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Algal biomass from wastewater: soil phosphorus bioavailability and plants productivity

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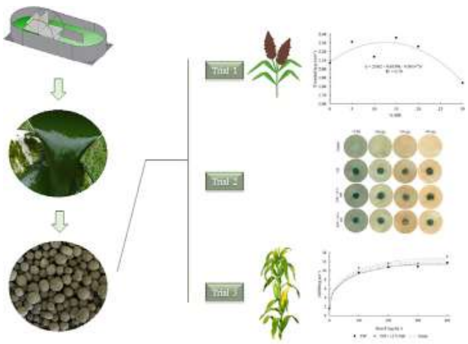
HIGHLIGHTS

- TSP fertilizer mixed with MB produced an environmentally friendly fertilizer;
- The addition of 12% of MB in the TSP had a positive effect on plant growth;
- The addition of 30% of MB in the TSP impaired phosphorus diffusion.

GRAPHICAL ABSTRACT



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ABSTRACT

The cultivation of microalgae in wastewater allows to obtain a biomass concentrated in nutrients and organic material. This biomass added to phosphate fertilizers can promote a slow release of the nutrient and consequently a higher absorption of phosphorus (P). The objective of this study was to investigate P uptake by plants subjected to triple superphosphate (TSP) fertilization, added with microalgae biomass (MB) grown in wastewater. TSP was added with different MB proportions in order to verify if there would be a different behaviour in P release for millet (*Pennisetum glaucum* L.) plants. With the proportion that maximized P accumulation in plants, a second experiment was carried out to investigate whether MB exerts influence of P diffusion in the soil. Finally, a third trial was conducted in a greenhouse, where TSP and TSP + 12% MB were applied to the soil under different phosphorus doses in corn (*Zea mays* L.). The proportion of MB in TSP that maximized the increase of P content and concentration in plants was approximately 12% MB. From this proportion, a reduction in the values of the variables analysed in the plant with the increase of the proportion of MB in the biofertilizer was observed. Similar behaviour was observed when evaluating P diffusion in sandy and clay soils. Fertilizers TSP and TSP + 12% MB showed no difference in P diffusion in the soil, while the ratio of 30% MB clearly impaired P diffusion. In a greenhouse, the P content presented significant difference for the tests carried out with TSP and TSP + 12% MB fertilizer, in which the latter provided higher P recovery rate by plants. Therefore, MB added to TSP had a positive influence on plant development and its P recovery capacity when applied in a proportion of 12% MB to the fertilizer mass.

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Nomenclature

Al	Aluminium
B	Boron
Ca	Calcium
mMS	Dry matter mass
EEFs	Enhanced efficiency fertilizers
EFFs	Environmentally friendly fertilizers
HRAPs	High-rate algal ponds
HCl	Hydrochloric acid
H + Al	Hydrogen plus aluminum
pH	Hydrogen potential
Fe	Iron
Mg	Magnesium
Mn	Manganese
MB	Microalgae biomass
N	Nitrogen
NT	Nutrient concentration
NC	Nutrient content
OM	Organic Matter
P	Phosphorus
K	Potassium
Effective CEC	Effective cation exchange capacity
DBC	Randomised blocks
P_{rem}	Remaining phosphorus
SEM	Scanning electron microscope
SDMM	Shoot dry mass matter
Na	Sodium
NaOH	Sodium Hidroxide
S	Sulfur
Potential CEC	Potential cation exchange capacity
TSP	Triple superphosphate
Zn	Zinc

1. Introduction

In many situations, the soil is unable to meet all crop nutrient requirements, which requires the use of fertilizers to achieve higher agricultural productivity. Among the most used fertilizers are nitrogen, phosphate and potassium, which contain the main macronutrients for plants. The production of synthetic fertilizers contributes significantly to different impact categories, such as acidification potential, eutrophication potential and global warming potential (Murphy et al., 2014).

Among the main macronutrients used in agricultural cultivation, P stands out as presenting a peculiar management difficulty due to its rapid adsorption to soil particles. It is estimated that when a soluble source of P is applied as fertilizer in a soil, >90% of the element is adsorbed in the first hour of contact and is unavailable to plants (Novais et al., 2007).

Despite its importance, the efficiency of P use does not reach 20%, and the remainder ends in wastewater or reaches surface water through runoff from cultivated fields (Solovchenko et al., 2016). Due to these losses, which increase the cost, waste of energy and pollution of the environment (Chen et al., 2018), it is necessary to adopt technologies that increase the efficiency of the use of phosphate sources resulting in reduced fertilizer costs and lower use of non-renewable natural resources.

In the case of phosphate fertilizer, efforts are made to increase P bioavailability after its application to soil. For this purpose, technologies have been adopted and added to fertilizers, aiming to increase their efficiency. There are different types of fertilizer technologies, which have various names, such as slow-release fertilizers-compounds that have low water solubility (Chalk et al., 2015); controlled-release fertilizers (González et al., 2015), which consist of coating highly soluble granules with water-insoluble material (Chalk et al., 2015) and “enhanced efficiency fertilizers” (EEFs), a junction of groups called slow-release and controlled-release materials (Timilsena et al., 2015). Generally, all these groups can be called environmentally friendly fertilizers (EFFs), which is a name for fertilizers that aim to reduce environmental pollution by nutrient losses by slowing or even controlling the release of nutrients into the soil (Chen et al., 2018).

Among these technologies, the use of materials of synthetic or organic origin, mixed with the fertilizer mass, which reduce the speed of nutrient release to the soil, can be highlighted. The goal is to promote a synchronisation between nutrient release and plant demand, thus preventing the phosphorus provided by the fertilizer from being quickly adsorbed to the soil particle. The increase of organic material to fertilizers has been a recent target of research (Purnomo et al., 2018; Sharkawi et al. 2018).

Soil organic matter can correlate with P adsorption both positively, mainly due to the anionic character of organic matter, and negatively, blocking P adsorption sites in soil (Novais et al., 2007). Therefore, it should be stated that each material, whether animal waste, biochar or any biomass rich in organic material may, by virtue of its constitution, behave differently when added to the fertilizer mass.

Waste, especially biomass, is a large reservoir of nutrients that can be recovered through different technologies and used to manufacture fertilizers. In this aspect, a subject that has been widely studied is the cultivation of microalgae due to the countless possibilities of using this biomass in the production of biofuels, biofertilizer and animal feed (Javed et al., 2019; Zhang et al., 2019). Moreover, when this cultivation is carried out in wastewater, this practice allows the recovery of N, P and, therefore, an obtaining of a nutrient-concentrated algae biomass (Cai et al. 2013) and organic carbon (Costa et al., 2016). This practice, besides promoting the wastewater treatment, allows the obtaining of a MB with different potential for use. However, when recovered from wastewater by chemical or biological processes, P is often present in a form that does not meet specifications for agricultural use (Solovchenko et al., 2016), as it is in its organic form.

In this context, we highlight the possibility of microalgae biomass grown in wastewater to be added to phosphate fertilizers, in order to increase the efficiency of P uptake by plants. Although several works related to microalgae biorefinery cite the route of biofertilizers as potentially advantageous, there are few published studies referring to this subject. Favourable results regarding plant growth have already been found in the literature when applying moist microalgae biomass to the soil (Castro et al., 2017; Marks et al., 2017). However, data proving the increased efficiency of phosphate fertilizer in plants by the addition of dry microalgae biomass have not yet been obtained. Therefore, the objective of this study was to investigate, through different experiments, the bioavailability of P in soil, with the fertilizer source being TSP added from MB grown in wastewater.

2. Materials and methods

2.1. Algal biomass production

The algal biomass was produced at the experimental wastewater treatment and biomass production plant of the Sanitation and Environmental Engineering Laboratory of the Federal University of Viçosa (UFV), Minas Gerais, Brazil (UTM co-ordinates 722924 E, 7702003 S, zone 23 K). The municipality of Viçosa, with an average altitude of 648 m above sea level, is characterised by an average annual rainfall of approximately 1221 mm and an average annual temperature ranging between 19 °C and 20 °C. Relative humidity is, on average, 81%. The local climate, according to the Köppen classification, is Cwa type-tropical in altitude with hot and rainy summers and cold and dry winters (Rocha et al., 2012).

The microalgae biomass was cultivated as a by-product of the wastewater treatment of a meat processing industry in high-rate algal ponds (HRAPs). The main activity of this industry is the production of sausages (salami and hams, among others) and the production of shredded desalted codfish. The wastewater of industrial origin is generated in the various stages of the production process, especially in the discarding of the cooking and cooling tanks of sausages and of the desalting tanks of cod and also the washing of the floors and equipment at the end of the production processes.

As an initial evaluation, the wastewater characterization should be carried out aiming at obtaining a biomass suitable for the intended use. Therefore, in this study, a nitrogen rich effluent from a primary flotation unit was chosen. Its characteristics are better described in previous studies (Castro et al. 2017; Souza et al. 2019).

The pilot scale HRAPs used for biomass production have the following characteristics: width = 1.28 m, length = 2.86 m, total depth = 0.30 m, useful depth = 0.30 m, surface area = 3.30 m^2 and useful volume = 1.00 m^3 (Fig. 1). The HRAPs were made of fibreglass and the pedals of stainless steel, with six blades. During operation, pedalwheels were powered by a 1 hp electric motor. The rotation was reduced by a reducer coupled to the motor and controlled by inverter frequency (series WEG CFW-08), which ensured a liquid velocity between 0.10 m s^{-1} and 0.15 m s^{-1}. Similar values were used in different research with HRAPs (Picot et al., 1991; Park et al., 2011) and ensured the necessary revolving.

A CO_2 injection system was also used in order to control the pH of the pond, maintaining it between 7 and 8. The biomass was produced in a 14 days' batch in October 2017. In the operation, 40% of inoculum was used in relation to the total volume of the pond, which consisted of microalgae biomass previously adapted to the conditions of the wastewater.

At the end of the operation, the biomass was concentrated by chemical flocculation, with the addition of 50% NaOH solution (m v^{-1}) to raise the pH to 12. Then, the wastewater in the HRAP was stirred with the aid of a plastic container for 3 min and the sedimentation performed in the HRAP itself during the night. The concentrated biomass at the bottom of the HRAP was collected manually using plastic containers after the wastewater disposal. After collection, the pH of the concentrated biomass was neutralised with the addition of 1:1 HCl solution (v v^{-1}). Then the biomass was concentrated by a high speed refrigerated centrifuge, at 10,000 rpm for 3 min, and finally dried in a forced circulation oven at 60 °C. The characterisation of algal biomass obtained after drying is described in Table 1.

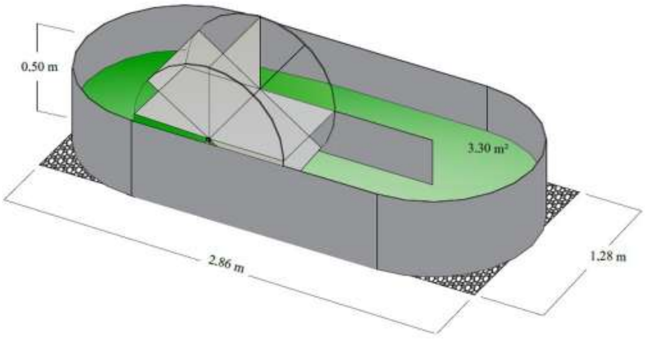

Fig. 1. HRAP scheme used for biomass production.

2.2. Biofertilizer production

The production of fertilizer granules was carried out in a granulator plate, whose working principle is based on the formation of granules obtained from continuously fed powder material with the addition of liquid (H_2O). The rotary movement of the plate and its inclination, at an angle ranging from 45 and 60°, enables the formation of the granules. Fertilizers consisting of TSP were produced with the addition of different MB proportions.

The granules were initially separated according to their particle size using 2 to 3 mm sieves. In each experiment, the granules were previously weighed to obtain minimal variability between replicates. Finally, fertilizers were visually analysed using a JSM-6010LA scanning electron microscope (SEM).

2.3. Experimental design

There were three trials carried out to evaluate the influence of MB on soil phosphorus adsorption/desorption and as a source of nutrients for plants. The first trial was conducted in a plant growth chamber. TSP was added with different MB proportions in order to verify if there would be a different behaviour in the phosphorus release to plants. The second test was performed to evaluate the phosphorus adsorption/desorption in TSP with 12% (ratio that maximized dry matter mass production (mMS) from the previous experiment) and 30% (maximum ratio tested in the previous experiment, tended to decrease phosphorus release to soil) MB added. And the third trial was conducted in a greenhouse in which TSP and TSP + 12% MB were applied to the soil under different doses (0, 100, 200, 300 and 400 mg dm^{-3}P).

In all trials, the 2 dm^3 soil samples were placed in plastic bags for liming by the Al_3^+ neutralisation method and Ca_2^+ and Mg_2^+ elevation (Alvarez 1999), with commercial calcitic limestone (Ca:Mg ratio 4:1). Then, the soil moisture was adjusted to 60% of field capacity, determined by the porous plate extractor 10 kPa pressure (Reichardt, 1988). After 21 days of incubation, the soil was air dried, deforested and passed through a 2 mm sieve. Chemical and physical soil analyses were performed according to Nelson and Sommers (1982), EMBRAPA (2009) and Almeida et al. (2012) Table 2.

2.3.1. Phosphorus absorption under different BM Concentrations—Trial 1

To set up this experiment, subsurface samples (20–40 cm) from a very clayey Red-Yellow Latosol, collected in the region of Viçosa, MG, were used. The collected material was air dried, soil clusters were breaked up and passed through a 2.0 mm sieve to obtain air dried soil.

The soil samples were weighed (200 cm^3) and placed in 500 dm^3 pots. Fertilization with 300 mg dm^{-3} of P was made with TSP at MB proportions of 5, 10, 15, 20 and 30% (w/w). TSP without the addition of biomass was used as a control. The experimental design was randomised blocks (DBC), with six treatments and five replications, totalling 30 experimental units.

A basic fertilization with N, K, S and micronutrients was performed according to Novais et al. (1991) on all soil volume before

Table 1
Chemical characterization of algal biomass after drying.

N	P	K	C	H	O	Ca	Mg	S	Na
(%)									
5.59	1.18	0.14	38.39	6.51	28.55	9.76	0.36	0.95	0.83
Zn	Fe	Mn	Cu	B	Cd	Pb	Cr	Ni	
(ppm)									
192.70	23,415	158.20	37.00	2.89	ND	ND	23.20	15.10	

ND = Not detectable.

Table 2
Chemical and physical properties of the soil – Trial 1.

Parameter	Unit	Value
Sand$^{1/}$	%	35
Silt$^{1/}$	%	10
Clay$^{1/}$	%	55
OM$^{2/}$	%	1.52
pH – $H_2O^{3/}$	–	5.73
H + Al$^{3+6/}$	cmol$_c$ dm^{-3}	2.50
Ca$^{2+4/}$	cmol$_c$ dm^{-3}	1.38
Mg$^{2+4/}$	cmol$_c$ dm^{-3}	0.28
K$^{+5/}$	mg dm^{-3}	13.00
P$^{5/}$	mg dm^{-3}	1.40
Base saturation	%	40.30
Sum of bases	cmol$_c$ dm^{-3}	1.69
$P^{5/}_{rem}$	mg L^{-1}	11.1
S$^{7/}$	mg dm^{-3}	2.50
Zn$^{7/}$	mg dm^{-3}	0.76
Effective CEC	cmol$_c$ dm^{-3}	1.69
Potential CEC	cmol$_c$ dm^{-3}	4.19

^{1}Pipette method (Almeida et al., 2012); ^{2}Nelson and Somers (1982); $^{3/}$Soil to water ratio 1:2.5 (EMBRAPA, 2009); $^{4/}$ Potassium chloride extraction method 1 mol L^{-1} (EMBRAPA, 2009); $^{5/}$Mehlich^{-1} Extractor (EMBRAPA, 2009); $^{6/}$Calcium acetate extraction method 0,5 mol L^{-1} - pH 7.0 (EMBRAPA, 2009); $^{7/}$Monocalcium phosphate in acetic acid extraction method (EMBRAPA, 2009).

Table 3
Chemical and physical properties of the clay soil – Trial 2.

Parameter	**Unit**	**Value**
Sand$^{1/}$	%	19.30
Silt$^{1/}$	%	0.80
Clay$^{1/}$	%	79.90
OM$^{2/}$	%	10.60
pH – $H_2O^{3/}$	–	6.00
H + Al$^{3+6/}$	cmol$_c$ dm^{-3}	1.30
Ca$^{2+4/}$	cmol$_c$ dm^{-3}	1.23
Mg$^{2+4/}$	cmol$_c$ dm^{-3}	0.35
K$^{+5/}$	mg dm^{-3}	10.00
P$^{5/}$	mg dm^{-3}	0.40
Base saturation	%	55.30
Sum of bases	cmol$_c$ dm^{-3}	1.61
$P^{5/}_{rem}$	mg L^{-1}	10.00
S$^{7/}$	mg dm^{-3}	10.50
Zn$^{7/}$	mg dm^{-3}	0.44
Effective CEC	cmol$_c$ dm^{-3}	1.61
Potential CEC	cmol$_c$ dm^{-3}	2.91

$^{1/}$Pipette method (Almeida et al., 2012); $^{2/}$Nelson and Somers (1982); $^{3/}$Soil to water ratio 1:2.5 (EMBRAPA, 2009); $^{4/}$ Potassium chloride extraction method 1 mol L^{-1} (EMBRAPA, 2009); $^{5/}$Mehlich^{-1} Extractor (EMBRAPA, 2009); $^{6/}$Calcium acetate extraction method 0,5 mol L^{-1} - pH 7.0 (EMBRAPA, 2009); $^{7/}$Monocalcium phosphate in acetic acid extraction method (EMBRAPA, 2009).

planting. Then four pre-germinated millet seeds (*Pennisetum glaucum* L.), variety BRS 1501, were sown.

Pot irrigation was performed daily, keeping soil moisture at 70% of field capacity. The assay was conducted in a controlled environment (temperature ± 25 °C) for 30 days.

At the end of the experiment, the plants were sectioned about 0.5 cm from the ground, placed in paper bags and placed in a forced air circulation greenhouse at 65 °C, to determine the shoot dry mass matter (SDMM). Then, the samples were ground and, after nitroperchloric digestion, the P concentration in the extracts were determined according to EMBRAPA (2009). The nutrient content in the plant was calculated by multiplying the nutrient concentration by the respective dry matter weight values ($NC = mMS \times NT/1000$, where NC = nutrient content (mg pot^{-1}); DM = weight dry matter (g pot^{-1}) and NT = nutrient concentration (g kg^{-1}).

2.3.2. Phosphorus diffusion Experiment—Trial 2

In this experiment, in addition to fertilizer sources, two soil types were tested, as soil texture is also an important factor in influencing phosphorus diffusion. Soils of two different textures were used: clay and sand.

The chemical and physical properties of the clay soil used in the experiment after acidity correction were shown in Table 3.

The chemical and physical properties of the sandy soil used in the experiment after acidity correction were shown in Table 4.

Petri dishes of 8 cm in diameter by 1 cm high were filled with the same amount of soil (approximately 80 g), and a fertilizer granule was placed in the centre of them. In all, 24 experimental units were conducted. The design was randomised blocks, with three blocks, two soil textures and three fertilizer sources (TSP, TSP + 12 % MB, TSP + 30 % MB), besides the control treatment (without application of fertilizer sources). The plates were incubated for 30 days, with soil moisture maintained at field capacity and room temperature (~24 °C).

Table 4
Chemical and physical properties of the sandy soil – Trial 2.

Parameter	**Unit**	**Value**
Sand$^{1/}$	%	71.40
Silt$^{1/}$	%	1.40
Clay$^{1/}$	%	27.20
OM$^{2/}$	%	14.60
pH – $H_2O^{3/}$	–	6.30
H + Al$^{3+6/}$	cmol$_c$ dm^{-3}	0.50
Ca$^{2+4/}$	cmol$_c$ dm^{-3}	2.94
Mg$^{2+4/}$	cmol$_c$ dm^{-3}	0.35
K$^{+5/}$	mg dm^{-3}	2.00
P$^{5/}$	mg dm^{-3}	0.40
Base saturation	%	87.80
Sum of bases	cmol$_c$ dm^{-3}	1.61
$P^{5/}_{rem}$	mg L^{-1}	30.00
S$^{7/}$	mg dm^{-3}	33.60
Zn$^{7/}$	mg dm^{-3}	0.70
Effective CEC	cmol$_c$ dm^{-3}	3.61
Potential CEC	cmol$_c$ dm^{-3}	4.11

^{1}Pipette method (Almeida et al., 2012); ^{2}Nelson and Sommers (1982); ^{3}Soil to water ratio 1:2.5 (EMBRAPA, 2009); ^{4}Potassium chloride extraction method 1 mol L^{-1} (EMBRAPA, 2009); ^{5}Mehlich^{-1} Extractor (EMBRAPA, 2009); ^{6}Calcium acetate extraction method 0,5 mol L^{-1} – pH 7.0 (EMBRAPA, 2009); ^{7}Monocalcium phosphate in acetic acid extraction method (EMBRAPA, 2009).

Evaluations were performed at 1, 10, 20 and 30 days after incubation. On each day of evaluation, the technique of filter paper impregnated with Fe oxide to evaluate the diffusion radius of P was used, after reaction with the malachite green dye, according to methodology proposed by Degryse and McLaughlin (2014). After the filter paper has completely dried, the papers were scanned and analysed using imaging software (GNU Image Manipulation Program, v. 2.6.11, Free Software Foundation, Boston, MA).

2.3.3. Greenhouse Experiment—Trial 3

The experiment was conducted in a greenhouse in pots containing 2.5 dm3 of clay soil. The evaluated fertilizers were: TSP and TSP + 12% MB. The doses used were 0, 100, 200, 300 and 400 mg dm−3 of P. P fertilization occurred in a localised manner, using a cylinder as the basis for the arrangement of the granules in the centre of each pot. For this, the fertilizers were mixed to a volume corresponding to 20% of the soil of the pot. The soil (clay soil previously characterised in the phosphorus diffusion experiment, session 2.3.2) was moistened and six seeds of corn (Zea mays L.) were sown per pot. After germination, thinning was done to maintain two more uniform plants per pot.

Water was supplied daily to maintain soil moisture between 70 and 90% of field capacity. Coverage fertilization was divided into three plots, performed at 6, 13 and 20 days, totalling 200 mg dm-3N (urea and ammonium sulphate); 150 mg dm−3 K (KCl); 0.81 mg dm-3B (Boric acid); 50 mg dm−3 of S (ammonium sulphate); 3.0 mg dm−3 Zn (ZnSO4·7H2O); 1.3 mg dm−3 Cu (CuSO4·5H2O); 3.6 mg dm−3 of Mn (MnSO4·H2O) and 0.15 mg dm−3 of Mo ((NH4)6Mo7O24·4H2O).

At the end of the experiment (30 days), the plants were sectioned at about 0.5 cm from the soil, placed in paper bags and placed in a forced air oven at 65 °C to determine the SDMM. Then, the samples were ground, and after nitroperchloric digestion, the P concentration in the extracts determined according to EMBRAPA (2009).

The nutrient content in the plant was calculated by multiplying the nutrient concentration by the respective dry matter weight, according item 2.3.1. The growth rate of the trend line corresponding to the content, plotted in mg pot^{-1} × mg pot^{-1}, was analysed, and the P recovery rate was then obtained.

2.4. Data analysis

Data regarding P concentration and content in the plants of the first trial were submitted to regression analysis with the aid of the software R© version 3.5.3 (R Core Team, 2016). The averages for the diffusion radii of the second test were subjected to analysis of variance and the means were compared by Tukey test at 5% probability of error. Finally, the data regarding mMS and P content of the last test were submitted to regression analysis with the aid of the software R© version 3.5.3 (R Core Team, 2016). After regression analysis, the models were submitted to the model identity test according to Regazzi (1996) in order to verify if the models are statistically different.

3. Results

3.1. Characterization of biofertilizer

The MB in the fertilizer influenced the availability and absorption of P by millet plants. The results regarding the content and concentration of P in the shoots varied significantly ($p < 0.05$) with the increase of MB proportion following the quadratic regression model (Fig. 2 and Fig. 3). In the proportions of 12.42% and 12.82% of MB, the concentration and content of P, in the aerial part of the plant, reached the maximum (5.42 g kg^{-1} and 2.31 μg pot^{-1}, respectively). Up to these proportions, the organic material present in the fertilizer granule contributed to the availability of P in the soil solution. Above that, there was a reduction in this availability. In treatment without MB, P content was 10.39% lower than that found for MB that maximizes absorption. For the maximum proportion of MB tested (30%), the content was 20.35% lower when compared to the one that provided the highest accumulation of P.

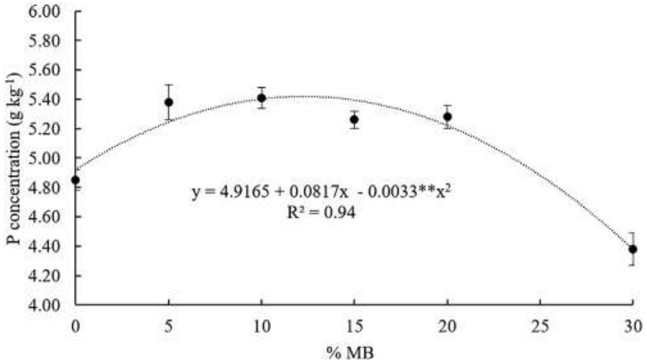

Fig. 2. P concentration of aerial part of millet (*Pennisetum Glaucum* L.) plants (g kg^{-1}), as a function of MB (%) doses with phosphate fertilizer (TSP). Error bars indicate standard deviation. ** significant at 5% probability by *t*-test.

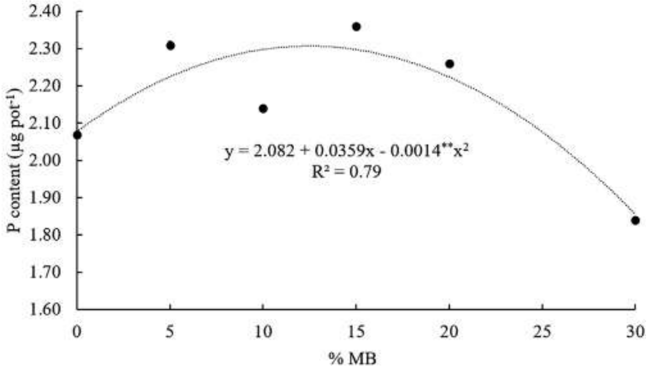

Fig. 3. P (μg pot^{-1}) content of millet (Pennisetum glaucum L.) shoot, as a function of MB (%) doses with phosphate fertilizer (TSP). Error bars indicate standard deviation. **significant at 5% probability by *t*-test.

Some factors may be associated with different P availability with an increasing MB ratio. One of these factors is that the organic material may have formed a "coating" on the fertilizer granule acting as a physical barrier to nutrient release. As the proportion of algal biomass in the fertilizer increased, the less visible P crystals in the fertilizer became (Fig. 4). Fig. 4a, corresponding to the SEM image obtained for the SPT, shows the presence of larger and more visible crystals when compared to the other images. Following images 4b (MB5), 4c (MB10), 4d (MB15) and 4e (MB20), it is still possible to notice the presence of crystals, but they are smaller and more camouflaged in the fertilizer mass. Finally, in Fig. 4f, it is not possible to observe them.

3.2. Phosphorus diffusion

In clay soil, P diffusion reached a smaller radius (up to 10.17 mm) when compared to sandy soil, which reached a maximum value of 12.02 mm (Figs. 5, 6, 7 and 8). The values obtained for TSP in clay soil were approximate to those obtained by Lustosa Filho et al. (2019), who observed approximate values at 8.20 mm for TSP applied in clay soil.

It was observed that there was no statistically significant difference of TSP and TSP + 12% MB ($p > 0.05$) fertilizer on any evaluation day for both soil textures. Fertilizer TSP + 30% MB was different from the others on all evaluation days, except for the 30th day of

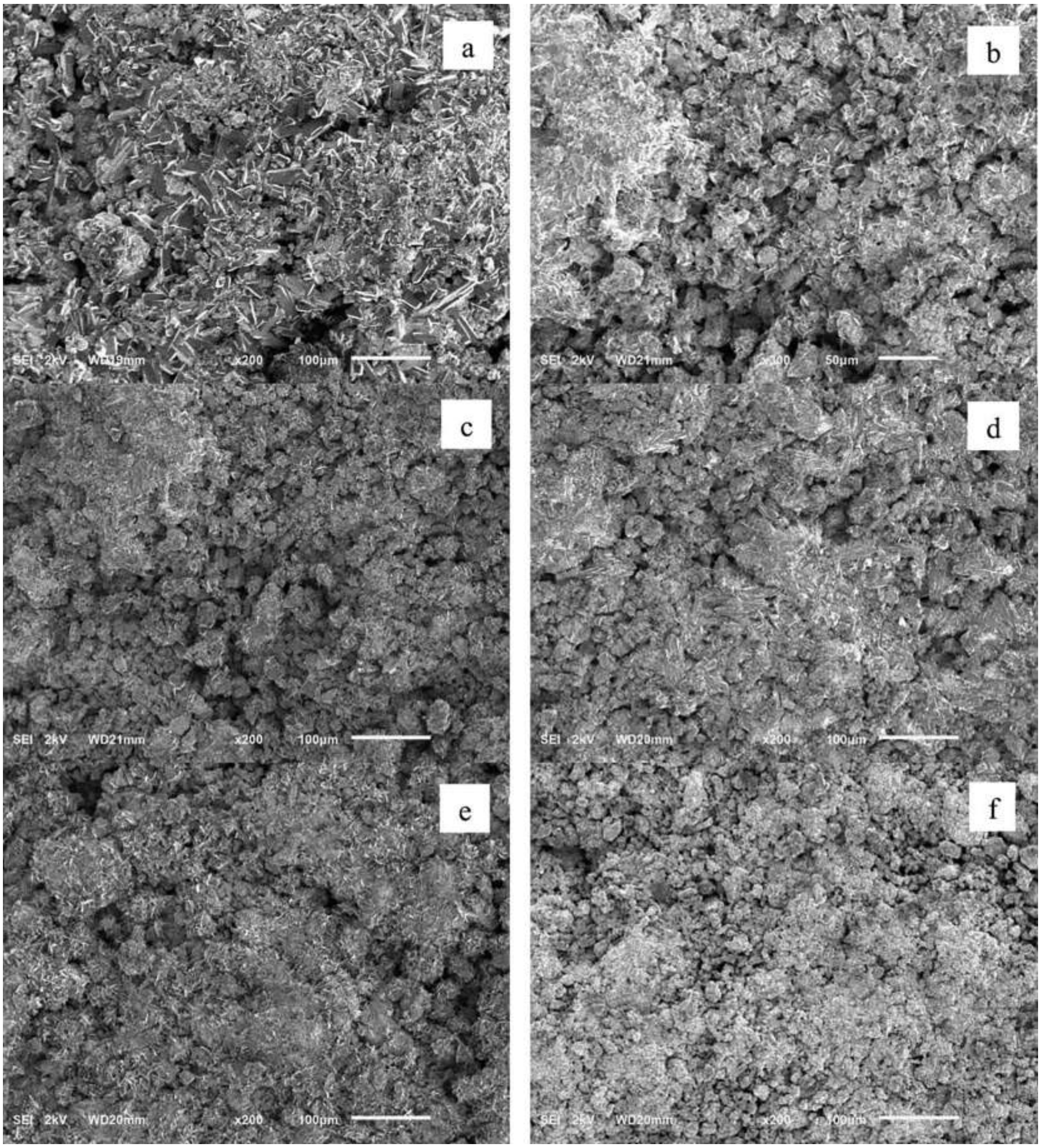

Fig. 4. SEM image of granules of sectioned fertilizers. a) TSP; b) TSP + MB5; c) TSP + MB10; d) TSP + MB15; e) TSP + MB20 and f) TSP + MB30.

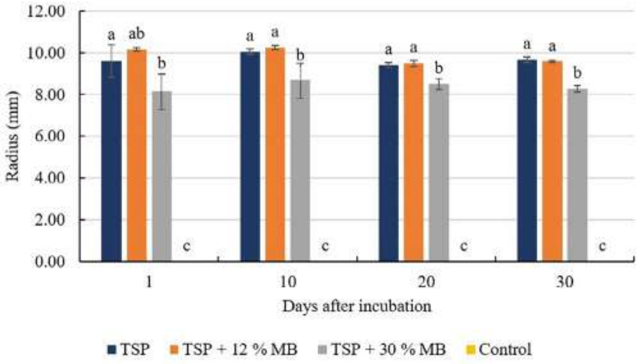

Fig. 5. P diffusion radius in clay soil. * Means followed by the same letter do not differ significantly from each other by the Tukey test at 5% probability.

the sandy soil, which, despite having a relatively smaller diffusion radius than the others, did not present statistical difference, probably due to greater variability of data (σ = ± 0.60).

The results are consistent with those obtained in the first trial, in which 30% MB treatment delayed P release in the soil. Chojnacka et al. (2019) mention that the main advantage of organo-mineral fertilizers is the slow release of nutrients due to the competition mechanism of organic binders and phosphorus in the soil adsorption sites. However, slow release is an advantage when it promotes synchronisation between nutrient availability and plant uptake. Such a result was not obtained when mixing proportions >12% MB in TSP for this study.

Within each treatment, a tendency of P diffusion stabilisation was observed from day one, as observed in the initial radius of the P diffusion zone (Figs. 6 and 8), which can be attributed to saturation of sorption sites they limit the movement of P in soil (Degryse and McLaughlin, 2014).

3.3. Greenhouse experiment

The models used to represent the SDMM data presented better fit in the square root function (Table 5). In this scenario, the dose that maximized function for TSP fertilizer was 354 mg dm^{-3} and

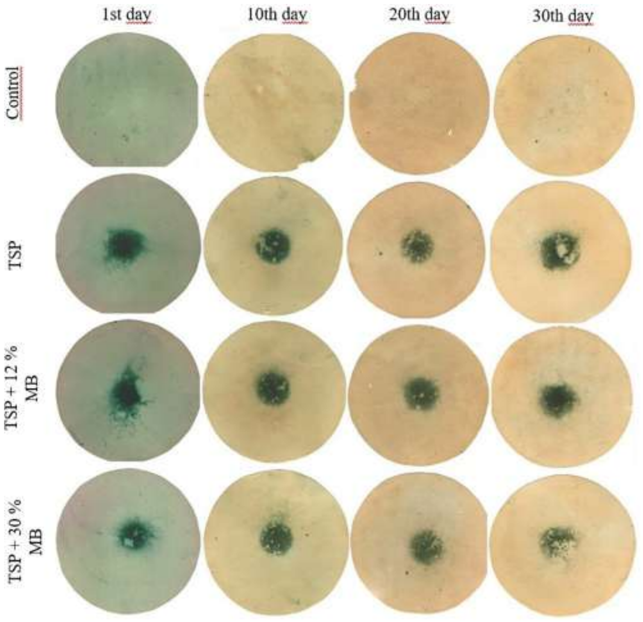

Fig. 6. P diffusion in clay soil.

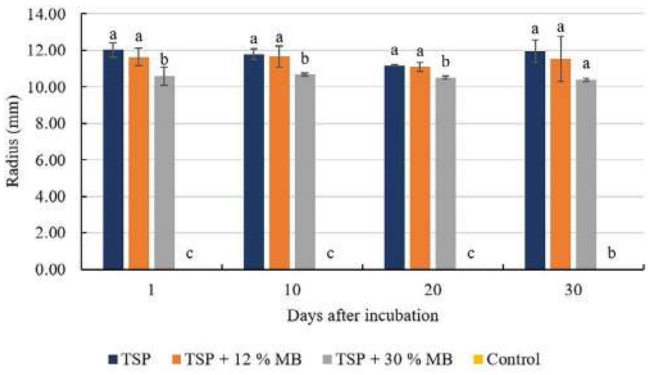

Fig. 7. P diffusion radius in sandy soil. * Means followed by the same letter do not differ significantly from each other by the Tukey test at 5% probability.

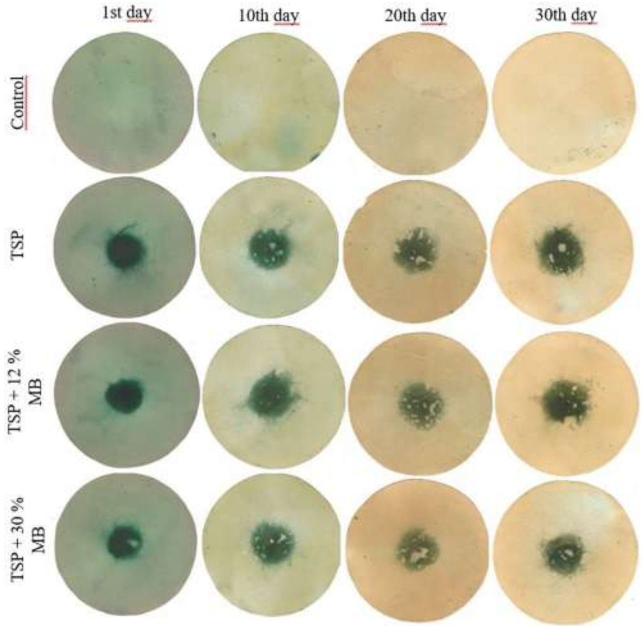

Fig. 8. P diffusion in sandy soil.

Table 5
Regression models for description of SDMM and P content.

Fertilizer	Regression equation	R^2
SDMM (g pot^{-1})		
TSP	$\hat{y} = 1.6314 + 1.0425^{***}\sqrt{[dose]} - 0.0277^{*}\,[dose]$	0.99
TSP + 12% MB	$\hat{y} = 1.6825 + 1.1323^{***}\sqrt{[dose]} - 0.0294^{**}\,[dose]$	0.98
P content (mg pot^{-1})		
TSP	$\hat{y} = 8.3522 + 0.0546^{***}\,[dose]$	0.93
TSP + 12% MB	$\hat{y} = 0.8745 + 0.0740^{***}\,[dose]$	0.99

*Significant at the 10% probability of error level by the *t*-test; ** significant at the 5% probability of error level by the *t*-test; *** significant at the 1% probability of error level by the *t*-test.

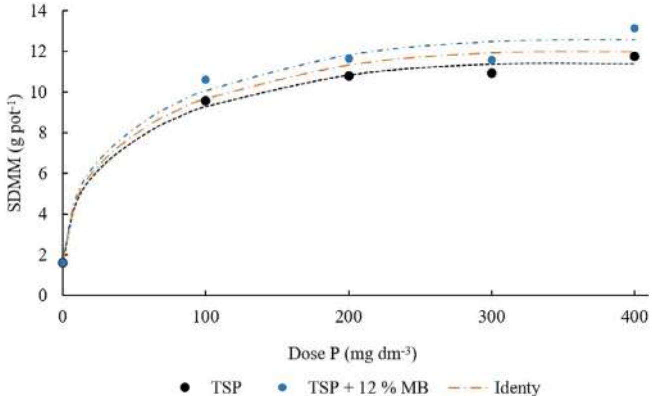

Fig. 9. Production of SDMM according doses of P fertilizers and fertilizer sources.

for TSP + 12% MB, 370 mg dm^{-3}, and the maximum dry matter yields were 11.44 g pot^{-1} and 12.58 g pot^{-1}, respectively (Fig. 9).

When applying the model identity test for the square root function parameters, no significant difference (p-value > 0.05) was observed between the models.

Regarding the P content in plants, the data from both sources tested fit the linear model better (Fig. 10). Up to the dose of approximately 160 mg dm^{-3}, the TSP fertilizer was superior in accumulated P content and, from then on, the TSP + 12% MB started to give a higher accumulation of P.

Moreover, it can be inferred that TSP + 12% MB fertilizer has a P recovery rate of 7.40%, with the addition of one unit of dose. TSP provides a 5.50% recovery rate. When comparing the linear models in the model identity test, it can be inferred that there was no statistically significant difference at the 5% level (p-value > 0.05). The significant difference was observed at the 15% probability of error level.

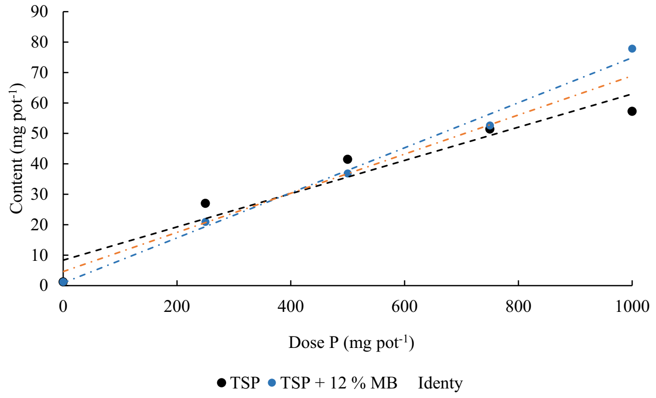

Fig. 10. P content as a function of P doses and fertilizer sources.

Table 6
Literature results of shoot dry weight of corn plants.

Fertilization method	N and P doses (mg kg^{-1} of soil)	Time until harvesting	Shoot dry weight (plant^{-1})	Reference
TSP with microalgae biomass	200 N and 100P – without MB	4 weeks	4.78 g	This study
	200 N and 100P – with 12% MB		5.29 g	
Inoculation with communities of whole rhizosphere microorganisms	71.43 N and 128.57P – without inoculation	8 weeks	~3 g	Carmona et al., 2019
	71.43 N and 128.57P – species obtained in Michoacán		~5 g	
	71.43 N and 128.57P – species obtained in Guanajuato		~3 g	
	71.43 N and 128.57P – species obtained in Estado do México		~6 g	
NPK and inoculated with biofertilizer microorganisms	187.50 N and 187.50P – without inoculum	9 weeks	5.66 g	Larsen et al., 2017
	187.50 N and 187.50P – inoculated with *Rhizophagys irregularis*		5.28 g	
	187.50 N and 187.50P – inoculated with *Trichoderma harzianum*		5.56 g	
	187.50 N and 187.50P – inoculated with *Azospirillum brasiliense*		5.36 g	
Organomineral inoculated with Mycorrhizal fungi	300 N and 92.3P – inoculated with *G. mosseae*	~12 weeks	~7 g	Wu et al., 2005
	300 N and 92.3P – inoculated with *G. intraradices*		~4.50 g	

Shoot dry weight results corroborated with values obtained in the literature (Table 6).

4. Discussion

Although the purpose of the study was not to promote the formation of a coated fertilizer, this occurred when the TSP was added with ratios above 12% MB. Coatings are used to promote a synchronisation between nutrient release and absorption by plants. EEFs coatings are generally formulated so that nutrients are coated with environmentally friendly materials that can be degraded in the soil and converted to carbon dioxide, water, methane, inorganic compounds or microbial biomass (Chen et al., 2018). This is a more common and commercially available standard (Naz and Sulaiman, 2016).

In general, the coatings may be of synthetic or organic origin. Particles have advantages over synthetic ones because they are biodegradable, totally releasing the encapsulated nutrient (Schneider et al., 2016), as well as having less soil disposal time until its complete degradation.

More studies should be performed with MB biofertilizer before its classification as EEFs, since in the proportion of MB in TSP that maximizes the adsorption of P by plants, no effect on P diffusion was observed. That is, this source behaved similar to that in which MB was not applied. In contrast, higher proportions of MB (above 12%) showed a reduction in nutrient release as well as a decrease in its absorption by plants, but they did not promote synchronisation.

However, the results showed that the microalgae biofertilizer is desirable for a smart fertilizer, since the promoted phosphorus adsorption higher to conventional fertilizer. Experiments need confirmation with long-term field data. However, if this biofertilizer are proved of higher efficiency, they might be an alternative for organomineral fertilizer production, which help to recycle and add value to organic residues and reduce the pressure on finite reserves of phosphate rocks.

Regarding the adsorption of P by OM, present in biomass, the main reason is its anionic character, which, through cations such as Al, Fe and Ca adsorbed to it, would retain P. However, there are studies that show the negative participation of OM, reducing P adsorption by means of organic acids that block active adsorption sites and or solubilise these oxide oxides, reducing their adsorption surfaces (Novais et al., 2007). The addition of up to 12% MB in the fertilizer seemed to block the phosphorus adsorption sites in the soil due to organic matter presence and allowed for greater availability of macro and micronutrients present in the microalgal biomass.

The recovery rate of P in TSP + 12% MB may have been higher due to the relationship that macronutrients and micronutrients present in the biomass (Table 1) may have exerted in plant development and consequently in their ability to assimilate P in soil solution. Micronutrients may also have influenced the highest concentration and content at 12% MB in the first experiment.

Among the essential micronutrients present in the biomass are Zn, Fe, Mn and B. At these low concentrations, micronutrients are critical for plant growth and development, acting as constituents of cell walls (e.g. B) and membrane integrity (e.g. B and Zn), redox systems in cells and enzyme activators (e.g. Fe and Mn), detoxification of superoxide radicals and synthesis of proteins and the phytohormone (Indole-3-acetic acid) (e.g. Zn) (Broadley et al., 2012). Despite their low concentrations in plant tissues and organs, micronutrients are just as important as macronutrients for their nutrition.

Another possibility is that phytohormones present in the biomass may have had a positive effect up to 12% MB in the fertilizer mass. Although the functional role of the phytohormones present in microalgae is still controversial, molecular evidence of the performance of these phytohormones begins to be evidenced in the literature (Lu and Xu, 2015).

5. Conclusion

The proportion of MB in the triple superphosphate that maximized P content and concentration in plants was approximately 12% MB. From then on, a reduction in the values of the analysed variables was observed with the increase in the proportion of MB in the biofertilizer. Similar behaviour was observed when evaluating phosphorus diffusion in sandy and clay soils. At 30% MB, the diffusion was clearly impaired by the increase of OM in the fertilizer mass, which promoted the formation of a physical barrier in the granule.

It is suggested that the positive result exerted by MB in plants is related to the macro and micronutrients constituting the biomass. Among the macronutrients, there are N, P and K. Among the essential micronutrients present in the biomass stand out Zn, Fe, Mn and B, besides Na, which is a non-essential but beneficial micronutrient.

The addition of an organic source of nutrients produced from the treatment of an environmental liability, which even in small proportions produces positive effects on plants, is of extreme interest to what is expected from the circular economy. It is an effective strategy for nutrient enhancement that contributes to resource savings, as well as environmental and social benefits.

It is recommended to extend the temporal and spatial scale (experimental plots) to make more accurate inferences related to the technical and financial feasibility of using MB in addition with mineral fertilizers. Additionally, experiments that focus on the influence of micronutrients present in biomass in plants, as well as the characterisation of MB for the presence of phytohormones.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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