PHOSPHORUS TRANSFORMATION IN POULTRY LITTER AND LITTER-TREATED OXISOL OF BRAZIL ASSESSED BY ^{31}P-NMR AND WET CHEMICAL FRACTIONATION[1]

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SUMMARY

Large quantities of poultry litter are being produced in Brazil, which contain appreciable amounts of phosphorus (P) that could be of environmental concern. To assess the immediate environmental threat, five poultry litters composed of diverse bedding material were incubated for 43 days under greenhouse conditions. The litters consisted of: coffee bean husk (CH); wood chips (WC); rice husk (RH); ground corn cobs (CC) and ground napier grass (NG) (*Pennisetum purpureum* Schum.), in which the change in forms of soluble P was evaluated using ^{31}P NMR spectroscopy. On average, 80.2 and 19.8 % of the total P in the extract, respectively, accounted for the inorganic and organic forms before incubation and 48 % of the organic P was mineralized to inorganic P in 43 days of incubation. Wide variation in the organic P mineralization rate (from 82 % -WC to 4 % - NG) was observed among litters. Inorganic orthophosphate (99.9 %) and pyrophosphate (0.1 %) were the only inorganic P forms, whereas the organic P forms orthophosphate monoesters (76.3 %) and diester (23.7 %) were detected. Diester P compounds were mineralized almost completely in all litters, except in the CH litter, within the incubation period. Pyrophosphates contributed with less than 0.5% and remained unaltered during the incubation period. Wood-chip litter had a higher organic P (40 %) content and a higher diester: monoester ratio; it was therefore mineralized rapidly, within the first 15 days, achieving steady state by the 29th day. Distinct mineralization patterns were observed in the litter when incubated with a clayey Oxisol. The substantial decrease observed in the organic P fraction (Po) of

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the litter types followed the order: CH (45 %) > CC (25 %) > RH (13 %) ≈ NG (12 %) > WC (5 %), whereas the Pi fraction increased. Incubation of RH litter in soil slowed down the mineralization of organic P.

Index terms: pyrophosphates, organic phosphorus, phosphorus fractionation, bedding material.

RESUMO: TRANSFORMAÇÃO DE FÓSFORO EM CAMAS DE AVIÁRIO E EM LATOSSOLO TRATADO COM CAMA DE AVIÁRIO AVALIADAS POR MEIO DE ^{31}P-RMN E FRACIONAMENTO QUÍMICO

A avicultura intensiva tem se expandido bastante nos últimos anos no Brasil e, com isso, gerado grandes quantidades de cama de aviário. Esses materiais contêm quantidades substanciais de fósforo (P) - nutriente altamente limitante ao crescimento de plantas nos solos mais intemperizados, mas que pode causar impactos ambientais negativos se inadequadamente manejado. A fim de melhor subsidiar o uso mais sustentável desses materiais orgânicos, o presente trabalho teve como objetivo avaliar as formas e as transformações de P de camas de aviário obtidas com cinco diferentes materiais de forração do piso: casca de café, maravalha, casca de arroz, sabugo de milho triturado e capim-napier triturado. As camas de aviário foram incubadas por 43 dias, e as mudanças nas frações de P foram avaliadas por meio de espectroscopia de ^{31}P RMN. Na média das cinco camas de aviário, 19,8 % do P total nos extratos encontrava-se na forma orgânica e 48 % do P dos compostos orgânicos foi mineralizado durante os 43 dias de incubação. Foi observada grande variação na mineralização desse nutriente entre as camas de aviário, de 4 % na cama de capim-napier até 82 % na cama de maravalha. Com exceção da cama de casca de café, os fostatos diéster foram quase totalmente mineralizados em todas as demais camas de aviário durante o período de incubação. O ortofofato inorgânico (99,9 %) e o pirofosfato (0,1 %) foram as únicas formas de P inorgânico detectadas, enquanto o P orgânico era composto por monoéster fosfatos (76,3 %) e diéster fosfatos (23,7 %). A contribuição do pirofosfato, menor que 0,5 %, permaneceu inalterada durante o período de incubação. A cama de maravalha apresentou a maior proporção de P na forma orgânica (40 %) e maior relação P diéster:P monoéster, resultando em rápida mineralização nos primeiros 15 dias de incubação, com estabilização a partir de 29 dias. Quando as camas de aviário foram incubadas com solo (Latossolo Vermelho-Amarelo), foram observados, na mineralização, distintos comportamentos entre elas. O decréscimo da fração de P em compostos orgânicos (Po) aplicados ao solo seguiu a ordem: casca de café (45 %) > sabugo de milho (25 %) > casca de arroz (13 %) ≈ capim-napier (12 %) > maravalha (5 %). Ao longo do período de incubação, foram observados incrementos no P inorgânico (Pi). A incubação da cama de casca de arroz com o solo reduziu a taxa de mineralização do seu Po. Portanto, a aplicação de cama de aviário em solos argilosos mais intemperizados pode ser uma alternativa segura de disposição e ciclagem das camas de aviário. No entanto, aplicações repetidas de doses elevadas de camas de aviário devem ser monitoradas para avaliar o risco de eutroficação de corpos de água devido ao P.

Termos de indexação: pirofosfatos, fósforo orgânico, fracionamento do P, materiais de forração.

INTRODUCTION

Brazil is the 3rd world leading producer of chicken meat, exporting 3.65 million tons in 2008 (ABEF, 2008). The production of poultry and related products generates massive amounts of litter, consisting of a mixture of faeces, feed, feathers, and bedding materials such as straw, peanut or rice hulls and wood shavings (Gupta et al., 1997). In the past, poultry litters were mostly used as ruminant feed. Since the prohibition of their use as animal feed by Brazilian authorities in 2001, they have been mainly used as organic manure. The total production of swine and poultry manure in Brazil was estimated at 65 million tons, based on the number of swine and poultry animals in 2003 (IBGE, 2003), and since then this number has increased tremendously. This material generally contains high levels of plant macronutrients, ranging from 39 to 49 g kg^{-1} N, 3.6 to 9.9 g kg^{-1} P, and 10 to 20 g kg^{-1} K (Kingery et al., 1994; Williams et al., 1999; Sauer et al., 2000). Poultry litters in the Zona da Mata in Minas Gerais contain on average 34 g kg^{-1} N, 16 g kg^{-1}

P and 27 g kg^{-1} K. An estimated 2.49 million tons of P were produced in swine and poultry manures in Brazil in 2003, which is 1.67 times the P used as mineral fertilizer (1.49 million tons). If the P used in fertilizer were replaced by swine and poultry manure, the large surplus of P would become an environmental concern (Shigaki et al., 2006).

One of the main environmental risks of poultry production is the imbalance of N and P in poultry manure. These two nutrients in poultry litter are not in the appropriate proportion for crops (Edwards & Daniel, 1992; USDA-ERS, 2000). When poultry litter is applied to agricultural areas in amounts based on the recommended N rates, P is over-supplied, leading to P accumulation in soil (Sims et al., 2000). This P has the potential to leave the fields as soluble P in runoff water and can lead to eutrophication of water bodies (Ribaudo et al., 2003; Sharpley et al., 2007). Basically, two factors exacerbate the risk of environmental degradation caused by intensive poultry production in Brazil. Firstly, these farms are concentrated in strategically important river basins, and secondly, the region is characterized by high rainfall and wavy landscapes that can accelerate runoff and leaching losses (Ghosh et al., 2011).

Phosphorus solubility is controlled by the chemistry of liquid and solid phases of the manure matrix and the surface chemistry of inorganic and organic particulates in that environment (Lindsay, 1979). Most agricultural soils in Brazil are Oxisols, which are rich in Al and Fe oxides, capable of sorbing large amounts of P (Fontes & Weed, 1991; Novais & Smyth, 1999). Repeated applications of poultry litter, however, can lead to N and P accumulation and to elevated levels of one or both nutrients in surface runoff and subsurface water (McLeod & Hegg, 1984; Sharpley & Menzel, 1987; Kingery et al., 1994). Moreover, the amount of P loss that would cause water quality problems is usually very low, compared to the amounts required by crops or contained in typical manure or fertilizer P applications. For example, lake water concentrations of P above 0.025 mg L^{-1} generally accelerate eutrophication. These values are an order of magnitude lower than P concentrations in soil solution critical for plant growth (0.2 to 0.3 mg L^{-1}), which illustrates the vulnerability to eutrophication of fresh waters.

In recent years, there has been a rapid increase in poultry industry in the state of Minas Gerais, Brazil, and very distinct alternative bedding materials, such as coffee bean husk, corn cob, napier grass, wood chips, and rice husk have been used on broiler farms and their use is expected to increase in the near future, due to their easy availability. However, it is estimated that 50 % of the litter from areas with high concentrations of poultry production facilities cannot be applied to agroecosystems in these same areas, due to environmental or economical constraints (Wimberly, 2002). This excess litter is usually stored and a time lag exists before it can be transported to other areas for application. This large quantity of manure P, without careful management, also represents a considerable potential source of P losses to ground and surface waters, especially if run-off occurs during this storage period (Sherwood & Fanning, 1981; McLeod & Hegg, 1984; Sharpley et al., 1994).

The normally alkaline pH of poultry litter limits the solubility of P forms, especially the inorganic ones. Poultry litter acidification after field applications could result in increased P solubilization, thus releasing inorganic P (Tasistro et al., 2004). Oxisols are highly acidic and the pH of poultry litter will likely decrease after soil application, thereby releasing additional water-soluble P. Little is known about the fate of P applied via poultry litter with diverse bedding materials to soils with high P sorption capacity, as for example to the Oxisols of Brazil. The development of better management practices to optimize the recycling of manure P and to minimize the adverse environmental effects of animal manure application is of significant public interest. For this purpose, a better understanding of manure P chemistry is needed to determine the potential for transport of bioavailable P to ground and surface waters. In this study, sequential extraction and ^{31}P nuclear magnetic resonance (^{31}P-NMR) spectroscopy have been used to characterize P forms and their transformation in manure and manure applied to an Oxisol of Brazil. This technique was chosen for enabling the speciation of soil P, which is a clear advantage over other chemical fractionation protocols.

MATERIAL AND METHODS

Soil and poultry litters

The soil used in the study was a clayey typic haplic Oxisol (Red-Yellow Latosol), sampled in Viçosa, region of Zona da Mata in Southeastern Minas Gerais, Brazil, in the Atlantic Rainforest domain (Ab'Saber, 1969). The climate is tropical with moist summers and dry, mild winters. The soil parent material is gneiss. The topography is steep with slopes from 20 to 45 % and average altitudes from 200 to 1,800 m (Golfari, 1975). The soil types in the Zona da Mata are predominantly Oxisols, which are deep and well-drained, but acidic and poor in available nutrients. The most important crops are pasture and coffee, the latter often intercropped with maize and/or common bean. To minimize the influence of organic P in the undisturbed topsoil, soil was sampled from the sub-surface layer 20-40 cm. The samples were air-dried and ground to pass through a 2 mm sieve and analyzed (Embrapa, 1997). The P concentration in the equilibrium solution was determined by shaking samples with $CaCl_2$ 0.01 mol L^{-1}, containing 60 mg L^{-1} P (1:10 soil:solution ratio) (Alvarez V. et al, 2000). The maximum P adsorption capacity was determined as described by Olsen & Watanabe (1957), following the protocol adapted by Alvarez V. & Fonseca (1990). The main chemical and physical properties are presented in table 1.

Five types of poultry litters (PL) containing different bedding materials were used, namely: coffee bean husk (CH); wood chips (WC); rice husk (RH); ground corn cobs (CC) and ground napier grass (*Pennisetum purpureum* Schum.) (NG). These litter types were collected in poultry houses in Guiricema County, Minas Gerais, Brazil, a region in which poultry industry has expanded quickly in recent years and a great diversity of bedding materials is being used. The collected litter had been used once as bedding material for 48 days, at a bird density of 15 m^{-2}. After collection, it was air-dried, passed through a hay shredder and stored in plastic bags at room temperature until subsequent use. Litter subsamples were oven-dried at 70 °C, ground in a Wiley mill (0.5 mm sieve) and analyzed for organic carbon (Yeomans & Bremner, 1988), total N (by dry combustion in an elemental analyzer CHNS/O 2400 (Perkin Elmer, USA). and P (by colorimetry after nitro-perchloric acid digestion; Murphy & Riley, 1962). Chemical properties of poultry litter are presented in table 2.

Experiment 1. Phosphorus transformation in poultry litters evaluated with ^{31}P-NMR spectroscopy

The five poultry litters (CH, WC, RH, CC, and NG) were incubated for 43 days without soil, to simulate the conditions in litter dumps and to study the changes in P forms that could be of potential environmental concern. The litters were incubated in 2 dm^3 plastic pots in a greenhouse (30 °C ± 5) at -33 kPa moisture content, maintained by weighing the pots daily and replacing the lost water. Litter samples were collected at the beginning (0 d) and the end of the incubation period (43 d). The WC litter was sampled more frequently (after 0, 15, 22, 29, 36 and 43 days) to monitor the complete pathway of P transformation. The experiment was arranged in a completely randomized block design, with three replicates.

The litter subsamples were dried at 70 °C and stored at room temperature for ^{31}P-NMR analyses. This technique was chosen because the P chemical species can be assessed not only in relation to their behavior in the soil, but also to their relative abundance. All P species in the sample may frequently be characterized without extensive sample preparation (Cade-Menun, 2005). Organic P compounds were extracted with a NaOH-EDTA mixture to minimize the hydrolysis of more labile Po compounds (Turner et al., 2003). In adddition, water was also used to extract P from poultry litter because water-soluble P (WSP) is readily released from the litter and represents a more immediate environmental concern (Sharpley & Moyer, 2000; Vadas et al., 2004). Samples were prepared by treating 5 g of litter with 100 mL of water or NaOH-EDTA mixture and shaken for 16 h at 4 °C to minimize any potential breakdown of Po compounds. Following the extraction, the samples were centrifuged for 10 min (509 *g*, 4 °C) and the supernatant was then filtered through Whatman 42 filter. The filtered extracts were lyophilized and stored at -20 °C for further analysis (usually within a week). For ^{31}P-NMR spectroscopy analysis, 200 mg of the freeze-dried material was reconstituted in 1 mL NaOD (0.2 mol L^{-1}) and transferred to 5 mm NMR tubes. The spectra were acquired in a liquid-state NMR spectrometer (Varian, Mercury 300 MHz) with acquisition time 1 sec, spectral width 10.000, pulse width 6; delay time 0.8 number of pulses 10.000 (Turner et al., 2003), spending approximately 5 h 20 min for the analysis of a sample. The identification of P compounds was based on the chemical shift of phosphoric acid (85 % v/v). The general classes of P compounds and the respective chemical shifts were: phosphonates 19 ppm, inorganic orthophosphate 6.1 ppm (PO_4^{3-}), orthophosphate monoesters 3-6 ppm, orthophosphate diester 0.5-2 ppm; pyrophosphate 4 ppm and inorganic polyphosphates 20 ppm (Turner et al., 2003; Chen et al., 2004; Hansen et al., 2004; Leytem et al., 2008). The relative contribution of each class of P compounds was obtained by dividing the integrated spectral area for each band by the total integrated area.

Table 1. Chemical and physical properties of the Oxisol under study

Property	Unit	Value
pH H_2O (1:2,5)		4.80
Al^{3+}	$cmol_c$ dm^{-3}(1)	1.10
Ca^{2+}	$cmol_c$ dm^{-3}(1)	0.02
Mg^{2+}	$cmol_c$ dm^{-3}(1)	0.01
H+Al	$cmol_c$ dm^{-3}(2)	6.70
K^+	mg dm^{-3}(3)	4.10
Mehlich1-P	mg dm^{-3}(3)	0.30
Equilibrium P	mg L^{-1}(4)	4.20
Max. P adsorption capacity	mg g^{-1}(5)	2.48
Organic C	dag kg^{-1}(6)	1.38
Coarse sand	%(7)	13.00
Fine sand	%(7)	7.00
Silt	%(7)	2.00
Clay	%(7)	78.00
Moisture at field capacity	kg kg^{-1}(8)	0.35

(1) Extracted with KCl 1 mol L^{-1}. (2) Extracted with calcium acetate 0.5 mol L^{-1}, pH 7.0. (3) Extracted with Mehlich-1. (4) P concentration in solution after 1h shaking with a 60 mg L^{-1} P (1:10 soil:solution ratio) (Alvarez V. et al, 2000). (5) (Olsen & Watanabe, 1957) adapted by Alvarez V. & Fonseca (1990). (6) Walkley & Black. (7) Pipet method. (8) -30 kPa.

Experiment 2: Transformation of poultry litter-treated soil as evaluated with wet chemical fractionation

The five types of poultry litter (CH, WC, RH, CC and NG) were mixed with 1.5 dm^3 of the clayey Oxisol at 40 Mg ha^{-1} on a dry-weight basis in plastic pots to study the transformation of P after litter application. The pots were incubated in a greenhouse (30 ± 5 °C) and maintained at -30 kPa, with daily replacement of water lost by evaporation. An additional treatment with wood chips only (no soil) was added to compare results with the previous study. Samples of litter-treated soil were collected at the beginning (0 days) and 15 days after incubation, since in this period most P transformation is expected to occur (Koopmans et al., 2003). The WC litter and litter-treated soil were incubated for 43 days and sampled after 0, 15, 22, 29, 36, and 43 days to monitor the P transformation kinetics. The collected samples were chemically fractionated (Bowman, 1989) into inorganic (Pi) and organic phosphorus (Po) fractions. The change in Pi and Po was calculated as the difference in P forms during the incubation period and the rate of P mineralization was calculated from the percentage increase in Pi during incubation per unit of Po. The experiment was set up in a completely randomized block design, with three replicates.

RESULTS AND DISCUSSION

The soil used in this study contained 78 % clay, 13.8 g kg^{-1} organic carbon and had a high P fixation capacity (2.48 mg P g^{-1} soil). The total and organic soil P content was 184.6 and 122.4 mg kg^{-1}, respectively, and 80 % of the total P was in organic form. Mehlich-1 available P was 0.3 mg dm^{-3}. In Brazil, the predominance of Al and Fe-rich Oxisols that can sorb large amounts of P has been reported (Fontes & Weed, 1991; Novais & Smyth, 1999). These soils occupy most of the areas under agricultural use and are therefore extremely important for crop and animal production and the overall economic viability of Brazilian agriculture (Fontes, 1996).

The chemical characteristics of the five types of poultry litters are listed in table 2. The total P content in the poultry litter ranged from 13.7 to 18.1 g kg^{-1}, with a higher P content and consequently lower N/P ratio in the CC than in the other litters. The average C and N content of the litters were 446 and 33 g kg^{-1}, respectively, similar to the values reported elsewhere (Kingery et al., 1994; Williams et al., 1999; Sauer et al., 2000), except that the P content was considerably higher in our study. There was no significant difference in the total C, N and C/N ratio among the litters. The P of the poultry litters was fractionated to find the respective contribution of inorganic and organic to total P. An average 61 and 39 % of total P in the poultry litter was in inorganic and organic forms, respectively (Table 3).

Changes in P forms in incubated poultry litter: ^{31}P-NMR spectroscopy

Solution-state ^{31}P NMR spectroscopy was used to elucidate P forms in poultry litter at the beginning and end of the 43-day incubation (Figure 1). The majority of P analyzed was in the form of either orthophosphate or phytic acid. Similar results were reported by Leytem et al. (2008) in poultry litter with fresh pine shavings as bedding for 42 days . A strong peak at 6.1 ppm (inorganic orthophosphate) and weaker peak area between 3-6 ppm (orthophosphate monoesters) and -0.5 - 2 ppm (orthophosphate diester) were observed in this study after incubation for 43 days, indicating mineralization of organic to inorganic P forms. In some litters, a complete peak absence at -0.5 to 2 ppm after incubation suggested complete mineralization of diester P (Figure 1 and Table 4). The more intensive mineralization of orthophosphate diesters may be attributed to the presence of compounds such as phospholipids, nucleic acids and sugar phosphates (Turner & McKelvie, 2002). Their

Table 2. Total organic C (TOC), nitrogen (N), phosphorus (P) concentration, and C/N, C/P and N/P molar ratios in five types of poultry litters

Poultry litter type	TOC	N	P	C/N	C/P	N/P
	g kg^{-1}					
Coffee bean husk	391 a	32.8 a	14.4 b	13.9	70.0	5.0
Ground corn cob	510 a	34.2 a	18.6 a	17.4	70.7	4.1
Wood chip	391 a	30.9 a	13.7 b	14.8	73.6	5.0
Rice husk	491 a	34.7 a	15.9 b	16.5	79.6	4.8
Ground napier grass	449 a	34.8 a	15.1 b	15.0	76.7	5.1
Mean	446	33.0	16.0	13.3	28.8	

Means followed by the same letter within each column do not differ by Tukey's test (p = 0.05).

Table 3. Total, inorganic (Pi), organic (Po) P concentration and relative contribution of Po in poultry litters

Poultry litter type	Total P	Pi	Po	Po contribution
	g kg^{-1}			%
Coffee bean husk	14.4 c	9.8 c	4.7 b	32
Ground corn cob	18.2 b	10.8 bc	7.4 ab	40
Wood chip	17.1 c	10.5 bc	6.7 ab	39
Rice husk	21.9 a	13.1 a	8.8 a	40
Ground napier grass	19.0 ab	10.9 b	8.0 ab	42
Mean	18.1	11.0	7.1	39

Means followed by the same letter within each column do not differ by Tukey's test (p = 0.05).

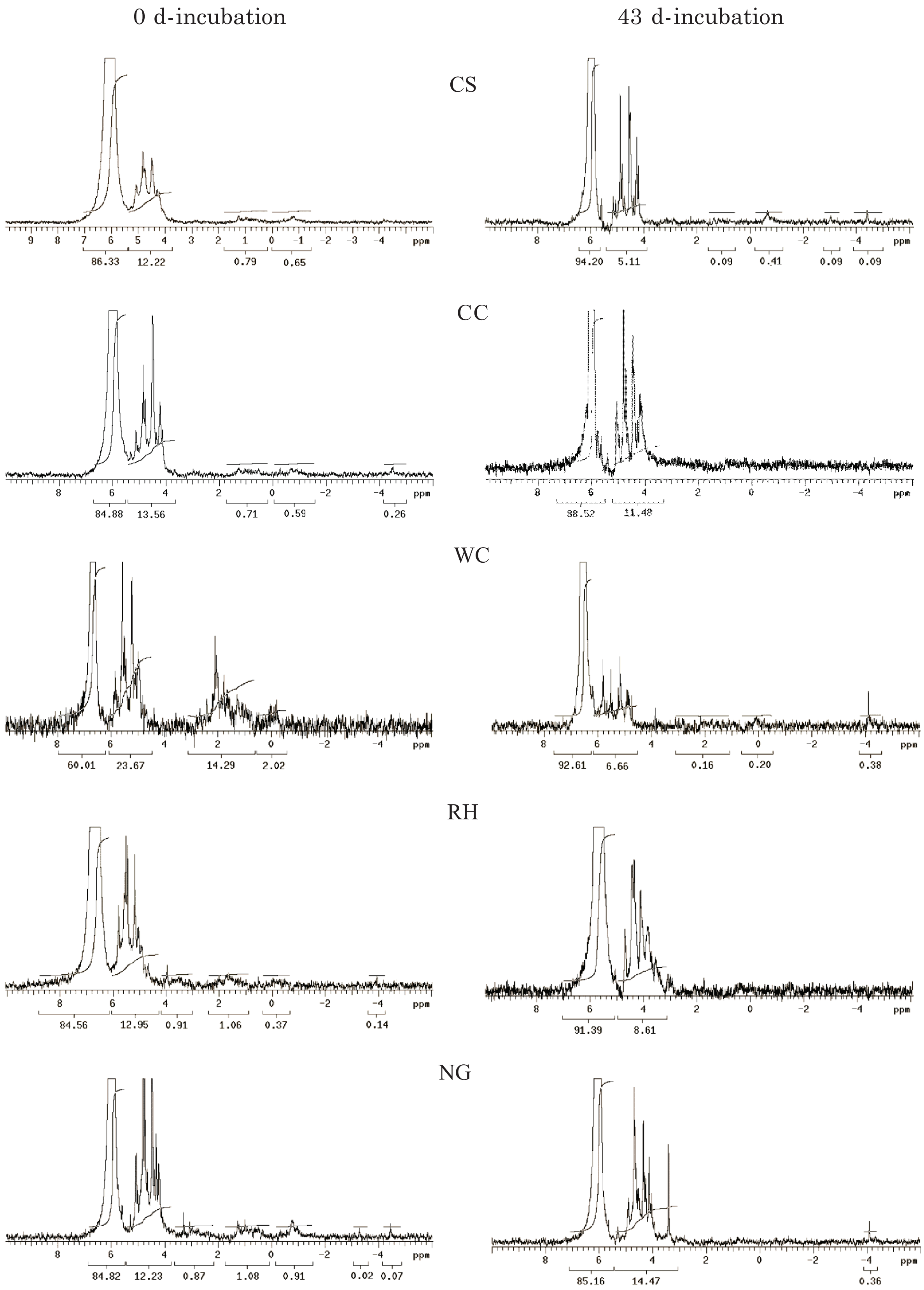

Figure 1. ^{31}P-RMN spectra of water-soluble P compounds found in poultry litters 0 and 43 days after incubation.

low charge density reduces the energy of interaction with soil colloids and they become more easily accessible to decomposers (Turrión et al., 2001). In semi-arid Northern Tanzania, clearing and continuous low input agriculture reduced both organic and inorganic P in the soil. Homestead cultivation led to a 53 % depletion of orthophosphate diester P, whereas only 30 and 39 % reduction of orthophosphate monoester P occurred after 3 and 15 years of cultivation, respectively (Solomon & Lehman, 2000). Thus, despite the smaller proportion of orthophosphate diesters, they may be a more readily available P source to plants than the dominant orthophosphate monoesters (Hayes et al., 2000).

The P distribution in the poultry litter extracts was calculated by integrating the signal areas, and P concentrations were computed by multiplying the proportion of the total spectral area assigned to a specific signal by the total P concentration in the original extract (Table 4). On average, 80.2 and 19.8 % of the total P in the extract was distributed in inorganic and organic forms before incubation. After 43 days of incubation, Pi increased to 90.4 % and Po decreased to 9.5 %. This very large quantity of inorganic P, without careful management, also represents a potential threat as source of P losses to ground and surface waters by incidental losses through surface and sub-surface flow, directly after surface application or indirectly, by soil P enrichment (Smith et al., 2001). P loss in surface run-off from livestock manures has been shown to depend not only on the rate and timing of manure application but also, more importantly, on the interval between the application and the run-off event (Sherwood & Fanning, 1981; McLeod & Hegg, 1984; Sharpley et al., 1994).

In the Pi fraction, the main component was inorganic orthophosphate (99.9 %) and the remaining small proportion was pyrophosphate (0.1 %), whereas in the organic P fraction, orthophosphate monoesters predominated (76.3 %), which contributed to 15.1 % of total P, while the orthophosphate diesters represented 4.7 % of the total P. Among the organic P compounds, monoesters have been reported to be predominant (Dalal, 1977). Our results are similar to those reported by Koopmans et al. (2003) for poultry litter, who found that 78.5 % of total P was in inorganic (Pi) forms (77 % inorganic orthophosphate and 1.5 % pyrophosphate) and 21.5 % was P in organic forms (17.9 % monoester phosphates, 0 % diester phosphates and 3.6 % aromatic diester phosphates).

Among the evaluated litter types, the WC litter differed from the others with a lower inorganic (60 %) and higher organic P (40 %) content. It also contained the highest proportion of orthophosphate monoesters (23.7 %) and the largest proportion of orthophosphate diesters (16.3 %) among the litters studied (Figure 1 and Table 3). The WC litter also had a considerably higher diester: monoester ratio than the others. Diester phosphates, although found in small amounts, are reported to be chemically more labile than monoester P (Tate & Newman, 1982), with higher susceptibility to microbial or enzyme attack in the soil environment (Dai et al., 1996). Because the monoester is considered more stable and less accessible to plants than diester, the diester: monoester ratio is a measure of organic P lability (Chapuis-Lardy et al., 2001; Turrión et al., 2001).

The incubation of WC litter samples was observed in detail (Figure 2). It was found that Po mineralization was highest in the first 15 d, achieving steady-state by the 29th day. The increase in inorganic orthophosphate was concomitant to the decrease in orthophosphate monoesters and diesters. Pyrophosphate was present at a low proportion and remained unaltered. Phosphonates and polyphosphates were not detected in the analyzed samples.

The measurement of net Po mineralization is complicated by the high reactivity of released inorganic P with the soil solid phase. However, since only poultry litters with similar C/N ratios were studied, a similar adsorption of the released Pi and calculated gross Po

Table 4. ^{31}P-NMR-based relative P distribution in distinct inorganic (Pi) and organic (Po) P forms in poultry litter before and after 43d-incubation, and relative mineralization of the Po forms (MPo)

Poultry litter	0 day				43 days				Change		MPo
	Pi		Po		Pi		Po				
	Ort[(1)]	Pyro[(2)]	Mon[(3)]	Dies[(4)]	Ort	Pyro	Mon	Dies	Mon	Dies	
	%										
Coffee bean husk	86.3	0.0	12.2	1.5	94.2	0.2	5.1	0.5	58	67	59.1
Ground corn cob	84.9	0.2	13.6	1.3	88.5	0.0	11.5	0.0	15	100	22.8
Wood chip	60.0	0.0	23.7	16.3	92.6	0.3	6.7	0.4	72	97	82.3
Rice husk	84.6	0.1	13.9	1.4	91.4	0.0	8.6	0.0	38	100	43.8
Ground napier grass	84.8	0.1	12.2	2.9	85.2	0.4	14.5	0.0	0	100	4.63
Mean	80.1	0.1	15.1	4.7	90.4	0.2	9.3	0.2	38	96	42.5

(1) Ort: Orthophosphate; (2)Pyro: pyrophosphate; (3)Mon: monoester P; (4)Dies: diester P.

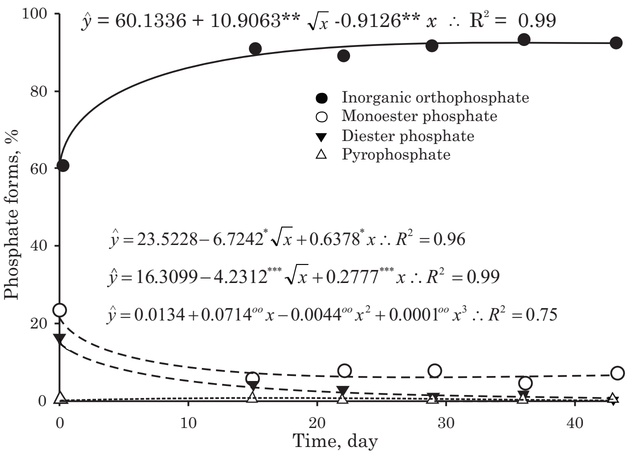

Figure 2. Dynamics of P fractions in wood chip-based poultry litter during the 43-d incubation period. *, **, *, and ⁰ indicate that coefficients are significant at 0.1, 1, 5 and 10 %, respectively.**

mineralization was assumed (Preusch et al., 2002). There was a wide variation in the organic P mineralization rate observed among litters; the highest mineralization (82 %) was detected in WC, followed by CH (59 %), RH (43 %), CC (22 %) and the lowest in NG (4 %). This trend followed the decrease in monoester content before and after incubation. Comparing the litter types, it was observed that in the CC, RH and NG litters, 100 % of orthophosphate diesters was mineralized during the 43 d-incubation, whereas in the CH and WC litters, 67 and 97 % of orthophosphate diesters, respectively, were mineralized.

Results of this study indicate that poultry litter contains a considerable amount of soluble Pi, which increases during incubation. Even WC litter, which initially contained less Pi, was rapidly mineralized during the incubation period and produced Pi equivalent to other litter types. Readily bio-available inorganic P represents a potential threat of leaching and runoff losses in the case of rainfall. Water-soluble Po is also mobile in the soil profile (Anderson & Magdoff, 2004). The open dumping of poultry litter or repeated applications of high poultry litter rates should therefore be avoided to minimize environmental risks of P contamination of fresh water bodies.

Soil-litter incubation study: chemical fractionation

The chemical fractionation of litter using Bowman's fractionation scheme (Bowman, 1989) revealed that the concentration of Pi was higher in the RH litter and lowest in the CH litter, but did not differ from Pi in the WC and CC litters (Table 3). The same pattern was observed for the Po concentration. The Pi was the dominant fraction, corresponding to an average of 61% of total P. The smallest proportion of Po was found in the CH litter (32 %) and the highest in the NG litter (42 %). The results found here show that a substantial proportion (39 %) of P present in the poultry litters is in organic forms, which is higher than the 21% Po reported by Peperzak et al. (1959) and 25 % reported by Cassol et al. (2001) in Southern Brazil, based on Hedley's fractionation scheme (Hedley et al., 1982). Using a similar fractionation procedure, Sharpley & Moyer (2000) however reported the contribution of a Po fraction of 9 % of total P in a litter consisting of pine bark (three lots raised, 1.31 birds m^{-2}). In the last decade, several methods have been used to characterize the different P forms in soils and animal manures (Hedley et al., 1982; Bowman, 1989; Dou et al., 2000; Sharpley & Moyer, 2000).These differences might be due to the distinct methods used in the assessment of P fractions (Koopmans et al., 2003), as well as the bird's diet, origin and management of the litters (Maguire et al., 2006).

The distribution of P fraction at the beginning (0 days) of the incubation period revealed no difference in the Po concentration among the poultry litters (Table 5). However, the litter differed in relation to the Pi concentration, which was highest in the RH litter and lowest in CH litter. The Pi concentrations in the other litters were intermediate. After 15 days of incubation with a clayey Oxisol, there was a substantial decrease in the Po fraction and an increase in the Pi fraction (Table 5). The litter with the highest relative decrease in Po was the CH litter (45 %) followed by the CC (25 %), RH (13 %), NG (12 %) and WC (5 %). However, no concomitant increase in Pi was observed in the CC litter, probably because of transformation to un-extractable forms in the soil.

The smallest decrease in Po and consequently smallest increase in Pi was observed in WC litter. Although the C/N and C/P molar ratios were similar as in the other poultry litters, factors such as elevated concentration of lignin and phenolic compounds present in the wood chips may have limited the decomposer activity of soil microorganisms (Oglesby & Fownes, 1992; Entry & Backman, 1982; Cortez et al., 1996). Possibly, high concentrations of orthophosphate monoesters, such as inositol hexaphosphate (phytic acid), with a high affinity for the soil colloids (Leytem et al., 2002; Gebrim et al., 2004), could have reduced the availability to soil microbes and thus decreased decomposition (Bowman & Cole, 1978; Evans, 1985; Turner & McKelvie, 2002). It is suspected that this phenomenon may favor the persistence of P in organic compounds for a longer period of time and be more prone to be transported to water bodies (Eghball et al., 1996; Chardom et al., 1997), increasing the risk of eutrophication.

The RH litter was incubated for a longer period (43 d) with and without soil (Figure 3). It was found that in the absence of soil, Po mineralization was higher in the first 15 days of incubation, with about 50 % of Po being mineralized (Figure 3a). Afterwards, the mineralization rates tended to stabilize. The

Table 5. Change in phosphorus (P) fractions of poultry litter incubated with soil

Poultry litter type	Pre-incubation		Post-incubation	
	Po	Pi	Po	Pi
		mg kg^{-1}		
Coffee bean husk	105.2 a	210.4 b	58.2 b (-44.7)	256.1 ab (21.7)
Ground corn cob	143.2 a	245.0 ab	107.5 a (-24.9)	255.2 ab (4.2)
Wood chip	105.1 a	217.9 ab	100.0 ab (-4.90)	220.0 b (1.0)
Rice husk	131.4 a	252.0 a	114.8 a (-12.6)	281.3 a (11.6)
Ground napier grass	122.4 a	214.7 ab	107.7 a (-12.0)	246.5 ab (14.8)
Mean	121.5	228.0	97.6 (-19.7)	251.8 (10.4)

Numbers in parentheses represent the relative change in comparison to the pre-incubation period. Means followed by the same letter within each column do not differ by Tukey's test (p = 0.05).

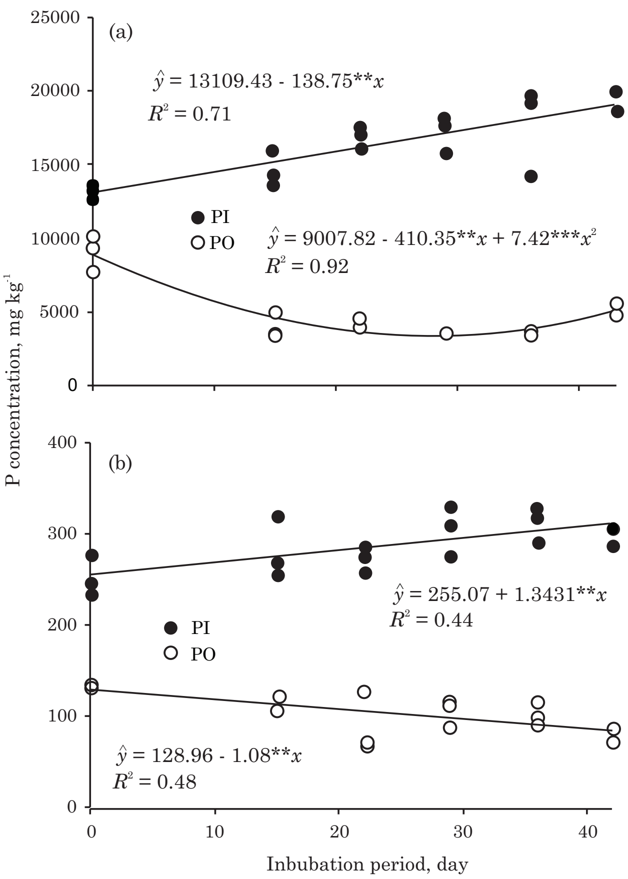

Figure 3. Organic (Po) and inorganic (Pi) P concentration in the rice husk-based poultry litter incubated without (a) and with (b) soil. *, **, * indicate that regression coefficients are significant at 0.1, 1, and 5 %, respectively.**

reduction in the Po mineralization rate after 15 days of incubation was possibly due to the high buildup of Pi, inhibiting microbial activity (Bahl & Toor, 2002). On the other hand, when RH litter was incubated with soil, the decrease in Po concentration was linear and slower (Figure 3b). At the end of the incubation period, the proportion of Po decreased by 41 % (decreased from 34 to 20 %) when incubated with soil and by 49 % (decreased from 41 to 21 %) when incubated without soil. The decrease in the Po mineralization rate in soil could possibly be due to a protection of organic phosphate compounds by adsorption on soil colloids (Cross & Schlesinger, 1995; Turrión et al., 2001; Conte et al., 2003), leading to the preferential preservation of some Po forms, especially orthophosphate monoesters (Koopmans et al., 2003). Pi increased linearly, irrespective of whether the RH litter was incubated with or without soil. However, the slope of the curve was steeper for incubation without soil, indicating quicker mineralization in the absence of soil.

Results of the chemical fractionation indicate distinct patterns of Po mineralization among litter types. The WC and CH litters, with fast Po mineralization, could be used to fertilize rapidly growing crops, with a high initial P demand. On the other hand, the CC and GN litter, with a slower Po mineralization rate, would better meet the demands of perennial crops or plants with a smaller initial P demand. Although some organic P forms, such as phytic acid, are more stable in the soil environment (Turner & McKelvie, 2002), it appears that some perennial plants are able to acquire them. Chen et al. (2004) reported that pine (*Pinus radiata*) trees are able to acquire P from phytic acid from soils, but faster-growing plants, including some annual grasses and legumes, are not efficient in acquiring P from such organic sources (Hayes et al., 2000).

CONCLUSIONS

1. Inorganic orthophosphate is the dominant species found in the water-soluble fraction of the poultry litters, but a portion of P in the poultry litters also occurs as organic forms.

2. There is a large variation among poultry litters in terms of the organic P mineralization rate in the the 43-day incubation period; the highest decomposition was 82 % for wood chip and the lowest 4 % for napier grass litter.

3. Among the organic P forms, the monoesters are predominant, but diester P forms are also present and are more rapidly mineralized. The mineralization rate of organic P in poultry litter is rapid in the first 15 days after incorporation into the soil and then slows down.

4. A higher mineralization rate of organic P was observed in the poultry litters, without soil.

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