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SOIL AND PLANT NUTRITION - Article

Phosphorus fractions in soil with a long history of organic waste and mineral fertilizer addition

Rafael da Rosa Couto[1]*, Paulo Ademar Avelar Ferreira[1], Carlos Alberto Ceretta[1], Cledimar Rogério Lourenzi[2], Daniela Basso Facco[1], Adriele Tassinari[1], Rogério Piccin[1], Lessandro De Conti[1], Luciano Colpo Gatiboni[3], Denis Schapanski[1], Gustavo Brunetto[1]

1. Universidade Federal de Santa Maria - Centro de Ciências Rurais - Departamento de Solos - Santa Maria (RS), Brazil.
2. Universidade Federal de Santa Catarina - Engenharia Rural - Florianópolis (SC), Brazil.
3. Universidade do Estado de Santa Catarina - Solos e Recursos Naturais - Lages (SC), Brazil.

ABSTRACT: Applications for long periods of organic animal waste as a source of nutrients to crops can contaminate the soil and the surface and subsurface waters. This study aimed to evaluate how the utilization of organic waste, after successive applications, affects P fractions in the soil and consequently environmental contamination. In an experiment conducted for 8 years, totaling 12 applications with pig slurry manure (PSM), liquid cattle manure (LCM), swine deep bedding (SDB), and mineral fertilizer (NPK), 460; 505; 1.111; and 535 kg P·ha^{-1} were added to the soil through PSM, LCM, SDB, and NPK, respectively. In September 2012, soil samples were collected in the layers 0.0 – 0.04; 0.04 – 0.08; 0.08 – 0.12; 0.12 – 0.16; 0.16 – 0.20 m, prepared and subjected to a sequential chemical fractionation of P. The 12 applications of pig slurry, liquid cattle manure, swine deep bedding and NPK fertilizer for eight years (urea + triple superphosphate + potassium chloride) provided accumulations of P fractions in the soil that were above the necessary for a proper nutrition to plants considering the available P fractions (AER + organic NaHCO$_3$ + inorganic NaHCO$_3$) in the soil. In addition, they showed that the use of swine deep bedding, followed by pig slurry, means higher levels of available P fractions in the soil. In this sense, the establishment of a dosage of organic waste to meet the nitrogen need of crops is not an environmentally appropriate parameter. It may cause P accumulation in the soil, increased absorption ability of plants and soil support, contributing to environmental contamination.

Key words: animal manure, availability, P fractionation, environmental contamination.

*Corresponding author: rrccouto@hotmail.com

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INTRODUCTION

The use of organic waste such as pig slurry manure (PSM), liquid cattle manure (LCM), and swine deep bedding (SDB) has increased over the years in annual crops or cultivation of perennial crops, due to the fertilizer potential of these wastes (Guardini et al. 2012; Ciancio et al. 2014; Lourenzi et al. 2014). Usually, the dose of organic wastes to be applied to the soil is established based on the percentage of dry matter (DM), the concentration of macronutrients such as nitrogen (N), phosphorus (P), potassium (K), and the index of efficiency, which is related to the entire amount of nutrients contained in waste that can be processed from an organic to a mineral form (CQFS-RS/SC 2004). However, when organic sources are used and the dosage is set from a percentage of DM and N concentration, the accumulation of other soil nutrients such as P may occur and, consequently, the distribution of its fractions in the soil is modified (Ceretta et al. 2010a; Couto et al. 2015).

The SDB has typically a higher C/N ratio, lignin, N and P contents and a smaller contact area with the soil as compared to LCM and PSM, which delays mineralization and changes the dynamics for nutrients such as P. Thus, there may be an increase in fractions considered bioavailable (Guardini et al. 2012), providing greater P availability to plants. However, when the addition of P is excessive, higher than the absorption capacity of plants and the soil adsorption, there may be losses of P by runoff or percolation in the soil profile (Rubæk et al. 2013; Schoumans et al. 2014; Gatiboni et al. 2015).

To evaluate the accumulation of forms of P in soil, fractionation techniques are used, for example, the methodology described by Hedley et al. (1982). The fractionation technique uses extractors sequentially from a lower to a higher desorption power and dissolution, allowing the separation of P fractions according to their nature (organic or inorganic) and ease of desorption/dissolution (Cross and Schlesinger 1995).

There is a lack of long-term studies under subtropical conditions assessing differences among organic sources, as well as comparing these organic sources with traditional mineral fertilization. This allows one to know the actual potential P contamination and to be able to develop appropriate strategies for the use of different organic sources. Therefore, this study aimed to evaluate how the utilization of organic waste for a period of 8 years affects the distribution of P fractions in the soil, affecting plant nutrition and environmental contamination.

MATERIAL AND METHODS

Characterization of the experimental area and the applied waste

The study was conducted in the experimental area, Santa Maria, Rio Grande do Sul, southern Brazil (lat 29°42′50.92″S and long 53°42′25.55″E; altitude: 100 m). The climate is humid subtropical (Cfa), with an average annual temperature of 19.3 °C, an average rainfall of 1.561 mm and a relative humidity of 82%. The soil is classified as a Typic Hapludalf soil (Soil Survey Staff 2006), which is observed in all regions of Brazil and is considered of great agricultural importance. At the beginning of the experiment, the soil had the following attributes: 180 g·kg^{-1} of clay; 193 g·kg^{-1} of silt; 627 g·kg^{-1} of sand; 19 g·kg^{-1} of organic matter; pH in water: 4.9; 20.3 mg·kg^{-1} of P and 60 mg·kg^{-1} of K (extracted by Mehlich-1); 0.03 cmol$_c$·kg^{-1} of exchangeable Al; 0.8 cmol$_c$·kg^{-1} of exchangeable Ca; and 0.3 cmol$_c$·kg^{-1} of exchangeable Mg (both extracted by KCl 1 mol.L^{-1}); 3.7 cmol$_c$·kg^{-1} of H + Al; 1.3 cmol$_c$·kg^{-1} of CEC$_{effective}$; 5.0 cmol$_c$·kg^{-1} of CEC$_{pH7.0}$; saturation by Al of 2% and base saturation of 25%.

The experiment was installed in 2004 in an area previously under no-tillage (NT). The experimental design was a randomized block with four replications with 5 × 5 m plots (25 m^2). The treatments were the addition of pig slurry manure (PSM), liquid cattle manure (LCM), swine deep bedding (SDB), mineral fertilizers (urea + triple superphosphate + potassium chloride: NPK) and a control (C) without the addition of nutrients. The crop rotation used, from the implementation of the experiment, was composed of black oat (*Avena strigosa* Schreb), corn (*Zea mays* L.), and black beans (*Phaseolus vulgaris* L.). The treatments were annually applied on the soil surface and on crop residues without incorporation and always before the corn crop. From 2010, it was decided to make two applications of each type of waste: being one before the sowing of the winter crop and another before the sowing of the summer crop. As a result, during the 8 years of experiment, 12 applications of treatments were made.

The waste applied in liquid form (PSM and LCM) was composed of feces, urine, food remains and washing water

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from the installations. It remained stored in anaerobic tanks. The SDB was composed of waste from rice processing, feces, urine and food remains. Before the collection of organic sources, a homogenization of lagoons was held and samples were collected to determine the chemical composition of each source.

The dose of organic and mineral sources to be applied prior to planting of each crop was established based on the requirement of N by the culture (CQFS-RS/SC 2004). For this, each source of nutrients was analyzed in order to quantify the total N present in each waste. The total contents of N, P, and K in the waste were determined on a wet basis using methodology proposed by Tedesco et al. (1995). To determine dry matter (DM), samples from organic sources were dried to constant weight in a forced-air oven at 65 °C until constant weight.

From the total N content of each source, the amount of each residue to be applied was calculated to meet the demand of N by the crops. Therefore, the efficiency ratio for each type of organic residue was considered, as recommended by CQFS-RS/SC (2004).

Thus, the amount of N applied before the implementation of each crop was: 30 kg N·ha^{-1} for black oats (dry matter production expected: 2,000 kg·ha^{-1}), 105 kg N·ha^{-1} for corn (grain production expected: 5,000 kg·ha^{-1}), and 50 kg N·ha^{-1} for black beans (grain production expected: 1,500 kg·ha^{-1}). The amounts of P and K applied to each crop were calculated from the total content of each source and the added dose. For the mineral source (NPK), 30; 60; and 25 kg P_2O_5·ha^{-1} (triple superphosphate source) and 20; 70 and 30 kg K_2O·ha^{-1} (source chloride K) were applied to black oats, corn, and black beans, respectively. Thus, from the beginning of the experiment until the agricultural year 2012/2013, the total amount of 1.153 kg N·ha^{-1}, 460 kg P·ha^{-1}, and 580 kg K·ha^{-1} were applied via PSM; 1.705 kg N·ha^{-1}, 1.111 kg P·ha^{-1}, and 1.656 kg K·ha^{-1} via SDB; 1.697 kg N·ha^{-1}, 505 kg P·ha^{-1}, and 1.276 kg K·ha^{-1} via LCM; and 920 kg N·ha^{-1}, 535 kg P·ha^{-1}, and 580 kg K·ha^{-1} via mineral fertilizer.

Chemical soil analyses

In September 2012, i.e. 8 years after the experiment had been initiated, 2 soil sub-samples were collected in each plot. For this, a trench with 0.10 × 0.10 × 0.60 m was opened at the center of each trench and, with the aid of a cutting blade, soil was collected at the 0.0 – 0.10 m depth. The soil was air-dried, manually crushed using a roller to pass through a 2-mm sieve, and stored in a screw-cap jar at room temperature. Subsequently, the pH values in water (1:1) and the exchangeable contents of Al, Ca and Mg (extracted by KCl 1 mol·L^{-1}) were determined (Tedesco et al. 1995). The Ca and Mg contents were determined by atomic absorption spectrometry (AAS) and Al titration with NaOH 0.0125 mol·L^{-1}. From the data obtained, the cation exchange capacity at $pH_{7.0}$ ($CEC_{pH7.0}$), Al saturation, base saturation, and potential acidity (H + Al) were calculated (CQFS-RS/SC 2004). Total organic carbon (TOC) content was determined by digestion with a sulfochromic solution (Embrapa 1997). and multiplied by 1.724 (Van Bemmelen factor) to estimate soil organic matter (SOM), assuming 58% TOC in SOM. Available P and K contents were extracted with a Mehlich-1 solution (Tedesco et al. 1995), followed by spectrophotometric determination of P and flame photometric determination of K (Table 1).

Table 1. Chemical characteristics of a Typic Hapludalf soil in the layer 0.0 – 0.10 m of the control, swine deep bedding, liquid cattle manure, pig slurry manure, and chemical fertilizer soils.

	C	SDB	LCM	PSM	NPK
Clay (g·kg^{-1})$^{(1)}$	180	180	180	180	180
SOM (g·kg^{-1})$^{(2)}$	13.5	27.2	22.8	21.1	21
pH-$H_2O^{(2)}$	4.8	5.6	5.2	5.2	5
P available (mg·dm^{-3})$^{(3)}$	15.5	116.1	62.1	65.3	28.8
K exchangeable (mg·dm^{-3})$^{(3)}$	24.5	70.4	66.2	61.5	47.5
Ca exchangeable ($cmol_c$·dm^{-3})$^{(4)}$	1.5	3.35	3.00	2.9	2.3
Mg exchangeable ($cmol_c$·dm^{-3})$^{(4)}$	0.7	1.30	1.0	1.2	1
Al exchangeable ($cmol_c$·dm^{-3})$^{(4)}$	0.3	0.04	0.1	0.1	0.5
H + Al ($cmol_c$·dm^{-3})$^{(5)}$	5.8	3.51	4.0	5.2	4
CEC_{ef} $cmol_c$·dm^{-3})$^{(5)}$	3.2	4.4	4.2	3.7	3.9
CEC_{pH7} ($cmol_c$·dm^{-3})$^{(5)}$	8.7	7.9	8.1	8.8	7.3
Al saturation (%)$^{(2)}$	1.9	1	3.2	2.9	13.8
Base saturation (%)$^{(2)}$	29.2	57.1	51.0	45.0	46

$^{(1)}$Pipette method (Embrapa 1997); $^{(2)}$Determined by Tedesco et al. (1995); $^{(3)}$Extracted by Mehlich-1 (Tedesco et al. 1995); $^{(4)}$Extracted by KCl 1 mol·L^{-1} (Tedesco et al. 1995); $^{(5)}$Calculated according to CQFS-RS/CS (2004). C = Control; SDB = Swine deep bedding; LCM = Liquid cattle manure; PSM = Pig slurry manure; NPK = Mineral fertilization; SOM = Soil organic matter.

Chemical fractionation of P

From the same trench sampled for soil characterization, samples were collected incrementally (0 – 0.04; 0.04 – 0.08; 0.08 – 0.12; 0.12 – 0.16; and 0.16 – 0.20 m) for chemical P fractionation. Samples were air dried, organic waste was removed manually, and then they were ground and passed through a 2 mm mesh sieve. Afterwards, 0.5 g of soil was added to 15 mL Falcon tubes and P fractionation was carried out in the following order: anion exchange resin — AER (inorganic fractions); $NaHCO_3$ 0.5 $mol{\cdot}L^{-1}$ (organic and inorganic fractions); NaOH 0.1 $mol{\cdot}L^{-1}$ (organic and inorganic fractions); HCl 1.0 $mol{\cdot}L^{-1}$ (inorganic fractions), and NaOH 0.5 $mol{\cdot}L^{-1}$ (organic and inorganic fractions). At each step of the fractionation, the soil was centrifuged at 4,000 rpm for 20 min to separate the supernatant from the soil sample. After the extractions, the remaining soil was dried at 45 °C in an oven and subjected to digestion with $H_2SO_4 + H_2O_2 + MgCl_2$ (residual fraction). The inorganic P of alkali extracts ($NaHCO_3$ and NaOH) was determined by the method proposed by Dick and Tabatabai (1977). In these alkaline extracts, the P was also determined by digestion with ammonium persulfate + sulfuric acid in an autoclave (total P) (USEPA 1971). The organic P was obtained by the difference between total phosphorus and inorganic phosphorus. The P of acid extracts was determined according to the method described by Murphy and Riley (1962). Each sample was subjected to chemical P fractionation with five repetitions.

Statistical analysis

Data obtained by P fractionation were tested for normality using the Kolmogorov-Smirnov method and subjected to analysis of variance. The forms of the P fractions were subjected to analysis of variance by the statistical program SISVAR (Ferreira 2003), with the model:

$$y_{ijk} = \mu + B_i + W_j + \text{error (i,j)} + Dk + \text{error (i,k)} + W.D_{jk} = \text{error (i,j,k)}$$

where: μ is the general mean; B = represents block (i = 1, 2, 3); W represents treatments (j = 1, 2, 3, 4, 5); error represents the experimental error; D means depth (k = 1, 2, 3, 4, 5).

When significant, the data were compared by the Scott-Knott test ($p < 0.05$). A principal component analysis (PCA) was performed on the fractions of P (AER, inorganic $NaHCO_3$, organic $NaHCO_3$, inorganic NaOH 0.1, organic NaOH 0.1, inorganic NaOH 0.5, organic NaOH 0.5 and HCl). PCA is used to reduce the data size, facilitating analysis by means of a correlation circle graph (Jolliffe 2002; Herlihy and McCarthy 2006).

RESULTS AND DISCUSSION

In all fractions of extracted P, the levels were significantly higher in the SDB treatment in relation to the others, showing that the use of the defining criterion of dosage from organic sources, based on the N requirements of crops, means to assume that the contents of P will be greatly increased when SDB is used in the soil with successive applications over the years (Tables 2,3). Decisive for this was the fact that, with the use of SDB, 1.111 kg $P{\cdot}ha^{-1}$ were applied over the years, against 460, 505, and 535 kg $P{\cdot}ha^{-1}$ in PSM, LCM, and mineral fertilizer sources, respectively. Thus, upon using SDB, the P content was higher in all fractions at least up to 0.16 m deep compared to other organic sources. However, when compared with the use of mineral fertilizers, the increases in depth are restricted up to the 0.04 m layer, when the extraction was carried out with NaOH 0.5 $mol{\cdot}L^{-1}$, i.e. both sources were more effective in accumulating in the soil considering the fraction of P physically protected within micro aggregates (Guardini et al. 2012; Schmitt et al. 2014) (Table 3).

The highest levels of P fractions extracted by AER, inorganic and organic P of $NaHCO_3$, NaOH 0.1, NaOH 0.5, HCl and residual P were observed at the 0.0 – 0.04 m layer with the addition of organic waste (SDB, LCM and PSM) and mineral fertilization (NPK), decreasing in depth (Tables 2,3). The highest levels of P fractions in the upper layers of the soil occurred because the organic waste and the mineral fertilizer were added to the soil surface without incorporation, since it was a no-tillage system (Rodrigues et al. 2016).

Among the extractors used, the AER is relevant because it represents the fraction available, which emphasizes its contents when using SDB. This is because the P contents showed levels of 8; 57; 25; 40 and 26 $mg{\cdot}kg^{-1}$ for the control, SDB, LCM, PSM, and NPK, respectively, considering the average of P concentrations extracted by AER in the layer 0.0 – 0.12 m, which is close to the sampled layer recommended for fertilization and liming when the soil management is no-tillage (0.0 – 0.10 m), in the states of Rio Grande do Sul and Santa Catarina. According to the Commission of

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Chemistry and Soil Fertility of RS/SC (CQFS-RS/SC 2004), the levels observed in the control soil are considered low (5.1 – 10.0 $mg\cdot kg^{-1}$), high in LCM, PSM and NPK (20.1 – 40.0 $mg\cdot kg^{-1}$) and very high in the soil with SDB (> 40 $mg\cdot kg^{-1}$). These results can be explained by the fact that over eight years and 12 applications, 55% more P were added to the SDB soil treatment in relation to the average LCM, PSM, and NPK. Besides the large amount of P supplied through organic sources, the harvesting were carried out only in corn and bean crops, reducing exports of P by crops and contributing to the increases of the contents available. For all these reasons, it is usual to find in the literature an increase in values of available P in the surface layers of soils with pig slurry, cattle manure and soluble mineral fertilizer applications in agricultural areas, especially under no-tillage (Adeli et al. 2008; Veiga et al. 2012; Sartor et al. 2012; Lourenzi et al. 2014; Schmitt et al. 2014).

An effect similar to that of the fraction extracted by AER was observed in the P fractions extracted with $NaHCO_3$ and NaOH 0.1, both in inorganic and organic forms. The highest content of these fractions occurs in SDB followed by PSM (Tables 2,3). In general, the lowest inorganic and organic contents extracted by AER and $NaHCO_3$ were observed in the control soil without fertilization, especially at the 0.0 – 0.12-m layer. The inorganic fraction extracted by $NaHCO_3$ is considered as an available form because it is balanced with the P of the soil solution (Condron et al. 1985; Guardini et al. 2012). The organic fraction extracted with $NaHCO_3$, despite being contained in organic compounds and therefore considered at first unavailable, is an easily decomposable form formed by diester phosphates and regulated by the soil microbial activity (Chen et al. 2002; Xavier et al. 2009). Increases in its contents in the soil are closely related to the increase of readily available inorganic fractions, constituting the ‘pool’ of labile P in the soil (Ceretta et al. 2010a; Guardini et al. 2012; Schmitt et al. 2014). Although this is agronomically desired, it raises the risk of an environmental contamination (Stewart and Tiessen 1987; Ceretta et al. 2010b).

Thus, like the P fractions extracted by AER and $NaHCO_3$, the P extracted with organic and inorganic NaOH 0.5 also

Table 2. Phosphorus contents in a Typic Hapludalf soil extracted by anion exchange resin and $NaHCO_3$ 0.5 $mol\cdot L^{-1}$ in the different layers of the control, swine deep bedding, liquid cattle manure, pig slurry manure, and chemical fertilizer soils.

Phosphorus fraction	Layer (m)	C	SDB	LCM	PSM	NPK
				$mg\cdot kg^{-1}$		
Inorganic phosphorus extracted by AER	0.0 - 0.04	14.43 aC[(1)]	60.04 bA	40.96 aB	63.21 aA	41.21 aB
	0.04 - 0.08	4.78 bD	72.00 aA	21.95 bC	40.04 bB	22.69 bC
	0.08 - 0.12	4.87 bD	38.78 cA	11.61 cC	19.56 cB	15.30 cC
	0.12 - 0.16	4.13 bC	33.91 dA	7.83 cC	14.82 dB	10.43 dC
	0.16 - 0.20	3.60 bC	26.26 eA	9.06 cB	12.97 dB	8.74 dB

Phosphorus fraction	Layer (m)	C	SDB	LCM	PSM	NPK
				$mg\cdot kg^{-1}$		
Inorganic phosphorus extracted by $NaHCO_3$ 0.5 $mol\cdot L^{-1}$	0.0 - 0.04	14.57 aE	134.20 aA	54.45 aC	74.38 aB	46.01 aD
	0.04 - 0.08	7.67 bE	93.56 bA	30.67 bC	60.55 bB	19.42 bD
	0.08 - 0.12	5.11 bD	42.43 cA	14.05 cC	29.14 cB	14.31 bC
	0.12 - 0.16	4.34 bC	30.93 dA	12.78 cB	15.33 dB	9.71 cB
	0.16 - 0.20	4.34 bC	23.51 eA	11.92 cB	11.50 dB	9.20 cB

Phosphorus fraction	Layer (m)	C	SDB	LCM	PSM	NPK
				$mg\cdot kg^{-1}$		
Organic phosphorus extracted by $NaHCO_3$ 0.5 $mol\cdot L^{-1}$	0.0 - 0.04	6.67 aD	122.20 aA	41.81 aC	52.59 aB	39.85 aC
	0.04 - 0.08	2.82 aD	86.29 bA	20.57 bC	41.08 bB	16.27 bC
	0.08 - 0.12	2.17 aC	23.82 cA	9.16 cB	41.08 bA	12.78 bB
	0.12 - 0.16	2.17 aB	18.78 dA	7.57 cB	9.18 cB	6.55 cB
	0.16 - 0.20	1.88 aB	15.75 dA	6.36 cB	6.77 cB	5.88 cB

[(1)]Data followed by the same lowercase letter in columns and uppercase in lines are not different from each other by Scott-Knott test at 5% error probability. C = Control; SDB = Swine deep bedding; LCM = Liquid cattle manure; PSM = Pig slurry manure; NPK = Mineral fertilization; AER = Anion exchange resin.

Table 3. Phosphorus contents in a Typic Hapludalf soil extracted by NaOH 0.1 mol·L^{-1} and NaOH 0.5 mol·L^{-1} in the different layers of the control, swine deep bedding, liquid cattle manure, pig slurry manure, and chemical fertilizer soils.

Phosphorus fraction	Layer (m)	C	SDB	LCM	PSM	NPK
				mg·kg^{-1}		
Inorganic phosphorus extracted by NaOH 0.1 mol·L^{-1}	0.0 - 0.04	74.27 aE(1)	250.77 aA	166.93 aC	208.33 aB	130.43 aD
	0.04 - 0.08	46.84 bE	219.72 bA	102.48 bC	170.03bB	91.10 bD
	0.08 - 0.12	44.77 cE	135.61 cA	62.63 cD	85.92 cB	76.08 cC
	0.12 - 0.16	41.15 dC	113.35 dA	52.53 dB	69.62 dB	55.64 dC
	0.16 - 0.20	37.27 dD	94.46 eA	47.35 dC	60.04 eB	58.75 dB
Phosphorus fraction	**Layer (m)**	**C**	**SDB**	**LCM**	**PSM**	**NPK**
				mg·kg^{-1}		
Organic phosphorus extracted by NaOH 0.1 mol·L^{-1}	0.0 - 0.04	48.85 aE	220.46 aA	127.61 aC	162.22 aB	107.85 aD
	0.04 - 0.08	28.98 bD	197.93 bA	72.17 bC	132.61 bB	75.94 bC
	0.08 - 0.12	28.20 bD	117.29 cA	44.16 cC	59.23 cB	61.72 cB
	0.12 - 0.16	25.37 bC	97.88 dA	29.36 dC	45.93 dB	46.90 dB
	0.16 - 0.20	23.53 bC	78.83 eA	26.95 dC	38.25 eB	41.90 dB
Phosphorus fraction	**Layer (m)**	**C**	**SDB**	**LCM**	**PSM**	**NPK**
				mg·kg^{-1}		
Inorganic phosphorus extracted by NaOH 0.5 mol·L^{-1}	0.0 - 0.04	37.03 aC	135.07 aA	49.83 aB	46.02 aC	48.47 aB
	0.04 - 0.08	33.77 aC	49.84 bA	36.22 bC	38.40 bC	42.75 bA
	0.08 - 0.12	26.96 bB	35.95 cA	30.50 cB	28.32 cB	35.67 cA
	0.12 - 0.16	23.96 bC	34.58 cA	24.51 dC	24.78 cC	29.41 dB
	0.16 - 0.20	16.07 cB	25.87 dA	24.23 dA	18.24 dB	22.06 eA
Phosphorus fraction	**Layer (m)**	**C**	**SDB**	**LCM**	**PSM**	**NPK**
				mg·kg^{-1}		
Organic phosphorus extracted by NaOH 0.5 mol·L^{-1}	0.0 - 0.04	30.54 aD	123.47 aA	45.81 aB	39.37 aC	41.98 aC
	0.04 - 0.08	28.35 aC	42.41 bA	32.50 bB	34.22 bB	37.96 aA
	0.08 - 0.12	22.93 bB	33.63 cA	27.09 cB	26.15 cB	30.26 bA
	0.12 - 0.16	19.17 cB	28.86 dA	22.03 dB	22.30 cB	25.38 cA
	0.16 - 0.20	11.11 dC	22.62 eA	22.07 dA	16.39 cB	17.10 dB

(1)Data followed by the same lowercase letter in columns and uppercase in lines are not different from each other by Scott-Knott test at 5% error probability. C = Control; SDB = Swine deep bedding; LCM = Liquid cattle manure; PSM = Pig slurry manure; NPK = Mineral fertilization.

showed higher levels in the soil with the addition of SDB, but followed by the NPK soil (Table 3). Fractions extracted with NaOH 0.5 represent a moderately labile P bonded to Fe and Al oxides by monodentate, bidentate and binucleated bonds (Hedley et al. 1982). This phenomenon may be occurring due to the greater availability of inorganic P in the soil with the application of SDB and to the lowest concentration of compounds dissolved in NPK, since these compounds may be adsorbed to the functional groups of the surface of the mineral constituents of the solid phase, increasing P desorption (Ceretta et al. 2010a). P added above the demand of the plants tends to be initially adsorbed on the surface of functional groups more avid for P, causing an accumulation in soil fractions with an intermediate lability such as those extracted by NaOH 0.5 (Ceretta et al. 2010a; Couto et al. 2015). The increase of the organic fraction extracted by NaOH 0.5 occurred because of the increase of SOM due to 8 years of organic waste applications, primarily by the addition of organic carbon (CO) through the application of SDB and PSM and the deposition of waste produced by the crops over the soil. SOM allows the formation of metallic cation bonds such as Al, Fe and Ca. P, in small quantities, is capable of binding with these metals (Sanyal and De Datta 1991).

The highest P contents extracted by HCl in the 5 layers of the soil were observed in the soil that received the applications of SDB, followed by LCM, in the layer of 0.0 – 0.04 m and PSM in the other layers (0.04 – 0.08; 0.08 – 0.12; 0.12 – 0.16 and 0.16 – 0.20) (Table 4). Although the

Table 4. Phosphorus contents in a Typic Hapludalf soil extracted by HCl 1.0 mol·L^{-1} and $H_2SO_4 + H_2O_2 + MgCl_2$ in the different layers of the control, swine deep bedding, liquid cattle manure, pig slurry manure, and chemical fertilizer soils.

Phosphorus fraction	Layer (cm)	C	SDB	LCM	PSM	NPK
				mg·kg^{-1}		
Inorganic phosphorus extracted by HCl 1.0 mol·L^{-1}	0.0 – 0.04	9.91 aE(1)	83.00 aA	43.30 aB	33.65 aC	20.74 aD
	0.04 – 0.08	6.34 bE	43.96 bA	17.91 bC	29.08 bB	13.47 bD
	0.08 – 0.12	4.78 bC	27.52 cA	7.04 cC	11.95 cB	7.25 cC
	0.12 – 0.16	3.34 cC	10.56 dA	5.35 cC	7.56 dB	5.21 cC
	0.16 – 0.20	2.26 cB	6.21 eA	5.04 cA	5.47 dA	7.21 cA
Phosphorus fraction	**Layer (cm)**	**C**	**SDB**	**LCM**	**PSM**	**NPK**
				mg·kg^{-1}		
Residual phosphorus extracted by $H_2SO_4 + H_2O_2 + MgCl_2$	0.0 – 0.04	153.34 aC	156.52 aC	197.24 aA	182.43 bB	205.70 aA
	0.04 – 0.08	141.18 aB	151.76 aB	178.20 bA	186.13 bA	181.90 bA
	0.08 – 0.12	118.97 bD	149.12 aC	186.136 bB	220.50 aA	176.05 bB
	0.12 – 0.16	102.05 cC	165.51 aA	151.76 dB	169.74 bA	166.57 bA
	0.16 – 0.20	78.79 dC	157.05 aB	170.79 cA	180.90 bA	168.15 bA

(1) Data followed by the same lowercase letter in columns and uppercase in lines are not different from each other by Scott-Knott test at 5% error probability. C = Control; SDB = Swine deep bedding; LCM = Liquid cattle manure; PSM = Pig slurry manure; NPK = Mineral fertilization.

experimental soil does not have high amounts of non-apatite minerals (Ceretta et al. 2010a), sources of P added to the soil have in their composition P bound to Ca, which may explain the increase in the contents extracted by HCl.

Except for the residual, all P fractions evaluated in the soil with the addition of organic waste and mineral fertilizer showed increases in the contribution of the sum of fractions of P (total P) compared to the control (Figure 1). However, the highest increases were observed in the P fractions extracted by AER and organic and inorganic $NaHCO_3$ (Figure 1). The P extracted by AER comprised 2% of the total P compared to the control. However, in SDB and PSM soils, the fraction of P extracted by AER represented 6 and 5%, respectively, of the total content. In the soil with the addition of LCM and NPK, the fraction of P extracted by AER accounted for 4% of the total P. The addition of organic waste to the soil can decrease the sorption P because of the competition between phosphate ions and organic compounds (such as phenolic compounds) in P sorption sites (Xavier et al. 2009), favoring the accumulation of P in the soil's available fractions. In addition, this study's Typic Hapludalf soil has low levels of Fe and Al oxides, indicating that this soil, when subjected to a high addition of P, can become quickly saturated, favoring the soil profile migration and the increase of contents in depth (Ceretta et al. 2010a).

The fractions extracted by organic and inorganic $NaHCO_3$ showed similar effects to those observed with the fraction extracted by AER. However, the fraction of organic and inorganic $NaHCO_3$ represented 8 and 7% of the total P in the soil with the addition of SDB, which means contents 4 and 6 times higher than those observed for the control soil. Other studies conducted in fertilized soils with animal waste also found P accumulation mainly in labile inorganic fractions (Ceretta et al. 2010a; Guardini et al. 2012; Couto et al. 2015).

The increase in the organic fraction extracted by $NaHCO_3$ is associated with the accumulation of SOM (Aguiar et al. 2013). The 8-year application of PSM, LCM, NPK, and especially SDB induced higher levels of SOM, which may explain the increase of the organic fraction of P extracted by $NaHCO_3$ relative to the control soil. In addition, organic P fractions tend to increase in systems with high levels of P, above that required by plants (Aguiar et al. 2013; Couto et al. 2015; Ye et al. 2015). They may be immobilized on the microbial biomass if there are supply sources of C (Lukito et al. 1998; Heuck et al. 2015).

The organic and inorganic fractions extracted with NaOH 0.1 and 0.5 represented 18; 11; 10 and 8%, respectively, of the total P compared to the control (Figure 1). In soils that received organic and mineral fertilizers, the organic and inorganic fractions of NaOH 0.1 represented an average of 20 and 15%, respectively, of the total P (Figure 1). However, the fractions of organic and inorganic NaOH 0.5 showed a decrease in the contribution of the sum of fractions if compared to the control. The content of the organic fraction of NaOH 0.5

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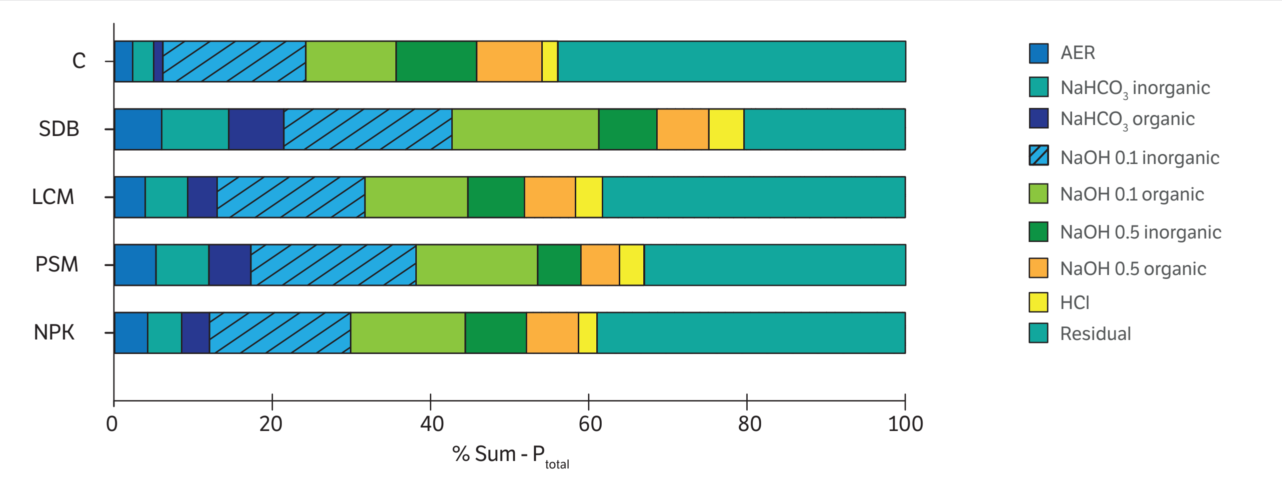

Figure 1. Relative percentage of the average of P fractions in the layers 0.0 – 0.04; 0.04 – 0.08; 0.08 – 0.12; 0.12 – 0.16; and 0.16 – 0.20 m in a Typic Hapludalf soil with swine deep bedding (SDB), pig slurry manure (PSM), liquid cattle manure (LCM), chemical fertilizer (NPK), and the control soil (C) using the sequential extraction method of Hedley et al. (1982): inorganic P extracted by anion exchange resin (AER), inorganic P extracted by $NaHCO_3$ 0.5 mol·L^{-1}, inorganic and organic P extracted by NaOH 0.1 mol·L^{-1}, inorganic P extracted by HCl 1.0 mol·L^{-1}, inorganic and organic P extracted by NaOH 0.5 mol·L^{-1}, and residual phosphorus extracted by $H_2SO_4 + H_2O_2 + MgCl_2$.

ranged from 5% in the soil with PSM to 7% in the soil with NPK, while the fraction of inorganic NaOH 0.5 ranged from 5% in the soil with PSM to 8% in the soil with NPK (Figure 1). The decrease in the proportion of organic and inorganic fractions extracted by NaOH 0.5, in the sum of all fractions, can be an indicative of a saturation of functional groups of P adsorption, indicating that the ability of the soil to be used for the application organic waste was exceeded (Couto et al. 2015). Fractions extracted by NaOH 0.5 represent P coordinated to Fe and Al oxides and clays, with a moderate binding energy (Gama-Rodrigues et al. 2014). This fraction can act as a drain of P in fertilized soils, especially in soils with a high clay and oxide content or as a source of P in the short and medium term under low nutrient availability conditions. However, the Typic Hapludalf soil of the experiment has a clay loam texture and it is derived from granite sediments. Thus, it has a reduced concentration of crystalline iron oxides and a low crystallinity in relation to soils derived from basalt, for example. Therefore, the ability to adsorb a moderate P lability is lower, causing P to accumulate in more labile forms, which are extracted by AER and $NaHCO_3$. Consequently, it does not accumulate in recalcitrant forms (Wang and Liang 2014). This behavior of P in the soil was confirmed by the results obtained by Pavinato et al. (2009), who observed an accumulation of P in labile fractions in soils with phosphate fertilizers. In this sense, the fact of not observing an increase of inorganic and organic fractions extracted with NaOH 0.5 relative to the total P means an increase in the transfer potential of P by percolation, which is evidenced by an increase of the more labile fractions in deeper layers (Table 2). Thus, it is unwise to carry out new additions of P, since P levels that meet the demand of plants are satisfactory, as recommended for corn crops, beans and winter hibernation coverage plans (CQFS-RS/SC 2004), in soils with the addition of LCM and NPK. However, in the soils with PSM and SDB, pollution risks are even higher: P levels in the layer of 0.0 – 0.10 m are very high. In assessing the losses of P losses by runoff, under no-tillage, Girotto et al. (2013), after 6 years and 16 applications of pig slurry, which meant adding to the soil 0; 1,128; and 2.256 kg·ha^{-1} of P with doses of 0, 40 and 80 m^3·ha^{-1}, respectively, of waste, observed the greatest losses of total P and free forms (HPO_4^{2-} and H_2PO_4) in the soil that received the highest contents P. It should be noted that organic waste, such as animal waste, which has its doses established to meet the N demand of crops, promotes the accumulation of P and favors the loss of nutrients by erosion more than with the use of mineral fertilizers due to the higher amount applied and the ease of transport of organic constituents by the flood stream caused by its lower density and greater concentration in the surface (Ceretta et al. 2010b; Couto et al. 2015). According to the first author, this fact can be explained by the selectivity of the soil's erosion process by rain water, which preferably carries the finest (smaller diameter) and lighter (less dense) sediments, consisted primarily of organic and mineral colloids, which are typically rich in nutrients.

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The fraction extracted with HCl represented 2% of the total P in the control soil (Figure 1). The biggest change in the P extracted by HCl in relation to soil without fertilization was observed in the SDB soil, which represented 4% of the total P. However, the residual fraction represented 44% of the total P observed in the control soil, whereas in the areas with the addition of P through organic waste, the residual P ranged from 20% in the SDB soil to 39% in the NPK soil (Figure 1). P fractions extracted by HCl and residuals represent a P strongly bound to the adsorption sites or to a component of the structure of soil minerals, therefore considered unavailable to plants and/or susceptible to be transferred (Cross and Schlesinger 1995; Ranatunga et al. 2013).

The sum of available P fractions (AER + organic $NaHCO_3$ + inorganic $NaHCO_3$) presented the highest levels in the layer of 0.0 – 0.04 m in all treatments, decreasing in depth, while in the control soil there was no difference in the layer 0.0 – 0.04 – 0.16 – 0.20 m (Figure 2a). With the addition of LCM, the lowest contents in the sum of available P fractions were observed in the layer 0.04-0.20 m and in the layer 0.08 – 0.12 m with the use of NPK and PSM. As it stood out regarding the addition of P in the soil upon adding SDB, the lower levels of the sum of fractions of available P were observed only in the layer of 0.16 – 0.20 m (Figure 2a).

Besides SDB, the highest levels of the sum of fractions of available P in the layers of 0.0 – 0.04; 0.04 – 0.08; and 0.08 – 0.12 m were also observed when using PSM (Figure 2a). However, in deeper layers (0.12 – 0.16 and 0.16 – 0.20 m) only with the addition of SDB, the content of the sum fractions of available P remained higher than the other sources, which showed no difference from each other (Figure 2a).

Considering the 12 applications for eight years of organic and mineral waste, amounts equivalent to 2,544; 1,053; 1,156 and 1,225 kg $P_2O_5 \cdot ha^{-1}$ were added to the soil with SDB, PSM, LCM, and NPK, respectively. However, considering the export by corn and bean grains, an average of 360 kg $P_2O_5 \cdot ha^{-1}$ were exported considering the yield of 1.8 $Mg \cdot ha^{-1}$ of beans and 6 $Mg \cdot ha^{-1}$ of corn (CQFS-RS/CS 2004). Therefore, it is estimated that there was a surplus of 2; 184; 693; 796 and 865 Mg $P_2O_5 \cdot ha^{-1}$ with the addition of SDB, PSM, LCM, and NPK, respectively. Despite P having little mobility in the soil, doses higher than those supported by the system may increase P transfer risks by runoff (Gatiboni et al. 2015), and it may also migrate to deeper soil layers (Guardini et al. 2012). In an experiment with the application of 0; 20; 40 and 80 m^3 PSM·ha^{-1} over a sandy Typic Hapludalf soil, Ceretta et al. (2010a) observed the levels of the sum of fractions of available P of 226 $mg \cdot kg^{-1}$ in the layer 0.0 – 0.16 m. These high levels on

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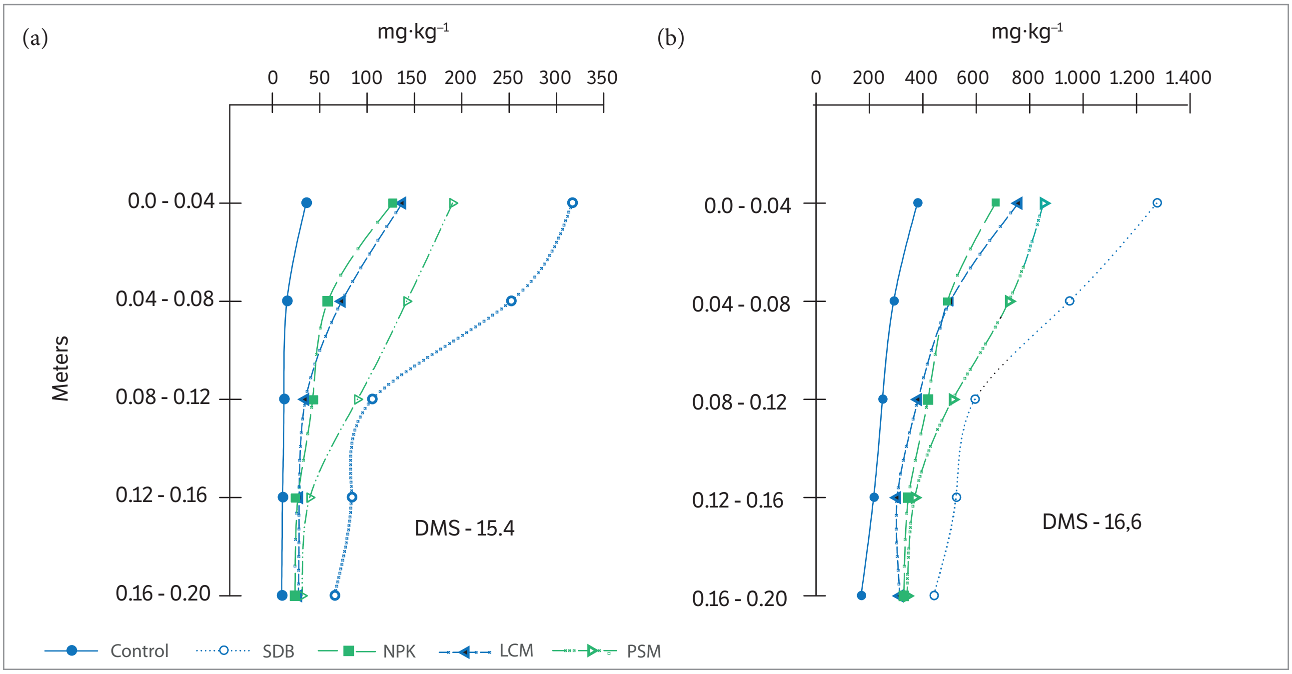

Figure 2. Sum of P fractions available (anion exchange resin — AER + inorganic $NaHCO_3$ + organic $NaHCO_3$) (a) and total P (b) in the layers 0.0 – 0.04; 0.04 – 0.08; 0.08 – 0.12, 0.12 – 0.16; and 0.16 – 0.20 m of a Typic Hapludalf soil, control soil, and soil with swine deep bedding (SDB), pig slurry manure (PSM), liquid cattle manure (LCM), and chemical fertilizer (NPK). Available fractions were obtained by sequential extraction method of Hedley et al. (1982): inorganic P extracted by AER, inorganic P extracted by $NaHCO_3$ 0.5 $mol \cdot L^{-1}$, and inorganic P extracted by NaOH 0.1 $mol \cdot L^{-1}$.

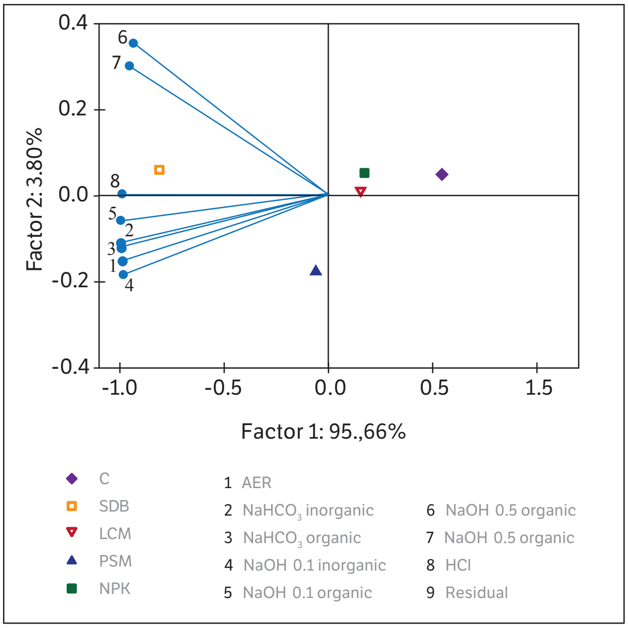

Figure 3. Relation between principal component 1 (Factor 1) and the principal component 2 (Factor 2) of P fraction averages in the layers 0.0 – 0.04; 0.04 – 0.08; 0.08 – 0.12; 0.12 – 0.16; and 0.16 – 0.20 m in a sandy Dystrophic Red Argisol, control soil (C), and soil with swine deep bedding (SDB), pig slurry manure (PSM), liquid cattle manure (LCM), and chemical fertilizer (NPK) using the sequential extraction method of Hedley et al. (1982): inorganic P extracted by anion exchange resin (AER), inorganic P extracted by $NaHCO_3$ 0.5 mol·L^{-1}, inorganic and organic P extracted by NaOH 0.1 mol·L^{-1}, inorganic P extracted by HCl 1.0 mol·L^{-1}, inorganic and organic P extracted by NaOH 0.5 mol·L^{-1}, and residual phosphorus extracted by $H_2SO_4 + H_2O_2 + MgCl_2$.

the surface allowed the P content of the runoff to exceed the maximum permissible concentration for water class 3 (0.15 mg of P·L^{-1} in lotic environments), i.e. water which could be used for human consumption after an appropriate treatment (Conama 2005). It is noteworthy that the average of the sum of fractions of available P in the soil with the addition of SDB was 190 mg·kg^{-1}, values very close to those observed by the authors above mentioned. Furthermore, Ceretta et al. (2010b) observed that the transfer of P via runoff was positively correlated with the PSM doses applied to the soil in the same experiment.

The principal component analysis showed through the relation between the principal component 1 (Factor 1) and the principal component 2 (Factor 2) that there was a separation among treatments (Figure 3). Using the Factor 1, which explains 96% of the data, it appears that there was a separation of the control, NPK and PSM treatments from the LCM and SDB treatments. For this result, all P fractions evaluated in the principal component analysis (AER, organic and inorganic $NaHCO_3$, organic and inorganic NaOH 0.1, organic and inorganic NaOH 0.5 and HCl) showed a high contribution and were related to SDB and PSM treatments. The fertilization of all treatments was performed in order to meet the demands of N to plants. However, organic wastes, such as LCM, but mainly PSM and SDB, have an imbalance between the amount of nutrients needed to meet the demand of the plants and the amount added. In the soil with SDB, 62 and 70% more P were added when compared to soils with LCM and NPK. However, in the soil with addition of PSM, 27 and 43% more P were added when compared to the soil with LCM and NPK. Furthermore, the behavior of the available fractions in the soil of the LCM and NPK treatments were very similar (Figure 1). In this case, both treatments outweigh the P needs of the plant and presented a low environmental impact compared to the other treatments.

CONCLUSION

During 8 years, all 12 applications of pig slurry manure, liquid cattle manure, swine deep bedding and NPK fertilizer (via urea + triple superphosphate + potassium chloride) provided accumulations of P fractions in the soil, which were above the necessary for a proper nutrition to plants.

Special attention should be given when using swine deep bedding, followed by pig slurry manure, as these organic sources provided higher levels of P in available fractions (AER + organic $NaHCO_3$ + inorganic $NaHCO_3$) in the soil.

The establishment of the dose of organic waste used to meet the nitrogen need of crops is not an environmentally appropriate parameter. It may cause P accumulation in excess to crop requirements and soil retention capacity, contributing to environmental contamination. However, whenever necessary, the use of liquid cattle manure is recommended compared to pig slurry manure.

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