




Characterization of the solid residue generated in the detoxification step of sugarcane bagasse hemicellulosic hydrolysate and behavior in agricultural soils

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ABSTRACT: Overliming is an effective way of neutralizing and reducing the toxicity of the hydrolysates generated from acidic pretreatment of lignocellulosic biomass for ethanol production and others biotechnological products. Overliming generates a solid residue whose inadequate disposal may represent an environmental problem. This work aimed at the chemical characterization of the solid residue generated during the detoxification of the sugarcane bagasse hemicellulosic hydrolysate and evaluation of its behavior in soil. The solid residue was submitted to physicochemical and granulometric analysis and determination of the contents of sugars, metals and toxic compounds (acetic acid, furfural, hydroxymethylfurfural and phenols). The potential of the residue as corrective of soil acidity was determined by analyzing the quality attributes of soil correctives (neutralizing power – NP, relative total neutralizing power – RTNP, Ca and Mg contents and granulometry). The residue was also evaluated for its influence on soil microbial communities and toxicity through bioassays with *Lactuca sativa* seeds. The residue contributed to the overall improvement of soil chemical attributes. The levels of Ca, Mg, C, OM and CEC were increased, nutrients such as Zn, Fe, Ni, Cr and Mn were detected, and microbial communities were stimulated. Besides, the residue showed no significant values of toxic compounds and no toxicity to *L. sativa* seeds. The residue was able to reduce the soil pH and to keep it stable throughout the study period. As a higher amount of the residue was necessary to reach pH 7 than limestone, it should be used as an auxiliary corrective of acidity.

Key words: overliming, limestone, soil acidity, soil quality, toxicity.

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INTRODUCTION

The growing demand for fuel has led to an increase in the use of liquid fuels, however, the availability of energy from nonrenewable sources is limited and their exploration, transformation and use can generate serious impacts on the environment. In this sense, biofuels can be a viable alternative to increase the ethanol production required to meet world demand, being a renewable source of energy widely accepted as an alternative to fossil fuels (Sudhakara et al. 2016).

The lignocellulosic biomass consists basically of cellulose, hemicellulose and lignin (Raud 2016) and its use in the production of second-generation ethanol and other biotechnological products depends on the availability of sugars present in their composition. The process of chemical hydrolysis, in which diluted acid is used, is a technique commonly used as a pretreatment to increase the enzymatic accessibility of cellulose and, at the same time, to obtain a hemicellulosic hydrolysate rich in xylose, glucose and arabinose, which can be fermented to ethanol (Camargo et al. 2014). The hemicellulosic fraction has also been used in bioprocesses to obtain other products of industrial interest, such as xylitol (Hernández-Pérez et al.

2016), poly-3-hydroxybutyrate (Dietrich et al. 2018) and coproduction of ethanol and butanediol (Sharma et al. 2018), in addition to transforming aliphatic alcohols derived from hemicellulose into alkanes for better carbon utilization (Sun et al. 2018). These processes contribute to the efficient utilization and valorization of this fraction as biorefinery feedstock (Arora et al. 2018).

One of the main problems related to the biomass pretreatment is the generation of toxic compounds during the biomass hydrolysis process, which negatively interfere in the microbial metabolism, affecting the conversion of sugars into the product and therefore need to be removed (Sudhakara et al. 2016). During hemicellulose hydrolysis, inhibitors such as furfural and hydroxymethylfurfural are generated from pentoses and hexoses degradation, respectively and formic and levulinic acids can also be formed as a result of the degradation of these inhibitors (Jönsson and Martín 2016). The partial decomposition of lignin leads to the formation of furfural and hydroxymethylfurfural (Mathew et al. 2016).

The detoxification method known as overliming, which consists in raising the pH to 10 with calcium hydroxide ($\text{Ca}(\text{OH})_2$) or calcium oxide (CaO), is effective in reducing the toxicity of hemicellulosic hydrolysates (Zhang et al. 2018; Guan et al. 2018). However, this method generates a solid residue rich in calcium salts, which requires an adequate allocation. Until now, there are no reports of its chemical characterization, risk analysis for the environment and possible ways to use it.

Brazilian soils are mostly acidic and the effects of this acidity lead to low crop productivity (Serrat et al. 2002; Roscoe 2006). Among the benefits of soil acidity correction are the supply of calcium (Ca) and magnesium (Mg) for plants, reduction or elimination of the toxic effects of aluminum (Al) and the excess of manganese (Mn), iron (Fe) and copper (Cu), increased availability of nutrients such as phosphorus (P), sulfur (S) and nitrogen (N) present in organic matter (Serrat et al. 2002).

Therefore, the objective of this work was to carry out the chemical characterization of the solid residue generated in the detoxification process of the sugarcane bagasse hemicellulosic hydrolysate and to evaluate its potential in correcting soil acidity, as well as its influence on the chemical and microbiological quality of soil for agricultural activity. The results were compared to limestone, commonly used in the practice of soil liming.

MATERIAL AND METHODS

Obtainment and characterization of the solid residue

Sugarcane bagasse hydrolysate was obtained by acid pretreatment (Silva et al. 2014) and detoxified by overliming with CaO (commercial degree) by raising the pH to 8.0, followed by filtration. The recovered solid residue was characterized and used in the experiments.

Physicochemical characterization of the solid residue

Chemical and particle size analysis

The chemical characterization of the solid residue was performed using the Mehlich extractor for K, Fe, Mn, Cu, and Zn and the extractor KCl for Ca, Mg and Al (Embrapa 2009). For Ni, Cr, Pb and Cd, the samples were submitted to nitroperchloric digestion, according to the method described by Johnson and Ulrich (1959). After all the analytical procedures for opening the samples, a 5 mL volume was transferred into 5 mL vials and, then, subjected to analysis in an atomic absorption spectrophotometer (Shimadzu, Model 6300). Determinations of carbon content (C), organic matter (OM), cation exchange capacity (CEC) and granulometric analysis were performed according to the methodology described by Embrapa (2009).

Determination of the concentration of sugars and acetic acid

For the quantification of the sugars (glucose, xylose and arabinose) and acetic acid, 1.0 g of the residue was added to 5 mL of distilled water, then the mixture was allowed to stand for 30 min and centrifuged for 10 min at $1500 \times g$. Then the supernatant was purified in a Sep Pak C18 cartridge and analyzed in a liquid chromatograph (Shimadzu 20A), with refractive index detector, using a Phenomenex Rezex ROA-Organic Acid H^+ (8%) 300×7.8 mm column, eluent H_2SO_4 $0.005 \text{ mol}\cdot\text{L}^{-1}$,

flow $0.6 \text{ mL} \times \text{min}^{-1}$ and oven temperature $65 \text{ }^\circ\text{C}$. The concentrations of the compounds were determined from standard curves obtained with high purity standards (98-99%, Sigma and Vetec).

Determination of the concentration of furfural and hydroxymethylfurfural

For the determination of the concentration of the compounds furfural and hydroxymethylfurfural, the samples (prepared according to the previous item) were filtered through HAWP $0.45 \text{ }\mu\text{m}$ membrane (Millipore) for total solid particles removal. The samples were analyzed in a liquid chromatograph (Shimadzu 20A) employing the following conditions: column Waters Resolve C18 $5 \text{ }\mu\text{m}$ 90 \AA $3.9 \times 300 \text{ mm}$, room temperature, UV detector at 276 nm , eluent acetonitrile/water (1:8) with 1% of acetic acid, flow $0.9 \text{ mL} \times \text{min}^{-1}$ and sample injection volume $20 \text{ }\mu\text{L}$. The concentrations of furfural and hydroxymethylfurfural were determined from standard curves obtained with high purity standards (99%, Sigma).

Determination of the concentration of phenols

The concentration of total phenols was determined according to the Folin-Ciocalteu method (Singleton et al. 1999). In this process, 0.2 mL of the Folin-Ciocalteu reagent were added to 3 mL of the residue (prepared according to the procedure for the chromatographic analysis) in glass tubes. After 5 min , 0.8 mL of sodium carbonate solution ($150 \text{ g}\cdot\text{L}^{-1}$) were added, followed by vortexing. Samples were kept in the dark for 30 min and then the absorbance values were measured in a spectrophotometer (UV-VIS FEMTO 700 Plus), at 760 nm . The phenols concentrations were determined from a standard curve of vanillin (98%, Synth).

Evaluation of the utilization of the solid residue in soil

Soil collecting

The soil came from an uncultivated area in Cascavel-PR, Brazil. Subsamples (1.0-kg^{-1}) were collected at a depth of 15 cm , from 10 different points, following a zigzag geometric pattern, identified by means of a global positioning system (GPS, Garmin Etrex- Vista). Subsamples from each point were placed in individual sterile polypropylene bags ($15 \times 20 \text{ cm}$), then pooled in a single bag and thoroughly mixed to obtain a homogeneous mixture. The samples were transported to the laboratory in polystyrene boxes and stored at $4 \text{ }^\circ\text{C}$ for further analyses and experiments.

Soil characterization

The physicochemical characterization of the soil was performed as described for the residue characterization, according to Embrapa (2009). Chemical characteristics of the soil before the application of the residue and the limestone are shown in Table 1. To determine soil moisture, 10 g of soil were oven-dried at $105 \text{ }^\circ\text{C}$ for 24 h .

Table 1. Physicochemical characterization of soil used in the experiments.

K^+	Ca^+	Mg^{2+}	$\text{H}^+ + \text{Al}^{3+}$	Al^{3+}	P	C	N	pH (water)
		$(\text{cmol}\cdot\text{dm}^3)$			$(\text{mg}\cdot\text{dm}^3)$	$(\text{g}\cdot\text{dm}^3)$		
0.06	0.86	0.14	3.69	Not detected	0.50	6.30	0.50	5.30
	Cu		Zn	Fe	Mn	Pb		Cd
				$(\text{mg}\cdot\text{dm}^3)$				
	10		0.80	77	16	Not detected		Not detected
	Granulometry (%)							
	Clay		Silt		Sand		Class Clay	
	63		19		18		Soil type 3	

Residue characterization for acidity correction

Analyses were carried out to determine the residue potential as a corrective of soil acidity. For this, the quality of the correctives attributes such as neutralization power (NP), relative total neutralization power (RTNP) and particle size analysis were determined. All analyses were performed following the methodology described by Embrapa (2009). The quantity of oxides present in the solid residue were determined by an X-ray fluorescence spectrometer (Panalytical, model Axios Max. X-ray tube: rhodium).

The results were compared to those of dolomitic limestone, characterized as to the neutralization power (NP), relative total neutralization power (RNTP), quantity of oxides and particle size.

Soil acidity correction tests

The soil pH correction tests were conducted in 3 L capacity pots, in quadruplicate, at 24 °C and humidity 40%. To achieve the desired pH value (7), 150 g of solid residue were added to 600 g of soil. In order to compare the results with the limestone, commonly used in the practice of liming in Brazil, vessels containing 600 g of soil + 6 g of limestone (pH 7), in quadruplicate, were used. The test lasted 45 days, with samples collections for pH monitoring on days 10, 20, 30 and 45. For pH monitoring soils samples of 10 mL were mixed with 25 mL of deionized water, stirred with a stick, left standing for 1 h and then measured with a pHmeter, according to the methodology described by Embrapa (2009).

Bioassays with lettuce (*Lactuca sativa* L.) seeds

The soil extracts were obtained from the addition of sufficient water to reach a 60% soil moisture. Then, the soil samples were allowed to stand for 30 min and were centrifuged at $3400 \times g$, for 15 min. The germination tests with lettuce seeds were carried out in Petri dishes, containing two overlapping filter paper discs, moistened in the proportion of 2.5 times the mass of the paper in relation to the volume of water (control) or soil extracts of both the treatments (soil + residue and soil + limestone, incubated for 45 days at 24 °C and humidity 40%), as described in the previous item.

Twenty-five seeds were used for each treatment, which were carefully distributed on the filter paper inside the plates. The soil extracts were added only once, and then only distilled water was added to maintain the moisture at 40%. The plates were incubated at 25 °C under a 12-h photoperiod in a BOD-type greenhouse (Technal TE-401). The seeds were daily observed.

Germination speed index (GSI)

The counts of germinated seeds were daily performed and started from the day the first seed germinated until the day the germination stabilized, following the methodology described by Nakagawa (1999). Seeds with radicle protrusion were considered germinated. The GSI was calculated according to Eq. 1:

$$GSI = (E_1/N_1) + (E_2/N_2) + \dots + (E_n/N_n) \quad (1)$$

where E_1, E_2, \dots, E_n = number of seeds germinated on the first count, on the second count (...) until the last count; N_1, N_2, \dots, N_n = number of days of sowing on the first count, on the second count (...) until the last count. The results were expressed as number of germinated seeds per day.

Germination speed (GS)

The data used to evaluate the mean germination time were the same as those used for the evaluation of GSI. The GS was calculated according to Edmond and Drapala (1958), who considered that the treatment with the lowest mean took the shortest period in days to germinate, therefore, it presented the highest germination speed, according to Eq. 2:

$$\text{MGT} = [(N_1 E_1) + (N_2 E_2) + \dots (N_n E_n)] / N_1 + N_2 \dots + N_n \quad (2)$$

where $E_1 + E_2 + \dots + E_n$ = number of normal seedlings computed on the first count, on the second count (...) until the last count; $N_1 N_2 \dots N_n$ = number of days of sowing on the first count, on the second count (...) until the last count. The results were expressed in number of days the seeds took to germinate.

Percentage of germination (%)

The percentage of germination was calculated considering the total number of seeds germinated and seeded, according to Eq. 3:

$$\% \text{ Germination} = \text{total germinated} / \text{total seeded} \cdot 100 \quad (3)$$

Seedling growth

Growth analyses of lettuce seedlings were performed with 10 normal lettuce seedlings of each replicate. The length of the primary root and height of the aerial part of the seedlings were evaluated on the seventh day of incubation, using a millimeter ruler, and the results were expressed in centimeters.

Statistical analysis

Statistical analysis of the data (% of germination, GSI and MGT) was performed by analysis of variance (ANOVA, $\alpha = 0.05$) using R software 3.2.1 for Windows (R Core Team 2015). The averages were submitted to Tukey's multiple range test, to detect the occurrence of significant differences ($\alpha = 0.05$).

Microbiological analysis

Microbiological analyses were performed before and after the application of the residue in the soil in order to evaluate whether it exerted influence in the soil microbial community. The analyses consisted in the total quantification of bacteria and fungi. Soil samples treated with limestone, commonly used in the practice of liming, were also analyzed for results comparison.

The experiment was conducted for 45 days, with counts performed on days 10, 20, 30 and 45.

Fungal counts were performed on potato dextrose agar medium (pH 3.5, adjusted with sterile 10 % w/v tartaric acid aqueous solution). Soil samples of 10 g were weighed and added in sterile Erlenmeyer flasks containing 90 mL of 0.1% peptone water. After vigorous mixing, serial dilutions were made up to 10^{-4} in tubes containing 9 mL of sterile 0.1% peptone water. Aliquots of 0.1 mL from the 10^{-1} to 10^{-4} dilutions were inoculated on the surface of the Petri dishes, in triplicate. After inoculation, the plates were incubated at 25 °C for 5 days and the number of colonies were registered. The results were expressed as number of colonies forming units per sample volume unit (CFU·g⁻¹). To estimate the number of viable bacteria, the same technique was used, except that the culture medium was the nutrient agar. The incubation period was 3 days at 32 °C.

RESULTS AND DISCUSSION

Physicochemical characterization of the solid residue

The solid residue generated in the detoxification step of the sugarcane bagasse hemicellulosic hydrolysate was submitted to physicochemical characterization, whose results can be observed in Table 2. The analysis found concentrations

of 291.17 and 44.83 $\text{cmol}\cdot\text{dm}^{-3}$ of secondary macronutrients such as Ca and Mg, respectively, important elements for acidity correction in soils. According to Abreu Júnior et al. (2001), these cations are related to the values of index (V%) and potential soil acidity. The value of potential acidity ($\text{H}^+ + \text{Al}^3$) of the residue was 9.935 $\text{cmol}\cdot\text{dm}^{-3}$.

The presence of micronutrients was determined for Zn, Mn, Ni, Fe and Cr (101.89; 171.96; 320.48; 798.79 and 1.006.43 $\text{mg}\cdot\text{dm}^{-3}$, respectively). According to Carvalho et al. (2012), micronutrients such as Zn, Fe, Mn and Ni are essential for plants and animals because they participate directly and indirectly in important metabolic reactions and, therefore, it is necessary to evaluate their contribution in soil, because tropical soils often have micronutrient deficiencies.

The inhibitor compounds showed values of 0.0005 g furfural $\cdot\text{g}^{-1}$, 0.001 g hydroxymethylfurfural $\cdot\text{g}^{-1}$ and 0.001 g phenols $\cdot\text{g}^{-1}$ of residue. Since the detoxification process may also remove quantities of sugars from the lignocellulosic biomass, the residue was also characterized for sugar concentrations, whose values were 0.005 g glucose $\cdot\text{g}^{-1}$, 0.091 g xylose $\cdot\text{g}^{-1}$ and 0.08 g arabinose $\cdot\text{g}^{-1}$ of residue.

Table 2. Physicochemical characterization of the solid residue generated in the detoxification of the sugarcane bagasse hemicellulosic hydrolysate.

K^+	Ca^+	Mg^{2+}	$\text{H}^+ + \text{Al}^{3+}$	Al^{3+}	N ($\text{g}\cdot\text{dm}^3$)	pH (water)	
0.06	291.17	44.83	9.94	1109.95	1925	5.60	
Cu	Zn	Fe	Cr	Pb	Cd	Mn	Ni
($\text{mg}\cdot\text{dm}^3$)							
Not detected	101.89	798.79	1006.43	Not detected	Not detected	171.96	320.48
Granulometry (%)							
Sieve 10			Sieve 20			Sieve 50	
91.82			61.57			23.91	

Soil acidity correction tests

Analysis of the quality attributes such as neutralization power (NP) and relative total neutralization power (RTNP) of the studied residues was based on the evaluation of Ca and Mg oxides (Table 3). These values were lower than those of the limestone, product commonly used for liming in Brazil, which are also shown in Table 3. From the characterization of the oxides present in the solid residue, the NP of 52.65%, RTNP of 27% and the sums of CaO and MgO of 29.5%, were obtained.

Table 3. Technological characterization of the residue and limestone for soil acidity correction.

Oxides present in the solid residue						
SO_3 (%)	CaO (%)	Fe_2O_3 (%)	Al_2O_3 (%)	SiO_2 (%)	Cr_2O_3 (%)	P_2O_5 (%)
33.20	29.30	3.50	1.40	1.00	0.40	0.30
K_2O (%)	MgO (%)	NiO (%)	SrO (%)	MnO (%)	Na_2O (%)	Loss to fire (%)
0.20	0.20	0.10	0.10	< 0.10	< 0.10	30.42
Total sum of oxides (MgO + CaO)					29.50%	
NP ²	52.65%		RTNP		27%	
Oxides present in the limestone						
CaO (%)			MgO (%)			
44.90			4.90			
Total sum of oxides (MgO + CaO)					49.88%	
NP ²	92.50%		RTNP		77.20%	

Equivalent in CaCO_3 ².

The values found for the limestone were: NP of 92.52%, RTNP of 77.17% and sums of CaO and MgO of 49.88%. Therefore, to reach pH 7, it was necessary that the soil was mixed with limestone in the proportion of 600 g of soil + 6 g of limestone; for the residue, the proportion was 600 g of soil + 150 g of residue. Therefore, to reach the same pH values, the amount of limestone necessary to correct the initial pH from 5.3 to 7.0 differed from the amount of residue used, a fact that can be explained by the NP value, since the higher the NP, the higher is the amount of acids it neutralizes. Thus, as the NP value observed for the residue was lower than that observed for the limestone, a higher amount of solid residue was necessary to reach the desired pH.

After correction of the soil pH to 7.0 with the residue and limestone, 45 days were waited for stabilization and a new soils characterization was performed. The results are shown in Table 4. The utilization of the solid residue promoted an increase in the Ca and Mg contents, both in a much more pronounced way when compared to the soil with limestone. According to Medeiros et al. (2008), the variation in the Ca:Mg in soil acidity correctives is one of the main ways to affect the availability of these nutrients to plants in acid soils. The authors state that high concentrations of exchangeable Ca in soil, caused by the addition of acidity correctors with high Ca:Mg contents, inhibit the absorption of Mg and K by corn plants, decreasing dry matter production and plant height at the initial development stage. Rigon et al. (2015) also observed that the increase of Ca in the Ca:Mg ratio consequently decreased the availability of Mg limiting the productive properties of sunflower and the physiological quality of seeds.

Regarding K, the values in the soil and soil with limestone were low and remained unaltered for 45 days. The addition of the residue in soil increased the value from $0.04 \text{ cmol}_c \cdot \text{dm}^{-3}$ to $0.6 \text{ cmol}_c \cdot \text{dm}^{-3}$, considered very high according to SBCS (2017), with no change after 45 days. Potassium is one of the nutrients most required by plants for stomatal response of the epidermis, with the content in cells correlated to closure and opening of stomata (Rehman and Yun 2006). In addition, it acts as a cofactor for more than 40 enzymes, participating in protein and photosynthetic metabolism and, also, in the transport of assimilates. It is also very important for the control of cellular electroneutrality (Van Raij 2011).

Another aspect that should be observed are the values of C and OM, which considerably increased when the residue was applied to soil (28.08 and $48.25 \text{ cmol}_c \cdot \text{dm}^{-3}$ after 45 days, respectively). This behavior was not observed with limestone, which presented values of 7.65 and $13.16 \text{ cmol}_c \cdot \text{dm}^{-3}$ after 45 days, respectively. Organic matter performs fundamental functions for the proper functioning of the soil and is involved in physical, chemical and biological processes. In tropical environments, the soil degradation process is closely related to the organic matter dynamics (Roscoe et al. 2006). These results suggest that by raising the soil organic matter, residues can contribute to the prevention of soil degradation processes as well as to fertility.

The cation exchange capacity (CEC) is an attribute of great practical interest in fertility studies and indispensable for the characterization of soil units. It was observed that both soils presented very high values, according to the classification of SBCS (2017). However, the addition of the residue to the soil promoted a five-fold CEC increase ($55 \text{ cmol}_c \cdot \text{dm}^{-3}$) when compared with limestone ($12.55 \text{ cmol}_c \cdot \text{dm}^{-3}$), which can be attributed to the organic matter content. Scherer et al. (2007) attributed relatively high values of CEC ($17\text{-}20 \text{ cmol}_c \cdot \text{dm}^{-3}$) to high levels of OM in the superficial layers (0-20 cm) of a soil under application of organic amendments (swine manure) and the higher clay content in deeper layers. The values of CEC found in the soil analyzed can be attributed to the clay content (63%).

Moreover, CEC values are influenced by Ca and Mg, which are of great importance with regard to soil fertility, since they indicate the soil capacity to adsorb cations in exchangeable form that, in general, will serve as nutrients for plants. Liming raises the availability of nutrients, increasing the exchange complex for plants and the value of the effective CEC. Liming, in addition to saturating the exchange complex with Ca and Mg, raises the pH to a level where Al becomes virtually unavailable for crops (Ronquim 2010).

Soil characterization before and after the residue addition suggests that, although the residue has a lower potential for soil acidity correction than limestone, it can be used as a complement to limestone and as a source of macro and micronutrients, contributing to the improvement of the quality of degraded soils.

Table 4. Chemical analysis of the soil, soil + residue, soil + limestone in the initial condition and after 45 days of incubation at 24 °C.

Soil ²			Soil + Residue (initial) ²			Soil + Limestone (45 days) ²		
Element	cmolc.dm ³		Element	cmolc.dm ³		Element	cmolc.dm ³	
Ca	0.86	Low	Ca	52.20	Very High	Ca	41.10	Very High
Mg	0.14	Very Low	Mg	0.79	Medium	Mg	0.80	Medium
K	0.04	Very Low	K	0.60	Very High	K	0.60	Very High
Al	0	Very Low	Al	0	Very Low	Al	0	Very Low
H + Al	3.69	Medium	H + Al	1.40	Low	H + Al	1.21	Low
C1	6.30	Low	C1	25.50	Very High	C1	28.10	Very High
OM1	10.84	Low	OM1	43.90	Very High	OM1	48.30	Very High
CEC	4.73	High	CEC	55	Very High	CEC	44	Very High
pH	5.30	Medium	pH	7	Very High	pH	7.60	Very High

Soil ²			Soil + Residue (initial) ²			Soil + Limestone (45 days) ²		
Element	cmolc.dm ³		Element	cmolc.dm ³		Element	cmolc.dm ³	
Ca	0.86	Low	Ca	8.01	Very High	Ca	8.01	Very High
Mg	0.14	Very Low	Mg	0.53	Medium	Mg	0.42	Medium
K	0.04	Very Low	K	0.04	Very Low	K	0.04	Very Low
Al	0	Very Low	Al	0	Very Low	Al	0	Very Low
H + Al	3.69	Medium	H + Al	2.03	Low	H + Al	1.63	Low
C1	6.30	Low	C1	7.05	Low	C1	7.65	Low
OM1	10.84	Low	OM1	12.13	Low	OM1	13.16	Low
CEC	4.73	High	CEC	10.61	Very High	CEC	12.55	Very High
pH	5.30	Medium	pH	7	Very High	pH	7.60	Very High

¹The values of C and OM are expressed as g.dm³. ²Contents of elements in soil, soil + residue and soil + limestone (initial and after 45 days), were classified as low, very low, medium, high and very high, according to ^{SBCS 2017}.

Microbiological analysis

Table 5 shows the total counts of bacteria and fungi expressed in CFU.g⁻¹.

The results revealed a growth of the fungal and bacterial communities in the soil with limestone and with the solid residue. Furthermore, there was a statistically significantly increase of both bacteria and fungi in the soil with the solid residue, suggesting that it was not toxic to the microorganisms and stimulated their growth. In fact, as demonstrated, the inhibitors levels found in the residue were low. Branco et al. (2013) used alkaline residues from the pulp and paper industry (dregs) in agriculture and evaluated their effect on soil chemical attributes and in the leaching of phenolic compounds. The results obtained showed that the application of dregs led to the increase of compounds above the maximum allowed by legislation: 0.01 mg.L⁻¹ (ABNT 2004) and 0.5 mg.L⁻¹ (Brazil 2008), which did not occur in the present study.

The increase in both fungal and bacterial communities in soil containing residue can be explained by the study of Ma et al. (2015), which suggests that the neutralization of soil acidity may reduce the stress of an acid soil to microorganisms. In addition, the increase in C and OM in soil after the residue application may have stimulated the development of microorganisms, as well as may also be related to the high levels of Ca and Mg, as shown in Table 4. Faoro et al. (2010) analyzed the bacterial biodiversity in the ecosystem of the Atlantic Forest and verified a high level of diversity. Statistical analysis showed that the diversity of bacteria was influenced by factors such as altitude, Ca, Mg, Al and the content of P, which also affected diversity within the same species.

Table 5. Total count of bacteria and fungi in the soil in the initial condition and after application of the residue and limestone, during incubation for 45 days at 24 °C.

Treatment	Average count Bacteria (Log CFU·g ⁻¹)	Collect (days)	Average count Fungi (Log CFU·g ⁻¹)
Soil	4.24	0	3.06
Soil + residue	6.46 a	10	6.04 a
Soil + limestone	6.22 a	10	3.12 b
Soil + residue	6.05 a	20	6.47 a
Soil + limestone	4.32 b	20	2.54 b
Soil + residue	6.89 a	30	6.42 a
Soil + limestone	4.79 b	30	2.58 b
Soil + residue	7.70 a	45	6.41 a
Soil + limestone	5.02 b	45	2.75 b

Equivalent letters in the columns, within each collected period, correspond to the same means, by the Tukey's test, at 5% significance.

Bioassay with lettuce (*Lactuca sativa* L.) seeds

The phytotoxicity of soluble elements extracted from the residue generated in the detoxification process of sugarcane bagasse hemicellulosic hydrolysate was assayed with lettuce seeds. The results were compared to those of the soil + limestone extract and control (distilled water). Bioassays with lettuce seeds are applicable in the measurement of the phytotoxicity as an inexpensive, quick and reproducible way to determine soil toxicity (Valerio et al. 2007). According to the bioassays, all treatments showed normal plants (Fig. 1a and b) and similar leaf development (Fig. 2).

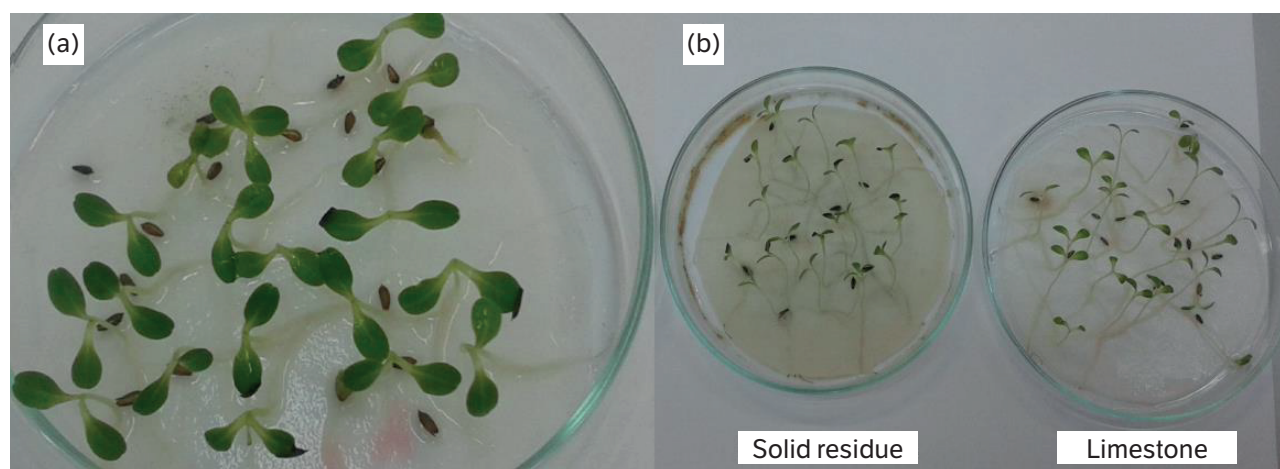


Figure 1. Aspect of lettuce seedlings (a) Lettuce seedlings grown in the control treatment (distilled water). (b) Lettuce seedlings grown in soil + residue extract and soil + limestone extract.

The germination percentage, the germination speed index (GSI) and germination speed (GS) are described in Table 6. For the variable germination percentage, there was no significant difference among treatments after 7 days of cultivation. However, a different response was observed for GSI when the soil + residue extract treatment was used, showing the lowest mean when compared to the control (distilled water) and the soil + limestone extract treatment, which had similar GSI values and no statistically significant difference.

The germination speed (GS) corresponds to the period in days the seeds take to germinate. In the present work, the use of soil + residue extract reduced significantly the GS of lettuce seeds, which took on average 3 days, compared to the GS of

control (distilled water) and to the treatment with soil + limestone extract. There was no statistically significant difference between control and soil + limestone extract treatment, which presented a GS of approximately 1 day.

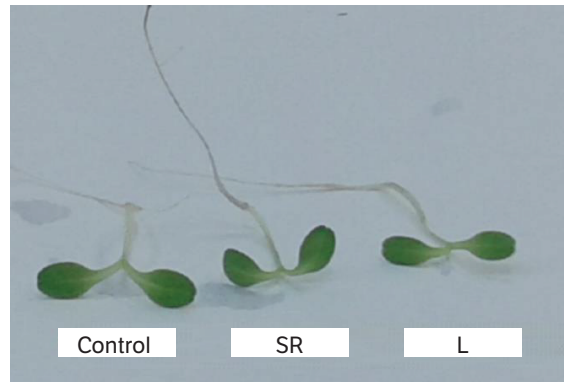


Figure 2. Leaf development of lettuce seedlings, after 7 days of cultivation with the treatments: control (distilled water), soil + residue extract (SR) and soil + limestone extract (L).

Table 6. Percentage of germination, germination speed index (GSI) and germination speed (GS) of lettuce seeds submitted to the treatments: control (distilled water), soil + residue extract and soil + limestone extract.

Treatment	Germination (%)	GSI	GS
Control (distilled water)	94 a	17.04 a	1.59 a
Soil + limestone extract	92 a	15.09 a	1.66 a
Soil + residue extract	82 a	3.09 b	3.09 b

Equivalent letters in the columns correspond to the same means, by the Tukey's test, at 5% of significance.

The results concerning the root and aerial part length of lettuce (Fig. 3) show that the incorporation of the solid residue into the soil did not interfere with root growth nor with the aerial part of the lettuce seedlings, with no statistically significant difference between treatments. This suggests that even though the residue showed a lower GSI and a higher GS compared to other treatments, it can be used in the soil, because it did not inhibit the growth of either the shoot or the roots of lettuce seedlings. According to Souza et al. (1997), the root is more sensitive to aqueous extracts than germination because the plant depends on a healthy root system, mainly for absorption of water and nutrients. The inhibition of the root system is detrimental to plant development, and thus will compromise the entire life cycle.

According to these results, the solid residue can be incorporated into the soil for cultivation of less sensitive plants than lettuce.

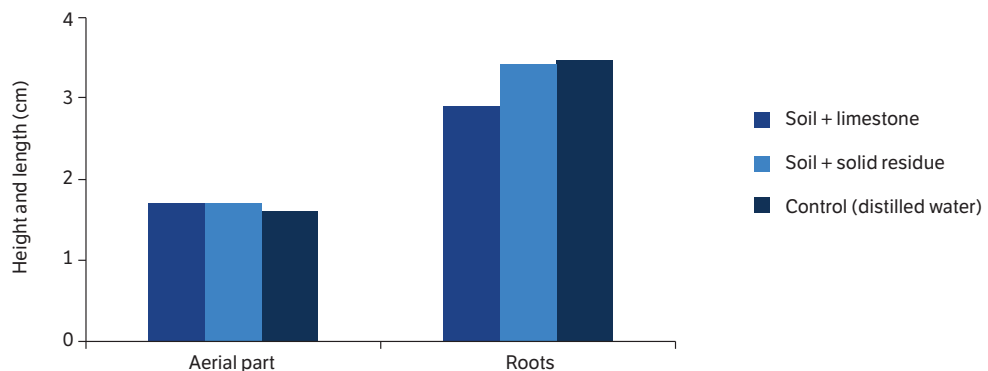


Figure 3. Aerial part height and root length of the lettuce seedling, submitted to the treatment with soil + limestone extract, soil + residue extract and control. Notes: * No significant differences were observed for the variables, aerial part (AP) and root by ANOVA F test, because of this, the mean comparison test was not applied.

CONCLUSION

The solid residue generated during the detoxification step (overliming) of the sugarcane bagasse hemicellulosic hydrolysate contributed to overall improvement of the soil chemical attributes, mainly the levels of Ca, Mg, C, OM and CEC, important indicators of soil quality. Although a larger amount of this residue was needed for soil pH correction than limestone, it was able to maintain the soil pH stable during the 45 days of the experiment.

The utilization of the residue in the soil contributed positively to the increase of bacteria and fungi, indicating no toxicity to these microorganisms. The bioassays with lettuce seeds showed that the soil + residue extract increased the germination time compared to the control and soil + limestone extract treatments. However, the soil + residue extract did not negatively influence the germination percentage and the development of lettuce seedlings, indicating that the residue can be applied to soil for the cultivation of lettuce and of less sensitive plants.

Therefore, the results obtained in this study suggest that residues from the detoxification of sugarcane bagasse hydrolysates can be used as auxiliary corrective of soil acidity and as source of nutrients, representing a useful management strategy to restore degraded agricultural soils.

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