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Original Article

Microbial-inoculated remineralizers as source of potassium and other nutrients[1]

Desempenho de remineralizadores inoculados com microrganismos como fonte de potássio e outros nutrientes

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HIGHLIGHTS:

Potassium aluminosilicate mineral weathering depends more on mineral structure than potassium content.
Olivine melilitite is a competitive natural source for supplying potassium and calcium to plants.
The BiomaPhos® inoculant enhances phosphate solubilization for plant uptake.

ABSTRACT: The consumption of potassium (K) fertilizers in Brazilian agriculture exceeds the national production of this input by more than ten times, indicating the need for alternatives to reduce imports of these products. The objective of this study was to evaluate the performance of the remineralizing rocks phonolite and olivine melilitite as sources of K and other nutrients, as well as the effects of inoculation with *Bacillus subtilis* and *Bacillus megaterium* on the solubilization of these rocks in the soil. A greenhouse experiment was conducted using a randomized block design, with four replications, in a factorial arrangement consisting of three K sources (potassium chloride, phonolite, and olivine melilitite), three rates (150, 300, and 600 kg ha^{-1} K_2O), with and without inoculant, and two controls without K (one with and one without inoculant). The experimental units consisted of pots containing 3 kg of soil and a strawberry plant grown for eight months. The treatment with olivine melilitite inoculated with rhizobacteria favored phosphate solubilization when applied the highest rate. Olivine melilitite remineralizer, alone or inoculated with rhizobacteria, increased K^+ and Ca^{2+} contents in the soil when applied at the intermediate and highest rates (300 and 600 kg ha^{-1} K_2O), highlighting this remineralizer as an alternative potassium source for plants.

Key words: rock dust, potassium fertilization, rhizobacteria

RESUMO: O consumo de fertilizante potássico pela agricultura brasileira supera em mais de dez vezes a produção nacional desse insumo, fato que indica a necessidade de alternativas para diminuir a dependência de sua importação. Diante disso, o objetivo deste trabalho foi avaliar o desempenho dos remineralizadores fonolito e olivina melilitito, como fonte de potássio e outros nutrientes, bem como o efeito da inoculação dessas rochas com as rizobactérias *Bacillus subtilis* e *Bacillus megaterium*, no incremento da solubilização destes materiais. O experimento foi realizado em casa de vegetação, sendo conduzido em delineamento blocos casualizados em arranjo fatorial, sendo três fontes (cloreto de potássio, fonolito e olivina melilitito), três doses (150, 300 e 600 kg ha^{-1} de K_2O) com a presença ou ausência da inoculação, além de dois controles, sem adição de potássio, um com e outro sem inoculação, aplicados em 4 repetições. Foram avaliados os teores de potássio e outros nutrientes no solo. O tratamento com olivina melilitito inoculado com rizobactérias favorece a solubilização de fosfato na maior dose. O remineralizador olivina melilitito, isolado ou inoculado com rizobactérias aumentou os teores de K^+ e Ca^{2+} no solo nas doses intermediária e máxima (300 e 600 kg ha^{-1} de K_2O), destacando esse remineralizador como fonte alternativa de potássio para as plantas.

Palavras-chave: pó de rocha, adubação potássica, rizobactéria

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Introduction

Agricultural production is a highly important economic activity in Brazil, but it faces several challenges related to soil fertility management and environmental sustainability (Rabel et al., 2018). This activity is predominantly carried out under conventional systems, with intensive use of agrochemicals and fast-release fertilizers. However, the efficiency of these fertilizers may be low, as a part of the nutrients is lost by leaching or runoff, affecting their utilization by plants (Resende et al., 2006; Fiedler et al., 2020).

Exclusive use of soluble fertilizers for providing nitrogen (N), phosphorus (P), and potassium (K) to plants can result in reduced availability and absorption of other nutrients, including calcium (Ca) and magnesium (Mg), and micronutrients, which can limit the production of demanding crops such as strawberry (Marschner, 2012).

Considering a scenario of scarcity of nutrient sources, high fertilizer costs, and environmental degradation, the use of soil remineralizers combined with rhizobacteria is an alternative to stimulate plant growth and enhance productivity (Anjanadevi et al., 2016; Sattar et al., 2019; Manning & Theodoro, 2020).

Despite several studies have been conducted on the use of silicate rock compounds for soil remineralization in cultivation systems, the efficiency of these materials in Brazil is still poorly proven (Silva et al., 2012; Gotz et al., 2019; Almeida et al., 2022; Brasil et al., 2023), especially regarding their interaction with solubilizing rhizobacteria and the recommended rates of these materials for different crops (Sattar et al., 2019; Silva et al., 2023).

In this context, the objective of this study was to evaluate the performance of the remineralizing rocks phonolite and olivine melilitite as sources of potassium and other nutrients, as well as the effects of inoculation with *Bacillus subtilis* and *Bacillus megaterium* on the solubilization of these rocks in the soil.

Material and Methods

The experiment was conducted from May to December 2021 in a greenhouse, in Lages, Santa Catarina (SC), Brazil, at the following geographical coordinates: 50° 18’ 10.80” W and 27° 47’ 31.82” S, with a geometric altitude of 920 m in the SIRGAS 2000 coordinate system.

The treatments were designed using a 3 × 3 × 2 factorial arrangement, consisting of a commercial soluble potassium source (KCl) and two powdered rocks (phonolite [Pho] and olivine melilitite [OM], three rates (150, 300, and 600 kg K_2O ha^{-1}), with and without inoculation with rhizobacteria, and two controls (one with and one without inoculation). A randomized block design with four replications was used. The experimental units consisted of polyethylene pots containing 3 kg of soil, each cultivated with a strawberry plant for eight months.

The rock powders were processed from materials collected in Lages and Palmeira, SC. Both rocks were subjected to granulometric and elemental chemical analysis for the quantification of elements/minerals (Table 1).

The inoculant used was BiomaPhos®, developed by the Brazilian Agricultural Research Corporation (EMBRAPA); it contains two strains of phosphate-solubilizing rhizobacteria species: *Bacillus subtilis* (CNPMS B2084) and *B. megaterium* (CNPMS B119). This inoculant is recommended for seed treatment or direct spraying to sowing furrows (Oliveira et al., 2020). The product has a fluid physical nature, with a density of 1.01 g mL^{-1} and contains rhizobacteria at concentration (CFU mL^{-1}) of 4×10^9 viable cells mL^{-1}, with a recommended rate of 100 mL ha^{-1} (Oliveira et al., 2020).

The soil used was a Nitossolo Bruno Distrófico típico, according to the Brazilian Soil Classification System (Santos et al., 2018), corresponding to a Humic Xantic Kandiudox (USDA, 2014). It was collected from the 0-20 cm layer in an area close to the BR-116 highway, in southern Lages. A soil sample was air-dried under greenhouse conditions, ground, passed through 2.0 mm mesh sieves to obtain the bulk soil fraction, and subjected to analysis to determine chemical attributes. The results obtained were: pH in water of 4.95 and pH SMP of 5.12, both determined by potentiometry; organic matter content of 27.8 g kg^{-1}; exchangeable Ca^{2+}, Mg^{2+}, and Al^{3+} contents, and potential acidity of 1.25, 0.42, 1.75, and 12.0 $cmol_c$ dm^{-3}, respectively; and P and K (Mehlich-1) contents of 3.27 and 85 mg dm^{-3}, respectively. The clay content, determined by the pipette method, was 608 g kg^{-1}. The analyses were carried out following the methodologies described by Tedesco et al. (1995) and Murphy & Riley (1962).

Soil preparation for the experiment consisted of application of dolomitic limestone to correct soil acidity to pH 5.5, homogenization of samples, and adjustment of soil moisture to 80% of field capacity, determined by the method of Casaroli & van Lier (2008). At this stage, the treatments with powdered rocks were mixed, and N, P, and micronutrients with soluble fertilizers were applied at 1.5 times the recommended rates in the liming and fertilization manual for growing strawberry crops in the states of Rio Grande do Sul and Santa Catarina (CQFS, 2016).

Table 1. Elemental chemical composition of the rocks

Rock	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	MnO	P_2O_5	Cu	Zn
	(%)									(ppm)	
Pho	53.6	22	3.96	0.88	0.11	9.63	5.71	0.17	0.02	15	146
OM	38.2	8.84	11.26	13.55	15.05	2.98	2.84	0.19	0.19	101	107

Toxic elements	Arsenic (As)		Cadmium (Cd)		Mercury (Hg)		Lead (Pb)	
	Real	Limit	Real	Limit	Real	Limit	Real	Limit
	(mg kg^{-1})							
Pho	4.7	15	<0.5	10	0.034	0.1	37	200
OM	2.8	15	<0.5	10	0.038	0.1	16	200

Pho - phonolite; OM - olivine melilitite. Table containing the results of the chemical analysis of the primary and secondary elements in the rocks, which was conducted at the ALS Global Laboratory in Vespasiano, Minas Gerais, Brazil, using ICP methods, with quantification of 13 primary elements and 52 secondary elements

The pots were weighed every 2 days and manually irrigated with deionized water, maintaining soil moisture close to 80% of the field capacity. The weights corresponding to plant growth in each pot and treatment were discounted for water replacement and moisture control.

The experiment was conducted from May to December 2021, using the strawberry cultivar Pircinque®, which is considered hardy and resistant to soil pathogens (Fagherazzi et al., 2021).

Soil samples were collected by quartering after removing plant roots. These samples were air-dried and ground to obtain the bulk soil. Exchangeable Ca^{2+}, Mg^{2+}, K^+, and Al^{3+} contents were determined using methods described by Tedesco et al. (1995). P and K contents were determined by Mehlich-1 solution; K contents were also extracted by extraction with 1 mol L^{-1} ammonium acetate solution at pH 7.0. P was quantified by colorimetry (Murphy & Riley, 1962), whereas K was quantified by flame photometry.

Statistical analysis of the results was performed using the program SISVAR 5.6 (Ferreira, 2019). The data were subjected to analysis of variance by the F test, data normality test (Shapiro Wilk), and regression analysis.

Results and Discussion

The analysis of variance showed significant patterns in soil K^+_{M1} and K^+_{AA} contents, which represent the available potassium extracted by Mehlich 1 and ammonium acetate extractors, respectively (Table 2). Additionally, patterns were found for P, Ca^{2+}, Al^{3+}, and pH $CaCl_2$ in relation to source and rate. However, Mg^{2+} did not show a significant correlation with the applied rate. Regarding inoculation with rhizobacteria, only K^+_{M1} and K^+_{AA} had significant variations, whereas P, Ca^{2+}, Mg^{2+}, Al^{3+}, and pH $CaCl_2$ remained consistent. The interaction between source and inoculation was significant for all these elements.

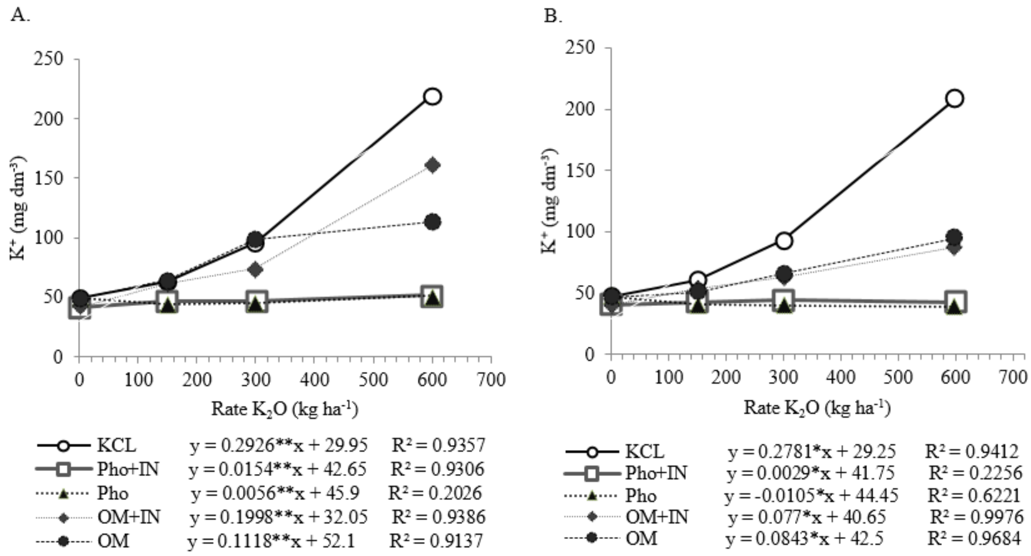
Exchangeable potassium contents in the soil increased as the rates of KCl and powdered olivine melilitite remineralizer (OM) were increased, fitting to linear models (Figure 1). K contents increased with increasing KCl rates and were significantly higher than those found with increasing OM rates, as KCl is a soluble fertilizer and OM is a silicate mineral that partially dissolves in the soil. Despite this partial dissolution, this material has potential for use as an alternative K source, as it increased K contents by more than 2 times when using the second and third rates. However, the Pho remineralizer did not increase K contents, indicating that it has a low potential for K solubilization in the soil.

The K^+ contents obtained by both extractors were similar, except for the source OM. The Mehlich 1 method resulted in higher K^+ values than ammonium acetate. This was also observed by Resende et al. (2006), who highlight that Mehlich-1 can overestimate K contents due to its acidic nature, which favors the dissolution of silicate compounds. However, the literature still lacks information on K^+ contents extracted by the acetate method from soils treated with remineralizers.

Table 2. Summary of analysis of variance for K^+_{M1}, K^+_{AA}, P, Ca^{2+}, Mg^{2+}, Al^{3+}, and pH $CaCl_2$ contents in the soil after strawberry cultivation

Sources of variation	K^+_{M1}	K^+_{AA}	P	Ca^{2+}	Mg^{2+}	Al^{3+}	pH $CaCl_2$ (0.01M)
Source (S)	**	**	**	**	*	**	**
Rate	**	**	**	**	ns	**	**
Inoculation (I)	**	**	*	ns	ns	ns	ns
Interaction (S × I)	**	**	**	**	*	**	**

K^+_{M1} - available potassium extracted by Mehlich 1; K^+_{AA} - available potassium extracted by ammonium acetate. ns - not significant; ** and * - significant at $p \leq 0.01$ and $p \leq 0.05$ by the F test, respectively



ns - not significant; ** and * - significant at $p \leq 0.01$ and $p \leq 0.05$ by the F test, respectively

Figure 1. Exchangeable K contents, extracted by Mehlich-1 (A) and ammonium acetate pH 7 (B), in a Humic Xantic Kandiudox soil as a function of KCl, phonolite (Pho), and olivine melilitite (OM) rates, without and with inoculation (IN) with *Bacillus subtilis and B. megaterium bacterium*

The lowest K^+ contents were found in the control, powdered phonolite (Pho), and Pho+Inoculation (Pho+IN) treatments, regardless of the extractor used, denoting that the solubilization or reactivity of phonolites is very low. Low K^+ release in the soil was also observed when Pho was inoculated with rhizobacteria, presenting similar K contents to those in the control. The trend line of K^+ content extracted by acetate showed a slight decrease depending on the applied rate. This absence of K^+ release by Pho may be due to the greater resistance of its minerals to weathering and the coarser grain size of phonolite compared to olivine melilitite. Cunha & Almeida (2021) also found a low response for this rock powder in the first crop (common bean), with a slight increase in the second crop (oats), indicating that it requires more time for K release, as also reported by Almeida et al. (2022).

Regarding inoculation with rhizobacteria, no consistent effect was observed, except for the highest OM rate, which increased K^+ contents. Microorganisms could contribute to the solubilization of potassium minerals through the production of organic acids (Meena et al., 2014). However, this lack of effect may be due to the composition of the tested remineralizing materials. Phonolites contain alkaline feldspars of the sanidine type, in addition to nepheline and clinopyroxenes of the aegirine type, while olivine melilitites predominantly consist of mellilites (approximately 40%), phlogopite (30%), clinopyroxenes (15%), olivine (10%), and opaque minerals (5%).

According to Uroz et al. (2009), redox reactions and production of chelating molecules and organic acids by bacteria can favor the weathering of rocks rich in K^+. A study about the effects of bacteria on the solubilization of phonolites in vitro showed a thirteen-fold increase in soluble K rate and a strong positive correlation between the medium acidity and K solubilization (Florentino et al., 2017). Some microorganisms may also favor the release of K^+ from remineralizers in the soil through indirect mechanisms, such as chelation of cations present in silicates and direct adhesion of mineralizing colonies to mineral surfaces (Sattar et al., 2019). However, there is still a lack of available information on the solubilization of potassium minerals by rhizobacteria.

Mancuso et al. (2014) applied a commercial phonolite rock powder (Ekosil®), containing K-feldspar, andesine, and nepheline, to a Oxisol in Brazil at a rate of 150 kg ha^{-1} of K_2O and found similar coffee yield to that found with application of KCl, in two crop seasons. However, they found that both K^+ sources resulted in lower crop yields when using a K_2O rate of 300 kg ha^{-1} compared to those obtained with a rate of 150 kg ha^{-1}, attributing this result to an excess supply of K^+ and to imbalances in relation to other nutrients. Furthermore, Tavares et al. (2018) evaluated the application of phonolite containing feldspar and feldspathoids (without further specifications on mineralogy) to an Oxisol cultivated with *Urochloa decumbens* and found no effect of this material on grass yield; however, the treatment with phonolite resulted in a greater residual effect of K^+ and Si in the soil compared to that with KCl.

According to Duarte et al. (2013), the application of olivine melilitite, syenite, and phonolite rocks as sources of K^+ can provide benefits, such as increases in soil K^+ availability and crop yield. This indicates the potential of this rocks as K sources for soil application, corroborating with the results found for the application of the OM remineralizer in the present work.

Field studies have also shown that the application of rocks containing micas (illite, muscovite, and/or biotite), combined with inoculation with bacterial strains of *Bacillus pasteurii*, *B. cereus*, and *B. mucilaginosus* increases K^+ absorption and the yields of wheat, pepper, and peanut crops (Supanjani et al., 2006; Youssef et al., 2010).

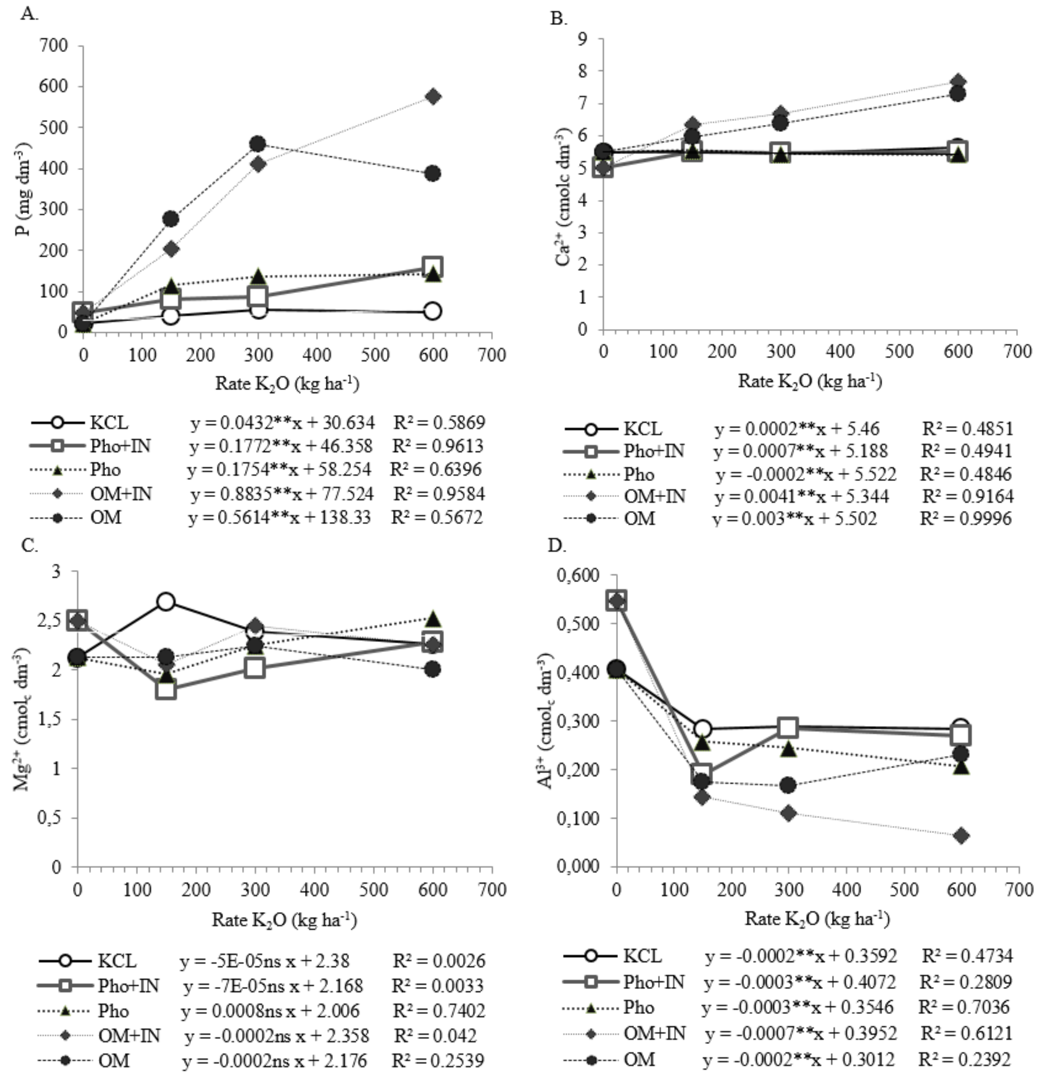
The conventional treatment used in the present work, consisted of KCl, liming, and phosphorus applications, resulted in a slight increase in soil P (Mehlich) content, significantly contrasting with the treatments with phonolite, known for its low P contents. The treatments with powdered rocks resulted in high P contents, especially those with OM; additionally, the inoculant used can solubilize phosphate and the evaluated remineralizing rocks contain P in their mineral matrix. Thus, it is important to consider the possibility that the acid Mehlich extractor can solubilize forms of P not available to plants. This is supported by previous studies and denotes the need for accurate evaluations when interpreting the effectiveness of soil remineralizers in agronomic contexts.

The addition of the OM remineralizer increased soil P availability, especially in the treatments that included bacterial inoculation (Figure 2A). However, this increase was more pronounced at the highest rate. The presence of apatites in the OM composition, combined with the action of organic acids produced by solubilizing bacteria (Uroz et al., 2009; Duarte et al., 2013), may have contributed to this increase. In this sense, the solubilizing effect of the BiomaPhos® product was confirmed, as shown by Milléo et al. (2023), who reported increased P uptake in soils cultivated with corn and soybeans.

Considering that all treatments were subjected to liming for soil acidity correction, Ca^{2+}, Mg^{2+}, and Al^{3+} contents were affected by the application of the remineralizing powdered rocks, as shown in Figures 2B, C, and D, respectively. Ca^{2+} and Mg^{2+} contents increased from 1.25 and 0.42 to values ranging between 5 and 5.5 and between 2 and 2.5 $cmol_c$ dm^{-3}, respectively, in all treatments. However, little change in their contents was found when using KCl and Pho as K sources, denoting the limited effectiveness of phonolite in supplying Ca^{2+} and Mg^{2+} to the soil. However, increasing OM rates, applied alone or combined with inoculation, resulted in a significant increase in Ca contents, with contents surpassing those in the treatments with Pho. This denotes the effectiveness of OM in making Ca available.

The OM remineralizer did not increase Mg^{2+} contents, despite the presence of olivine, pyroxenes, and phlogopite in its mineral composition. This indicates that, although OM is rich in Mg, there may be soil factors preventing its effective release; thus, further investigation is needed.

Al^{3+} contents decreased from 1.75 to values between 0.5 and 0.55 $cmol_c$ dm^{-3} after liming, as expected. However, the OM+IN



In the regression test: ns, **, and *, respectively, indicate non-significance and significance at $p \leq 0.01$ and $p \leq 0.05$

Figure 2. Extractable P (Mehlich 1) and exchangeable Ca^{2+}, Mg^{2+} and Al^{3+} contents in a Humic Xantic Kandiudox soil as a function of KCl, phonolite (Pho), and olivine melilitite (OM) rates, with and without inoculation (IN) with *Bacillus subtilis and B. megaterium* bacterium

treatment resulted in additional reducion, indicating positive interaction between the remineralizer and inoculation. This result indicates that combining remineralizers with inoculants might be an effective approach for soil management.

The results of pH evaluations (preferably in a 0.01M $CaCl_2$ solution) are essential to explain the observed increases in Ca^{2+} and decreases in Al^{3+} (Figure 3). The different pH levels found for the evaluated treatments can be explained by differences in the chemical and mineralogical properties of the studied rocks, as the ultramafic rock is an alkaline igneous rock, while phlogopite is a metamorphic rock. These intrinsic characteristics can affect the ability of microorganisms to solubilize minerals, resulting in variations in soil pH.

The results found under greenhouse conditions indicated that the application of olivine melilitite remineralizer provides agronomic and environmental benefits, as it releases nutrients slowly, potentially reducing leaching and environmental impact compared to synthetic fertilizers. Its combination with bacterial inoculants might enhance these benefits. However, these findings should be validated under field conditions, considering environmental variables and farming practices.

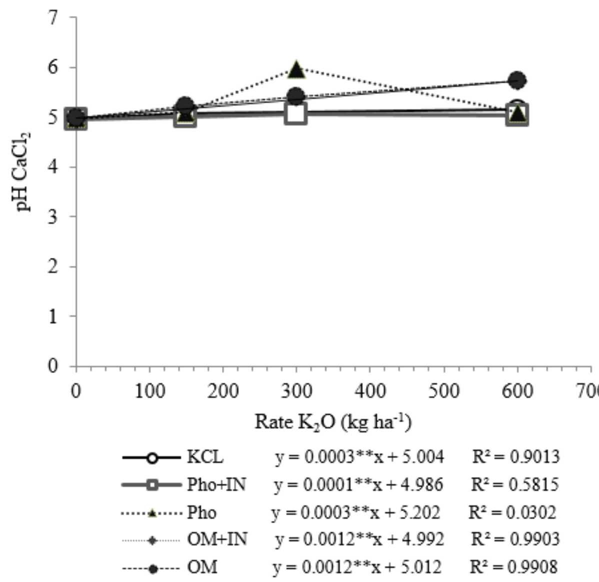


Figure 3. Contents of exchangeable pH ($CaCl_2$) in a Humic Xantic Kandiudox soil as a function of KCl, phonolite (Pho), and olivine melilitite (OM) rates, with and without inoculation (IN) with *Bacillus subtilis and B. megaterium* bacterium

Conclusions

1. The remineralizing rock olivine melilite increases exchangeable K^+ and Ca^{2+} contents in the soil and their availability to plants, mainly when inoculated with the rhizobacteria *Bacillus Subtilis* and *Bacillus Megaterium*. However, phonolite rock does not have this effect.

2. Olivine melilitite may have potential to release phosphorus in the soil, increasing its availability to plants. However, this contribution might not be as significant as initially thought. The source of this released phosphorus can be attributed to minerals such as apatite.

3. The positive interaction between olivine melilitite and specific strains of rhizobacteria increases soil nutrient contents, contrasting with the limited impact of phonolite. This highlights the importance of selecting the right combination of powdered rock and bacteria for an optimal soil remineralization.

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