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Cultivation of vetiver in saline tailings contaminated with arsenic under phosphorus doses

Lorena A. de O. P. Guimarães[1], Luiz E. Dias[2], Igor R. de Assis[2] & Amanda L. Cordeiro[3]

[1] Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural/Centro Regional de Desenvolvimento Rural Centro Serrano. Domingos Martins, ES. E-mail: lorena.prata@hotmail.com (Corresponding author)
[2] Universidade Federal de Viçosa/Departamento de Solos. Viçosa, MG. E-mail: ledias@ufv.br; igor.assis@ufv.br
[3] Instituto Nacional de Pesquisas da Amazônia. Manaus, AM. E-mail: amanda_longhi@hotmail.com

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ABSTRACT
The processing of gold ores exploited in Paracatu, MG, generates tailings that are challenging for revegetation, mainly because of the high content of arsenic and salinity. Aiming at the revegetation of the area of disposal of these tailings, the objective of this study was to evaluate the effects of phosphorus doses on vetiver plants (*Chrysopogon zizanioides*) and on the planting substrate, which consisted of tailings from the processing of ore named B1, exploited in Paracatu, with water restriction. Vetiver was grown for four months in the substrate under doses of 0, 140, 280, 560 and 1280 mg kg^{-1} of P_2O_5. Increasing doses of phosphorus improved the chemical characteristics of the substrate. However, the highest dose (1280 mg kg^{-1} P_2O_5) resulted in higher toxicity of arsenic for the plants. Under the evaluated conditions, the dose of 560 mg kg^{-1} of P_2O_5 is the most suitable for the fertilization of vetiver and, therefore, also for the revegetation of the substrate. Vetiver survives under low availability of water in the tailings.

Palavras-chave:
substrato sulfetado
mineração de ouro
recuperação ambiental
Chrysopogon zizanioides

Cultivo de vetiver em um rejeito salino contaminado com arsênio sob doses de fósforo

RESUMO
O beneficiamento dos minérios de ouro explotados em Paracatu, MG, gera rejeitos com características limitantes à revegetação, principalmente pelo elevado teor de arsênio e salinidade. Visando à revegetação da área de depósito desses rejeitos, o objetivo deste trabalho foi avaliar os efeitos de doses de fósforo sobre plantas de vetiver (*Chrysopogon zizanioides*) e sobre o substrato de plantio, que consistiu em um rejeito proveniente do beneficiamento do minério denominado B1, explotado em Paracatu, com restrição hídrica. O vetiver foi cultivado durante quatro meses no substrato sob as doses de 0, 140, 280, 560 e 1280 mg kg^{-1} de P_2O_5. Com o aumento das doses de fósforo houve melhoria nas características químicas do substrato, porém a dose mais elevada (1280 mg kg^{-1} de P_2O_5) propiciou maior toxicidade de arsênio nas plantas. Nas condições avaliadas, a dose de 560 mg kg^{-1} de P_2O_5 é a mais adequada para a adubação do vetiver, visando à revegetação do substrato. O vetiver sobrevive à baixa disponibilidade de água no rejeito.

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Introduction

Some ores exploited for gold production may contain toxic elements, such as arsenic (As), in their composition, causing environmental problems. In Brazil, gold and iron mining has contributed to the dispersion of As and its entry in the food chain (Campos et al., 2006).

The tailings produced in the processing of gold ores exploited in Paracatu-MG, Brazil, have limiting characteristics to plant growth, especially acidity, salinity and high As concentrations, which compromise the process of revegetation of the basin where they are disposed. A practice that can facilitate the establishment of plants in these tailings is phosphate fertilization.

The addition of P has been reported as inhibitor of As absorption (Lei et al., 2012). Phosphate and arsenate are chemically similar and share the same membrane transporters in root cells, and the transporters have higher affinity for the phosphate (Tu & Ma, 2003; Zhao et al., 2008).

Besides phosphate fertilization, plants tolerant to As and to salinity may have promising responses for the revegetation of tailings. One species with desirable characteristics to start the process of revegetation is *Chrysopogon zizanioides* (vetiver). The species, besides having high biomass production, has high tolerance and capacity of accumulation of contaminants (Singh et al., 2007).

In this context, this study aimed to evaluate the effects of P doses on vetiver plants and on the planting substrate, which consisted of tailings from the processing of one of the gold ores exploited in Paracatu, MG, Brazil, subjected to water restriction.

Material and Methods

The substrate consisted of tailings from the processing of B1 ore, which is a highly weathered phyllite, with sulfide contents around 3.0 g kg^{-1}, collected in Paracatu, MG, Brazil. Three samples of the substrate were collected for its initial characterization, through the determination of pH in water, available contents of P, K, Fe, Mn and S, exchangeable contents of Na, Ca^{2+}, Mg^{2+} and Al^{3+}, CEC at pH 7.0, exchangeable sodium percentage, content of organic matter (OM) (EMBRAPA, 1997), reminiscent P (Farias et al., 2009), field capacity (FC) at -10 kPa, substrate density (Ds) and particle density (Dp), through the methods of graduated cylinder and volumetric flask (EMBRAPA, 1997), respectively, and granulometry (Ruiz, 2005). The content of available As was extracted with Mehlich 3 extractor and quantified through inductively coupled plasma optical emission spectrometry (ICP-OES), at the wavelength of 193.696 nm.

Each experimental plot consisted of a column made of PVC pipe (45 x 15 cm, height x diameter), filled with substrate. The 5 cm of the upper portion were not filled with substrate and only served as a protection against the loss of material. Each column had a utilizable volume of 7.07 dm^3.

The substrate for the filling of the columns was air-dried and passed through a 4-mm-mesh sieve. In each column, 7.8 kg of dried substrate were added, deducting the residual moisture obtained by the thermogravimetric method (EMBRAPA, 1997), to obtain the density of 1.10 kg dm^{-3}.

The substrate of each plot was homogenized with $CaCO_3$ and $MgCO_3$ (Analytical Reagent - AR), at the Ca:Mg proportion of 4:1. The amount of acidity corrective was determined by the method of Al^{3+} neutralization and the increase of Ca^{2+} + Mg^{2+} contents, corresponding to 1 t ha^{-1}. Then, the substrate was moistened with deionized water in amount equivalent to 60% FC and incubated in a plastic bag for fourteen days.

After the incubation period, the doses of 0, 140, 280, 560 and 1280 mg kg^{-1} of P_2O_5 were added in the form of monobasic ammonium phosphate and homogenized in the entire volume of the substrate. The substrate was then deposited in the columns and the vetiver seedlings, with previously washed roots, were planted (one plant per column). The seedlings were cut at the height of 30 cm to standardize their mass.

The substrate was fertilized through the addition of 340, 120, 0.833 and 0.266 mg kg^{-1} of N, K, B and Mo, respectively, in all treatments. The doses of N and K were divided into two applications, half at 7 days after planting (DAP) and half at 15 DAP. Fertilization with B and Mo was performed in a single dose, at 15 DAP. In the N fertilization, the amount of N added in the monobasic ammonium phosphate was deducted. The nutrients were supplied with ammonium nitrate (AR), potassium chloride (AR), sodium borate (AR) and ammonium molybdate (AR).

The experiment was conducted for four months in a greenhouse with randomized block design and four replicates. In the first, second, third and fourth months, 2.06, 0.85, 0.20 and 0.065 L of deionized water were added to each column, divided in 12, 9, 2 and 1 application, respectively. The reduction in the amount and frequency of irrigation aimed to promote water restriction.

After the experimental period, plants were separated into shoots and roots. Since it was not possible to measure root length (because they fragmented during the separation from the substrate), the root system was analyzed separately in the layers of 0-20 and 20-40 cm, which allowed to observe where the roots were concentrated.

The roots were washed with deionized water with a 0.1% neutral detergent solution and again with deionized water; then, they were washed with 3% HCl solution and with deionized water. The shoots were washed only with deionized water; after washing, the plant material was dried in a forced-air oven at 60 ºC until constant mass for the determination of dry matter.

The plant samples were subjected to nitric-perchloric digestion (Tedesco et al., 1995) for the determination of As and P contents in the biomass; the quantification was performed through ICP-OES and the total amounts of the elements in the biomass were calculated based on their contents and on the dry matter production. The analyses of the samples of the substrate collected in the layers of 0-10, 10-20 and 20-40 cm were performed using the same methodologies of the initial characterization, with alteration in the determination of the content of available P, which was extracted using the Mehlich 3 extractor and quantified through ICP-OES, at the wavelength of 214.914 nm. In addition, the electrical conductivity (EC) was determined in the saturation paste extract (EMBRAPA, 1997).

The data were subjected to analysis of variance through F test and regression to verify the response of the variables to the P doses. Linear and quadratic regression models were tested.

Results and Discussion

Due to its mineralogical nature, the substrate showed low fertility with low contents of P, Ca^{2+}, Mg^{2+} and B, although Zn, Fe, Mn and S contents were high (Alvarez V. et al., 1999) (Table 1). The As content was very high, well above the prevention value of 15 mg kg^{-1} established by the CONAMA Resolution nº 420 (Brasil, 2009), above which there may be harmful alterations in the quality of the soil and groundwater. In addition, the predominance of silt fraction and the low CEC promotes low capacity of adsorption of cations in the substrate.

The OM content in the initial characterization of the substrate was possibly overestimated. The method used in the determination of the organic carbon content is based on the oxidation of this element (EMBRAPA, 1997) and, because of this, the result may suffer interference from other elements that are in reduced forms and that can be equally oxidized, such as Fe, Mn and S, overestimating the content of organic carbon.

The dry matter production of vetiver did not respond significantly to the applied P doses (Table 2). Root dry matter tended to be higher in the surface layer, 0-20 cm.

The characteristic symptoms of As toxicity in plants are wilting of leaves, slow growth, leaves with necrosis and purple color and, finally, the death of the plant (Melo et al., 2009; Schneider et al., 2012). Plants subjected to the highest P dose showed the greatest damages similar to those of As toxicity, with chlorosis and necrosis in young leaves and necrosis in all older leaves. In the other treatments, especially at the dose of 0, the leaves showed dark green color, which can be a symptom of P deficiency (Choi et al., 2013), and necrosis only on the tips. In spite of that, the plant constantly produced new leaves and remained alive.

Visually, it was observed that, in plots with doses lower than or equal to 560 mg kg^{-1} of P_2O_5, the main root was thicker and managed to grow until 40 cm depth, becoming denser at the bottom of the columns. Differently, the roots at the highest P dose did not become denser at the bottom of the columns, remaining concentrated in the surface - 91% of the total root dry matter concentrated in the layer of 0-20 cm. In the treatments with 0, 140, 280 and 560 mg kg^{-1} of P_2O_5, respectively, 76, 58, 61 and 65% of the total root dry matter remained concentrated in the surface layer.

The damage on root growth may be an indication of the sensitivity of the plant to a toxic element. The reduction in the length of roots and shoots and the loss of root ramifications are symptoms of exposure of plants to As (Liu et al., 2005; Moreno-Jiménez et al., 2012). In the present study, the lower proportion of root biomass in the layer of 20-40 cm, at the highest P dose, may result from the toxicity of As.

The lower damages observed in the leaves and the greater deepening of the roots in the substrate, although it is a strategy of the plant to increase its ability to access and absorb P (Hinsinger et al., 2015), suggest that doses lower than or equal to 560 mg kg^{-1} of P_2O_5 reduced As toxicity.

The contents and total amounts of As in the dry matter increased with the doses of P (Figure 1), corroborating Tu & Ma (2003), who observed that the addition of phosphate to the soil increased the accumulation of arsenate by *Pteris vittata*. The dose of 1280 mg kg^{-1} of P_2O_5 may have resulted in increase of As toxicity due to the increment in its absorption and translocation, which means that plants can become less tolerant to As when subjected to the highest P dose, since the tolerance is related to the capacity of preventing the absorption and translocation of the pollutant.

The study was based on the hypothesis that the highest P dose would reduce As absorption, since the transporters in the roots have greater affinity for the phosphate (Zhao et al., 2008), which was not confirmed. Although P was absorbed in greater amounts compared with As, the increase in P doses resulted in increments in the absorption not only of P, but also of As, which may have occurred due to the mass effect

Table 1. Initial characterization of the substrate used for the planting of vetiver

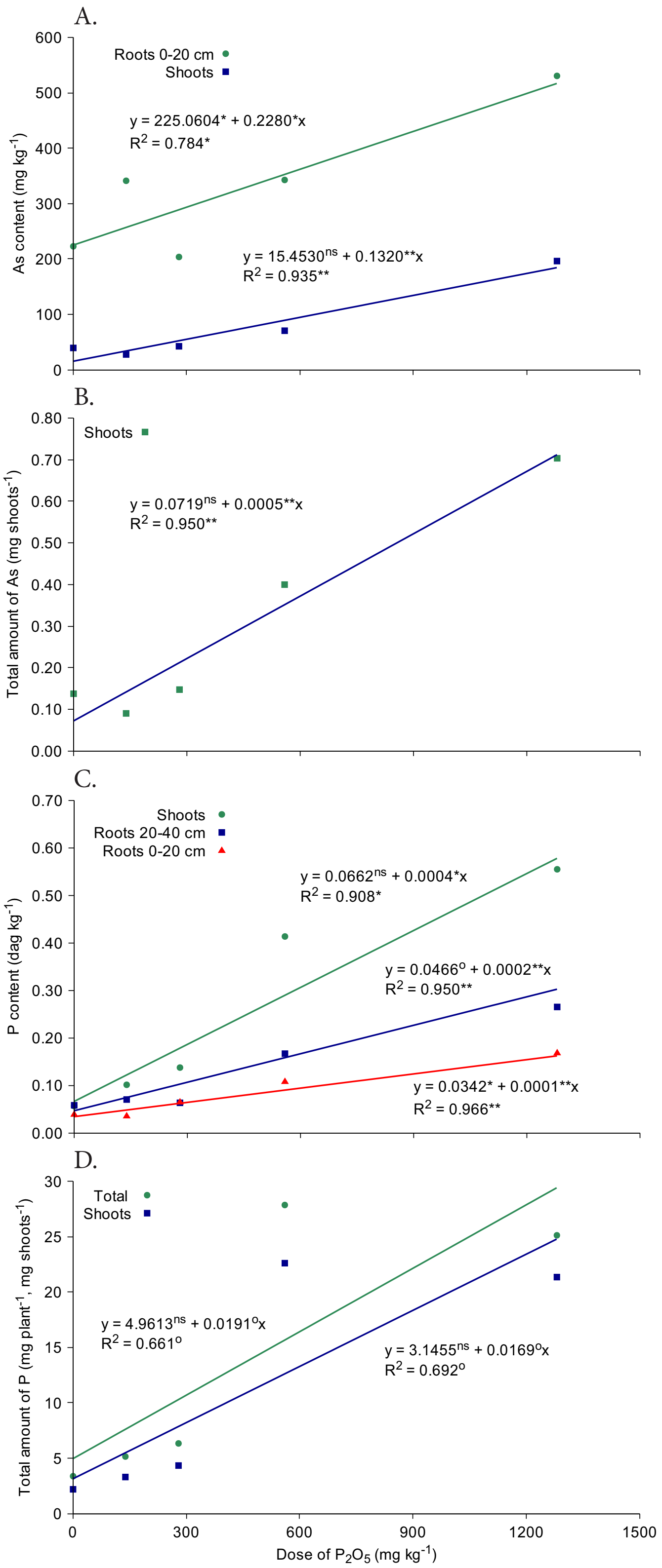
pH (H_2O)	P	K (mg dm^{-3})	Na	Ca^{2+}	Mg^{2+} (cmol$_c$ dm^{-3})	Al^{3+}	CEC	ESP (%)	OM (dag kg^{-1})	P-rem (mg L^{-1})
4.57	21	68	15.2	0.79	0.35	0.10	1.78	3.71	0.91	55.1
Fe	**Mn (mg dm^{-3})**	**S**	**As**	**FC (g g^{-1})**	**Ds (kg dm^{-3})**	**Dp**	**CS**	**FS**	**Silt (kg kg^{-1})**	**Clay**
1.026	13	142	112	0.391	1.10	2.40	0.020	0.239	0.705	0.036

CEC - Cation exchange capacity at pH 7.0; ESP - Exchangeable sodium percentage = Na x 100 / CEC; OM - Organic matter; P-rem - Reminiscent phosphorus; FC - Field capacity; Ds - Substrate density; Dp - Particle density; CS - Coarse sand; FS - Fine sand. pH - relation 1:2.5; P, K, Na, Fe and Mn - Extracted with Mehlich 1; Ca^{2+}, Mg^{2+}, Al^{3+} - Extracted with 1 mol L^{-1} KCl; OM - Organic carbon x 1.724; S: - Extracted with monocalcium phosphate in acetic acid; As - Extracted with Mehlich 3 and determined through ICP-OES

Table 2. Mean values of shoot and root dry matter (in the layers of 0-20 and 20-40 cm) of vetiver plants under applied phosphorus doses

Dose of P_2O_5 (mg kg^{-1})	Shoots	Roots 0-20 cm	Roots 20-40 cm (g plant^{-1})	Roots total	Total
0	3.87 ± 1.26	2.20 ± 0.86	0.68 ± 0.27	2.88 ± 1.03	6.75 ± 2.28
140	3.34 ± 0.31	2.11 ± 0.12	1.55 ± 0.37	3.66 ± 0.37	6.99 ± 0.68
280	3.53 ± 1.93	1.96 ± 1.97	1.24 ± 1.06	3.20 ± 2.98	6.73 ± 4.80
560	5.30 ± 2.51	2.56 ± 1.38	1.35 ± 0.90	3.92 ± 2.28	9.22 ± 4.78
1280	3.57 ± 2.06	1.94 ± 0.83	0.21 ± 0.20	2.14 ± 1.01	5.71 ± 3.05

Mean ± standard deviation



ns not significant. o, *, ** significant at 0.10, 0.05 and 0.01 probability level, respectively

Figure 1. Contents and total amounts of As and P in shoot and root dry matter (in the layers of 0-20 and 20-40 cm) of vetiver plants evaluated as a function of the applied phosphorus doses

generated by the addition of P, as occurs with the cations of the soil (Ferreira et al., 2009).

Due to the mass effect, the P may have displaced As from the sorption sites of the particles, increasing the concentrations of the metalloid in the solution (Smith et al., 2002). Consequently, greater amounts of As were absorbed by the plants with the increment in P doses.

The plants accumulated As predominantly in the roots (Figure 1), corroborating with Moreno-Jiménez et al. (2012). Unlike P, As has low mobility in the plants, except in hyperaccumulator species (Zhao et al., 2008). The explanation for the limited translocation is that the arsenate is rapidly reduced to arsenite in the roots and, then, is complexed and sequestered in the vacuoles of root cells, which may reduce its translocation (Su et al., 2008; Zhao et al., 2008). Thus, the lower translocation may be associated with mechanisms of defense of the plants.

The tolerance of plants is related to the maintenance of the photosynthetic functions. The lower the As translocation, the lower the damage on photosynthetic rate and the higher the tolerance of the plant (Nascimento, 2007). In addition, arsenate compromises ATP biosynthesis, altering the energetic flow of plant cells (Meharg & Hartley-Whitaker, 2002).

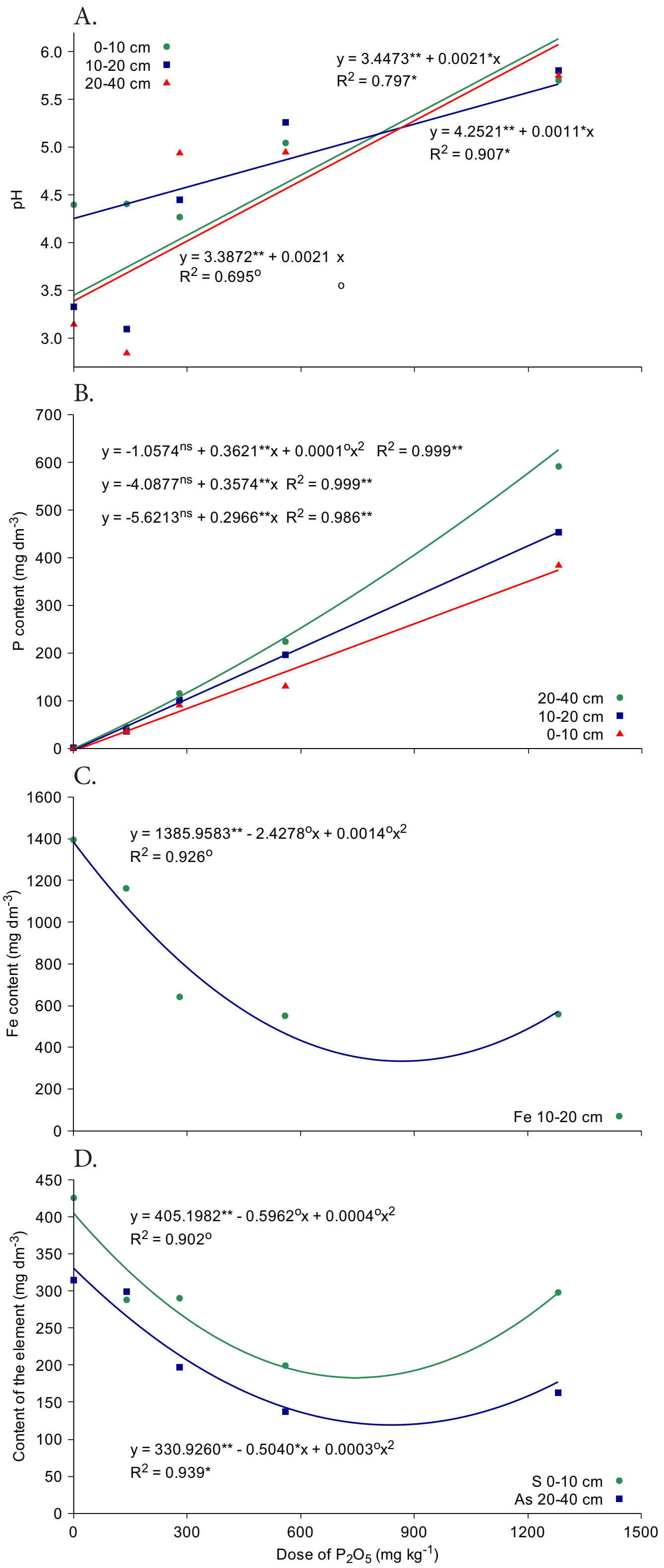
At the end of the experimental period, the content of As available in the substrate decreased with the increase in P doses (Figure 2). Such reduction can be attributed mainly to the greater absorption by the plants and the increment in pH with the increase in the doses. Higher pH values favor iron precipitation and, consequently, the reduction in As mobilization due to the adsorption or coprecipitation in the iron oxyhydroxides (Andrade et al., 2008). Thus, plants absorbed more As even with the reduction of its availability at the highest P doses, which reinforces the theory of the mass effect.

The oxidation of sulfide minerals accelerates the process of acidification. The reduction in pH, in turn, accelerates the oxidation of sulfide minerals, increasing the activity of the elements As, Fe and S in solution. Higher pH inhibits the oxidation of pyrite (FeS_2) and reduces the solubility of the metals (Simmons et al., 2002). Therefore, the reduction in the available contents of As, Fe and S in the substrate due to the increment in the doses of P, at least in part, results from increments in pH.

The EC did not show significant responses to the P doses, varying from 5.78 to 9.32 dS m^{-1} in the layer of 0-10 cm, from 2.53 to 3.00 dS m^{-1} in the layer of 10-20 cm and from 2.56 to 3.29 dS m^{-1} in the layer of 20-40 cm. The higher values in the surface layer may be a result of the capillary rise of the saline solution. Different from what normally occurs in cultivated soils, sulfate salts are the main responsible for the EC in the studied substrate and come from the oxidation of sulfide minerals.

In dry periods in which the evaporation exceeds rainfall, there is the crystallization of salts on the surface of the tailings in Paracatu, which is a limitation to the revegetation of the disposal area. In various plots, there were saline accumulations forming a whitish crust of calcium sulfate on the surface.

The water restriction was imposed in order to verify whether the vetiver crop would survive to the low water availability, as occurs in Paracatu. The plants responded to the water restriction through the wilting of the leaves, but this symptom was not severe to the point of compromising the survival of the plants.



°, *, ** significant at 0.10, 0.05 and 0.01, respectively

Figure 2. Values of pH and contents of P, Fe, S and As in the studied substrate, in the three layers evaluated, as a function of the applied phosphorus doses

Conclusions

1. The highest P dose tested (1280 mg kg^{-1} of P_2O_5) increases As toxicity in vetiver; despite the improvements in the chemistry of the substrate, the highest dose favors As absorption even with the reduction of its availability in solution.

2. Under the evaluated conditions, the dose of 560 mg kg^{-1} of P_2O_5 is the most adequate for the fertilization of vetiver aiming at the revegetation of the substrate.

3. Under the evaluated conditions, vetiver survives to the low water availability and possibly will also survive in the driest period of the year in Paracatu.

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