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Alkalinized sewage sludge affects nutrition and growth of common bean cultivated in Ferralsols

Lodo de esgoto alcalinizado afeta a nutrição e o crescimento do feijoeiro cultivado em Latossolos

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Abstract: The application of alkaline sewage sludge to agricultural soils has increased, but nutritional implications for crops are still poorly explored. In order to evaluate the effect of alkalinized sewage sludge on nutrition and growth of common bean (*Phaseolus vulgaris* L.), a greenhouse experiment was carried out using three Ferralsols and three sewage sludge types. Soil samples of loam, very clayey, and clayey Ferralsol were collected from three geologically distinct regions (sandstone, basalt, and argillite, respectively) of Paraná State, Brazil. Samples of sewage sludge were stabilized with lime (Ca-Mg oxide) and subsequently mixed with the soil samples. Alkaline stabilized sewage sludge and lime were applied to soils at rates of 50, 100, 150 and 200% the amount necessary to reach pH 5.5. Plants were sown and on the 67th day after sprouting were harvested. Shoot dry matter (DM) and height, and elemental composition of leaves were measured. Comparing to lime, the alkalinized sewage sludge had a greater effect on the shoot DM in the clayey soils. The alkaline sewage sludge applied to soils resulted in higher Zn and P contents in plant shoots than did the lime, while both amendments reduced K and Mn contents in shoots. The relationship of Fe/Mn showed very effective to explain foliar symptoms occurrence under very acid condition. The alkaline sewage sludge showed a superior performance to nourish and promote the growth of common bean in the clayey soil compared to agriculture lime.

Key words: acidity, liming, biosolids, iron manganese balance, *Phaseolus vulgaris* L

Resumo: A aplicação de lodo de esgoto alcalino em solos agrícolas aumentou, mas as implicações nutricionais para as culturas ainda são pouco exploradas. Com o objetivo de avaliar o efeito do lodo de esgoto alcalinizado na nutrição e crescimento do feijoeiro (*Phaseolus vulgaris* L.), foi realizado experimento em casa de vegetação com três Latossolos e três tipos de lodo de esgoto. Amostras de Latossolos (texturas franca, muito argilosa e argilosa) foram coletadas em três regiões geologicamente distintas do estado do Paraná, Brasil: arenito, basalto e argilito. Amostras de lodo de esgoto foram estabilizadas com calcário e posteriormente misturadas com as amostras de solo. Lodo de esgoto alcalinizado e calcário foram aplicados aos solos nas taxas correspondentes a 50, 100, 150 e 200% da quantidade necessária para o solo atingir pH 5,5. Aos 67 dias após emergência, foram medidas a altura das plantas, matéria seca da parte aérea e composição elementar das folhas. Em comparação ao calcário, o lodo alcalinizado promoveu maior produção de matéria seca da planta nos solos argilosos. O lodo de esgoto alcalinizado aplicado aos solos resultou em maiores teores de Zn e P na parte aérea da planta do que o calcário, enquanto ambos os corretivos reduziram os teores de K e Mn na parte aérea. A relação Fe/Mn mostrou ser muito efetivo para explicar sintomas foliares que foram observados sob condição de elevada acidez. O lodo

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alcalinizado apresentou desempenho superior quanto à nutrição e promoção do crescimento do feijoeiro no solo argiloso.

Palavras-chave: acidez, calagem, biossólidos, balanço nutricional, *Phaseolus vulgaris* L

Introduction

Sewage sludge is a by-product of wastewater treatment that contains significant concentrations of organic matter, nitrogen, phosphorus and other plant nutrients (Corrêa et al., 2012). Several studies have shown the benefits of sewage sludge application to agricultural soil (Abreu-Junior et al., 2019; Melo et al., 2018; Poggere et al., 2012; Urbaniak et al., 2017). Analysis of total contents of the main elements in sewage sludge has revealed high concentrations of P and low levels of K, probably due to the high water solubility of this last element, which remains in the liquid phase during the sewage sludge dewatering process (Leila et al., 2017). High contents of organic matter and nitrogen reinforce the potential use of sewage sludge as soil amendment and source of plant nutrients (Bai et al., 2017; Leila et al., 2017).

Although the sewage sludge contains significant levels of nutrients that can be absorbed by plants (Shaheen et al., 2014), it can also present high levels of potentially toxic elements (PTEs) (Yang et al., 2018) , and pathogens (Zdybel et al., 2019). Once the activity of pathogens and plant availability of PTEs are strongly dependent on medium pH, sewage sludge stabilization processes are employed to eliminate or decrease concentrations of human pathogens and minimize metal availability in soils treated with this residue. The treatment of sewage sludge with Ca and Mg oxide to increase its pH is considered an appropriate treatment process prior to applying it to soils (Barbosa et al., 2017; Poggere et al., 2012).

Sewage sludge generation is an unavoidable consequence of wastewater treatment, and the amount of this residue is expected to increase in Brazil as only 55% of the sewage is collected in the country and from that, only 28% are treated. The state of Paraná is the third in the country in relative amount of sewage collected and treated (IBGE, 2010), and the beneficial use of residues complies with the current policy of waste reuse and recycling, as long as it is done according to sanitary and environmental regulations (Corrêa et al., 2012).

The application of domestic sewage sludge to agricultural soils in Brazil must abide by the Resolution n° 375/ 2006 of the National Environmental Council (Conama). According to this resolution, application rates must consider i) neutralization/acidification capacity, ii) heavy metal content, and iii) N content and availability. Once these three criteria are evaluated, the lowest rate must be applied to the soil. In Paraná state, procedures, standards, and requisites to apply sewage sludge in agricultural soils are established in the Resolution n° 021/09 of the Secretary of State for the Environment and Water Resources (SEMA, 2009).

Alkaline sewage sludge has been regionally utilized as an alternative to lime and it has been presented positive results in crops under different conditions (Adjei & Rechcigl, 2002; Barbosa et al., 2007; Tamanini et al., 2008). Between 2011 and 2013, sewage sludge was applied to several crops, benefiting over one hundred farmers, in 41 municipalities of Paraná. In these farms, alkaline sludge has provided 90% of the lime, 69% of the N, 83% of the P_2O_5 and 35% of the K_2O demanded by the crops in this period (Bittencourt et al., 2018). However, the chemical composition of the sewage sludge varies according to several factors (i.e. sewage processing, domestic habits, and presence of industries) (Głąb et al., 2020; Praspaliauskas et al., 2020; Skowrońska et al., 2020; Yang et al., 2018). Therefore, local studies are necessary to attest the efficiency of alkaline sewage sludge under the regional soil conditions and most relevant crops.

To study the effects of alkalinized residues on plant nutrition and growth is more complex than to evaluate lime effects on soils and crops because i) the reaction speed depends on the amount and kind of alkaline material (CaO, $CaOH_2$) used in the residue treatment and ii) significant amounts of plant nutrients may be added to soils along with the

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residues (Dalpisol et al., 2017), raising their availability to plants (Nascimento et al., 2013). Considering the great potential for the expansion of sewage sludge use in agriculture, our aim was to evaluate the response of an agricultural crop to application rates of alkalinized sewage sludge to soils with tree different textures using lime as reference.

Material and methods

Sampling and properties of soils and sewage sludge

Three soil classes of agricultural importance and representativity in the Paraná state were selected in the municipalities of Umuarama, Pato Branco and Pinhais. Soil samples were collected from areas under secondary vegetation at 0-20 cm depth (A horizon). Soil samples were air-dried, passed through 2.0 mm sieves (10 Mesh) and analyzed for granulometry and chemical attributes (Table 1).

Table 1. Chemical and physical properties of three soils from Paraná, Brazil

[1]pH		[2]Al^{3+}	H+ Al	Ca^{2+}	Mg^{2+}	K^+	[3]CEC	P	C	[4]V	[5]m	[6]Cu	Mn	Fe	Zn	[7]Clay
$CaCl_2$	SMP	---------- $cmol_c\ dm^{-3}$ ----------						$mg\ dm^{-3}$	$g\ dm^{-3}$	---- % ----		-------- $mg\ kg^{-1}$ --------				$g\ kg^{-1}$
LVdf - Rhodic Ferralsol (Pato Branco)																
3.9	4.6	2.9	14.1	0.5	0.1	0.14	14.8	1.3	46.9	5	80	2.8	23	46	7.4	850
LBw - Umbric Ferralsol (Pinhais)																
4.0	4.7	3.7	15.8	3.1	1.6	0.09	20.6	1.3	65.4	23	44	0.8	25	70	--	650
Lvd - Rhodic Ferralsol (Umuarama)																
4.1	5.8	0.7	5.8	1.2	0.4	0.13	7.5	3.1	13.3	23	29	0.5	72	44	1.7	200

[1]pH $CaCl_2$ = soil/$CaCl_2$ 0.01 M ratio 1: 2.5; pH_{SMP} = SMP buffer solution; [2]H + Al extraction acetate Ca (0.5 mol L^{-1}); Al, Ca and Mg extraction KCl (1 mol L^{-1}); K and P extraction Mehlich I; C – organic carbon (volumetric method potassium dichromate); [3]CEC = Cation Exchange Capacity at pH 7,0; [4]V= base saturation of CEC pH 7.0 (Cation Exchange Capacity); [5]m= aluminum saturation of effective CEC; [6]Cu, Mn, Fe and Zn – extraction Mehlich I ; [7]clay determined by the hydrometer method.

In Umuarama municipality the collected soil was classified as Rhodic Ferralsol (Dystric, loamic) (from now on named as loam LVd), Caiuá Formation and Bauru Group (sandstone). The local climate is Cfa, according to Köppen-Geiger classification. Soil from Pato Branco municipality was classified as a Rhodic Ferralsol (Dystric, clayey) (from now on named as very clayey LVdf). Local geology is classified as Serra Geral Formation, São Bento Group (basalt). Soil from Pinhais municipality (metropolitan region of Curitiba city) is characterized as Gerric Ferralsol (Dystric, clayey) (from now on named as clayey LBw). Parent material is Guabirotuba Formation (claystone). The climate in the municipalities of Pinhais and Pato Branco is Cfb (Köppen-Geiger) (Alvares et al., 2013).

Considering that, due to logistic and cost optimization, the sewage sludge is usually applied in areas near by the treatment plant, samples of sewage sludge from anaerobic treatment were collected from local wastewater treatment plants close from the soil sample points (in the municipalities of Umuarama, Pato Branco, and Almirante Tamandaré). Total solids (TS) of samples were determined, and then the material was alkalinized in the laboratory by the prolonged alkaline stabilization process. The process consisted of the incorporation of the Ca-Mg oxide at 50% (w/w) of the content of dry solids determined in the sludge, and a subsequent curing period of thirty days. Elemental concentrations in the sewage sludge samples followed Martins and Reissmann (2007). Neutralizing equivalence of $CaCO_3$ reaction or neutralization power (NP) was determined based on Tedesco et al. (1995). NP values for Ca-Mg oxide and NP for lime used to stabilize the samples of sewage sludge were respectively 105.1% and 101.3 % (Table 2).

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Table 2. Values of pH, neutralization power e total concentration of macro and micronutrients present in the alkalinized sewage sludges from Pato Branco, Almirante Tamandaré and Umuarama applied in the soils from the same regions

Alkalinized sewage	pH	NP[1]	C	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
	H_2O	% Eq. $CaCO_3$	---------- g kg^{-1} ----------						------ mg kg^{-1} ------			
Pato Branco	9.5	31	144.8	14.0	2.6	1.3	49.0	28.0	80	8038	123	168
Almirante Tamandaré[2]	9.7	29	113.2	8.8	1.5	0.8	54.4	31.2	112	8560	124	84
Umuarama	11.2	39	152.2	12.4	1.8	0.9	63.9	34.1	112	5498	135	113

[1]NP = neutralization power. [2]Soil samples from Pinhais (metropolitan region of Curitiba city). Sewage sludge from the same region was collected in the municipality of Almirante Tamandaré

Experimental design

Soil samples were air-dried, sieved (<4 mm) and placed in pots of 2.3 dm^3. The greenhouse experiment followed a randomized block design with three replications in a 2 x 5 factorial scheme for each soil, two acidity correctives (lime and alkaline sewage sludge) and five rates, including the control. Each soil type filled 30 pots, which received 0% (control), 50%, 100%, 150%, and 200% the amount necessary to raise soil pH to 5.5 in 60 days, previously obtained from soil pH neutralizing curves (Poggere et al., 2012) (Table 3).

Based on the rates of alkaline sewage sludge applied to the soils (Table 3), and the total concentration of macro and micronutrients present in the sludges (Table 2). We determined the nutrients added to the soils (Table 4).

Table 3. Rates of alkaline sewage sludge and lime applied to the three soil types, having as reference the level of 100% correction obtained from pH neutralizing curves

Amendment level	loam LVd (Umuarama)		very clayey LVdf (Pato Branco)		clayey LBw (Pinhais)	
	Sewage	Lime	Sewage	Lime	Sewage	Lime
%	T ha^{-1}					
0	0.00	0.00	0.00	0.00	0.00	0.00
50	3.20	1.56	17.43	5.39	18.15	6.20
100[1]	6.40	3.12	34.86	10.77	36.29	12.40
150	9.60	4.68	52.29	16.16	54.44	18.60
200	12.80	6.24	69.72	21.56	72.60	24.80

[1]100 % of the quantity calculated to reach pH 5.5, obtained by the curve of elevation of pH of each soil

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Table 4. Total amount of nutrients added to the soil as function of the alkaline sewage sludge application.

Amendment level (%)	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
					kg ha^{-1}				
					loam LVd (Umuarama)				
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50	39.7	5.8	2.9	204.5	109.1	0.4	17.6	0.4	0.4
100	79.4	11.5	5.8	409.0	218.2	0.7	35.2	0.9	0.7
150	119.0	17.3	8.6	613.4	327.4	1.1	52.8	1.3	1.1
200	158.7	23.0	11.5	817.9	436.5	1.4	70.4	1.7	1.4
					very clayey LVdf (Pato Branco)				
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50	244.0	45.3	22.7	854.1	488.0	1.4	140.1	2.1	2.9
100	488.0	90.6	45.3	1708.1	976.1	2.8	280.2	4.3	5.9
150	732.1	136.0	68.0	2562.2	1464.1	4.2	420.3	6.4	8.8
200	976.1	181.3	90.6	3416.3	1952.2	5.6	560.4	8.6	11.7
					clayey LBw (Pinhais)				
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50	153.4	26.1	13.9	948.2	543.8	2.0	149.2	2.2	1.5
100	306.8	52.3	27.9	1896.4	1087.6	3.9	298.4	4.3	2.9
150	460.2	78.4	41.8	2844.6	1631.4	5.9	447.6	6.5	4.4
200	613.5	104.6	55.8	3792.8	2175.3	7.8	596.8	8.6	5.9

Common bean (*Phaseolus vulgaris* L.) cultivar IPR Tiziu was selected as crop test due to its regional importance. Basal fertilization was applied to pots upon seeding at 15 kg ha^{-1} of N. LVdf and LVd soils received equivalent rates of 70 kg ha^{-1} of P_2O_5 and 30 kg ha^{-1} of K_2O, while LBw received equivalent rates of 80 kg ha^{-1} of P_2O_5 and 40 kg ha^{-1} of K_2O. Nutrient sources used were potassium nitrate, sodium phosphate monobasic monohydrate, diabasic potassium phosphate. Twenty days after seeding, all pots received 60 kg ha^{-1} of urea as recommended in Paraná state (IAPAR, 2003) based on results of soil analysis (Table 1).

Seven seeds were sown per pot, and five days after sprout (DAS) plants were thinned out to two plants per pot. Pots were daily watered with deionized water to keep soils at 80% field capacity, and plants were tutored later on. All senescent leaves were collected, washed, and dried before nutritional analysis. On the 67th DAS, was observed a modified staining pod in 50% of plants (development stage R9); then, the plants were harvested.

Morphological evaluation and nutritional analysis of the common bean plants

The plant height was measured at the end of the experiment. Plants were harvested by cutting it 1 cm above the soil surface, washed with tap water, rinsed with purified water and oven-dried (65°C) in order to obtain aboveground biomass. Aboveground biomass was milled (< 1 mm) prior to chemical analysis.

Analysis of P, K, Ca, Mg, Fe, Mn, Cu, and Zn in plant tissue was performed by dry digestion, according to Martins and Reissmann (2007). Atomic absorption spectrophotometer was used for the determination of Ca, Mg, Fe, Mn, Cu, and Zn. Spectrophotometer and flame spectrophotometer were used to respectively determine concentrations of P and K. Analysis of total N and C were performed by dry-digestion using elemental analyzer (Vario EL III – Elementar®).

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Statistical analysis

Data displaying normality of the residuals (Shapiro-Wilk test) and homogeneous variances (Bartlett test) were subjected to regression analysis. When the variable did not present normality and homogeneity, box-cox transformation was carried out to attend those requirements. Tests were performed for each soil and acidity corrective source. All statistical analyzes were performed using software R, version 3.4.1 (Team, 2014).

Results and discussion

Aboveground biomass and plant height

The aboveground biomass and plant height responses to increased rates of alkaline sewage sludge and lime varied among soils (Figure 1). The increase of aboveground biomass and plant height followed the application rates of alkaline sewage sludge in the very clayey LVdf and clayey LBw soils. Increment in the aboveground biomass in the very clayey LVdf soil was approximately four times higher compared to the clayey LBw soil, indicating that the degree of limitation imposed by the exchangeable acidity in the very clayey soil was higher than in soils with less clay content. Besides, the very clayey LVdf presented higher concentration of nutrients like N, P, and K, in comparison to the clayey LBw (Table 4). Comparing the amendments, the alkaline sewage sludge promoted a dry mass production almost 2-fold higher than the lime. The higher nutrient availability probably boosted the dry matter production of the common bean plants.

An excessive amount of soluble salts can cause unbalanced conditions in soil solution and thereby reduces plant growth (Gondek et al., 2018). This phenomenon could explain the result observed on plant height in the loam LVd soil, in which plant growth was negative in response to both amendments. In this soil, with low buffering power, nutritional imbalance may have been more evident and impaired the development of the common bean plants.

Since Al^{3+} is considered the most toxic element to plant and root development in acidic soils (Foy, 1984), the different responses of biomass and height to the amendments in the three soil types suggest that the relative availability of toxic Al^{3+} in comparison with soil bases (Table 1) may represent the common bean response (Figures 1A, 1B, and 1C). The differences in the responses to the use of alkaline sludge and lime in the soils contrasted with the similar initial soil pH (Table 1).

The degree of plant responses to the use of the soil amendments followed the relative values of Al saturation (m%). In other words, the degree of limitation imposed by soil acidity was more evident in the relative values of the element. According to the classification established by Sobral et al. (2015), the levels of m% in the LVdf, LBw, and LVd soils can be considered high, medium and low, respectively. Therefore, the effect of the treatment is more pronounced in the soil with higher saturation levels (very clayey LVdf soil). In soils with a low level of m%, the amendments did not result in increments of biomass production.

Poggere et al. (2012) have established a maximum annual application rate of 68, 74 and 10 t ha^{-1} of sewage sludge in the respective soils of Pato Branco, Pinhais, and Umuarama municipalities. In our study, maximum efficiency rates based on biomass production of alkaline sludge were 54 t ha^{-1} (equivalent to 151%) for the very clayey LVdf soil from Pato Branco, and 68 t ha^{-1} (equivalent to 186%) for the clayey LBw soil (Pinhais). Such results demonstrate that the ideal rate of alkaline sewage sludge and lime to reach pH 5.5 (100% level) was not sufficiently high to reach the maximum plant production in the evaluated soils. This may indicate that in clayey soils with higher CEC, the cultivar used in the present study has growth potential at pH above 6.25.

The amount of heavy metals and N applied to the soil through alkalinized sewage sludge is a concern (Yang et al., 2018). The use of 54 t of the alkalinized sewage sludge would represent the apport of 4.3 kg of Cu, 434 kg of Fe, 6.6 kg of Mn, 9.1 kg of Zn, and 756 kg of N per hectare of application in the very clayey LVdf soil. For the clayey LBw soil, the use of the maximum efficiency rate would contribute with 7.6 kg of Cu, 582 kg of Fe, 8.4 kg of Mn, 5.3 kg of Zn, and 598 kg of N per hectare. The Resolution n° 375/ 2006 of the National Environmental Council (Conama)

presents the threshold value for Zn (445 kg ha^{-1}). The level of Zn apported in the soils from the recommend doses presented in this study is more than 50 times smaller than the value provided by the legislation.

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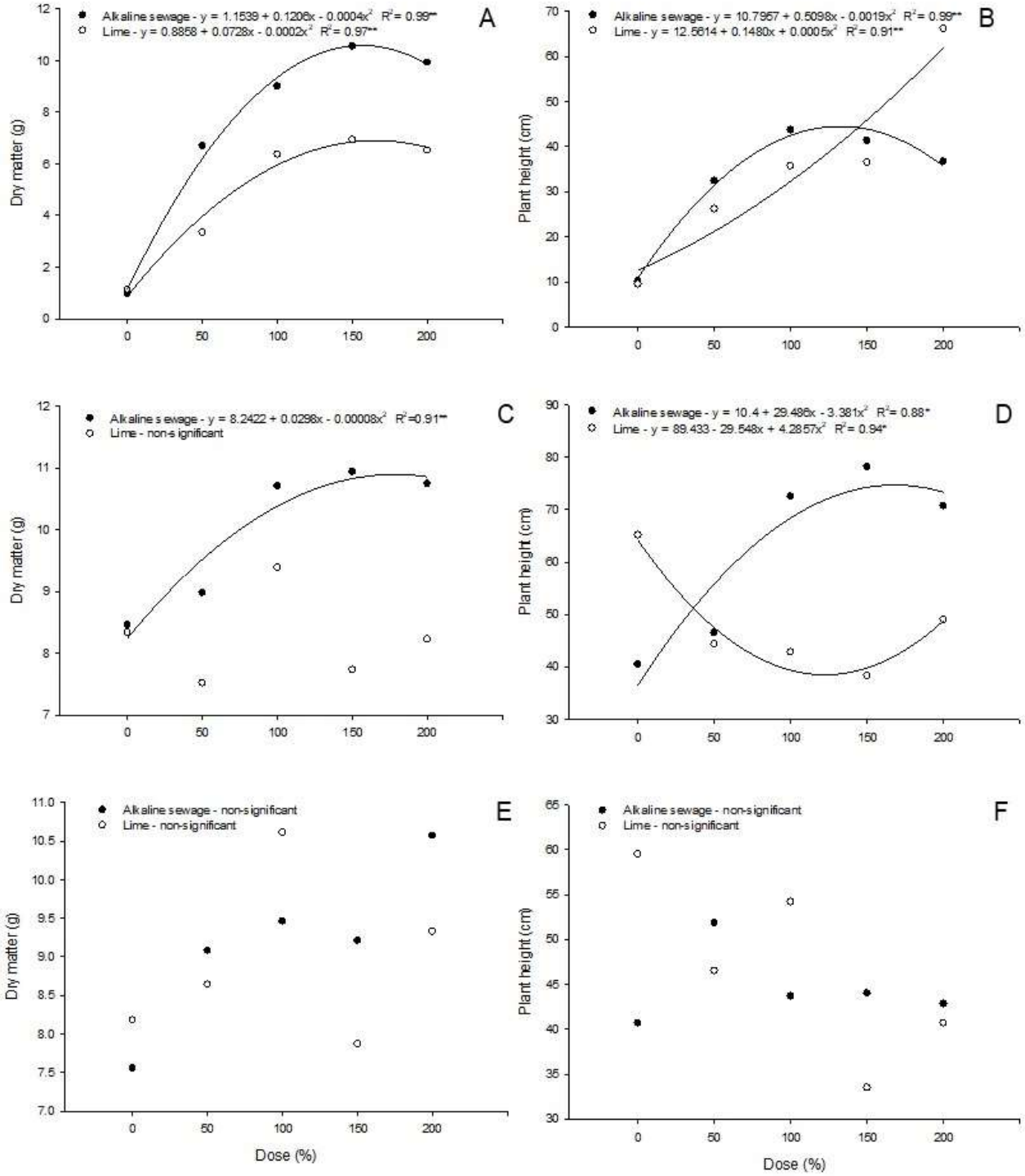

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Figure 1. Aboveground biomass [(dry matter (g)] and plant height of common bean growing under five rates of alkalinized sewage sludge and lime for the very clayey LVdf soil (A, B), clayey LBw soil (C, D) and loam LVd soil (E, F)

Nutritional content in plant tissues

In Average, the effect of alkaline sewage sludge and lime promoted a quadratic effect on the N content and extraction in the plant cultivated on the very clayey LVdf soil (Table 5). While the highest doses of both amendments promoted a 2-fold decrease in the N content, the alkaline sewage sludge application resulted in a 5-fold increase in the N extraction. In the treatments with lime, the increase of N extraction was solely of 2-fold. These results corroborate the differences in dry mass production (Figure 1A).

In the very clayey LVdf soil, P content in plant tissue increased about 60% with the application of the lowest rate of alkaline sewage sludge (Table 5), while the application of the lowest rate of lime promoted a 3-fold increase in P content in plant tissue when compared to the control treatment. The levels of P in plants cultivated in the clayey LBw soil showed a slight decrease in response to increased rates in relation to the control with both amendments. In the loam LVd soil, which presented an initial P level three time greater than the other soils, the application of the amendments did not cause changes in P absorption by the plants.

Although soil mineralogy has not been analyzed, LVdf soil presents a high concentration of Fe in the form of oxides in relation to the other soils due to its parent material – basalt – has a high concentration of Fe compared to argillite (clayey LBw) and sandstone (loam LVd) (Poggere et al., 2018). Thus, a combination of high contents of clay and Fe oxides in the LVdf soil causes significant P adsorption applied by soil, leading to a minor availability to the plants, while in the loam soil (LVd) there was more available P for bean plants.

The quadratic effect observed in the very clayey soil (Table 5) indicates that the rate of 50% of alkaline sewage sludge seems to be the ideal rate of P absorption by the common bean plant. On the other hand, P absorption decreased in response to the amendments in the LBw soil, especially for lime application. This soil presented high initial Ca, and more Ca was added by the correctives. Summed to this, the increased soil pH may have led to the precipitation of P in the form of calcium phosphate (Nascimento et al., 2013), maintaining the availability of the element practically constant between the rates of corrective.

Both the use of alkaline sewage sludge and lime in the three soils increased Ca and Mg concentrations in plant tissue and decreased its K level. Sewage sludge usually presents low levels of K, probably due to the high solubility of this element, that remains in the aqua phase during the sludge treatment process (Barbosa et al., 2017; Nascimento et al., 2013). In the LVdf soil, which had the highest initial values of available K and where there was greater input of the nutrient from the sludge application, the concentration of K in shoot in the amended soil was up to 2 times smaller than in the plants from the control treatment. This soil presented effect of the amendment on the dry matter production (Figure 1A). Probably, the increased mass production resulted in a dilution effect, reducing the relative K concentration.

Apparently, the treatment with alkaline sludge and lime had a similar effect on the absorption and accumulation of Ca and Mg in plants, mainly in the very clayey soil, that also presented the lowest initial values. The use of alkaline sludge guaranteed greater concentrations of Ca and Mg in the plants when compared to lime application. In the case of LBw soil (which received the highest effective rates of Ca and Mg) and loam LVd soil, the average effect of the alkaline sewage sludge differed from the lime effect. For the LVd plants, the amendment represented a linear increase in the Ca and Mg uptake.

High levels of Fe and Mn observed in plants grown in the control treatments in the very clayey LVdf soil indicate the availability of these nutrients in high acidity conditions (Table 6). Results also do not show Zn and Cu deficiencies. Considering that the sludges provided similar amount of these micronutrients (Table 4), the pattern observed in the very clayey LVdf indicates

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natural high levels of these elements, a fact common to soils originated from basalt, such as the soil in question (MOTTA et al., 2007).

Table 5. Macronutrient concentration and extraction in the aboveground part of the common bean plants treated with alkaline sewage sludge, lime, and NPK in all treatments in the very clayey LVdf soil, clayey LBw soil and loam LVd soil

Treatment	N		P		K		Ca		Mg	
	Content	Extraction	Content	Extraction	Content	Extraction	Content	Extraction	Content	Extraction
	(g kg^{-1})	(mg pot^{-1})	(g kg^{-1})	(mg pot^{-1})	(g kg^{-1})	(mg pot^{-1})	(g kg^{-1})	(mg pot^{-1})	(g kg^{-1})	(mg pot^{-1})
very clayey LVdf (Pato Branco)										
Alkalinized sewage 0%	63.0	62.8	1.0	1.0	21.4	20.9	5.7	5.5	2.1	2.1
Alkalinized sewage 50%	36.7	196.6	1.7	9.4	16.7	87.2	8.8	44.2	2.6	13.0
Alkalinized sewage 100%	34.9	275.5	1.4	11.1	15.2	124.1	9.1	68.1	2.7	20.8
Alkalinized sewage 150%	32.9	342.8	1.4	15.8	12.3	129.9	8.6	97.4	2.7	29.6
Alkalinized sewage 200%	34.0	359.0	1.1	13.8	10.4	135.3	10.4	104.4	3.4	33.1
Lime 0%	53.2	120.9	0.6	2.0	28.7	39.5	10.6	32.2	2.2	9.6
Lime 50%	39.8	93.0	1.6	2.8	18.8	48.2	7.4	17.0	2.4	5.0
Lime 100%	37.7	233.9	1.6	10.2	14.1	95.8	8.8	53.2	2.6	15.6
Lime 150%	36.9	223.8	1.4	9.3	15.0	82.8	9.0	53.6	2.8	16.4
Lime 200%	37.2	244.1	1.4	9.2	15.6	92.5	8.3	57.1	2.6	17.8
Effect of the alkalinized sewage	ns	ns	Q**	ns	ns	ns	L**	ns	L**	ns
Effect of the lime	ns	ns	Q**	ns	ns	ns	Q**	ns	Q**	ns
Interaction amendment x dose	ns	ns	ns	ns	ns	ns	**	ns	**	ns
Average effect of alkalinized sewage and lime	Q**	Q**	ns	Q**	Q**	Q**	ns	Q**	ns	Q**
clayey LBw (Pinhais)										
Alkalinized sewage 0%	23.6	249.9	1.9	13.1	15.8	137.2	5.5	49.5	1.4	13.8
Alkalinized sewage 50%	31.3	280.7	1.4	13.8	14.0	130.0	11.4	73.1	2.7	19.1
Alkalinized sewage 100%	31.5	337.4	1.5	15.4	12.1	129.3	11.2	128.8	3.0	31.9
Alkalinized sewage 150%	34.4	340.6	1.6	14.8	12.6	133.3	10.8	112.2	3.0	31.6
Alkalinized sewage 200%	33.3	396.0	1.6	20.0	13.2	148.0	10.8	129.7	3.1	36.5
Lime 0%	29.2	210.3	2.4	17.1	14.6	118.3	5.7	58.8	1.6	17.2
Lime 50%	33.0	229.9	1.6	15.1	15.6	117.9	9.5	64.0	2.2	16.1
Lime 100%	32.3	280.0	1.5	13.8	12.6	123.0	13.4	90.6	3.1	21.1
Lime 150%	32.6	280.3	1.4	11.5	14.1	107.2	11.0	116.8	2.8	27.7
Lime 200%	34.2	283.0	1.5	13.4	13.6	120.7	10.8	91.7	2.9	24.0
Effect of the alkalinized sewage	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Effect of the lime	ns	ns	ns	L**	ns	ns	ns	ns	ns	ns
Interaction amendment x dose	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Average effect of alkalinized sewage and lime	L**	L**	Q**	ns	Q**	ns	Q**	Q**	Q**	Q**
loam LVd (Umuarama)										
Alkalinized sewage 0%	33.3	232.5	2.2	12.3	16.6	111.3	6.8	54.8	1.7	14.8
Alkalinized sewage 50%	28.3	271.9	2.3	19.9	15.3	132.6	9.6	77.0	2.3	18.1
Alkalinized sewage 100%	26.8	312.3	2.3	23.7	14.5	143.3	11.1	90.8	2.8	23.9
Alkalinized sewage 150%	30.5	253.2	2.5	21.1	15.2	129.5	12.4	108.7	3.1	25.9
Alkalinized sewage 200%	27.7	317.0	2.2	23.0	13.4	149.2	9.0	117.3	2.8	34.2
Lime 0%	32.0	288.7	2.2	19.8	18.3	148.3	6.3	60.1	1.6	16.7
Lime 50%	31.2	253.1	2.0	16.9	16.7	159.0	7.1	71.7	1.8	17.2
Lime 100%	31.0	263.0	2.0	18.6	13.6	127.5	7.8	56.4	2.2	16.5
Lime 150%	30.6	244.1	2.5	21.5	16.8	136.9	10.7	91.9	2.7	23.9
Lime 200%	30.4	246.5	2.4	20.2	14.8	133.8	10.1	86.6	2.9	24.7
Effect of the alkalinized sewage	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Effect of the lime	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

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Interaction amendment x dose	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Average effect of alkalinized sewage and lime	ns	ns	ns	ns	L**	ns	L**	L**	L**	L**

* P value < 0,05; ** P value < 0,01; ns = non-significant; Q = quadratic effect; L = linear effect

The maintenance of the Zn contents and the reduction of Mn and Fe concentrations with increased rates of alkaline sewage sludge applied to soils (Table 6) probably favored an increase in plant dry mass production in the very clayey LVdf soils. However, at the highest rate of alkaline sewage sludge, Mn contents in plant tissue had an expressive reduction, which may have promoted the negative effect on aboveground biomass of bean plants (Figure 1A). Once the sludge provided Mn to the soils (Table 2 and 4), the reduction in the Mn availability may be related to the increasing in the pH promoted by the highest dose.

The smallest doses of lime decreased the Fe and Mn availability probably due to apport of bases and increased pH. This may had contributed to the biomass production (Figure 1A). However, there was an increase in Fe levels in plant tissue at the higher rates of lime, indicating a nutritional imbalance. In general, it was observed little effect of the treatments on Fe content in the shoots tissue, probably due to the high contents of this element initially available in soils (Table 1) and in the alkaline sewage sludge (Table 2).

Asgari Lajayer et al. (2019) observed that increasing rates of sewage sludge promoted increased Fe concentration in roots, but there was no variation of the element concentration in the shoot of the basil (*Ocimum basilicum* L.). In our study, Fe deficiency symptoms, such as internerval chlorosis, were observed in common bean plants grown in the loam LVd soil in the control treatment. However, there was no variation in the levels of this element with the application of the amendments. Therefore, it is likely that there has been an interaction involving other elements, mainly Mn and P.

High levels of Mn in relation to Fe in the plant, summed with the adequate supply of P, have been indicated as inducing Fe deficiency in plants (Kohno & Foy, 1983), and may occur in acidic environments. In the present study, Fe/Mn ratio was lower than 0.3 (Table 6). According to Malavolta and Kliemann (1985), Fe/Mn ratio close to 0.16 is related to the occurrence of Fe deficiency symptoms in common bean plants. In addition to chlorosis, it was also observed shrinkage in plants leaves, a symptom related to Mn toxicity (Malavolta & Kliemann, 1985). The authors verified that the Fe/Mn and Fe/Zn ratio was useful in indicating Fe toxicity in corn plants.

Table 6. Average concentration and micronutrient extraction in the aboveground part of the common bean plants treated with alkaline sludge, lime, and NPK in all treatments in the very clayey LVdf soil, clayey LBw soil and loam LVd soil

Treatment	Fe		Mn		Cu		Zn	
	Content	Extraction	Content	Extraction	Content	Extraction	Content	Extraction
	(mg kg^{-1})	(mg pot^{-1})	(mg kg^{-1})	(mg pot^{-1})	(mg kg^{-1})	(mg pot^{-1})	(mg kg^{-1})	(mg pot^{-1})
very clayey LVdf (Pato Branco)								
Alkalinized sewage 0%	689.1	0.67	788.5	0.77	7.8	0.01	28.3	0.03
Alkalinized sewage 50%	121.8	0.81	102.8	0.71	6.3	0.04	22.8	0.12
Alkalinized sewage 100%	217.7	1.57	98.0	0.61	6.6	0.05	28.6	0.21
Alkalinized sewage 150%	169.7	1.91	38.9	0.40	6.6	0.07	29.9	0.29
Alkalinized sewage 200%	81.6	1.18	15.4	0.31	5.1	0.07	29.6	0.38
Lime 0%	1049.9	1.00	502.5	0.44	5.0	0.01	32.4	0.07
Lime 50%	175.1	0.69	106.0	0.47	5.8	0.01	17.7	0.05
Lime 100%	154.6	1.07	66.8	0.44	6.0	0.04	16.8	0.10
Lime 150%	486.9	1.62	51.6	0.19	6.5	0.04	16.5	0.10
Lime 200%	429.5	2.79	64.4	0.34	6.7	0.05	15.3	0.10
Effect of the alkalinized sewage	ns	ns	Q**	ns	L**	ns	ns	L**

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Effect of the lime	ns	ns	Q**	ns	L**	ns	Q**	L**
Interaction amendment x dose	ns	ns	**	ns	**	ns	**	*
Average effect of alkalinized sewage and lime	Q**	L*	ns	Q**	ns	Q**	ns	ns
clayey LBw (Pinhais)								
Alkalinized sewage 0%	144.4	3.16	136.4	1.08	8.7	0.07	26.6	0.20
Alkalinized sewage 50%	163.1	1.58	73.8	0.86	5.3	0.06	41.9	0.34
Alkalinized sewage 100%	141.4	1.33	31.2	0.44	5.4	0.05	42.1	0.44
Alkalinized sewage 150%	136.1	1.52	18.0	0.26	5.3	0.05	42.1	0.42
Alkalinized sewage 200%	129.6	1.42	15.7	0.18	5.0	0.07	41.8	0.48
Lime 0%	215.3	1.86	148.7	0.85	26.0	0.19	27.4	0.29
Lime 50%	140.2	1.09	58.8	0.71	11.2	0.10	24.8	0.19
Lime 100%	137.4	1.18	26.8	0.32	6.1	0.06	20.7	0.20
Lime 150%	195.2	1.48	16.4	0.16	5.1	0.04	18.0	0.15
Lime 200%	151.5	1.41	10.6	0.10	5.0	0.04	13.8	0.13
Effect of the alkalinized sewage	ns	ns	ns	Q**	ns	ns	Q**	Q**
Effect of the lime	ns	ns	ns	Q**	ns	ns	L**	L**
Interaction amendment x dose	ns	ns	ns	*	ns	ns	**	**
Average effect of alkalinized sewage and lime	ns	ns	Q**	ns	L*	L*	ns	ns
loam LVd (Umuarama)								
Alkalinized sewage 0%	162.3	1.20	621.4	3.12	5.8	0.04	37.9	0.21
Alkalinized sewage 50%	152.4	1.18	196.3	2.68	5.4	0.05	35.3	0.30
Alkalinized sewage 100%	139.1	1.42	77.0	1.10	6.3	0.06	30.8	0.31
Alkalinized sewage 150%	169.0	1.41	56.3	0.63	7.1	0.06	27.6	0.26
Alkalinized sewage 200%	138.5	1.50	40.1	0.46	6.2	0.07	24.9	0.27
Lime 0%	130.3	1.23	631.3	3.57	5.0	0.05	40.4	0.31
Lime 50%	140.1	1.35	278.5	4.27	6.5	0.05	31.9	0.33
Lime 100%	140.2	1.33	70.8	0.50	6.0	0.07	21.9	0.21
Lime 150%	212.3	1.37	57.5	0.57	6.9	0.05	28.1	0.22
Lime 200%	130.5	1.29	39.6	0.46	6.6	0.06	17.0	0.15
Effect of the alkalinized sewage	ns	ns	ns	ns	ns	ns	ns	ns
Effect of the lime	ns	ns	ns	ns	ns	ns	ns	ns
Interaction amendment x dose	ns	ns	ns	ns	ns	ns	ns	ns
Average effect of alkalinized sewage and lime	ns	ns	Q**	Q**	ns	L**	L**	L**

* P value < 0,05; ** P value < 0,01; ns = non-significant; Q = quadratic effect; L = linear effect

In contrast to the results from the very clayey LVdf soil, no variation was observed in nutritional contents of plants cultivated in the loam LVd soil treated with the alkaline sewage sludge and lime (Table 5 and 6), following what was observed with the production of biomass (Figure 1E). Plant leaves in control soils showed signs on Mn deficiency but such deficiency was not high enough to cause a decrease in the production of aboveground biomass. Izaguirre-Mayoral and Sinclair (2005) have reported similar results with soybean plants, being the influence of Mn toxicity on Fe uptake range among genotypes.

Mn in the vegetal tissue appeared to be the most sensitive parameter to alkaline sewage sludge and lime application, proving the high sensitivity of Mn to pH variations (Dalpisol et al., 2017; Motta et al., 2007). However, the decrease in Fe content in plants only occurred when the Fe plant content was high, as observed in the very clayey LVdf soil, proving that the pH increase can be an excellent tool in reducing the Mn and Fe toxicity typically present in high acidity conditions.

The Mn contents in plants grown in the very clayey LVdf soil were close to those of the loam LVd soil, which presented only shading and low developmental symptoms. The absence of chlorosis for LVdf can be associated with high levels of Fe and a Fe/Mn ratio higher than 0.63, considered normal for bean culture (Malavolta & Kliemann, 1985). According to Kohno and Foy (1983), the tolerance to excess of Mn is linked to

plant genetics and it is regulated by the reduced absorption capacity by roots, by the low translocation of the element and by the tolerance of high levels of Mn in the foliar tissue. The same authors verified that Mn toxicity symptom in susceptible and Mn-tolerant bean cultivars with levels of 334 and 1253 mg kg^{-1}, respectively. Possibly the cultivar used in the present study has intermediate tolerance.

The Zn supply (Table 5) may have contributed to the highest increase in the aboveground biomass with addition of alkaline sewage sludge compared to the addition of lime to the very clayey LVdf soil and to the clayey LBw soils. Unlike lime application, there was no decrease in Zn content in response to soil application of alkaline sewage sludge. Similar patterns were observed for the Zn extracted by plant in theses soils. This fact is due to the action of Zn is an enzymatic activator in plant metabolic processes, especially in the production of tryptophan, the precursor of auxin, responsible for plant growth (Taiz et al., 2015).

The increase in biomass and productivity with the use of sewage sludge has been attributed to the greater availability of nutrients, mainly N, P, Cu and Zn (Hussein, 2009; Martins et al., 2003; Suhadolc et al., 2010). With the application of both alkaline sewage sludge and lime in the very clayey LVdf soil, the increase of P, Ca and Mg contents in plant tissue was observed and promoted the decrease of N and Mn contents, while maintaining adequate levels of Fe and K (Table 4 and 5). However, higher levels of N, K, and Fe in the plants in the control soil can be explained by the nutrient concentration effect, considering the increase of the extraction of these nutrients in the plants cultivated in this soil.

Conclusion

The neutralization capacity is the more common criteria to calculate the dose of alkaline sewage sludge to be applied. Our results showed that alkaline sewage sludge was more efficient than lime to produce bean plant biomass in clayey soils. However, the use of the residue as a soil corrective presented more implications than the use of lime because the sludge also adds several plant nutrients. Once the elemental composition varies between the alkaline sewage sludges, the amendments provide different levels of nutrient availability and interactions, especially among the micronutrients.

The soil with the highest Al^{3+} saturation (m%), lower initial P content, and higher contents of Fe and Mn showed greater responses to alkaline sewage sludge application. In the opposite case, the soil with low Al^{3+} saturation and high initial P content, and unbalance Fe/Mn ratio had little or no response to the application of correctives. The soil texture was also important since greater responses were observed in soils with higher clay content, while the amendment of the loam soil had no effect on the plant development and little effect on the plant nutrition. Probably, the clay guarantees some buffer power, minimizing the deleterious effect of unbalanced nutrient availability.

Regardless of the source, the balance of nutrients was fundamental to better biomass production, once the increase in Ca and Mg availability probably reduced K content in the aboveground part of the common bean. Among the micronutrients, Zn content was determinant in the production of biomass in clayey soils, but excessively high rates of alkaline sewage sludge and lime may have limited the Mn uptake, decreasing the biomass production in these soils. Under acidic conditions, the combination of excessively high levels of Al^{3+} (available in the soil), Fe and Mn in plant tissue led to a negative effect on the production of biomass, indicating a nutritional imbalance and need for correction.

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