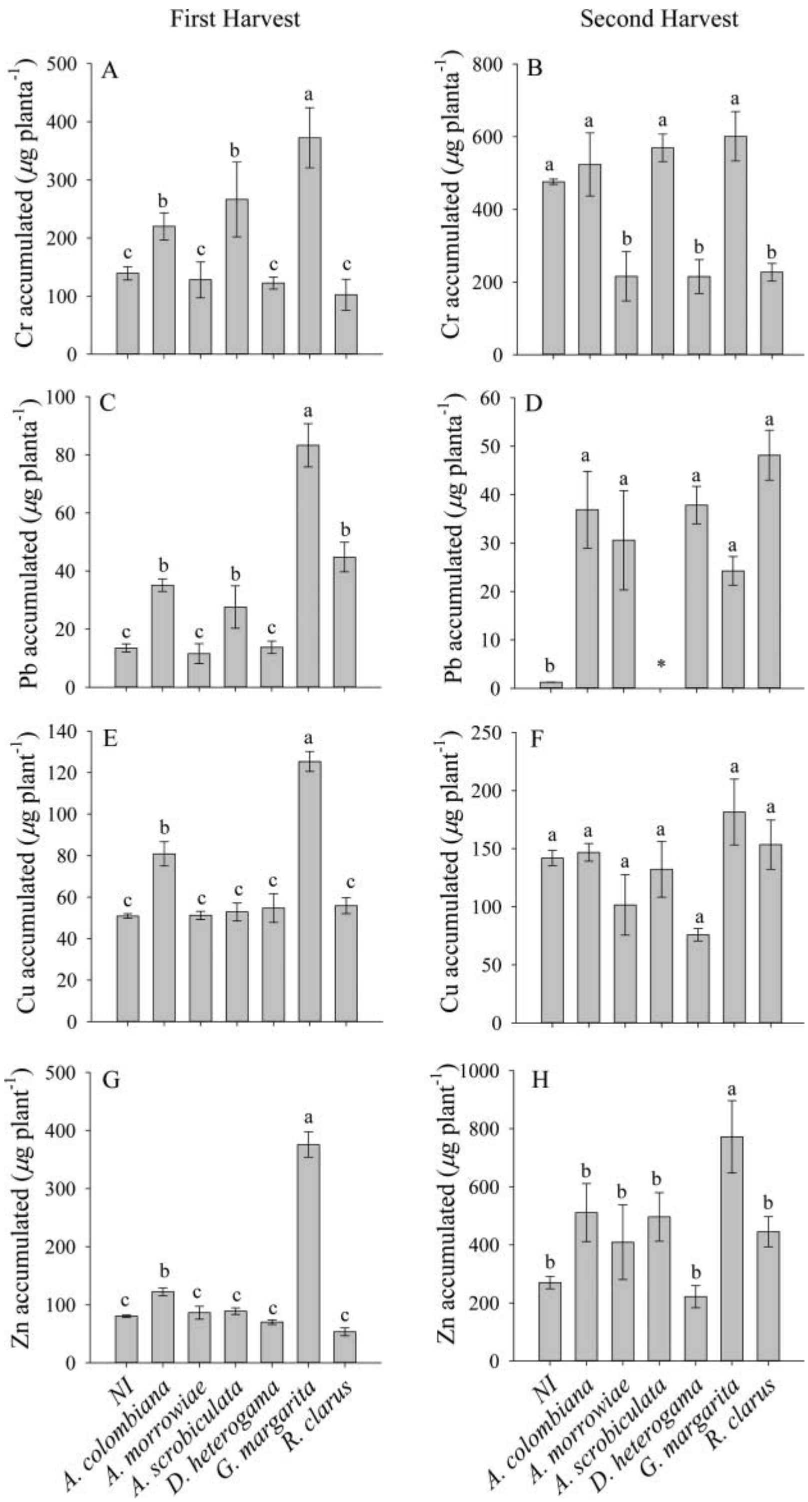

Figure 4. Accumulation of trace elements in the shoots of vetiver (*Chrysopogon zizanioides*) inoculated with AMF evaluated after 90 (first harvest) and 165 days (second harvest) of cultivation in a substrate containing coalmine wastes. NI = Non-inoculated. Different letters are significantly different (Scott–Knott test, $p < 0.05$). Vertical bars represent ± mean standard error (n = 4 replicates).* Not detected.

respectively (Figures 4B, 4D, 4F, and 4H). The accumulation capacity of *G. margarita* is not a function of the percentages of colonization, since these were low for both harvests.

Discussion

Several studies show that living organisms react differently to trace elements in soil (Shaw 1989). Tolerance to these compounds varies between species of microorganisms, such as AMF, and is different from those detected for plants. Plant responses to toxicity of trace elements include structural, physiological, and biochemical modifications and depend on the contaminant's chemical structure, concentration, and exposition length (Ferreira *et al.* 2014). Ye *et al.* (1997) verified that ecotypes of the plant species *Typha latifolia* from soils contaminated with trace elements showed higher accumulation levels of

Cd, Zn, and Pb in the roots than populations from non-contaminated environments. In a study evaluating the different tolerance behaviors of AMF to trace elements, Pawlowska and Charvat (2004) observed that *Rhizophagus intraradices* (*in vitro*) had higher density of spores and longer pre-symbiotic mycelia than *Claroideoglomus etunicatum* when supplemented with increasing concentrations of As, Cd, Cu, and Pb. The sensitivity of arbuscular mycorrhizal endophytes to high amounts of trace elements, expressed as a reduction or delay in their colonizing ability, has been observed. Seedlings of vetiver had reduced mycorrhizal colonization in Zn- and Pb-contaminated soils, reaching values lower than 20% when exposed to high concentrations of Zn (Karagiannidis and Nikolaou 2000; Wong *et al.* 2007). These studies demonstrate the complexity in the plant–microorganism interaction in environments contaminated with trace elements. The present study revealed distinct capacities for symbiotic establishment and tolerance of trace elements among species of AMF for vetiver grown on a substrate containing coal mine wastes rich in trace elements. For instance, *R. clarus* was less sensitive to the coal waste substrate than all other AMF species. This result may indicate higher tolerance levels of that genus to contaminated environments. Studies have shown the adaptive capacity of *R. clarus* to soils with high toxicity levels of Pb and Zn (Orlowska *et al.* 2012), suggesting the potential of this species for soils contaminated with trace elements.

Even though the highest colonization levels and tolerance to trace elements were detected for *R. clarus*, other fungi such as *G. margarita* and *A. colombiana* showed greater shoot biomass. Plants inoculated with those fungi had larger growth when compared to other inoculated AMF species. Apparently, the positive effects of such isolates are not related to higher colonization percentages or production of spores, indicating that independent mechanisms may be involved in the tolerance of plants to trace elements.

Among the phytoprotecting mechanisms associated with AMF are the dilution effect in the tissues of the symbiotic plants due to biomass increase, and the release of organic acids into the rhizosphere. These organic acids form complexes with the trace elements in the extracellular compartments, excluding or restricting their absorption by the plants (Christie *et al.* 2004). Furthermore, other characteristics important for phytoprotection have already been described—among them are the length of the extracellular mycelium and the presence of auxiliary cells, structures that store and isolate trace elements from the metabolism of the cell (Göhre *et al.* 2006).

The larger accumulation of P in the shoots of vetiver when inoculated with AMF (50% in average) creates the possibility to increase the pools of this element aboveground. The P in the biomass may become available to other species; as a result, it helps speed up the whole process of soil rehabilitation. Three AMF species, *A. colombiana, G. margarita*, and *R. clarus*, contributed the most in accumulation of P in the plants. For the former two this is the effect of a larger biomass production, while for the latter it is due to increments in the levels of that element in the plant's tissues, as discussed previously. On the other hand, *A. morrowiae, A. scrobiculata*, and *D. heterogama* were inefficient in absorbing P. The efficiency in P uptake is related to the volume of soil exploited and the AMF absorbing capacity (Newsham *et al.* 1995). Furthermore, it also depends on the production of phosphatases that help release P from organic complexes (Berbara *et al.* 2006). It is important to mention that the isolates promoting the largest accumulations of P were also those that promoted the most notorious increments in plant's biomass. Therefore, a proper supply of P is fundamental for plants to survive in soils contaminated with trace elements, even if the plant is tolerant to those metals (Soares and Siqueira 2008).

The results of this study show the great capacity that vetiver has in absorbing and tolerating trace elements found in the soil. Among the elements analyzed in plant tissues, Cr and Zn had the highest levels in the shoots, with values above 5–30 mg kg^{-1} for Cr and 100–400 mg kg^{-1} for Zn. These values are considered excessive or toxic to the majority of plant species (Kabata-Pendias 2011). Overall, the roots presented higher levels of trace elements than the shoots for both harvest. Elements such as Cu and Pb showed reduced levels in the shoots of the plants, and this is due to the high affinity these elements have for the roots, avoiding translocation to the shoots (Marschner 2012). This result is similar to that found by Roongtanakiat and Sanoh (2011), working with two vetiver ecotypes in soils contaminated with Cd, Pb, and Zn. The results from this study corroborate those of Wang *et al.* (2007), working in soils contaminated with trace elements, showing that higher AMF colonization rates do not necessarily result in higher rates of trace element absorption by the plants. The concentration of trace elements in the plant's tissue is related to an adsorptive or connecting capacity of the AMF hyphae, arbuscules, and vesicles (Mozafar *et al.* 2002). These can retain and immobilize the compounds in the fungal wall (chitin), in structures containing hydroxyl and carboxyl radicals and free amino acids. Additionally, polyphosphate granules in the fungal structures have a chelating effect on metallicions (Khan *et al.* 2000). Moreover, glomalins, which are glycoproteins produced by AMF, have high retention capacity for trace elements in the soil (González-Chavez *et al.* 2004). It has been also described that the mycorrhizal symbiosis may regulate the expression of genes responsible for the production of proteins, such as metallothioneins and antioxidizing enzymes, that increases the plant's tolerance to trace element stress, by the reduction of free metallic ions inside the plant (Miransari 2010; Bhalerao 2013). This behavior is dependent on the type of the element present in the soil. While some elements (Cd, Ni, V, and Zn) have increasing coefficients of transference from the soil to the plant, others (As, Co, Cr, Cu, Mn, and U) show reduction in that same coefficient when the plants are colonized by AMF (Paun *et al.* 2012). Indeed, studies by Jamal *et al.* (2002) in soils contaminated with Zn and Ni showed increases in the absorption of those elements by soybean and lentil inoculated with AMF. Results like these call attention to the need for further studies that unveil the real processes and mechanisms involved in plant stress alleviation when exposed to trace element contamination.

The large volumes of biomass, fast growth, and intrinsic capacity to tolerate trace elements make vetiver a potential candidate for soil rehabilitation programs. This study showed that these characteristics remain even after successive harvests of the aboveground biomass of the plants. Therefore, the species is a promising candidate for the revegetation of degraded areas. It not only tolerates trace elements, but also can be incorporated into the soil to increase the soil's organic matter. Consequently, it potentially alleviates the soil physical–chemical conditions, allowing the establishment of other species involved in the rehabilitation of compromised environments (Moreira and Costa 2004; Bhogal *et al.* 2009; Fujino *et al.* 2008). The inoculation with proper AMF can amend this task, permitting better establishment of the vetiver by improving the plant's P nutritional levels, aiming at extracting/inactivating soil trace elements contributing to soil rehabilitation.

Conclusion

The inoculation of vetiver with efficient AMF represents a promising technology to recover soils degraded by the mineral coal extraction activities. This study shows the benefits that certain AMF species (*G. margarita, R. clarus*, and *A. morrowiae*) have on the growth of vetiver cultivated in substrates containing coal mine wastes. The benefits include larger growth and high biomass production, aside from enhanced P uptake. These results extended to field trials will attest the effects of the association in real conditions, endorsing the utilization of the symbiosis for soil rehabilitation.

Funding

The authors thank the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq) for the grant financing a project (561819/2010-6) and granting a productivity subsidy to the senior author (306986/2012-4).

References

Aneel. 2008. Atlas de Energia Elétrica do Brasil. Agência Nacional de Energia Elétrica. Brasília. 236p.

Bhalerao SA. 2013. Arbuscular mycorrhizal fungi: a potential biotechnological tool for phytoremediation of heavy metal contaminated soils. Int J Sci Nat. 4:1–15.

Barnhisel RI, Powell JL, Akin GW, Ebelhar MW. 1982. Characteristics and reclamation of acid sulfate mine spoil. In: Kittrick JA, Fanning DS, Hossner LR. (eds), Acid sulfate weathering. Soil Science Society America, Madison (WI)., p. 37–56.

Bedini S, Pellegrino E, Avio L, Pellegrini S, Bazzoffi P, Argese E, Giovannetti M. 2009. Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. Soil Biol Biochem. 41:1491–1496.

Berbara RLL, Souza FA, Fonseca HMAC. 2006. III – Fungos micorrízicos arbusculares: muito além da nutrição. In: Fernandes MS. (eds), Nutrição mineral de plantas. Sociedade Brasileira de Ciência do Solo, Viçosa (MG)., p. 74–85.

Bhogal A, Nicholson FA, Chambers BJ. 2009. Organic carbon additions: effects on soil bio-physical and physico-chemical properties. Eur J Soil Sci. 60:276–286.

CETESB - Companhia Ambiental do Estado de São Paulo. 2014. Decisão de Diretoria n° 045/2014/E/C/I, de 20 de fevereiro de 2014. Valores Orientadores para Solos e Águas Subterrâneas no Estado de São Paulo – 2014 WEB. http://www.cetesb.sp.gov.br/userfiles/file/solo/valores-orientadores-2014.pdf. Accessed 08 May 2014.

Christie P, Li X, Chen B. 2004. Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc. Plant Soil. 261:209–217.

Ferreira DF. 1998. Sistemas de análise estatística para dados balanceados. DEX: Sisvar, UFLA, Lavras. 141p.

Ferreira PAA, Brunetto G, Giachini, AJ, Soares CRFS. 2014. Heavy metal uptake and the effect on plant growth. In: Gupta DK, Chatterjee S. (eds), Heavy metal remediation: transport and accumulation in plants. New York: Nova Science Publishers, Inc.p. 127–154.

Fujino C, Wada S, Konoike T, Toyota K, Suga Y, Ikeda JI. 2008. Effect of different organic amendments on the resistance and resilience of the organic matter decomposing ability of soil and the role of aggregated soil structure. Soil Sci Plant Nutr. 54:534–542.

Gerdemann JW, Nicolson TH. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc. 46:235–244.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorryhizal infection in roots. New Phytol. 84:489–500.

Göhre V, Paszkowski U. 2006. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta. 223:1115–1122.

González-Chavez MC, Carrillo-González R, Wright SF, Nichols KA. 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut. 130:317–323.

Hoagland D, Arnon DI. 1950. The water culture method for growing plants without soil. California Agriculture Experimental Station Circular. 347p.

Jamal A, Ayub N, Usman M, Khan AG. 2002. Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soybean and lentil. Int J Phytorem. 4:205–221.

Jastrow JD, Miller RM, Lussenhop J. 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biol Biochem. 30:905–916.

Kabata-Pendias A. 2011. Trace elements in soils and plants, 4th ed. Boca Raton (FL): CRC. 505p.

Karagiannidis N, Nikolaou N. 2000. Influence of arbuscular mycorrhizae on heavy metal (Pb & Cd) uptake, growth, and chemical composition of *Vitis vinifera* L. (cv. Razaki). Am J Enol Vitic. 51:269–275.

Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ. 2000. Role of plants, mycorrhizae and phytochelators en heavy metal contaminated land remediation. Chemosphere. 21:197–207.

Leake JR, Johnson D, Donnelly DP, Muckle GE, Boddy L, Read DJ. 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Can J Bot. 82:1016–1045.

Lovelock CE, Wright SF, Nichols KA. 2004. Using glomalin as an indicator for arbuscular mycorrhizal hyphal growth: an example from a tropical rain forest soil. Soil Biol Biochem. 36:1009–1012.

Marschner H. 2012. Marschner's mineral nutrition of higher plants. Academic press. 651p.

Marschner H, Dell B. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant Soil. 159:89–102.

Miransari M. 2010. Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. Plant Biol. 12:563–569.

Meeinkuirt W, Kruatrachue M, Tanhan P, Chaiyarat R, Pokethitiyook P. 2013. Phytostabilization potential of Pb mine tailings by two grass species, *Thysanolaena maxima* and *Vetiveria zizanioides*. Water Air Soil Pollut. 224:1–12.

Moreira A, Costa DG. 2004. Dinâmica da matéria orgânica na recuperação de clareiras da floresta amazônica. Pesq Agropec Bras. 39:1013–1020.

Mozafar A, Ruh R, Klingel P, Gamper H, Egli S, Frossard E. 2002. Effect of heavy metal contaminated shooting range soils on mycorrhizal colonization of roots and mineral uptake by Leek. Environ Monit Assess. 79:177–191.

Murphy J, Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. 27:31–36.

Newsham KK, Fitter AH, Watkinson AR. 1995. Multifuncionality and biodiversity in arbuscular mycorrhizae. Trends Ecol Evol. 10:407–411.

Nogueira MA, Soares CRFS. 2010. Micorrizas arbusculares e elementos-traço. In: Siqueira JO, Souza FA, Cardoso EJBN, Tsai SM. (eds), Micorrizas: 30 anos de Pesquisa no Brasil. Lavras, (MG): UFLA. 716p.

Orlowska E, Godzik B, Turnau K. 2012. Effect of different arbuscular mycorrhizal fungal isolates on growth and arsenic accumulation in *Plantago lanceolata* L. Environ poll. 168:121–130.

Paun A, Neagoe A, Baciu I. 2012. The role of fungi on alleviating the stress induced by heavy metals uptake in rye plants (*Secale cereale* L.)

cultivated in soil from a roumanian industrial area. Rev Roum Chim. 57:141–150.
Pawlowska TE, Charvat I. 2004. Heavy-metal stress and developmental patterns of arbuscular mycorrhizal fungi. Appl Environ Microbiol. 70:6643–6649.
Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular – arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc. 55158–55161.
Rangel WDM, Schneider J, Costa ETDS, Soares CRFS, Guilherme LRG, Moreira FMDS. 2014. Phytoprotective effect of arbuscular mycorrhizal fungi species against arsenic toxicity in tropical leguminous species. Int J Phytorem. 16:840–858.
Roongtanakiat N, Sanoh S. 2011. Phytoextraction of zinc, cadmium and lead from contaminated soil by Vetiver Grass. Kasetsart J: Nat Sci. 45:603–612.
Sanchez JCD, Formoso MLL. 1990. Utilização do carvão e meio ambiente. Porto Alegre (RS): CIENTEC. 34p.
Sharpley AN, Weld JL, Beegle DB, Kleiman PJA, Gburek WJ, Moore JPA, Mullins G. 2003. Development of phosphorus indices for nutrient management planning strategies in the United States. J Soil Water Conserv. 58:137–52.
Shaw AJ. 1989. Heavy metal tolerance in plants: Evolutionary aspects. New York: CRC Press. 355p.
Silva S, Siqueira JO, Soares CRFS. 2006. Fungos micorrízicos no crescimento e na extração de metais pesados pela braquiária em solo contaminado. Pesq Agropec Bras. 12:1749–1757.
Soares CRFS, Carneiro MAC. 2010. Micorrizas arbusculares na recuperação de áreas degradadas. In: Siqueira JO, Souza FA, Cardoso EJBN, Tsai SM. (eds), Micorrizas: 30 anos de Pesquisa no Brasil. Lavras (MG): UFLA. 716p.
Soares CRFS, Siqueira JO. 2008. Mycorrhiza and phosphate protection of tropical grass species against heavy metal toxicity in multi-contaminated soil. Biol Fertil Soils. 44:833–841.
Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ. 1995. Análise de solo, plantas e outros materiais. UFRGS, Porto Alegre, RS. 174p.
Truong P. 2000. Vetiver grass technology for environmental protection. In: Proceedings of the 2nd International Vetiver Conference: Vetiver and the Environment. Annals. Cha Am, Thailand.
Wang FY, Lin XG, Yin R. 2007. Effect of arbuscular mycorrhizal fungal inoculation on heavy metal accumulation of maize grown in a naturally contaminated soil. Int J Phytorem. 9:345–353.
Wong CC, Wu SC, Kuek C, Khan AG, Wong MH. 2007. The role of mycorrhizae associated with vetiver grown in Pb−/Zn-contaminated soils: greenhouse study. Restor Ecol. 15(1):60–67.
Ye ZH, Baker AJM, Wong MH, Willis AJ. 1997. Zinc, lead and cadmium tolerance, uptake and accumulation by *Typha latifolia*. New Phytol. 136:469–480.
Zasoski RJ, Burau RG. 1977. A rapid nitric-perchloric acid digestion method for multi-element tissue analysis. Commun Soil Sci Plant Anal. 3:425–436.