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Research Article

Phosphorus Speciation by ^{31}P NMR Spectroscopy in Leaf Litters and Crop Residues from Para Rubber, Cocoa, Oil Palm, and Banana Plantations in the Humid Forest Zone of Cameroon

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The release of nutrients, including phosphorus from agricultural residues, is an important potential source of nutrients for subsequent crops. To fully understand the contribution of this residue P as a source of plant P for agricultural production, its chemical nature needs to be understood. In this study P species were identified and quantified in leaf litters and crops residues from cocoa farms, oil palm, rubber, and banana plantations by ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Phosphorus in the crop residues was predominantly in the form of inorganic P mainly as orthophosphate and ranged from 45.9 to 89.2%. The highest relative percentage of P as orthophosphate was found in cocoa pod husk (89.2%) and the lowest percentage was found in decaying banana pseudostem (45.9%). Pyrophosphate was detected in trace amounts in all samples (less than 6%) except in fresh palm fronds. However, orthophosphate diester was detected only in fresh palm fronds (11.4%) and phytate was detected only in palm male inflorescence (6.7%). The result implied that cocoa pod husk, palm empty fruit bunch, and palm male inflorescence could be used as organic amendment, based on their high P content and release potential.

1. Introduction

Phosphorus deficiency in many tropical soils is a major constraint of crop production. This deficiency is primarily as a result of inherent low soil P, depletion of soil P by cropping, and sorption and precipitation involving Fe and Al oxides and hydroxides [1]. Phosphorus is a major plant nutrient which is involved in many metabolic processes [2]. It is often referred to as "energizer" since it helps to store and transfer energy during the process of photosynthesis. According to Frossard et al. [3] only a small proportion of P in soil is found in the soil solution (e.g., 0.01–3.0 mg P L^{-1}) or in forms available to crop plants at any given time. Thus to improve crop production, most farmers have depended on the use of P fertilisers. The release of nutrients, including P from litters and crop residues, is an important potential source of nutrients for subsequent crops [4, 5]. Total P in plant material ranges between 0.5 and 10 g kg^{-1}. Inorganic orthophosphate, which is the preferred source of P to plants, is also the major form of P found in green crops (60–80% of total P) during vegetative growth [3, 6, 7]. At physiological maturity, nutrients in crop residues can be released to the soil where they are incorporated into different labile and nonlabile pools [8]. For example, Seephueak et al. [9] showed that in the para rubber plantation ecosystem, leaf litter is a major contributor to nutrient cycling pathways. Studies by Khalid et al. [10] showed that oil palm residues during replanting contributed significant amount of nutrients that could be recycled in the plantation. According to Hartemink [11] rain wash and litter fall are key components in the cycling of nutrients of cocoa ecosystems. Wortman et al. [12] also demonstrated that the management of harvested (senescent) banana pseudostem was found to be very important in the nutrient use efficiency of banana plantations with most of their nutrients translocated to the attached growing pseudostems.

The major gap in soil fertility recommendations for the tropics is phosphorus management as crop production on many of the soils in the tropics is limited primarily by phosphorus. The understanding of soil phosphorus dynamics and indicators of phosphorus availability lags far behind that

Table 1: Description of sampling sites of the crop residues and litters used for the study.

Land use type	Location	Longitude	Latitude	Elevation (m)	Soil type
Banana	Mussaka	04° 11.468′	009° 19.25′	477	Volcanic soil
Cocoa	Ekona	04° 06.167′	009° 23.51′	34	Volcanic soil
Rubber	Misellele	04° 07.555′	009° 26.62′	35	Volcanic soil
Oil palm	Maumu	04° 12.29′	009° 19.76′	437	Volcanic soil

for nitrogen. Part of the problem in modelling phosphorus is in its complex biogeochemical cycle [13]. Optimizing P use efficiency is very important for agronomic, economic, and environmental benefits especially when adjusting agricultural production systems to meet future global food production targets [14]. Such optimization will rely on adequate knowledge of the dynamics of soil and residue P pools to enable accurate predictions of the required external P inputs to achieve optimum growth of subsequent crops.

The most commonly used measure of P in crop residues is total P, followed by the distinction between inorganic P and organic P. The carbon : phosphorus (C : P) ratio of crop residues has often been widely used to predict potential P immobilization or mineralization [15]. However, these measures do not identify or take into account the various P species found within crop residues. The employment of ^{13}P NMR spectroscopy has greatly improved the understanding of soil P species, particularly the organic P forms quantified in relative terms [16]. P speciation is also important for the estimation of P release from crop litters and residues in cropping soils, as some species of P in residues may be more recalcitrant than others. Few studies have used solution NMR to identify P species in fresh and mature plant materials and mature crop residues [8]. However, this technique has not been used to identify P forms in litters and residues of para rubber, cocoa, oil palm, and banana plantations. Phosphorus in soil is generally divided into organic P and inorganic P [17, 18]. Inorganic P is classified into orthophosphate, pyrophosphate, and polyphosphate. Typical organic P compounds are divided into phosphate monoesters, phosphate diesters, phosphonates, and organic polyphosphates. However, knowledge of how these P forms change as plants senesce is limited, since most research in this area focused on fresh or immature plant materials rather than the senesced materials that are returned to soil in most farming systems [19]. To fully understand the contribution of crop residue P as a source of plant P for agricultural production, its chemical nature needs to be understood. This is a prerequisite to further understand the dynamics of crop residue P in the soil ecosystem, and its bioavailability to plants and microorganisms. Proper identification of P species in crop litters and residues can improve the understanding of the potential turnover of these P species in soil, leading to a better assessment of the amount of P that may be provided for subsequent crops.

The objective of this study therefore is to identify and quantify the various phosphorus species in leaf litters and crop residues from cocoa farms, oil palm, rubber, and banana plantations.

Table 2: Samples used and code names.

Sample name	Description
JN-01	Cocoa pod husk
JN-02	Senescent cocoa leaves (litter)
JN-03	Fresh banana pseudostem
JN-04	Decaying banana pseudostem
JN-05	Fresh palm fronds
JN-06	Palm empty fruit bunches
JN-07	Palm male inflorescence
JN-08	Senescent rubber leaves (litter)

2. Materials and Methods

2.1. Site Description. Samples were collected from some banana, cocoa, rubber, and oil palm fields in Fako Division of the South West region of Cameroon. The banana, rubber, and oil palm fields belong to the Cameroon Development Cooperation (CDC) and the cocoa farm to a small holder farmer. The exact locations of the fields are presented in Table 1.

The study area (Fako division) has humid tropical climatic conditions and rich volcanic soils with two distinctive seasons, a long rainy season which ranges from mid-March to mid-November and a short four-month dry season expanding from mid-November to mid-March [21, 22]. In cocoa farms the main source of organic waste is the cocoa pod husk which is left in the fields after bean harvest. There are often in large quantities because for a cocoa pod, just 30% of its weight is made up of the cocoa bean and the rest is the husk. Another source of organic waste in cocoa farms is the leaf litter (senescent leaves). In mature oil palm plantations in Cameroon the fronds (branches) are often cut and left to rot in the fields which is the main source of organic waste. The empty fruit bunches and the male inflorescence is also residues of the oil palm plantation. In rubber plantations there is the annual leaf fall referred to as defoliation where the rubber tree shades all its leaves and this forms the main source of organic waste (leaf litter) in rubber plantations. In banana plantations, the main organic waste is the stems that remain after bunch harvest.

2.2. Sample Collection. For each agroecosystem, the main organic wastes were identified and samples of about 10 kg collected. The samples were sun dried to reduce the moisture content and reduce the risk of molds growing on the samples. The dried samples were ground into powder and packaged in polyethylene bags for analyses. Table 2 shows the sample codes and their description. These agricultural crop residues

Table 3: Some physical properties of the samples, pH, organic matter (%), moisture, ash (%), and organic carbon content.

Sample	pH(H_2O)	Moisture content (%)	Organic matter (%)	Ash content (%)	[a]Organic carbon (%)
JN-01	8.17	10.2	91.7	8.3	53.3
JN-02	6.52	5.3	85.7	14.4	49.8
JN-03	9.28	4.9	80.9	19.1	47.0
JN-04	10.29	7.4	68.8	31.2	40.0
JN-05	5.82	5.2	91.2	8.8	53.0
JN-06	7.55	5.1	93.3	6.8	54.2
JN-07	7.58	14.0	88.0	12.1	51.1
JN-08	6.75	6.00	94.5	5.5	55.0

[a]Estimated by a conversion factor of 1.72.

were distinguished into field residues (JN-02, JN-03, JN-04, JN-05, JN-07, and JN-08), which are materials left in an agricultural field after the crop has been harvested, and process residues (JN-01 and JN-06), which are materials left after the crop is processed into a usable resource.

All the crop residue samples were analyzed for pH, organic matter, ash content, moisture content, and P species.

2.3. Physicochemical Analysis of Crop Residues and Leaf Litters. The pH was measured in H_2O (ratio soil : water 1 : 2.5 w/v). Percentage of ash content and organic matter (OM) was determined by loss of ignition using 0.5 g of sample heated up to 550°C in a muffle furnace (Carbolite, UK) for 4 hours. Total P was determined in samples using a nitric acid (HNO_3) digestion method. 0.5 g of plant material was digested with 5 mL of HNO_3 (Sigma Aldrich reagent grade, 69%) for up to 4 hours. Phosphorus concentration in all digests was measured by Colorimetry [20].

2.4. Solution ^{31}P: NMR Spectroscopy Analysis

2.4.1. NaOH-EDTA Extraction. The sample preparation for solution ^{31}P NMR spectroscopy was performed using a modified procedure as described in Ebuele et al. [23]. 1 g of crushed freeze-dried crop residue sample was mixed with 25 mL of a solution of 0.25 M NaOH and 0.05 M EDTA and shaken at 250 rpm at 20°C for 6 hours. The extracts were then centrifuged for 20 minutes at 5000 rpm and filtered using Whatman number 42 filter paper. An aliquot of 0.5 mL was then diluted for colorimetric analysis and the remaining solution was freeze-dried.

Approximately, 100 mg of each freeze-dried extract was redissolved in 0.6 mL of D_2O, 0.5 mL 10 M NaOH, and 0.4 mL extracting solution (0.25 M NaOH + 0.05 M EDTA). Samples were centrifuged for 60 minutes at 2500 g (to remove particles that might contribute to line broadening) and then transferred to a 5 mm NMR tube and analyzed via ^{31}P NMR spectroscopy.

2.4.2. Identification and Quantification of Phosphorus Species Using ^{31}P NMR. Spectra were acquired on a Bruker Avance DRX 400 MHz NMR spectrometer (7.5 T, 161.9 MHz), equipped with a 5 mm broadband probe at 20°C. Instrument parameters were a 90° pulse, 0.68 s acquisition time, and recovery delay of 4.32 s to 15 s. Inverse gated proton decoupling was used and set to at least five times the T_1 (lattice relaxation time). In the experiment, between 3000 scans to 5000 scans (4–7 hours of running time) were required to achieve a good signal to noise ratio. The spectral width used was 8090.6 Hz and the number of data points was 11002. The chemical shift (ppm) of the signals was indirectly referenced to an external 85% H_3PO_4 standard via the lock signal. Peaks were defined by three parameters: chemical shift, line width, and peak height. Peak assignment was based on literature data [8, 24–27]. Integration of peak areas was calculated on spectra processed with a line broadening of 1–10 Hz using a Bruker Topspin 2.0 software and MestReNova v.7.0. Quantification of P species was done by spectra deconvolution analysis based on the chemical shift, peak width, and peak area, which proved to be successful in particular for areas such as the monoester region containing a number of peaks, sometimes overlapping; the relative P concentration in the NaOH-EDTA extracts was estimated, based on the total NMR signal area, and presented as percentages of each specie or group of species.

3. Results and Discussions

3.1. Physicochemical Properties of Crop Residues and Leaf Litters. A summary of some physicochemical properties of the samples is presented in Table 3. The characteristics of the plant residues vary which is due to differences in plant species, plant tissues, and soil chemical and physical properties [28]. The crop residues and leaf litters exhibited a wide range of pH (5.82–10.29). This can be categorized into 4 groups: weakly acid (JN-02, JN-05), neutral (JN-06, JN-08, JN-07), weakly basic (JN-01, JN-03), and basic (JN-04). The palm male inflorescence (JN-07) had the highest moisture content (14.0%) as compared to the other plant residues. The plant residues had relatively high ash content ranging from 5.5% (JN-08) to 31.2% (JN-04). These high ash content values can be attributed to the incorporation of inorganic materials in the residues [29]. On the other hand the samples were quite high in organic matter (85–94.5%) except for the decaying banana pseudostem which was low and this could account for its highest value of ash (31%). These high levels of organic matter is in agreement with the study of Naklang et al., [30] who concluded that crop residues,

Table 4: The relative percentage of total extractable inorganic and organic P species in the NaOH-EDTA extracts based on total NMR peak area. Total P and total NaOH -EDTA-P concentration in mg kg^{-1} and % recovery.

Samples	Inorganic P		Organic P			[a]Total P (HNO_3) mg kg^{-1}	[a]Total NaOH-EDTA-P mg kg^{-1}	Recovery %
	Ortho-P	Pyro-P	Phytate	Other monoesters	Other diesters			
	Relative percentage (%)							
JN-01	89.2	5.8	-	5.0	-	1436.0	1113.4	77.5
JN-02	79.6	4.6	-	15.9	-	729.8	688.4	94.3
JN-03	72.5	3.1	-	24.4	-	1252.1	1112.9	88.9
JN-04	45.9	14.3	-	39.9	-	986.1	821.9	83.4
JN-05	54.3	-	-	34.3	11.4	1221.9	968.9	79.3
JN-06	81.4	4.8	-	13.8	-	1449.4	1260.8	87.0
JN-07	85.5	2.6	6.7	5.1	-	4001.2	4229.8	105.7
JN-08	53.0	5.7	-	41.3	-	865.2	618.9	71.5

[a]Determined by Colorimetry [20].

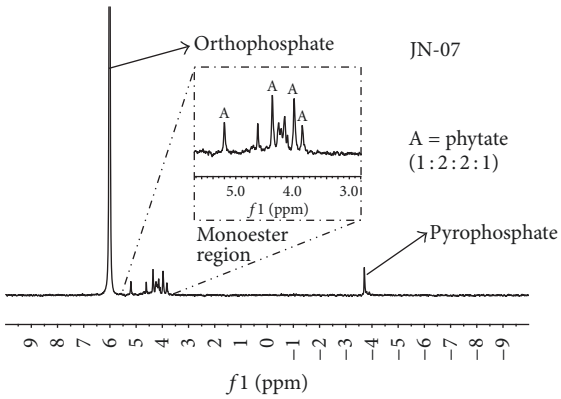

Figure 1: Solution ^{31}P NMR spectra of crop residue extracts (JN-07) showing orthophosphate, pyrophosphate, orthophosphate monoesters, and diesters. The inset shows the orthophosphate monoester region including peaks for phytate (A).

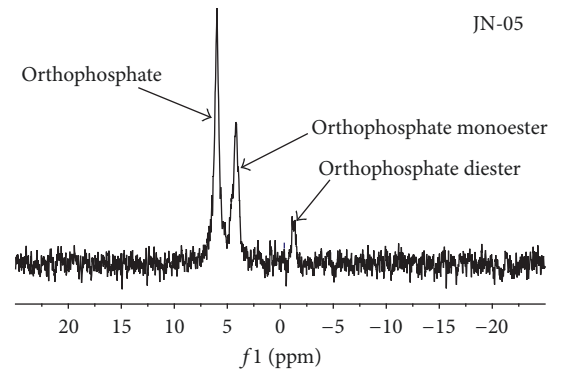

Figure 2: A solution ^{31}P NMR spectrum of crop residue extracts (JN-05) showing orthophosphate diesters.

leaf litters, and green manures are needed to rehabilitate soil carbon.

3.2. Phosphorus Species in NaOH-EDTA Extracts. Figure 1 shows the ^{31}P NMR spectra of the various P species found in the NaOH- EDTA crop residue extracts using JN-07 as an example. All samples showed a peak between 5.88 and 6.05 ppm, characteristic resonances for orthophosphate (Figures 1, 2, and 3). The other inorganic P species detected was pyrophosphate between −3.5 and −3.8 ppm, identified in all samples except JN-05 (Figure 2). The organic orthophosphate monoesters showed resonances from 3.8 to 5.2 ppm (Figure 1, inset) and the only diester P compound was detected in sample JN-05 (Figure 2) at −1.15 ppm at a very low resonance intensity and was attributed to nonhydrolyzed DNA [31, 32]. Phytate (*Myo*-Inositol hexakisphosphate) was identified at 5.2, 4.4, 4.0, and 3.8 ppm (Figure 1, inset peak A) with the signals occurring in a ratio of 1 : 2 : 2 : 1, corresponding to the phosphate ion group on the inositol ring confirming the phytate peak [31]. Other resonances in the monoester region, occurring between 3.8 and 5.2 ppm, were attributed to lower inositol phosphates, sugar phosphates, mononucleotides, and phospholipid degradation products α- and β-glycerophosphate. Sample JN-07 is male palm inflorescence and its highest phytate is similar to observations by Mitchell and Allsopp [33] who found phytate to be the main P species in seeds of *Hakea sericea*. The highest value of phytate P also agrees with the work of Reddy et al., [34] who confirmed that the majority of P stored in plant seed is in the form of phytate.

3.3. Phosphorus Species Quantification. The concentration of total P (Nitric acid digestion) and extractable NaOH-EDTA in the crop residue extracts is shown in Table 4. Extraction efficiency (or recovery%) ranged from 72 to 105%, with sample JN-08 giving the lowest extraction efficiency compared to other samples. In general, the results showed a high extraction efficiency which means that there is little P in the leaf litters and crop residues that were not included in the NMR analysis. The relative percentages of P species found in the NaOH-EDTA extracts are also listed in Table 4. ^{31}P NMR spectra showed the presence of orthophosphate, pyrophosphate, phosphate monoesters, and diesters, while phosphonates and polyphosphates were not detected in any of the NaOH-EDTA crop residue extracts (Figures 1, 2, and 3). Phosphorus in the crop residues was predominantly in

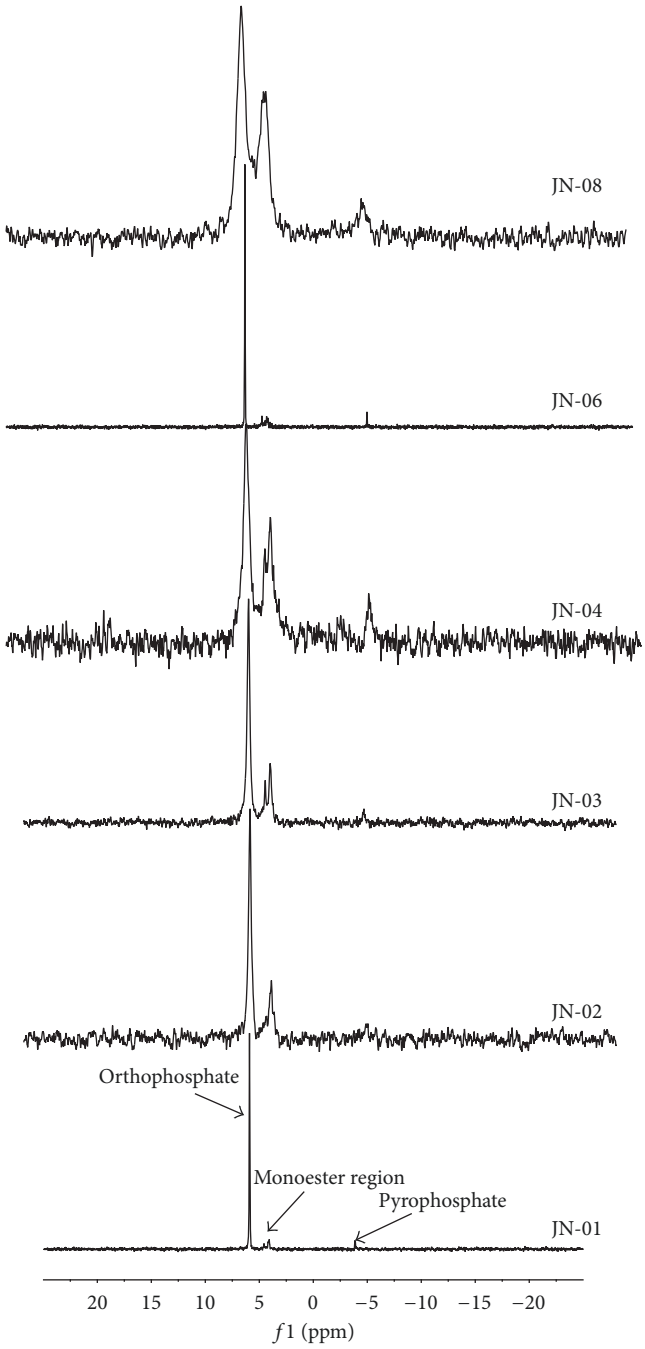

Figure 3: Solution ^{31}P NMR spectra of NaOH-EDTA leaf litters and crop residue extracts for JN-01, JN-02, JN-03, JN-04, and JN-06, JN-08.

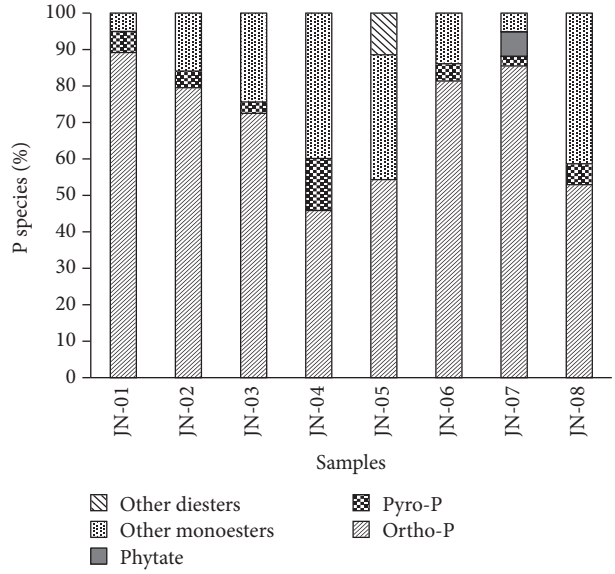

Figure 4: Relative percentages of P species in the crop residues.

the form of inorganic P mainly as orthophosphate and ranges from 45.9 to 89.2% with respect to total NaOH- EDTA extractable P (Table 4). This agrees with the results of Noack et al., [8] who reported that a very high proportion of P in crop residues exists as orthophosphate. This orthophosphate in the residues has the potential to be returned to the soil in a readily available form for plants (via root uptake) and microorganisms, as well as sorption onto soil minerals. The highest relative percentage of P as orthophosphate was found in sample JN-01 (89.2% of total NaOH-EDTA extractable P) and the lowest percentage was found in sample JN-04 (45.9% of total NaOH-EDTA extractable P). Sample JN-01 is cocoa pod husk and the results of this study are in agreement with those of Adejobi et al., [35] who found high concentrations of P in cocoa pod husk. The other major inorganic P species, pyrophosphate, was detected, in all samples except JN-05. Pyrophosphate was detected in trace amounts in all other samples (less than 6%) but was unusually high in sample JN-04 (14.2% of total NaOH-EDTA extractable P). Pyrophosphate is the simplest inorganic polyphosphate and is essential for cellular functioning of living organisms [8] and it absence in fresh palm fronds (JN-05) suggests that its availability may be governed by chlorophyll content. The second most abundant P species were the organic P forms mainly as orthophosphate monoesters. Organic P speciation varied across crop residue types. The highest percentage of orthophosphate monoesters was found in sample JN-08 (41.3% related to total NaOH-EDTA extractable P), while the lowest was in sample JN-01 (4.9% of total NaOH-EDTA extractable P). Phytate (myo-inositol phosphate) was detected in only sample JN-07 (6.7% of total NaOH-EDTA extractable P) (Table 4, Figure 1). The results of Table 4 also showed that total P was very high for JN-07 as compared to the other crop residue samples.

Other organic P species detected in the samples would likely include degradation products such as α- and β-glycerophosphate (most likely originated from phospholipid usually found in plants) and mononucleotides (likely from nucleic material found in plant cells), group under other monoesters (Figure 4 and Table 4). The only orthophosphate diester detected was in sample JN-05 (11.4% of total NaOH-EDTA extractable P).

Overall, the P species detected in all the crop residues were similar for all the different samples and in line with previously reported P forms in other plant based crop

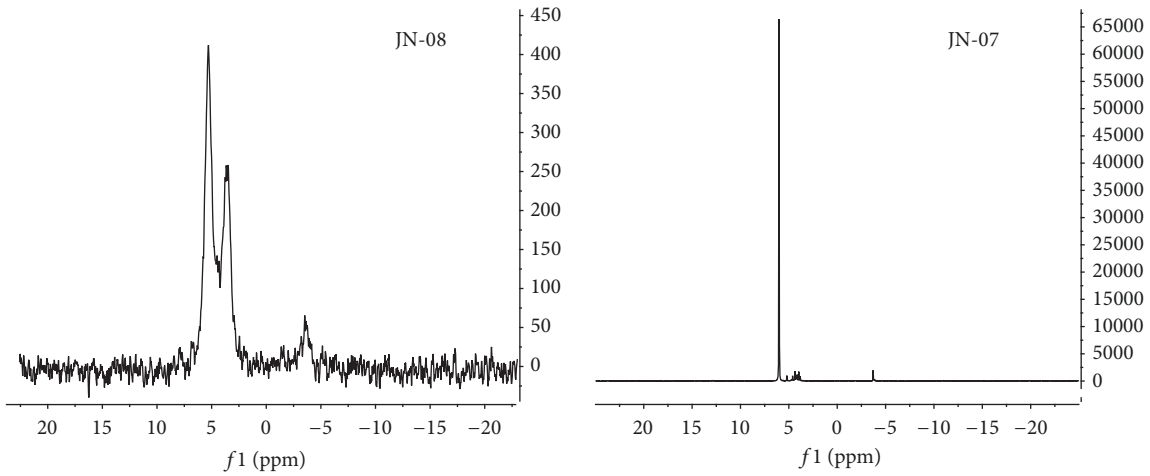

Figure 5: Comparison between the peak intensities of samples JN-07 and JN-08.

residues [8, 25, 27, 32]. The poor resolution shown by field residues samples (JN-02, JN-03, JN-04, JN-05, and JN-08) with the exception of JN-07 was evident in their relatively lower peak intensities, compared to process residues samples (JN-01 and JN-06) (Figure 5 for clarity). The higher peak intensities shown by samples JN-01, JN-06, and JN-7 also suggest that they would likely contain higher concentrations of P compared to the other samples; this was also supported by the elemental P data in Table 4.

The other inorganic P form detected pyrophosphates are the smallest group of inorganic condensed polyphosphates, found in nature. They have also been found in plant tissues such as stems [8]. Their main functions in plants include acting as a storage form of P when inorganic P is in excess, and as a sink or strong chelator of metals ions such as Ca^{2+}, Mn^{2+}, and Mg^{2+} [36]. Phytate detected only in sample JN-07 has been reported to be very stable under alkaline solutions and is also one of the primary storage forms of P in plants (most especially in seeds). The absence of stable diesters such as DNA in most of the ^{31}P NMR spectra was likely as a result of their low concentration and signals to noise ratio, making them relatively unquantifiable. However, the low concentration or complete absence of these diesters P compounds in the analyzed crop residue extracts can also be likely governed by their relative instability in NaOH-EDTA alkaline solution. This suggest that the higher percentages of monoesters relative to diesters detected in the crop residue extracts either are present in the samples or are degradation products of alkaline hydrolysis [37, 38]. This usually occurs during the extraction and redissolving processes required for ^{31}P NMR, thereby leading to an underestimation of diesters and overestimation of monoesters.

4. Conclusion

Eight crop residue samples were analyzed for P species using ^{31}P NMR spectroscopy to quantify the various P forms in the materials. The crop residues were distinguished as field residues (JN-02, JN-03, JN-04, JN-05, JN-07, JN-08) and process residues (JN-01 and JN-06). Based on P speciation data obtained, the recommended crop residues that should be used as phosphorus sources were samples JN-01, JN-06, and JN-07 (i.e., cocoa pod husk, palm empty fruit bunch, and palm male inflorescence). The results demonstrated that phosphorus in the crop residues was predominantly in the form of inorganic P mainly as orthophosphate which is a form readily available for plants uptake and microorganisms, as well as sorption onto soil minerals. The orthophosphate monoesters were the second major P group detected in all samples, with phytate detected only in sample JN-07. Orthophosphate diesters were detected in only one sample (JN-05), while pyrophosphate was detected in all samples except JN-05. The result also suggested that sample JN-07 was likely from a seed based plant. This study shows that field residues especially male inflorescence should be allowed to rot in the field. The process residues should be thrown back to the field to rot or composted as these materials are important sources of P.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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