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Mycorrhizal activity as a quality indicator in the use of mining slag as soil conditioner

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The main residue of iron and nickel mining is slag, a solid residue of low solubility and rich in magnesium silicate. A residue with potential use as a soil conditioner, however, it is still necessary to investigate whether its use brings impacts to the environment. Microorganisms are extremely sensitive to environmental changes, changes that can be used as environmental quality indicators. Among the organisms that act as microbiological indicators of soil quality, the activity of arbuscular mycorrhizal fungi stands out. With this, this study aimed to verify the mycorrhizal activity as a quality indicator under application of nickel iron mining slag as soil conditioner. The experiment was carried out in the greenhouses of the agronomy course and in the laboratory of agricultural microbiology of the Evangelical College of Goianésia. Magnesium silicate was supplied by Anglo American Mining Company, located in Barro Alto, Goiás. Agronomic and microbiological analyses were performed at the Evangelical College of Goianésia, Goiás. The experimental design used was completely randomized, arranged with five treatments and with five replications, where the treatments were composed of four doses of magnesium silicate: 9, 12, 15, and 18 g dm⁻³ of soil in addition to the control without application. Ten golden saw rice seeds were planted in 10-L pots. Soil analysis used in the experiment was performed to determine soil nutritional parameters. The application of iron and nickel mining slag does not influence the development of rice in the first application. The application of 9 dm³g⁻¹ of iron and nickel mining slag soil positively influences mycorrhizal activity on roots and soil.

Key words: Mycorrhiza, magnesium silicate, stress, cerrado.

INTRODUCTION

The mining industry generates a gigantic amount of solid and liquid waste, which is accumulated and causes great socio-environmental impact. According to the Brazilian Mineral Yearbook (2019),iron and nickel are among the main ores extracted in Brazil and occupy the range of 71.1% and 2.5% of metals extracted in the country, respectively, reaching the financial movement of 88.5 billion reais in 2017.

The state of Goiás is the third with the highest production of ores in Brazil, surpassed only by Minas

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> Table 1. Soil analysis used for the installation of the experiment.

М.О.	C.O.	Р	K+	Ca ²⁺	Mg ²⁺	Al ²⁺	H+AI	Ca+Mg
mg dm ⁻³				cmolc dm ⁻³			cmolc dm ⁻	
21.50	12.5	138.0	238.0	2.84	1.30	0	1.96	4.14

Gerais and Pará and is the main nickel producer in Brazil. Anglo American mining company, located in the municipality of Barro Alto, occupies the first place in the country in the production and commercialization of Nickel and the third in the production and commercialization of Iron (Brasil, 2020).

The main residue of iron and nickel mining is slag, a solid residue of low solubility and rich in magnesium silicate. The slag produced from iron and nickel mining at anglo american mining company's Barro Alto plant reaches 2.4 million tons per year. These residues are packed in the form of dried batteries and require a large physical space to be stored, which causes great socio-environmental impact (Corrêa et al., 2009).

In this respect, a good alternative to this problem would be the use of this waste, which would add economic value to the material and prevent it from being accumulated in large quantities, thus generating an environmental liability. Magnesium Silicate is one of the main components of iron and nickel mining slag, and this substance has potential for use as soil conditioners in agroecosystem (Fortes et al., 2008). For Prado et al., (2003b) slag can be an excellent source of Ca and Mg for plants, as well as an acidity corrective and soil conditioner in depth.

However, the chemical characteristics of each tailings vary from each extractor unit and more detailed analyses are fundamental. The use of these wastes as soil conditioners can prove to be an excellent ecological and economic alternative for the industries that produce them, adding value to a by-product with great potential for pollution and environmental impact (Prado et al., 2003a).

Mycorrhizal fungi are excellent indicators of environmental changes because they are sensitive to negative and positive variations in soils. By using mycorrhizal fungi as bioindicators of environmental quality, it is possible to evaluate the impacts that the use of mining slag cause on soil microbial activity, as well as to all edaphic biodiversity (Souza et al., 2016).

Magnesium is one of the most important elements in plant nutrition, acting in the structure of proteins and enzymatic components; it is also fundamental in the constitution of chlorophyll and photosynthesis (Faquin, 2005). Composing the group of secondary macronutrients, the main source of magnesium for agriculture is the application of limestone. The use of silicon in agriculture also brings benefits, especially in grasses. This element decreases the severity of the attack of diseases and pests, promotes increased productivity and increases resistance to water stress (Haridasan, 2000).

The use of magnesium silicate as a source of these elements can add value to a residue, which has caused an environmental impact in the place where it is stored. With this, this study aimed to verify mycorrhizal activity as a quality indicator under application of nickel iron mining slag as soil conditioner.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse of the agronomy course and in the laboratory of agricultural microbiology of the Evangelical College of Goianésia. Magnesium silicate was supplied by Anglo American Mining Company, located in Barro Alto, Goiás. Agronomic and microbiological analyses were performed at the Evangelical College of Goianésia, Goiás.

The experimental design used was completely randomized, arranged in five treatments and with 10 replications, where the treatments composed of four doses of magnesium silicate: 9, 12, 15, and 18 g dm³ of soil and the control without application. Ten golden saw rice seeds were planted in 10-L pots. Soil analysis used in the experiment was performed to determine soil nutritional parameters (Table 1).

As response variable, vegetative characteristics of the plants (root length, root volume, root fresh mass, root dry mass, shoot length, aerial fresh mass and shoot dry mass) and mycorrhizal activity (mycorrhizal colonization rate, soil spore density and Identification of associated genera) were evaluated. The collection and analysis of the response variables were performed at the beginning of the flowering stage.

The analyses were carried out at the Laboratory of Agricultural Microbiology of the Evangelical College of Goianésia. The spores of arbuscular mycorrhizal fungi (AMF) were extracted from 500 cm³ of rhizospherical soil by the wet sieving technique, according to the methodology described by Gerdemann and Nicolson (1963) followed by centrifugation in water and sucrose solution 50%. The spores were separated according to their phenotypic characteristics such as color, size and shape, composing the different morphotypes, under stereoscopic binocular magnifying glass.

To determine the percentage of colonization, the roots were clarified and colored with 0.05% Trypan Blue in lactoglycerol (Phillips and Hayman, 1970) under stereoscopic microscope, following the technique of intersection of the quadrants (Giovannetti and Mosse, 1980).

To identify the genera of AMF from morphological characteristics, the spores were separated according to their morphotypes and mounted on blades with pure polyvinyl-lactoglycol (PVLG) and PVLG mixed with Melzer (1:1 v/v). To support the identification work, original articles of the description of the species provided on the website of the "International Culture Collection of Arbuscularand Vesicular-Arbuscular Mycorrhizal Fungi" (INVAM, 2018) were used.

The data were submitted to analysis of variance by the Assistat program (Silva, 2008) and the graphs constructed by prism software (Swift, 2020) and canonical correspondence statistics were

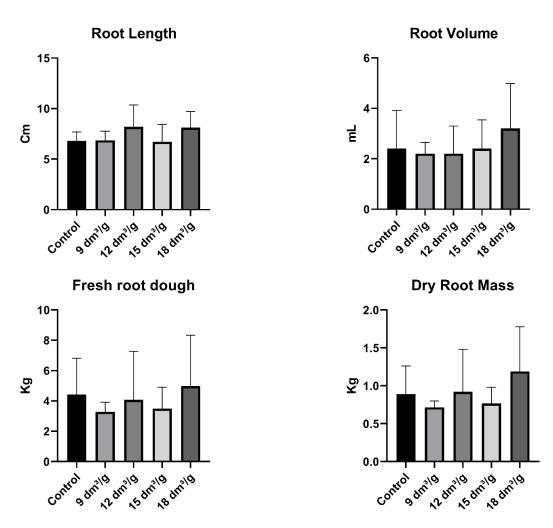


Figure 1. Length, volume, fresh mass and dry mass of rice root under application of iron and nickel mining slag soil conditioner.

performed by the Past software (Hammer, 2018).

RESULTS AND DISCUSSION

The application of iron and nickel mining slag in order to use it as a soil conditioner did not present a significant minimum difference in the statistics in vegetative parameters such as length, volume, fresh mass and root dry mass (Figure 1) and in the parameters plant height, fresh mass and shoot dry mass (Figure 2).

When analyzing mycorrhizal activity in rice rhizosphere under application of iron and nickel slag, it was possible to observe statistical difference in mycorrhizal colonization rate and spore density in rhizospherical soil (Figure 3).

The dose of 9 g/dm³ showed the highest values of mycorrhizal colonization rate and spore density. The other dosages presented values identical to the treatment and control for the two parameters evaluated.

The genera Acaulospora, Scutellospora, Sclerocystis, Glomus and Gigasporas were identified associated with rice rhizosphere (Table 2). The genera Acaulospora and Scutellospora were found in the rhizosphere of all treatments, except for control. The genus Glomus was found in all treatments, except in the treatment with application of 18 g/dm³ of iron and nickel mining slag. The genus Gigaspora was found only in the treatment with application of 18 g/dm³, and the genus Sclerocystis was identified only in the control treatments and with application of 9 g/dm³.

The analysis of main components of the genera of identified mycorrhizal fungi and the dosages of nickel iron mining slag (Figure 4) showed an approximation of some genera in relation to the applied treatments.

The genera *Acaulospora* and *Scutellospora* showed greater proximity to treatments with doses of 12 and 15 g/dm³ of mining slag. The genus *Glomus* approached the dose of 9 g/dm³ of mining slag. The genera *Slcerocystis* and *Gigaspora* did not present proximity to any of the

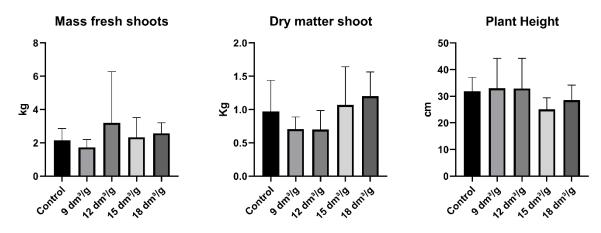


Figure 2. Plant height, fresh mass and dry mass of aerial part of rice under application of iron and nickel mining slag soil conditioner.

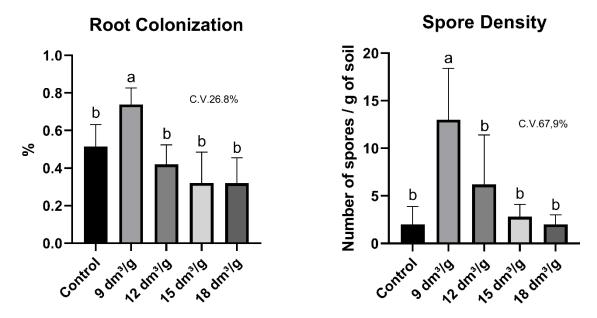


Figure 3. Mycorrhizal colonization rate and spore density in rice root soil under application of iron and nickel mining slag as soil conditioner.

treatments evaluated, demonstrating that they may have occurred casually in the samples evaluated. The treatments and control of 18 g/dm³ of slag were also not close to any identified mycorrhizal fungus genus.

Root colonization rate and spore density in the soil can be used as a parameter when measuring the level of impact caused by the application of slag. Mycorrhizal fungi act in the plant, aiding the absorption of nutrients and water in the soil (Moura and Cabral, 2019; Silveira and Freitas, 2007), working with organisms that promote growth and plant health. They act mainly when the plant is in a situation of stress, where the main triggers for the beginning of this symbiotic association is the low availability of water and nutrients (Johnson and Pfleger, 1992). High values of colonization in the roots and spores in the soil indicate a higher activity of the fungus because of a possible stress situation of the plant.

When comparing the behavior of the treatments in the vegetative characteristics (Figures 1 and 2), no statistical difference was observed in any of the response variables, demonstrating that the treatments did not influence the development of the plant, neither positively nor negatively. This is due to the high insolubility of the compounds found in slag, such as magnesium and silicon, which, if available, could positively influence plant development (Fortes et al., 2008).

Treatment	Acaulospora	Scutellospora	Sclerocystis	Glomus	Gigaspora
Control			+	+	
9 g/dm³	+	+	+	+	
12 g/dm ³	+	+		+	
15 g/dm ³	+	+		+	
18 g/dm ³	+	+			+

Table 2. Genera of arbuscular mycorrhizal fungi associated with rice rhizosphere under application of iron and nickel mining Slag as soil conditioner.

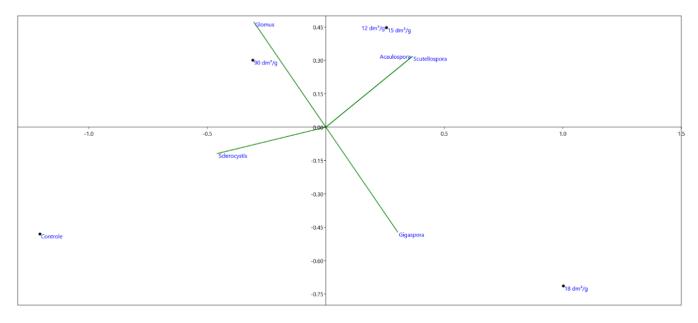


Figure 4. Analysis of main components of genera of arbuscular mycorrhizal fungi associated with rice rhizosphere under application of iron and nickel mining slag as soil conditioner.

Based on this interpretation, the plant did not suffer stress caused by the presence of the tailings, which is not responsible for the statistical difference verified when analyzing the mycorrhizal colonization rate and density of spores in the soil (Figure 3). These same insoluble compounds found in slag can be solubilized by soil microorganisms, among which mycorrhizal fungi are part (Sylvester-Bradley et al., 1982). The treatment of 9 g/dm³ of slag presented better conditions for the development of mycorrhizal fungi in soil and plant, being statistically superior to other treatments and that of control treatment.

This behavior can be explained by the solubilization of compounds beneficial to the development of microorganisms (Lapeyrie et al., 1991; Prado et al., 2003; 2003b), which in this dosage, worked more efficiently, bringing better development to fungi and indirectly can bring benefits to associated plants. If the slag can be solubilized over time by the action of microorganisms, the application over time may present good results in the production and development of plants.

Conclusion

The application of iron and nickel mining slag does not influence the development of rice in the first application. However, the application of 9 g/dm³ of soil of iron and nickel mining slag positively influenced mycorrhizal activity in roots and soil.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Brasil AN de M (2020). Anuário Mineral Brasileiro: Principais Substâncias Metálicas. Available in: https://www.gov.br/anm/ptbr/centrais-de-conteudo/publicacoes/serie-estatisticas-e-economiamineral/anuario-mineral-

brasileiro/copy_of_AMB2019_anobase2018_FINAL.pdf/view >. Access: 18 Jul. 2020. Carbone CAM, Edicarlos.

Corrêa JC, Bull LT, Moraes HM, Crusciol ACA (2009). Oxisol physical

attributes affected by surface application of flue dust, aqueous lime, sewage sludges and limestone. Revista Brasileira de Ciência do Solo 33(2):263-272.

- Faquin V (2005). Nutrição mineral de plantas. http://www.fisiologiavegetal.ufc.br/APOSTILA/NUTRICAO_MINERAL. pdf
- Fortes CA, Pinto JC, Neto AEF, Augusto RM, Antônio RE, Ronan MS (2008). Level of calcium and magnesium silicate in the yield of Marandu grass and Tanzania grass cultivated in one Quartzsandy Neosoil. Ciência e Agrotecnologia 32:267-274.
- Gerdemann JW, Nicolson TH (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society 46(2):235-244.
- Giovannetti M, Mosse (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. The New Phytologist 84(3):489-500.
- Hammer Ø (2018). Past 3.x-the Past of the Future. Oslo: Natural History Museum, University of Oslo. https://edisciplinas.usp.br/pluginfile.php/4407502/mod_resource/cont ent/1/past3manual.pdf
- Haridasan M (2000). Mineral nutrition of native cerrado plants. Revista Brasileira de Fisiologia Vegetal 12(1):54-64.
- INVAM (2018). International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi/ West Virginia University, Available in: <https://invam.wvu.edu/>. Accessed: 13 Oct. 2019.
- Johnson NC, Pfleger FL (1992). Vesicular-arbuscular mycorrhizae and cultural stresses. Mycorrhizae in Sustainable Agriculture 54:1-19
- Lapeyrie F, Ranger J, Vairelles D (1991). Phosphate-solubilizing activity of ectomycorrhizal fungi in vitro. Canadian Journal of Botany 69(2):342-346
- Moura JB, CabraL JSR (2019). Mycorrhizas in Central Savannahs: Cerrado and Caatinga. In: Pagano M, Lugo M (eds) Mycorrhizal Fungi in South America. Fungal Biology. Springer, Cham. https://doi.org/10.1007/978-3-030-**15**228-4_10
- Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55:158–161.

- Prado R de M, Corrêa MC de M, Cintra ACO, Natale W (2003a). Resposta de mudas de goiabeira à aplicação de escória de siderurgia como corretivo de acidez do solo. Revista Brasileira de Fruticultura 25:160-163.
- Prado R de M, Fernandes FM, Natale W (2003b). Efeito residual da escória de siderurgia como corretivo de acidez do solo na soqueira de cana-de-açúcar. Revista Brasileira de Ciência do Solo 27:287-296.
- Prado R, Fernandes FM; Natale W (2003). Residual effect of steel slag as soil acidity corrective in sugarcane soqueira. Revista Brasileira de Ciência do Solo 27(2):287-296.
- Silva F (2008). Assistat: Version 7.7 beta. Campina Grande-PB: DEAG-CTRN-Federal University of Campina Grande.
- Silveira A, Freitas S (2007). Soil Microbiota and Environmental Quality. Campinas: IAC. 312p.
- Souza BR, Moura JB, Oliveira TC, Ramos ML, Lopes Filho LC (2016). Arbuscular Mycorrhizal fungi as indicative of soil quality in conservation systems in the region of são Patrício valley, Goiás. International Journal of Current Research 8:43307-43311.
- Swift ML (2020). Prism GraphPad. https://www.graphpad.com/scientific-software/prism.
- Sylvester-Bradley R, Asakawa N, Torraca SL, Magalhães FMM, Oliveira LA, Pereira RM (1982). Quantitative survey of phosphate solubilizing microorganisms in the rhizosphere of forage grasses and legumes in the Amazon. Acta Amazonica 12(1):15-22.