Solubilization of diabase and phonolite dust by filamentous fungus[1]

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ABSTRACT

The objective of this study was to evaluate the effect of the fungus *Aspergillus niger* strain CCT4355 in the release of nutrients contained in two types of rock powder (diabase and phonolite) by means of *in vitro* solubilization trials. The experimental design was completely randomized in a 5 x 4 factorial design with three replications. It was evaluated five treatments (phonolite dust + culture medium; phonolite dust + fungus + culture medium; diabase powder + culture medium; diabase powder + fungus + culture medium and fungus + culture medium) and four sampling dates (0, 10, 20 and 30 days). Rock dust (0.4% w/v) was added to 125 mL Erlenmeyer flasks containing 50 mL of liquid culture medium adapted to *A. niger*. The flasks were incubated at 30°C for 30 days, and analysis of pH (in water), titratable acidity, and concentrations of soluble potassium, calcium, magnesium, zinc, iron and manganese were made. The fungus *A. niger* was able to produce organic acids that solubilized ions. This result indicates its potential to alter minerals contained in rock dust, with the ability to interact in different ways with the nutrients. A significant increase in the amount of K was found in the treatment with phonolite dust in the presence of the fungus. The strain CCT4355 of *A. niger* can solubilize minerals contained in these rocks dust.

Key words: *Aspergillus niger*, bio-solubilization, rock powder, stonemeal.

RESUMO

Solubilização de pós de diabásio e de fonolito por fungo filamentoso

Este trabalho objetivou avaliar o efeito do fungo *Aspergillus niger*, linhagem CCT4355, na liberação de nutrientes contidos em pós de dois tipos de rocha (fonolito e diabásio), por meio de ensaios de solubilização *in vitro*. O delineamento experimental foi o inteiramente casualizado, em esquema fatorial 5x4, com três repetições. Foram avaliados cinco tratamentos (pó de fonolito + meio; pó de fonolito + fungo + meio, pó de diabásio + meio, pó de diabásio + fungo + meio e fungo + meio) e quatro épocas de amostragens (0, 10, 20 e 30 dias). Adicionou-se pó de rocha (0,4% m/v) a Erlenmeyers de 125 mL, com 50 mL de meio de cultura líquido adaptado para *A. niger*. Os frascos foram incubados a 30 °C, por 30 dias, e foram feitas análises de pH em água, acidez titulável e teores de potássio, cálcio, magnésio, zinco, ferro e manganês solúveis. O *A. niger* foi capaz de produzir ácidos orgânicos que solubilizaram íons. Este resultado indica o potencial de *A. niger* para alterar minerais contidos em pós de rocha, com capacidade de interagir de diferentes formas com os nutrientes. Houve aumento significativo da quantidade de K solúvel, no tratamento com pó de fonolito, em presença do fungo. Conclui-se que a linhagem CCT4355 de *A. niger* pode solubilizar minerais contidos nesses pós de rochas.

Palavras-chave: biossolubilização, *Aspergillus niger*, pó de rocha, rochagem.

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INTRODUCTION

Brazil has tropical soils formed from different origin matter, often highly weathered, with accelerated removal of nutrients, which eventually promote high acidity and aluminum toxicity, besides the high capacity of phosphorus fixation (Lopes & Guilherme 2007). However, their low natural fertility can be modified by means of appropriate management techniques for conservation of their physical, chemical and biological characteristics. Noteworthy are the reduction of the intensity of tillage, crop rotation, intercropping cultivation, planting on contour lines, use of mulch, green manure and fertilizer to supply the nutrients demanded for the proper development of plants.

According to the Associação Nacional para Difusão de Adubos – ANDA (National Association for the Promotion of Fertilizers) (2014), Brazil imported more than 21.5 million tons of products to manufacture fertilizers in 2013. Because such fertilizer uses fossil fuel for its processing and transportation, its market value is directly influenced by currency fluctuations and fluctuations in the price of a barrel of oil. Moreover, because they are highly soluble, these fertilizers can be easily leached, both in rainfed agricultural systems as in irrigated systems. Furthermore, soluble fertilizers are not permitted under the current technical standards for organic agriculture, since soil biological activity and water quality must be maintained and incremented in this type of agriculture (Brasil, 1999; FAO, 2007).

Studies have been made in Brazil to alternative sources of nutrients, using organic matter or minerals that have not been commercially exploited due to the lack of knowledge on the potential use, lack of product positioning in the market or lack of interest by the fertilizer industry (Benites *et al*., 2010). Among these studies, the use of *in natura* rock powder directly in the soil has been considered of strategic importance for the country since it contributes to the reduction of negative impacts on the Brazilian trade balance, caused by the import of fertilizers (Martins *et al*., 2008; Nascimento & Loureiro, 2009).

However, the processes of chemical decomposition of minerals and the release of nutrients from the rock dust to the soil solution, in appropriate way form and amount for plant absorption, are relatively slow. In order to solve this issue, rock dust with finer grain size is searched because the stability of the minerals that compose rocks depends on its structure, its solubility, and its specific surface area among other factors (Kämpf *et al*., 2009). Promising results have been obtained in experiments with different micro-organisms (filamentous fungi, bacteria and yeasts) capable of promoting the solubilization of rock dust, accelerating chemical modification processes of silicate contained in the dust (Sheng, 2005; Sugumaran & Janarthanam, 2007; Hamdali *et al*., 2008; Lian *et al*., 2008; Xiao *et al*., 2008).

Saprophytic filamentous fungi present, as part of their metabolic activity, a potent secretory system of acids and enzymes able to solubilize materials and make nutrients available (Grimm *et al*, 2005; Guebel & Darias, 2001). Among the ascomycetes filamentous fungi, *Aspergillus niger* has been extensively used in studies of rock powder solubilization (Lopes-Assad *et al*., 2006; Lopes-Assad *et al*., 2010). The biossolubilization promoted by *Aspergillus niger* occurs in an indirect manner, by the action of reducing acids (citric and oxalic acids), which are produced by the metabolism of the fungus (Mehta *et al*., 2010) and released into the solution. It is found in leaf litter in the nature and its growth occurs in wide ranges of temperature (0 to 47°C) (Schuster *et al*., 2002).

The objective of this study was to evaluate the effect of the fungus *Aspergillus niger*, strain CCT4355, on the release of nutrients contained in phonolite and diabase dust by means of *in vitro* trials.

MATERIAL AND METHODS

The experiment was carried out in the Laboratory of Agricultural and Molecular Microbiology (LAMAM) and nutrient analysis was performed at the Soil and Plant Chemical Analysis Laboratory, at the Center for Agriculture Sciences of Universidade Federal de São Carlos, Araras Campus in the State of São Paulo.

The phonolite dust was provided by a mining company from Poços de Caldas, State of Minas Gerais, and diabase dust was supplied by a quarry from Araras, State of São Paulo. Both presented median amounts of silica (SiO_2) (Table 1).

In the solubilization tests, it was used the fungus *Aspergillus niger*, strain CCT-4355, isolated from sugar cane vinasse (Ceccato, 1989), of the LAMAM microorganism bank. Until the beginning of the tests, the fungus remained stored in the refrigerator (6-8°C) in test tubes, in MYPG inclined culture medium. Regarding production of inoculum, MYPG medium (3 g L^{-1} of meat extract, 3g L^{-1} yeast extract, 8 g L^{-1} peptone, 10 g L^{-1} glucose and 20 g L^{-1} agar) was used in culture in the test tube in inclined medium; the tubes were incubated at 30°C for 5 days. A volume of 4 mL Tween 80 (0.1%) was added to each culture tube and the spores were scraped with a platinum loop for their release. A suspension from 1.0 to 1.8 x 10^7 spores mL^{-1} was obtained, after counting in a Neubauer chamber. For setting up the experiment, it was used 50 mL of the liquid culture medium adapted for *A. niger* (2.85 g L^{-1} sodium citrate, 1 g L^{-1} ammonium phosphate, 0.5 g L^{-1} magnesium sulfate, 0.132 g L^{-1} calcium chloride and 10 g L^{-1} glucose and pH adjusted to 7.0), according to Cerezine *et al*. (1988). This means that in 50 mL of culture medium, there was 2.35 mg calcium, 38 mg sodium, 239.5 mg of carbon, 6.08 mg nitrogen, 13.48 mg phosphorus, 5 mg magnesium, 6.65 mg sulfur and 4.2 mg chlorine. The cultivation was performed in 125-mL Erlenmeyer flasks, with addition or not of 0.2 g of rock dust (0.4% w/v), with particle size smaller than 0.053 mm (270 mesh) and inoculation or not of 0.5 mL of the spore suspension, according to the treatment.

The *in vitro* experiment was carried out in a completely randomized design in a 5 x 4 factorial design with three replications. The treatments were as follows: *A. niger* + medium (MA); phonolite dust + medium (FM); phonolite dust + *A. niger* + medium (FMA); diabase dust + medium (DM) and diabase dust + *A. niger* + medium (DMA). The flasks were incubated at 30°C, 160 rpm, for 30 days, with four sampling times (0, 10, 20 and 30 days). The contents of the flasks were filtered under vacuum, using no. 1 Whatman filter paper. The following were analyzed in the filtrate: water pH and titratable acidity by samples titration up to pH 7, using NaOH 0.05 mol L^{-1} were analysed in the filtrate. Contents of potassium (K) were determined by flame emission photometry and contents of soluble calcium (Ca), zinc (Zn), manganese (Mn) and iron (Fe) by atomic absorption spectrophotometry.

The results of *in vitro* tests were submitted to analysis of variance (F test) and means were compared by Tukey test at 5% probability or by regression depending on the origin of the data, quantitative or qualitative, using the statistical software Statistica 6.0 (2001).

RESULTS AND DISCUSSION

The phonolite rock dust in the presence of the fungus provided greater amount of solubilized K (Table 2); however, the action of *A. niger* on the diabase rock powder did not provide significant difference in the amount of solubilized K. The amount of K from the phonolite rock dust is ten times greater than that of diabase rock dust (Table 1), which explains the result obtained in phonolite + medium + fungus treatment (AMF), which had a high release of the soluble K into the medium, demonstrating the solubilizing potential of the fungus.

Potassium present in feldspars is not readily available because it is strongly bound to oxygen molecules of tetrahedrons of SiO_4 and AlO_4 (Curi *et al*., 2005). Therefore, the release of K^+ ions requires the dissolution of feldspars, by the action of H^+ ions from the acids produced by *A. niger*. The reaction can be expressed in a simplified way, as $KAlSi_3O_8 + H^+ \rightarrow HAlSi_3O_8 + K^+$.

Contents of Ca were higher in treatments with rock powder, without the presence of fungus, with no difference between the two types of dust (Table 2), indicating a likely intake by the fungus. This is because Ca silicates and aluminosilicates are sufficiently solube in low pH, but the concentration of soluble Ca decreases as pH increases (Mello & Perez, 2009). The small amounts of Ca found in the treatment with phonolite rock dust, which showed no calcium oxide in its composition (Table 1), can be explained by the addition of calcium chloride in the culture medium.

The fungus *A. niger* had no effect on the solubilization of Mg, Zn, Fe and Mn in the treatments with both studied rock dust (Table 2), and the amount of solubilized Fe was higher in diabase rock dust treatment. Phonolite is an alkaline leucocratic rock whereas the evaluated diabase (geologically classified as a lati-andesite) is a basic mesocratic rock (Machado *et al*. 2005), a reflex of the mafic mineral contents that have dark color since they contain iron, magnesium , titanium, manganese, and so on. The iron content of the diabase rock was higher than that of the phonolite rock (Table 1). Therefore, the amount of that ion in the medium was higher in the diabase + medium + *A. niger* (DMA) treatment than in the phonolite + medium + *A. niger* (FMA) treatment.

Significant results at 5% of probability were found in the time and treatment interaction, for all attributes, except for pH and titratable acidity, which showed high variance. Data regarding treatments with diabase + *A. niger* + medium (DMA) and phonolite dust + *A. niger* + medium (FMA) are discussed in this paper.

The levels of soluble Ca (Figure 1A) decreased until the twentieth day of the trial; after this period, the concentration of Ca increased, but this increase was not

Table 1. Chemical characterization of phonolite and diabase dust used in *in vitro* solubilization trial

Rock dust	SiO_2	Fe_2O_3	K_2O	Al_2O_3	Na_2O	MgO	CaO	TiO_2	MnO
	%								
Phonolite[1]	54.8	3.29	8.46	21.3	6.07	-	-	-	-
Diabase[2]	50.31	12.62	0.84	13.94	2.24	6.91	10.16	1.02	0.19

[1] Supplied by minning company Curimbaba Ltda; [2] Supplied by Acme Analytical Laboratories LTD.

due to the action of *A. niger* (Table 2). This behavior is in part due to the consumption of this element by the microrganism, in part due to the presence of organic acids produced by *A. niger*, especially citric acid ($C_6H_8O_7$), which presents a chelating activity by Ca^{+2} (Carvalho *et al*., 2005).

The content of potassium showed an increase, over time, not reaching its maximum value (Figure 1B) being much more expressive in phonolite dust, richer in K_2O than diabase dust (Table 1). After 30 days, it was found that the amount of K, solubilized by *A. niger*, in the treatment with phonolite dust + medium + fungus (FMA) has not reached its maximum degree (Figure 1B). Because each flask with phonolite dust contained 7.2 $mmol_c\ dm^{-3}$ K, the content of soluble K, after 30 days of incubation, was 1.21 $mmol_c\ dm^{-3}$ in the phonolite + medium + fungus treatment. This corresponds to a solubilization rate of 16.8% whereas this rate remained at 7.2% in the medium + phonolite treatment. Using the same strain of *A. niger*, Lopes-Assad *et al*. (2006) obtained solubilization rates of 54.7% for the alkaline ultramafic rock dust, containing 3.32% of K_2O and 33.26% SiO_2, and 11.8% for phlogopitite dust, containing 5.13% of K_2O, and 47.12% SiO2 at 21 days of incubation. These results suggest that the rate of solubilization obtained with *A. niger* is probably related to the amounts of SiO_2 and K_2O, present in rocks and their mineralogical characteristics.

The behavior of ion Mg (Figure 2A) in the treatments with diabase dust was similar to the behavior presented by Ca, with a decrease in the content until day 20, probably due to consumption by the microorganism, and a subsequent increase in which it was not possible to state that the maximum solubilization was reached at the end of the trial. The behavior of the ions Zn, Fe and Mn in the treatments with diabase rock dust was similar over the 30 days of the trial (Figures 2b, c and d), with greater release between the tenth and twentieth day of the trial.

Fungus *A. niger* releases reducing acids (citric and oxalic acids), which convert the insoluble forms Fe (III) and MnO_2 into soluble forms Fe (II) and Mn (II) (Mehta *et al*., 2010). Some factors have an influence in the submerged fermentation of citric acid (type and

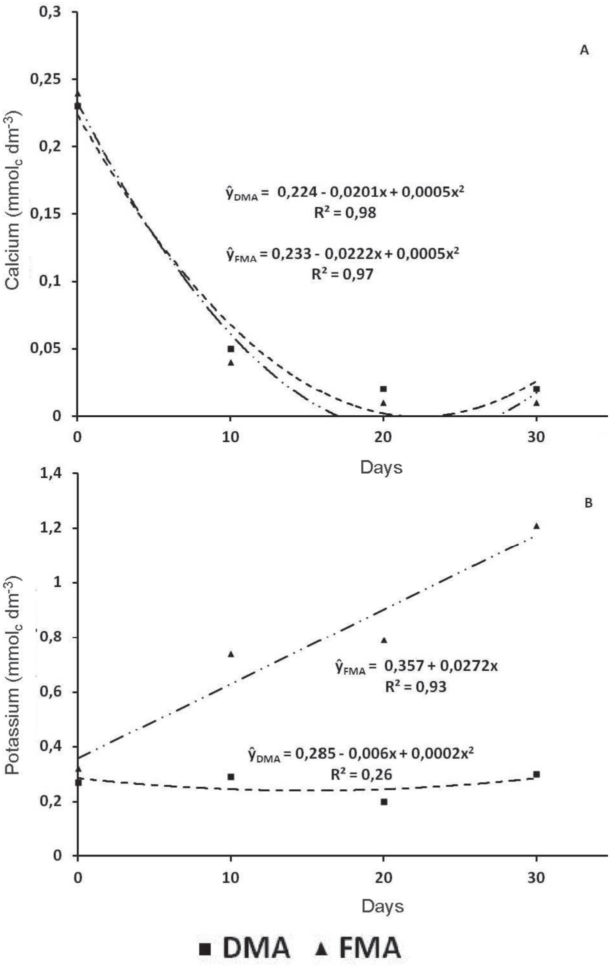

Figure 1. Variation in the concentration of calcium (A) and potassium (B) over 30 days of solubilization trials of diabase dust (DMA) and phonolite dust (FMA) using *Aspergillus niger* in liquid culture medium.

Table 2. Chemical characteristics determined at 30 days of *in vitro* trials of solubilization of phonolite and diabase dust by the fungus *Aspergillus niger* strain CCT4355

Characteristics	Treatments				
	DM	DMA	FM	FMA	MA
pH	6.64 b	7.15 a	6.71 b	5.95 c	3.53 d
Titratable acidity ($mmol_c\ dm^{-3}$)	2.76 bc	1.00 c	2.00 c	8.16 b	52.00 a
Potassium ($mmol_c\ dm^{-3}$)	0.36 c	0.26 c	0.50 b	0.77 a	0.30 c
Calcium ($mmol_c\ dm^{-3}$)	0.29 a	0.08 b	0.26 a	0.08 b	0.06 b
Magnesium ($mmol_c\ dm^{-3}$)	0.39 a	0.33 a	0.38 a	0.35 a	0.38 a
Zinc ($mg\ dm^{-3}$)	0.19 a	0.21 a	0.13 bc	0.16 ab	0.09 c
Iron ($mg\ dm^{-3}$)	64.40 a	64.47 a	12.25 bc	16.77 b	9.37 c
Manganese ($mg\ dm^{-3}$)	287 a	2.21 ab	2.58 ab	2.41 b	0.12 c

DM = diabase dust in liquid culture medium; DMA = diabase dust and *A. niger* in liquid culture medium; FM = phonolite dust in liquid culture medium; FMA = phonolite dust and *A. niger* in liquid culture medium; MA = *A. niger* in liquid culture medium. Means on the lines, followed by an equal letter at least, do not differ by Tukey test at 5% probability

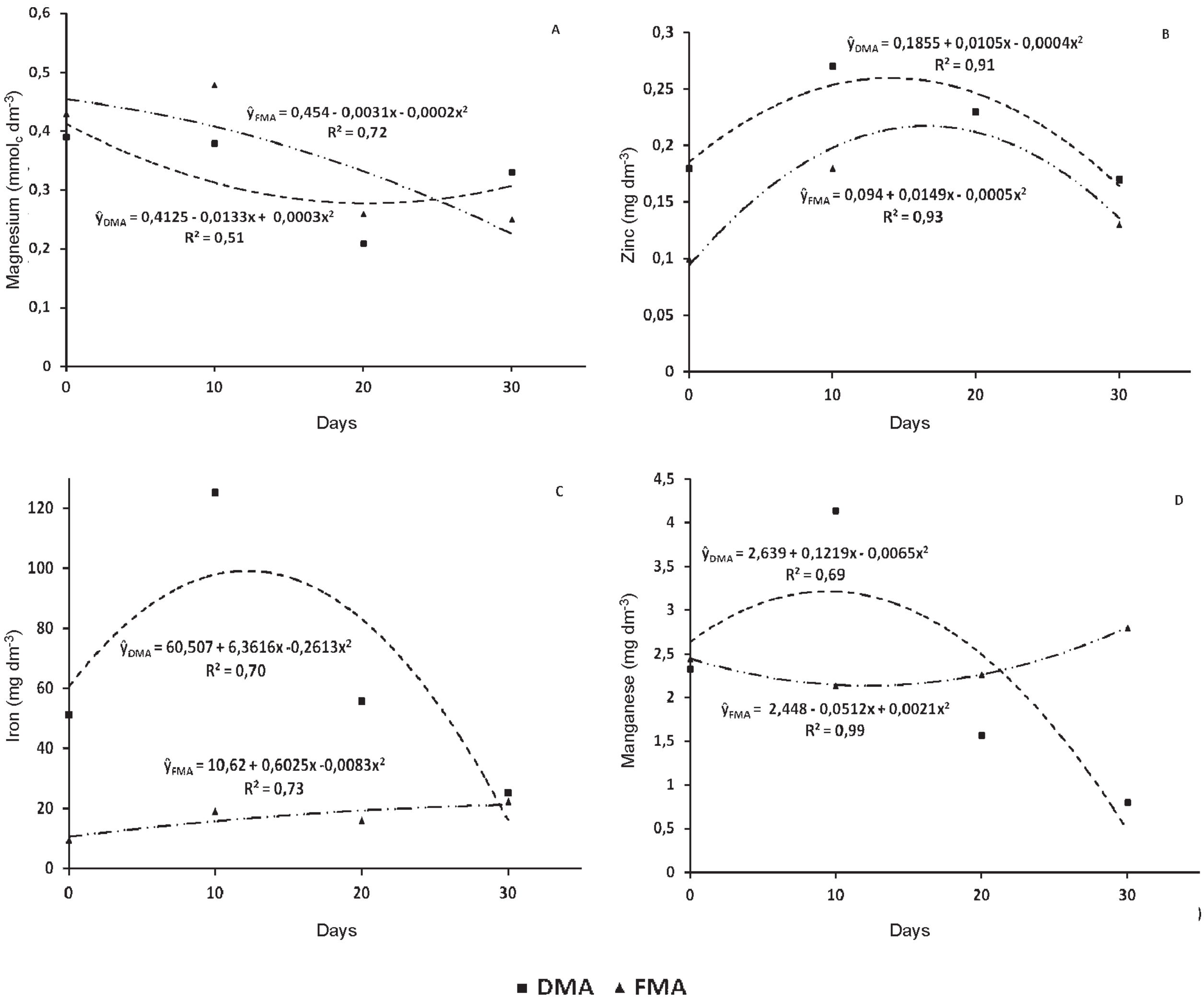

Figure 2. Variation in the contents of magnesium (A), zinc (B), iron (C) and manganese (D) over 30 days of solubilization trials of diabase (DMA) dust and phonolite dust (FMA) using *Aspergillus niger* in liquid culture medium.

concentration of the C source, N and phosphate limitation, pH, aeration, concentration of trace elements and morphology of the producer organism). Among the metals that need to be in a limit amount are Zn, Mn, Fe, Cu, alkali metals and heavy metals (Papagianni, 2007). In the solubilization of silicates contained in rock dust, the concentrations of Fe and Mn in the medium decrease at first, possibly due to the formation of oxides and hydroxides of Fe and Mn with low solubility (Oliveira Junior *et al.*, 2000; Mello & Perez, 2009).

Plant ashes are two sources of potassium commonly used in organic agriculture, with about 10% of K, and the decomposed cattle manure, with about 5% of K (Souza & Alcântara, 2008). Thus, phonolite dust, with about 8% of K, may be an alternative to organic fertilization. The biotechnology pathway by incubation of rock dust with *A. niger* and production of biofertilizer is a promising alternative in order to accelerate the release of nutrients.

CONCLUSIONS

The fungus *Aspergillus niger* promoted the solubilization of the elements contained in the dust of diabase and phonolite rocks.

The dust of the evaluated phonolite released soluble potassium in the culture medium in a significant way after 30 days of incubation.

Over the 30 days of trial, there were no significant variations in pH and exchangeable acidity in the performed treatments; however, there was effect on the levels of the soluble ions studied.

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