



Research article

Biomass production, metal and nutrient content in sorghum plants grown on soils amended with sewage sludge

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ABSTRACT

Sludge generation from wastewater treatment plants in Uruguay has increased in recent years. Agricultural soils may be a final destination. A greenhouse experiment was conducted to quantify the effect of this sludge on 1) plant biomass production and nutrient concentration of sorghum (*Sorghum bicolor* var. *vulgare*); 2) the chemical properties of amended soils; and 3) assess whether heavy metal concentrations in sludge are appropriate according to environmental regulations. Two soils (S1 and S2) were amended with pure sludge (PS) and limed sludge (LS), with low dose (LD) of 16.0 and 17.3 Mg ha⁻¹ and high dose (HD) of 32.0 and 34.6 Mg ha⁻¹, respectively. Sludge treatments increased plants' nutrient absorption and dry matter production. The LS treatments incremented plant biomass production, depending on soil pH and nutrient availability. The effect of sludge treatments on elemental concentration in aboveground biomass depended on the element, treatments, and soil type. Mineralized nitrogen (N) and plant available phosphorus (P-Bray 1) values increased with sludge addition without exceeding Uruguay's critical soil level of P-Bray 1 for the sorghum crop. The PS did not increase metal concentration in soils. The LS slightly decreased soil Pb and slightly increased Cr and Zn soil concentration; levels were according to Uruguayan environmental guidelines. Therefore, agriculture soils are a viable final destination for PS and LS. Land applied sludge has acceptable levels of metals and promotes crop development.

1. Introduction

In Uruguay, the generation of sludge from wastewater treatment plants (WWTP) increased with the building of new plants by the State Sewage and Water Works (OSE). Applying this waste to soils for agricultural purposes is one of the possible ways of disposal. While 36% and 77% of the generated sludge in the European Union and the United Kingdom have this destination (Rigby et al., 2016), it is not adopted in Uruguay. Currently, the sludge disposal from Uruguayan WWTPs takes place in municipal landfills.

The application of WWTP sludge on agricultural soils has three beneficial aspects: providing nutrients, improving soil's physical properties, and giving an alternative final destination to reduce human health risks. The sludge from WWTP is a source of macro and micronutrients essential for plant growth (Kominko et al., 2019) and contains high levels of organic matter (OM) (Malinowska et al., 2015). Several researchers

described an increase in soil OM when applying WWTP sludge to soils with reported concentrations of OM between 34 and 65 % (Angin et al., 2012; Bozkurt and Yarılgac, 2010; Fuentes et al., 2008; Sánchez-Monedero et al., 2004). This soil management practice also improved the soil's structure, porosity, permeability, and water retention capacity (Illera et al., 2001; Epstein et al., 1976). The improvement in soil fertility and structure also promotes improved crop yields (Bourrioug et al., 2014; Antolín et al., 2005; Fresquez et al., 1990).

Due to the potential sanitary risk of sludge application to soils, a recommended practice is liming the sludge using slacked lime [Ca(OH)₂] or quicklime (CaO) before soil application. Since liming provides an alkaline medium, the limed sludge usually attains a pH between 12 and 13. In particular, the addition of CaO to wet sludge causes an increase in its temperature and dehydration, helping eliminate pathogens (Zhu et al., 2012; Andreadakis, 1999). Other benefits include OM stabilization and metal precipitation (Samaras et al., 2008; Flores-Márgez et al., 2007; Araque

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Manrique, 2006). Land application of limed sludge (LS) is also an option to reduce soil acidity for crop production in acid soils (Sloan and Basta, 1995).

Although WWTP sludge is an excellent source of nutrients for plants, concentrations vary depending on the origin and treatment of the wastewater that produces it (Singh et al., 2012; Singh and Agrawal, 2008). Additionally, the WWTP sludge may contain heavy metals in unsuitable concentrations for application to agricultural soils, and knowing its elemental composition is required before using the sludge as an organic soil amendment. Therefore, the sludge as a soil amendment is prescribed in quantities that do not exceed nutrient crop demands, reducing the risks of nutrient losses from the soil-plant system into natural waters and their contamination.

This report aims to: 1) quantify the effect of WWTP sludge, with and without added CaO, on the production of plant biomass and the concentration of nutrients absorbed by sorghum plants (*Sorghum bicolor* var. vulgare) in a greenhouse experiment; 2) quantify the effect of sludge on soil chemical properties (pH, electrical conductivity, mineralized N, plant-available phosphorus, total exchangeable bases, and total heavy metal content: Cu, Fe, Mn, Zn, Cr, Pb, and Ni) at the end of the crop production cycle; and 3) evaluate whether the concentrations of heavy metals in the used sludge are adequate for agricultural use according to varying regulations in the European Union, the United Kingdom, the United States, and Uruguay.

2. Material and methods

2.1. Soil and sludge collection

The study used two different soils, a Typic Argiudoll (S1) with a light texture (sandy loam) from the Cuchilla Mangueras Unit (Latitude: 31°23'55.11"S, Longitude: 55°41'43.88"O) and a Typic Hapludoll (S2) with a heavier texture (silty clay loam) from the Tala Rodríguez Unit (Latitude: 34°26'54.07"S, Longitude: 56°3'10.49"O) (Altamirano et al., 1976). Soil samples collected from the top layer (0–15 cm depth) were used in this study. The physical and chemical properties of the two soils are presented in Table 1. The sludge was obtained from the WWTP located in Canelones, Uruguay. The sludge's chemical characteristics and elemental concentrations are presented in Table 2 and discussed in section 3.1.

Table 1. Chemical and physical properties of the soils used in the experiment †.

Parameters	S1†	S2
Sand (g kg ⁻¹)	521‡	145
Silt (g kg ⁻¹)	315	492
Clay (g kg ⁻¹)	164	363
Texture class	Sandy Loam	Silty Clay Loam
pH	4.9	7.7
Total organic C (g kg ⁻¹)	15.1	24.9
P-Bray 1 (mg kg ⁻¹)	6.80	3.50
Exchangeable Ca (cmc kg ⁻¹)	2.01	42.9
Exchangeable Mg (cmc kg ⁻¹)	0.91	1.78
Exchangeable K (cmc kg ⁻¹)	0.30	0.64
Exchangeable Na (cmc kg ⁻¹)	0.42	0.31
Exchangeable Acidity (cmc kg ⁻¹)	0.46	
Total Fe (g kg ⁻¹)	4.0	8.0
Total Zn (mg kg ⁻¹)	9.9	22.3
Total Mn (mg kg ⁻¹)	238	670
Total Pb (mg kg ⁻¹)	7.4	15
Total Cr (mg kg ⁻¹)	3.80	6.50
Total Cd (mg kg ⁻¹)	0.30	0.60
Total Cu (mg kg ⁻¹)	6.50	11
Total Ni (mg kg ⁻¹)	4.70	12

†S1: Soil 1, Typic Argiudoll; S2: Soil 2; Typic Hapludoll. ‡Values are the mean of duplicate samples.

2.2. Greenhouse pot experiment

Each soil received five treatments: pure sludge (PS) and limed sludge (LS), each applied at a low dose (LD) and high dose (HD), and a control (no sludge added). Each treatment had three replicates. The sludge-soil mixtures (3 kg dry weight) were placed in pots (4.5 L, height: 18 cm; diameter: 18 cm). The LS consisted of PS with added CaO in a proportion of 50% on a dry weight basis. On a wet basis, the LD was 16.0 Mg ha⁻¹ (19.2 g pot⁻¹) for PS and 17.3 Mg ha⁻¹ (20.8 g pot⁻¹) for LS. The HD was 32.0 (38.6 g pot⁻¹) for PS and 34.6 Mg ha⁻¹ (41.5 g pot⁻¹) for LS, respectively. In this way, the LS amendment contributed an equivalent of 1232 kg ha⁻¹ of lime with the LD and 2465 kg ha⁻¹ of lime with the HD (dry basis).

The PS contributed 102 kg ha⁻¹ P₂O₅ to the soil at the LD, whereas the HD was twice the LD. The LD of PS was selected considering that the initial levels of available P in the soils were low. Therefore, the LD would bring the available P closer to the critical P levels for the crop (14–16 mg kg⁻¹ P-Bray 1) (D'Alesandro Galain and Young Parietti, 2014; Quincke et al., 2008). The LS doses were equivalent to the PS doses of P₂O₅.

Five pre-germinated seeds were sown per pot, and seven days after emergence, the plants were thinned, leaving only two plants per pot. A soil moisture content equivalent to field capacity was maintained from sowing to harvest. The amended and control soils were placed in a nylon bag to prevent nutrient loss by percolating the excess water when irrigated.

2.3. Chemical soil analysis

Soil sampling was carried out at the crop cycle's start and end. The soil was dried at 40 °C, ground, and passed through a 2-mm mesh prior to analysis. Soil OM was quantified by the Walkley-Black wet combustion method (Nelson and Sommers, 1996). The soil's mineral N (NO₃-N + NH₄⁺-N) was extracted with a 2 M KCl solution, and colorimetric analysis was used to determine NO₃-N according to Mulvaney (1996) and NH₄⁺-N according to Rhine et al. (1998) in the KCl extract. The total exchangeable bases (TB), including Ca²⁺, Mg²⁺, K⁺, and Na⁺, were extracted with ammonium acetate buffered at pH 7 (Thomas, 1982). The K and Na were determined by atomic emission spectrometry, and Ca and Mg by absorption spectrometry. The P was determined by the Bray 1 method (Bray and Kurtz, 1945). The pH and electrical conductivity (EC) were determined by potentiometry in a suspension with a 1:1 v/v soil to water ratio. The extraction for the total content of heavy metals was performed according to the EPA method 3050B (U.S. EPA, 1996). The analysis of the extracted heavy metals was done by atomic absorption spectrometry (APHA, 2012).

The N mineralization in the soils was estimated by the sum of NO₃-N and NH₄⁺-N content (mg pot⁻¹) plus the total N content in the above-ground biomass of plants (mg pot⁻¹), hereafter called sum N_{min}.

2.4. Chemical analysis of sludge and plant biomass

Sludge and aboveground biomass samples from each pot (obtained before flowering) were dried at 65 °C for 48–72 h (until constant weight) and ground to pass through a 0.5-mm mesh. After that, the total N, P, K, Ca, Mg, Na, Fe, Cu, Zn, Mn, Pb, Cr, Cd, and Ni contents were determined in sludge and biomass samples. Subsequently, P, K, Ca, Mg, Na, Fe, Cu, Zn, and Mn were extracted with dilute HCl (20%) in samples calcined at 550 °C for 5 h. The molybdate blue method was used to determine the total P with ascorbic acid (Murphy and Riley, 1962). The Ca, Mg, Cu, Fe, Mn, and Zn elements were determined by atomic absorption spectrometry, and K and Na by atomic emission spectrometry (Isaac and Kerber, 1971). The total N content was determined by the Kjeldahl method (Bremner and Mulvaney, 1982). The heavy metals Pb, Cr, Cd, and Ni, were extracted according to EPA 3051 A (U.S. EPA, 2007), and their content in the extract was determined according to EPA 6010 D (U.S. EPA, 2014). The organic carbon of the sludge was determined using wet

Table 2. Chemical characteristics and total concentration of elements, in the sludge used in this study (PS, LS) and in other international studies.

Parameters	PS†	LS	Warman and Termeer (2005)	Wong et al. (2001)	Alvarenga et al. (2016)	Singh and Agrawal (2008)	Eid et al. (2021)	Bozkurt and Yarılgaç (2010)	Mendoza et al. (2006)
Countries			Canada	China	Portugal	Thailand; Spain; India	Saudi Arabia	Turkey	Chile
pH	7.5 ± 0.4**δ	12 ± 0.1		7.6; 8; 3‡	7.1	6.8; 8.6; 7.1	6.7	6.1	6.3
DM (g kg ⁻¹)	166 ± 3.1**	251 ± 3.1		146; 180					
OM (g kg ⁻¹)					675; 743	198; 434; 232	652	459	
Total C (g kg ⁻¹)	290	200		270; 280					307
C/N	7.9/1	7.3/1			5.4; 5.9				
P (g kg ⁻¹)	17.1 ± 0.1 ns	15.0 ± 0.4	8.7; 16.9	10.6; 19.4	59.30	nd; 10.6; 13.4	16.2	7.5	
N (g kg ⁻¹)	36.5 ± 0.2**	27.4 ± 0.2	32.5; 40.8	55.5; 65.0	62.0; 30.1	34; 25; 26	56.4	24.8	
Ca (g kg ⁻¹)	23.6 ± 1.1	132 ± 6.3	16.1; 25.5	0.82; 1.4	12; 27	nd; nd; 16.2			
Mg (g kg ⁻¹)	7.5 ± 2.5 ns	6.5 ± 0.6	3.0; 8.1	2.3; 2.7	4.6; 7.9				
K (g kg ⁻¹)	4.4 ± 1.44 ns	2.4 ± 0.1	2.5; 5.7	1.4; 1.6	7.1; 14.5	2.0; 4.2	6.1	4.3	
Na (g kg ⁻¹)	1.9 ± 0.46 ns	1.2 ± 0.1		0.8; 0.9	2.4; 1.6				
Fe (g kg ⁻¹)	21.2 ± 0.6 **	18.4 ± 1.1	15.3; 27.1				4.1	14.2	
Zn (mg kg ⁻¹)	667 ± 23 **	333 ± 18	390; 1450	1108; 4692	757; 581	1326; 445; 1900	402	1500	757
Mn (mg kg ⁻¹)	1077 ± 44 **	781 ± 10	420; 3150			2621; nd; 400	74.8	380	442
Pb (mg kg ⁻¹)	74	52			<5.6		7.1		81
Cr (mg kg ⁻¹)	28	7.30		53; 2239	<5.6		7.3	90	
Cd (mg kg ⁻¹)	1.5	<0.3		1.78; 2.34	1; <0.3	1.2; 1.0; 1.0		1.8	
Cu (mg kg ⁻¹)	253 ± 6.11	154 ± 4			140; 155	801; 174; 700	119	240	672
Ni (mg kg ⁻¹)	29	11		70; 52.5	7.3		38.9	80	30; 35

†PS: pure sludge, LS: limed sludge. ‡: Values cited by each author(s); nd: no data; δ: means ± standard error, n = 3); **: significant value with p < 0.01 according to t-Test.

oxidation following Mebius's technique (Mebius, 1960). For the experiments corresponding to S2, the limited primary productivity achieved in the plants did not allow measuring the treatment replications independently, so they were integrated into a single sample.

2.5. Statistical analysis

The data were analyzed by applying two statistical models. A mixed linear model was used when data were available for the two soil types due to possible independent variance between soils. A general linear model was used to analyze data from a single soil. The sludge dose, lime dose, and soil type were assumed as fixed effects. The data analysis followed a complete randomized design with three replications.

With the mixed linear model [1], the effects of the treatments and soil type were evaluated on the dependent variables measured in the soil and the plant.

$$Y = a + b_0 \times S_2 + b_1 \times PS + b_2 \times \text{lime} + b_3 \times S_2 \times PS + b_4 \times S_2 \times \text{lime} + b_5 \times PS \times \text{lime} + \varepsilon \quad (0, \sigma) \quad [1]$$

where Y represents the dependent variable in the plant: biomass (g pot⁻¹); content (mg pot⁻¹) of N, P, Ca, Mg, K, Na, Fe, Mn, Cu, and Zn. Likewise, in the soil: pH, EC, concentration (mg kg⁻¹) of P-Bray 1, TB (cmolc kg⁻¹), total concentration of Fe, Mn, Cu, Zn, Pb, Cr and Ni (mg kg⁻¹); sum N_{min} (mg pot⁻¹). The intercept (a) and the coefficients b₀, b₁, b₂, b₃, b₄, and b₅ represent regression coefficients adjusted by maximum likelihood with the statistical package InfoStat/Professional. S₂ represents the effect of the heavy texture soil; PS is the pure sludge dose (kg ha⁻¹); lime represents the dose of lime (kg ha⁻¹); S₂ x PS is the interaction of the heavy soil and the PS dose; S₂ x lime is the interaction

of the heavy soil and the lime dose added with the LS; PS x lime is the interaction of the PS dose and the added lime dose.

The general linear model [2] was used to evaluate the effects of the treatments on the plant variables measured only for S1. The effects on S₂ could not be quantified with the model [2] due to insufficient replicates.

$$Y = a + b_0 \times PS + b_1 \times \text{lime} + b_2 \times PS \times \text{lime} \quad [2]$$

where Y represents the dependent variable in the plant: concentration (% or mg kg⁻¹) of N, P, Ca, Mg, K, Na, Cu, Fe, Mn, Zn, Cr, Cd, and Ni; and content (mg pot⁻¹) of Cd, Cr, and Ni. The intercept (a) and the coefficients b₀, b₁, and b₂ represent regression coefficients adjusted by maximum likelihood with the statistical package InfoStat/Professional. PS represents the pure sludge dose (kg ha⁻¹); lime represents the dose of lime (kg ha⁻¹); PS x lime is the interaction of the PS dose and the added lime dose.

The years required to reach the maximum concentration admitted by Canada (CCME, 1999) were estimated for those metals in the soil that showed an increase in concentration at the end of the experiment. The estimation was carried out according to the equation [3].

$$\text{Years} = (\text{MAC} - \text{BC}) / \text{AMI} \quad [3]$$

MAC: maximum admitted concentration by the CME for the respective metal

BC: base concentration without treatment is the value that b₀ [1] takes for S₁ and b₀+b₁ [1] for S₂.

AMI: Annual metal increase is the metal content variation estimated by the model [1] for each treatment vs the control. The annual application of the treatments was used for this estimation.

3. Results and discussion

3.1. Sludge composition

The chemical characteristics of the used sludge are presented in Table 2. The PS presented an alkaline pH similar to Wong et al. (2001) for a sludge collected from a Hong Kong WWTP and another one generated in India reported by Singh and Agrawal (2008). The concentration of total C (290 g C kg^{-1}) of the PS was similar to the concentrations reported by Wong et al. (2001) and by Mendoza et al. (2006). The total N content was similar to Singh and Agrawal (2008) and Warman and Termeer (2005), 34 and 32 g N kg^{-1} , respectively. However, Wong et al. (2001) and Bozkurt and Yarılgac (2010) published higher (65 g N kg^{-1}) and lower N values (24.8 g N kg^{-1}), respectively (Table 2). The total concentration of P, Ca, Mg, K, and Zn in the PS was similar to those published by Warman and Termeer (2005) and Bozkurt and Yarılgac (2010). Mendoza et al. (2006) presented similar concentration values to those obtained in this study for Pb and Ni, while concentrations of Cr and Cd in our study were lower than those reported by Wong et al. (2001) and Bozkurt and Yarılgac (2010) (Table 2). The concentration of Cu, Zn, Pb, and Cr present in both sludges is below the maximum concentration allowed for agricultural soils according to international regulations (United States, Brazil, Switzerland, Sweden, Scotland, Belgium, Denmark, Germany, France, Italy, Holland, and Spain) (de Melo et al., 2015). For Cd and Ni, their concentrations in PS slightly exceeded the maximum limits established by Denmark and Scotland, which have the lowest limits for these two metals. The PS contents of Cu, Zn, and Ni levels are into Category B of Uruguayan legislation, requiring a management plan for land application (DINAMA, 2016).

3.2. Plant biomass production

Several research studies (Bozkurt and Yarılgac, 2010; Antolín et al., 2005; Castillo et al., 2003; Morera et al., 2002; Illera et al., 2001; Wong et al., 2001; Tsadilas et al., 1995) reported yield increases due to the addition of WWTP sludge to crops. For instance, Morera et al. (2002) reported increased production for sunflower in soils amended with anaerobically stabilized urban sludge, while Singh and Agrawal (2008) showed increased fescue production with sludge application to soil. In this study, the aboveground biomass production of sorghum across all treatments was, on average, 15.9 and 3.2 g pot^{-1} of dry matter (DM) in S1 and S2, respectively (Table 3). The biomass in the control treatment yielded 5.42 g pot^{-1} more in S1 than in S2 ($p < 0.0001$). Despite S2 having a higher concentration of nutrients (Table 1), the lower biomass production in S2 was attributed to the less favorable physical soil properties for root development (less porosity, increased bulk density, and compaction) due to prior exposure of S2 to water erosion of the surface layer.

Several pot experiments, specifically with sorghum plants grown in sludge amended soils at different doses, showed sludge amendment increased biomass production (Eid et al., 2021; Alvarenga et al., 2016; Hernández-Herrera et al., 2005). Mondal et al. (2015) indicated the increased biomass production is because sludge application dramatically improves soil fertility, stability of aggregates, and bulk density. In this pot study the application of PS or LS showed increased DM production in both soil types. Compared to the control, adding PS to S1 increased the biomass from 8.0 to 15.8 g pot^{-1} , representing an increase of 55 and 70% for LD and HD, respectively. The LS amendment generated an increase of 44 and 73% for LD and HD, respectively. In S1, the production of the LS-LD treatment was lower than that of PS-LD. However, the LS-HD treatment yielded more biomass than the PS-HD. These differing responses between doses could be explained by the negative effect of lime added to the positive effect of the sludge \times lime interaction (Table 3). Adding PS to S2 increased the DM production compared to the control by 2.6 and 5.2 g pot^{-1} , representing an 86% and 93% increase for LD and HD, respectively, while the LS amendment increased 78 and 91% for LD

and HD, respectively. The lower DM production LS treatment is likely related to increased alkalinity in S2, with pH as high as 7.7 , impairing the development of the crop. The increased availability of macro and micronutrients, reflected in the plant content, could be the factor responsible for increased production with the sludge treatments compared to the control in both soils (Table 3).

3.3. Nutrients and metal mass in aboveground biomass

The mass of nutrients N, P, Ca, Mg, K, Na, Fe, Mn, Cu, and Zn absorbed per pot by the crop in S1 surpassed the absorption in S2 ($b_0 < 0$). These results, presented in Tables 3 and 4, are attributed to a more significant plant biomass development in S1 (Table 3), possibly associated with physical soil properties, leading to better root system development. In S1, PS ($b_1 > 0$) increased the absorption of N, P, Ca, Mg, K, Na, Fe, Mn, Cu, and Zn. However, LS had a lower increase in the mass of nutrients due to liming ($b_2 < 0$). We observed similar behavior in S2 (Tables 3 and 4).

The lower increase of P, Ca, and K produced by adding LS in S2 is explained by the fact that the lime present in LS interacts negatively with S2 ($b_4 < 0$) to the detriment of the positive effect of PS. In the case of Fe, Mn, Cu, and Zn, the S2 \times lime interaction was not significant, but the lime effect and the sludge \times lime interaction were significant. In the case of P, it tends to form insoluble products with Ca, so the contribution of limed sludge can promote the low availability of soil P (Rabuffetti, 2017; Fageria, 2009). Similarly, Fe, Mn, Cu, and Zn were less available when acidic soil pH increased (Gondal et al., 2021; Neina, 2019). The increase in absorption of N, P, Ca, Mg, K, Na, Fe, Mn, Cu, and Zn due to PS addition compared to the control in S2 was lower (P, Mg, Fe, Mn, Cu, and Zn), equal (N, Ca, Na) or higher (K) than those observed in S1. Singh and Agrawal (2008) reported that the nutrient accumulation pattern in crops grown in sludge-amended soils varies with the type of soil, species, phenology, and the chelating effect on metals. In S1, the addition of PS increased the absorbed amount (mg pot^{-1}) of Cr, Cd, and Ni ($b_0 > 0$). The LS treatment also increased the absorbed amount of these metals, affected by sludge and not by lime since the lime (b_1 , ns) or the PS \times lime interaction (b_2 , ns) effects were not significant.

3.4. Nutrients and heavy metal concentration in aboveground biomass

In S1, the biomass concentration of N, K, Cu, and Mn decreased due to the PS treatment ($b_0 < 0$) compared to the control. On the other hand, the PS treatment did not significantly change the concentrations of P, Ca, Mg, Fe, Zn, Na, Cr, Cd, and Ni (b_0 , ns) (Table 5). Treatment with LS did not show differences in N, Ca, Mg, K, Cu, Fe, Zn, Cr, and Ni concentrations compared to PS (b_1 , ns), but decreased Mn concentration ($b_1 < 0$ and $b_1 + b_2 < 0$), even more than the PS treatment. Therefore, the effect of LS on these elements is somewhat similar to that of PS. However, LS increased P ($b_1 > 0$, $b_1 + b_2 > 0$). Indeed, the LS-LD in S1 had the highest P concentration in the biomass (Table 5). The increased soil P availability can explain the higher P concentration in the biomass of the LS-LD treatment compared to the control. The lower concentration of P in biomass with LS-HD treatment can be attributed to a nutrient dilution effect in biomass or impairment in absorption due to excessive Ca in the limed sludge. Although adding Ca promotes the solubilization of P retained by Al compounds in acidic soils, adding lime can also lead to P fixation due to the formation of sparingly soluble P compounds (Fageria, 2009).

The N concentration decrease of plants grown in S1 with PS compared to the control can also be explained by a dilution effect of biomass production (Abbate and Andrade, 2015; Echeverría et al., 2014; Ferreira and Ernst, 2014). On the contrary, Warman and Termeer (2005) reported greater N concentrations in the plant tissue (grass forage and corn stubble) of plants grown in a silty loam soil amended with sludge than the control. In S1, the concentration of N in the plant was lower than in S2

Table 3. Average dry matter production and macronutrient content in the biomass produced in the treatments and the significance of the effects for each nutrient.

Soil	DM	N	P	Ca	Mg	K	Na
	g pot ⁻¹						
S1†							
Control	6.6	65.1	7.3	23.2	22.9	137.8	1.3
PS-LD	14.5	69.0	16.5	37.6	39.6	148.7	2.9
PS-HD	22.4	129.4	32.7	85.6	85.6	188.5	4.5
LS-LD	11.7	87.5	20.4	50.2	40.3	170.0	2.4
LS-HD	24.0	159.3	32.8	116.3	87.7	169.6	0.8
S2							
Control	0.43	8.1	0.3	2.8	0.6	10.2	0.0
PS-LD	2.9	24.4	4.8	21.0	4.1	108.3	0.6
PS-HD	5.9	48.5	14.8	73.4	12.2	276.1	1.7
LS-LD	2.0	20.9	2.3	10.5	2.4	56.7	0.4
LS-HD	4.7	43.9	9.7	27.5	6.6	144.3	0.9
Fixed effects							
Regression model coefficients (bn) &							
a	5.84E + 00 **δ	5.16E + 01**	6.79E + 00**	1.42E + 01ns	1.47E + 01**	1.38E + 02**	1.68E + 00**
S 2	-5.42E + 00 **	-44.47**	-7.61E + 00**	-1.63E + 01*	-1.49E + 01**	-1.44E + 02**	-1.80E + 00**
PS‡	3.20E-03 **	1.00E-02**	4.70E-03**	1.00E-02**	1.00E-02**	1.00E-02**	5.20E-04**
Lime	-2.00E-03 **	1.10E-03ns	-2.70E-03ns	1.90E-03ns	-3.80E-03ns	-1.10E-03ns	-1.40E-03**
S2 x PS	-2.20E-03 *	-0.01ns	-1.90E-03**	6.00E-04ns	-1.00E-02**	4.00E-02**	-1.80E-04ns
S2 x lime	-8.70E-05 ns	-0.01*	-2.20E-03*	-3.00E-02**	-2.40E-04ns	-6.00E-02**	1.10E-03**
PS x lime	2.40E-07*	1.60E-06ns	4.00E-07ns	1.30E-06ns	2.60E-07ns	-7.10E-07ns	-1.10E-08ns

†S1: light-textured soil; S2: heavy-textured soil; LD: low dose; HD: high dose; ‡ PS: kg of dry sludge added in the treatment; lime: kg of lime added in the treatment. δ ns: non-significant value; *: significant value with $p < 0.05$; **: significant value with $p < 0.01$. & bn: regression coefficients. The values of these coefficients were obtained by adjusting the model [1]: $Y = a + b_0 \times S_2 + b_1 \times PS + b_2 \times \text{lime} + b_3 \times S_2 \times PS + b_4 \times S_2 \times \text{lime} + b_5 \times PS \times \text{lime}$.

Table 4. Content of heavy metals in the biomass produced and the significance of the effects in each treatment.

Soil	Fe	Mn	Cu	Zn	Cd	Cr	Ni
	$\mu\text{g pot}^{-1}$						
S1†							
Control	223	857	53	330	2.65	1.32	13.61
PS-LD	367	1477	50	551	6.26	17.07	23.16
PS-HD	1014	2396	105	890	7.54	15.52	45.27
LS-LD	417	751	47	422	5.44	7.24	21.23
LS-HD	852	1008	79	862	7.22	13.66	39.93
S2							
Control	16.9	33.9	5.6	39.5	nd §	nd	nd
PS-LD	140	215	17	146	nd	nd	nd
PS-HD	280	454	35	367	nd	nd	nd
LS-LD	52.3	109	8	72.4	nd	nd	nd
LS-HD	209	294	38	237	nd	nd	nd
Fixed effects							
Regression model coefficients (bn) &							
a	1.4E-01*	7.8E-01**	4.0E-02**	2.8E-01**	3.00E-03**	4.20E-03ns	1.00E-02*
S 2	-1.2E-01*	-7.5E-01**	-4.0E-02**	-2.6E-01**	nc	nc	nc
PS‡	1.5E-04**	3.0E-04**	1.0E-05**	1.1E-04**	9.30E-07**	2.70E-06*	6.00E-06**
Lime	-1.7E-04**	-7.2E-04**	-3.1E-05**	-1.6E-04**	-7.70E-09ns	-5.40E-06ns	-1.00E-05ns
S2 x PS	-1.0E-04**	-2.2E-04**	-4.4E-06ns	-5.1E-05**	nc	nc	nc
S2 x lime	3.2E-05ns	5.5E-04**	9.3E-06ns	-1.1E-05ns	nc	nc	nc
PS x lime	1.7E-08**	1.5E-08*	3.7E-09**	1.9E-08**	-8.80E-11ns	4.70E-10ns	1.20E-09ns

†S1: light-textured soil; S2: heavy-textured soil; LD: low dose; HD: high dose; ‡ PS: kg of dry sludge added in the treatment; lime: kg of lime added in the treatment. δ ns: not significant value; *: significant value with $p < 0.05$; **: significant value with $p < 0.01$. § nd: no data; nc: not applicable. & bn: regression coefficients. The values of these coefficients were obtained by fitting the model [1]: $Y = a + S_2 \times b_0 + b_1 \times PS + b_2 \times \text{lime} + b_3 \times S_2 \times PS + b_4 \times S_2 \times \text{lime} + b_5 \times PS \times \text{lime}$.

(Table 5). The lower plant biomass in S2 (Table 3) can explain the higher N concentration in S2 due to less dilution.

The concentration of K in plants grown in S1 with PS significantly decreased ($b_0 = -2.4E-4$) compared to the control (Table 5). This result can also be explained by the biomass production dilution effect and possibly by the low sludge contribution of K (Table 3). Warmar and

Termeer (2005) described that WWTP sludge contains small amounts of K because most of it is discharged with the effluent after the sludge passes through the wastewater treatment facility. Consequently, the soil-applied sludge does not provide enough K to meet the plant requirements. The K content in the harvest S1 was well below the critical level for the crop ($<0.34 \text{ cmc kg}^{-1}$), according to Barbazán et al. (2011). Opposite to these

Table 5. Nutrient and heavy metal concentration in aboveground sorghum at harvest (flowering) and significance of the effects in each treatment.

	N	P	Ca	Mg	K	Na	Cu	Fe	Mn	Zn	Cr	Cd	Ni
	%						mg kg ⁻¹						
S1†													
Control	0.98	0.11	0.35	0.35	2.08	0.02	8.0	34	130	50	0.20	0.40	2.06
PS-LD	0.47	0.12	0.27	0.28	1.07	0.02	3.3	25	105	39	1.20	0.43	1.60
PS-HD	0.58	0.15	0.38	0.38	0.84	0.02	4.7	45	107	40	0.70	0.33	2.00
LS-LD	0.76	0.18	0.43	0.35	1.47	0.02	4.0	38	64	36	0.70	0.46	1.80
LS-HD	0.67	0.14	0.49	0.37	0.71	0.01	3.3	35	42	36	0.60	0.30	1.66
S2													
Control	1.87	0.07	0.65	0.13	2.34	0.01	13.0	39	78	91	0.50	0.50	2.20
PS-LD	0.84	0.17	0.72	0.14	3.72	0.02	6.00	48	74	50	0.20	0.30	1.90
PS-HD	0.81	0.25	1.22	0.20	4.61	0.03	5.80	47	76	61	0.53	0.40	1.70
LS-LD	1.04	0.12	0.52	0.12	2.82	0.02	4.00	26	54	36	0.20	0.20	1.80
LS-HD	0.93	0.20	0.58	0.14	3.04	0.02	8.00	44	62	50	0.20	0.37	1.90
Fixed effects ‡													
	Regression model coefficients (b_n) &												
a	0.88**¶	0.11**	0.32**	0.32**	1.98**	0.02**	7**	29.22**	125.4**	47.9**	0.46*	0.42**	1.92**
LP δ	-7.7E-5**	6.4E-6ns	5.1E-6ns	6.4E-6ns	-2.4E-4**	0ns	-6.4E-4**	2.2E-3ns	-4.5E-3*	-1.9E-3ns	8.9E-5ns	-1.3E-5ns	-1.3E-5ns
Lime	8.1E-5ns	7.8E-5**	1.00E-4ns	1.1E-5ns	2.6E-4ns	6.8E6**	1.9E-3ns	1.0E-2ns	-6E-2**	-1E-2ns	8.6E-5ns	1.5E-4**	-6.4E-5ns
PS x lime	2.7E-9ns	-14E-9**	-8.3E-9ns	1.1E-9ns	-35.0E-9ns	-2.3E-9**	320E-9ns	-1.6E-6ns	5.5E-6*	1.6E-6ns	-4.3E-8ns	-2.9E-8**	-1.5E-9ns

†S1: light-textured soil; S2: heavy-textured soil; LD: low dose; HD: high dose; ‡ Statistical analysis was not performed in S2 due to the absence of repetitions; δ PS: kg of dry sludge added in the treatment; lime: kg of lime added in the treatment. ¶ ns: non-significant value; *: significant value with $p < 0.05$; **: significant value with $p < 0.01$; & bn: regression coefficients. The values of these coefficients were obtained by fitting the model [2]: $Y = a + b_0 \times PS + b_1 \times \text{lime} + b_2 \times PS \times \text{lime}$.

results, K concentration in plants grown in S2 were higher than those grown in S1. The low biomass production, higher initial K concentration in S2 (two-fold the critical crop level, Table 1), and the greater capacity of S2 to contribute K throughout the crop cycle due to its higher CEC (Korb et al., 2005) are the factors that explain these results.

The low concentrations of Cu and Mn in aboveground sorghum biomass presented in Table 5 for PS treatment in S1 match those reported by Mendoza et al. (2006). They reported low concentrations of Mn in sorghum stems grown in three soils with added sludge, compared to controls. These authors also reported that plant Cu concentrations did not increase compared to the control in agricultural soils with high Cu content amended with 100 t ha⁻¹ of sludge. They concluded that applying sludge to agricultural soils with high Cu content does not imply greater metal mobility, probably due to the sludge's high sorption capacity. Morera et al. (2002) attributed the lower availability of Cu in sunflower plants grown in acidic soils (pH = 5) amended with sludge to the high amount of soil OM. This tendency coincides with several case studies reported by Basta et al. (2005), where low solubility and phyto-availability of trace elements were observed due to the significant retention in the applied sludge. The lowest plant Mn concentration, in the LS treatment in S1, may reflect an increment of adsorption mechanisms of the LS.

Plant Cd concentration increased with LS-LD (in S1) due to the addition of lime ($b_1 > 0$), but it decreased with the HD (Table 5). This decrease observed with the HD could be due to the chemical retention of Cd by sludge components, the increase in pH, and the dilution effect of increased biomass production (Table 3). Soon (1981) reported that an increase in soil pH increased Cd adsorption due to three factors: the surface charge of the soil colloids becoming negative, the increase in the stability constant of the metal complexes -MO due to the ionization of functional groups (mainly -COOH), and the specific adsorption of heavy metals on the surface of hydrated oxides, such as the adsorption of Cd in CaCO₃ in soils amended with lime. This author concluded that Cd adsorption limited the concentration of Cd²⁺ in the soil solution rather than its precipitation as Cd₃(PO₄)₂. When studying the reaction of WWTP sludge in the soil, de Melo et al. (2015) reported that the di-hydroxy

phosphate anion could form sparingly soluble complexes with the metals Cu, Cd, Zn, and Pb and thus reduce their availability to plants.

3.5. Effect of sludge application on soil properties at harvest

3.5.1. Soil pH

The pH in the S1 with control treatment was 2.58 units ($b_0 = 2.58$, $p < 0.0001$) lower than S2 (Table 6). There were no significant differences in pH during the study for both control treatments in S1 and S2 since they had similar values to those at the beginning of the experiment (Table 1). The PS treatment on S1 decreased the pH ($b_1 = -2.50E-05$, $p = 0.098$) compared to the control, but the LS did not. The acidification caused by PS in S1 could be attributed to N mineralization, a process that produces acidity because the sludge initially had a high total N content (Table 1). However, the addition of LS increased the pH of S1 ($b_2 > b_1$, $p = 0.0001$), offsetting acidification of the N mineralization. Sloan and Basta (1995) reported increased pH of acidic soils (pH < 5) amended with sludge stabilized with Ca(OH)₂.

In S2, however, both PS and LS increased pH compared to the control (Table 6). Sloan and Basta (1995) reported better-aerated soils furthered N mineralization. The S2 used in our study came from a farm with a long history of agriculture and was affected by erosion and compaction, suggesting less aeration than S1. Besides, heavy soils like S2 have a greater capacity to offset acid generated by PS's N mineralization. Singh and Agrawal (2008) suggested that changes in pH can be attributed to calcium carbonate content and acid production during sludge decomposition. Other authors observed that the soil response to the PS amendment could increase (Tsadilas et al., 1995) or decrease the pH value (Epstein et al., 1976). Alvarenga et al. (2016) observed a decrease in pH with respect to the control when applying 24 Mg ha⁻¹ of sludge to sandy soils with alkaline pH (pH = 8.4) and low OM content.

3.5.2. Available N

A significant factor that favors biomass production is the sludge's contribution of Wong et al. (2001) reported that adding municipal sludge to an acid clay soil (pH = 4.7) caused a significant increase in NH₄⁺-N

Table 6. Average values of physical and chemical properties of the soil at the experiment harvest and the significance of the effects for each treatment.

Soil	pH (H ₂ O)	EC dS/m	P-Bray 1 mg kg ⁻¹	sum N _{min} (plant + soil) mg pot ⁻¹	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	TB ¶
					cmolc.kg ⁻¹				
S1†									
Control	5.13	0.09	3.00	94.0	2.15	0.77	0.17	0.43	3.52
PS-LD	5.01	0.09	4.78	91.4	2.42	0.77	0.15	0.43	3.77
PS-HD	4.97	0.08	9.59	154	2.39	0.77	0.13	0.44	3.73
LS-LD	5.37	0.11	6.84	107	2.94	0.83	0.13	0.29	4.18
LS-HD	5.89	0.15	12.07	184	4.7	0.72	0.17	0.43	6.00
S2									
Control	7.64	0.62	2.72	50.8	36.63	1.6	0.7	0.35	39.28
PS-LD	7.71	0.62	3.82	59.3	43.33	1.87	0.87	0.43	46.50
PS-HD	7.68	0.62	5.70	84.3	40.24	1.71	0.76	0.40	43.11
LS-LD	7.74	0.64	3.82	54.2	43.72	1.83	0.79	0.40	46.74
LS-HD	7.79	0.66	3.55	77.2	44.12	1.93	0.81	0.43	47.28
Fixed effects					Regression model coefficients (b_n) &				
a	5.09**δ	0.09 **	2.23E + 00 **	9.14E + 01**					3.56**
S2	2.58**	0.53 **	0.42ns	-4.01E + 01**					40.97**
PS‡	-2.50E-05*	-2.30E-06ns	1.30E-03 **	1.10E-02**					9.0E-05 ns
Lime	3.20E-04**	1.50E-05ns	1.60E-03 **	-2.49E-02*					2.1E-04ns
S2 x PS	2.90E-05*	2.30E-06ns	-7.70E-04 **	-4.90E-03*					9.7E-04ns
S2 x lime	-3.30E-04**	-1.00E-05ns	-1.60E-03 **	-8.10E-03ns					9.1E-04ns
PS x lime	9.30E-09ns	2.40E-09ns	-1.50E-07 *	5.60E-06*					2.6E-07*

†S1: light-textured soil; S2: heavy-textured soil; LD: low dose; HD: high dose; ‡ PS: kg of dry sludge added in the treatment; lime: kg of lime added in the treatment. δ ns: non-significant value; *: significant value with $p < 0.05$; **: significant value with $p < 0.01$. & bn: regression coefficients. The values of these coefficients were obtained by adjusting the model [1]: $Y = a + S2 \times b0 + b1 \times PS + b2 \times lime + b3 \times S2 \times PS + b4 \times S2 \times lime + b5 \times PS \times lime$. ¶ TB: Total exchangeable bases.

due to its high N content. Silva-Leal et al. (2013) evaluated the mineralization of N in WWTP biosolids and reported increased mineral N contents (from 93.8 to 439.8 mg kg⁻¹) in soils amended with thermally dried, alkalinized sludge. Yoneyama and Yoshida (1978) reported similar increases in mineral N contents in controlled soil incubations. Rasouli-Sadaghiani and Moradi (2014) reported that sludge induced the most significant net N mineralization among the organic residues applied to silty clay soils at pH 7.5. In our study, the increased biomass in S1 and S2 with PS and LS followed the increase in N mineralization quantified by the sum N_{min} (Table 6). The N content in the plant biomass increased at increasing sludge doses. This result is in line with other studies (Boyle and Paul, 1989; Stark and Clapp, 1980), which report that the total mineralized N increases with the dose of organic N applied with the sludge. Since sludge contributes most of the N in organic forms (Figueiredo et al., 2019; Yoneyama and Yoshida, 1978), increasing the sludge dose increases the soil mineralized N. The most significant fraction of sum N_{min} in this study is in the plant N and lowest in the residual mineral N in soils at harvest. The low concentrations of residual mineral N could be explained by sequential mineralization, which allows crops to meet their N needs by leaving low amounts of mineral N in the soil (Andrade et al., 2000). These low residual concentrations of mineral N imply that the sludge doses used were not excessive for the crop. The PS treatment increased production of sum N_{min} ($b1 = 0.011$, $p < 0.0001$) in both soils, but the effect tended to be lower in S2 ($b3 = -0.0049$, $p = 0.075$). LS treatment increased the sum N_{min} ($b5 = 5.60E-06$, $p = 0.0247$) more than the PS treatment. The increased mineralization in soils with sludge application is consistent with what Álvarez and Angelica (2004) and Quemada and Menacho (1999) reported. Besides the amount of sludge, its C/N ratio determines the net mineralization of the residue. The low C/N ratios of PS (7.9) and LS (7.3), shown in Table 2, favor the N mineralization process (Quemada and Menacho, 1999). Whereas C/N ratios over 20 favor N immobilization (Kuo and Sainju, 1998; Montero et al., 1997).

3.5.3. Exchangeable bases and EC

The TB content in S2 were eleven times higher than in S1 (Table 6). However, there was no effect on the TB content in any soils when amending with PS. When amending with LS, the TB concentration increased in both soils (average doses) mainly due to the Ca added with the lime treatment. EC values in S2 were six times higher ($b0 = 0.53$, $p < 0.0001$) than in S1. PS and LS treatments did not affect the soils' EC values (Table 6).

3.5.4. Available P

Residual available P levels (P-Bray 1) in the control treatments were similar in both soils (Table 6). The PS treatments increased the concentration of P-Bray 1 ($b1 = 1.30E-03$, $p < 0.0001$) at harvest, and the magnitude of this increase depended on the soil type ($b3 = -7.70E-04$, $p < 0.0001$). In S1, there was an average increase of 4.2 mg kg⁻¹ P-Bray 1 with the PS amendments and 6.5 mg kg⁻¹ P-Bray 1 with the LS amendments (Table 6).

The PS amendments increased P-Bray 1 in S2 ($b1+b3>0$, $p < 0.0001$). The average increase of the two PS doses was 2.0 mg kg⁻¹ P-Bray 1. The LS treatments in S2 increased the P-Bray 1 but less than PS. This dissimilar result is explained by the negative PS × lime interaction (Table 6). The increase in P available in soils with sludge application is consistent with that reported by several authors (Torri et al., 2017; Alvarenga et al., 2016; Singh and Agrawal, 2008; Epstein et al., 1976), which attributed the soil P increase to the high concentration of inorganic P in biosolids. Inorganic P is the predominant form of P in the sludge, accounting for between 70 and 90% of total Jenkins et al. (2000) estimated that almost 50% of phosphate in most biosolids is available for plant uptake during the first year. The lower increase of P-Bray 1 in the S2 compared to S1 can be attributed to a higher P retention capacity of the S2. Torri et al. (2017) and Kulhánek et al. (2009) reported that P adsorption commonly happens in clay minerals, Fe and Al organic complexes, and Fe and Al allophanes and oxides. In non-calcareous soils,

Table 7. Concentration of heavy metals in the soil (total fraction) at harvest and significance of the effects for each treatment.

Soil	Fe	Mn	Cu	Zn	Pb	Cr	Ni
mg kg ⁻¹							
S1†							
Control	3490	249	6.5	9.9	7.3	2.4	5.7
PS-LD	3792	249	6.5	10.7	6.9	2.1	6.4
PS-HD	3378	236	6.5	9.7	6.6	2.1	6.0
LS-LD	3395	234	6.1	9.7	6.3	2.9	5.9
LS-HD	3579	231	6.8	10.0	6.4	3.0	5.9
S2							
Control	3793	711	11.3	22.3	15.3	6.1	13.7
PS-LD	3312	702	10.6	21.3	14.0	5.2	12.7
PS-HD	3983	698	11.7	29.0	14.0	7.1	14.0
LS-LD	3649	679	11.7	36.3	14.0	7.2	14.3
LS-HD	3667	693	12.5	31.7	15.0	6.9	12.4
Fixed effects Regression model coefficients (bn) &							
a	3.6E + 03**	2.5E + 02**	6.4E + 00**	8.7E + 00**	7.3E + 00**	2.2E + 00**	5.6E + 00**
S2	5.0E + 01ns	4.5E + 02**	4.6E + 00**	1.4E + 01**	7.7E + 00**	3.5E + 00**	7.8E + 00**
PS‡	-1.0E-02ns	-3.0E-03ns	1.6E-05ns	3.2E-04ns	-1.5E-04ns	-2.9E-05ns	1.1E-04 ns
Lime	-1.4E-01ns	-3.0E-02ns	-4.9E-04ns	1.0E-02*	-1.2E-03*	1.3E-03*	9.6E-04 ns
S2 x PS	4.0E-02ns	1.0E-03ns	3.2E-05ns	6.1E-04ns	-9.0E-05ns	1.9E-04ns	-9.4E-05 ns
S2 x lime	-5.0E-02ns	-1.6E-03ns	4.4E-04ns	3.1E-03*	4.6E-04ns	-1.9E-04ns	-9.0E-05 ns
PS x lime	2.7E-05ns	4.2E-06ns	9.4E-08ns	-1.3E-06*	2.1E-07*	-1.6E-07ns	-2.1E-07ns

†S1: light-textured soil; S2: heavy-textured soil; LD: low dose; HD: high dose; ‡ PS: kg of dry sludge added in the treatment; lime: kg of lime added in the treatment. δ ns: non-significant value; *: significant value with p < 0.05; **: significant value with p < 0.01. & bn: regression coefficients. The values of these coefficients were obtained by adjusting the model [1]: $Y = a + b_0 \times S_2 + b_1 \times PS + b_2 \times \text{lime} + b_3 \times S_2 \times PS + b_4 \times S_2 \times \text{lime} + b_5 \times PS \times \text{lime}$.

Fe and Al phosphates are among the essential fractions retaining P (Rabuffetti, 2017; Fageria, 2009). A high iron content in the sludge, provided by FeCl₃ (flocculant added to the wastewater in the WWTP in the solid-water separation process), reduced P availability for crops. The Fe³⁺ derived from added soluble salt tends to form insoluble compounds that retain P (Hernández et al., 2013; Fernández, 2007; Hernández, 2004; Arambarri and Madrid, 1971).

However, soils derived from calcareous source material also have high P retention (Torri et al., 2017). The highest P availability in mineral soils occurs in the slightly acidic to neutral pH range. At low pH, P forms sparingly soluble compounds with Fe and Al and at high pH (7.5–8.5) with Ca (Rabuffetti, 2017; Ponnampertuma et al., 1967). This study, S2 presented an alkaline pH and higher contents of exchangeable Ca, clay, and OM (Table 1) that favor P retention more than S1. This research validates a contribution of P by sludge and a residual effect of this nutrient since treatments with PS or LS present the highest concentrations in the soil. In the case of S1, P residue is higher in the treatment with LS, which can be explained by the decrease in soil acidity, favoring P plant availability (Kisinyo et al., 2013; Tisdale et al., 1990).

Furthermore, when evaluating the potential negative impact of excess P fertilization, it is necessary to know the concentration of available P in the soil (Sharpley et al., 1996). In Uruguay, fertilization of agricultural and forage crops in dairy production systems must not exceed the maximum level of 31 mg kg⁻¹ P-Bray 1 as a requirement for nutrient management (DGRN, 2019). In this study, in all treatments, the concentrations of P-Bray 1 in the soil at harvest were below the critical level required by the crop (14–16 mg kg⁻¹ P-Bray 1) (Quincke et al., 2008); therefore, the available P (P-Bray 1) should be evaluated before sowing the next crop.

3.5.5. Metal concentration

The soil concentration of metals was higher (56, 46, 42, 39, 36, and 34% for Cu, Pb, Ni, Cr, Zn, and Mn, respectively) in the S2 control treatment than in the S1 control (b₀>0). Contrary to the increase in metal soil concentration reported by Eid et al. (2021), adding PS did not significantly affect the concentration at harvest of Fe, Mn, Cu, Zn, Pb, Cr,

and Ni in any of the soils (Table 7). The LS decreased the concentration of Pb in both soils, which was attributed to the lime effect (b₂ = -1.20E-03, p = 0.037).

In the LS treatment, the Zn and Cr increase in S2 is more significant than in S1, attributable to the lime effect (b₂ = 1.30E-03, p = 0.03; b₂ = 0.01, p = 0.043 for Cr and Zn, respectively). According to Shaltout et al. (2021), the bioconcentration factor (from soil to roots) associated with Cr and Zn is higher than 1.0. Additionally, they found a negative correlation between this bioconcentration factor and the soil pH, meaning that these metals in soils are less available when the soil pH increases. Silva and Gutiérrez (2010) mention that the increase in pH, through lime, oxides, and calcium hydroxide, up to pH 6–7, is a strategy to use in agricultural soils so that heavy and toxic metals such as Cr lose the ability to be absorbed by plants because of being insoluble. Despite the increase in the concentration of total Cr in soil, the levels reached are well below the maximum cited in the Canadian Environmental Quality Guidelines (CCME, 1999) and CONAMA (de Melo et al., 2015), of 64 and 152 kg ha⁻¹, respectively.

4. Conclusions

The addition of PS and LS favored plant development measured as aboveground biomass production.

The addition of PS and LS in S1 did not increase the plant concentration of any of the measured elements. These results demonstrate that the increase in the total concentration of a metal in soil by these amendments does not increase its concentration in plant materials. Still, the sludge amendments allowed the crop to achieve a greater extraction of elements, reducing the potential risk of accumulation in the soil and making land application of the sludge an option.

Apart from providing the soil with nutrients, the LS was an appropriate amendment to reduce the acidity of acidic soils, making it a better medium for crop development. The LS favored the availability and absorption of P in S1 (with higher acidity) more than by PS, so it would be more beneficial to use LS in light-textured soils with acidic pH (e.g., pH = 5). Critical concentrations of P-Bray 1 in the soil at harvest were not exceeded with the applied sludge doses.

Because of the low concentrations of Cr, Cd, and Pb in this sludge, it could be used as a soil amendment in crop production. The PS application, at the experimental doses, would not generate Cr or Pb accumulation in the soil. However, LS could cause Cr and Zn accumulation, therefore it is vital to monitor these metals. Taking the results obtained for S2 with LS-LD treatment as an example, it would take 46 and 8 years to reach the maximum concentration of Cr and Zn, respectively, according to Canada's protocols (CCME, 1999).

Declarations

Author contribution statement

Arlo, L.: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Beretta, A.; Szogi, A.A.: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

del Pino, A.: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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