

³¹P NMR Characterization and Efficiency of New Types of Water-Insoluble Phosphate Fertilizers To Supply Plant-Available Phosphorus in Diverse Soil Types

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ABSTRACT: Hydroponic plant experiments demonstrated the efficiency of a type of humic acid-based water-insoluble phosphate fertilizers, named rhizosphere controlled fertilizers (RCF), to supply available phosphorus (P) to different plant species. This effect was well correlated to the root release of specific organic acids. In this context, the aims of this study are (i) to study the chemical nature of RCF using solid-state ³¹P NMR and (ii) to evaluate the real efficiency of RCF matrix as a source of P for wheat plants cultivated in an alkaline and acid soil in comparison with traditional water-soluble (simple superphosphate, SSP) and water-insoluble (dicalcium phosphate, DCP) P fertilizers. The ³¹P NMR study revealed the formation of multimetal (double and triple, MgZn and/or MgZnCa) phosphates associated with chelating groups of the humic acid through the formation of metal bridges. With regard to P fertilizer efficiency, the results obtained show that the RCF matrix produced higher plant yields than SSP in both types of soil, with DCP and the water-insoluble fraction from the RCF matrix (WI) exhibiting the best results in the alkaline soil. By contrast, in the acid soil, DCP showed very low efficiency, WI performed on a par with SSP, and RCF exhibited the highest efficiency, thus suggesting a protector effect of humic acid from soil fixation.

KEYWORDS: Metal–humic–phosphates, RCF fertilizers, dicalcium phosphate, soil P fractionation, nutrient bioavailability, wheat

INTRODUCTION

A number of studies have shown that the rather low efficiency of water-soluble fertilizers as phosphorus (P) sources for plants grown in acidic and calcareous soils is a result of the precipitation of water-insoluble iron, aluminum, or calcium phosphates, respectively.^{1–4} One potentially effective way of improving P fertilizer efficiency is using P sources of limited water solubility but high solubility in solutions containing the main organic acids released to the rhizosphere by plant roots in response to nutrient-limiting conditions. In this way, P release from the fertilizer adapts itself partly to plant needs. This was the basis for the development of a new type of P fertilizer called “rhizosphere-controlled fertilizer” (RCF),^{5–7} which consists of a matrix involving the reaction of double-metal (Mg, Zn) phosphates with humic substances. Such a matrix is scarcely soluble in water but highly soluble in solutions containing the main carboxylic organic acids released to the rhizosphere by plant roots.^{6,7} Release of these exudates is an expression of nutritional requirements and boosted by nutrient-deficient conditions.^{8–10} The RCF matrix contains a low-P fraction that is soluble in water (the starter fraction) and a high-P fraction that is soluble in organic acids released by plant roots (citric acid, mainly).⁶ When an NPK RCF granulated fertilizer is prepared, N and K nutrients are inserted in the RCF matrix during granulation of the product. After application, N and K are released from the fertilizer as the RCF matrix dissolves in the rhizosphere.⁶ This fact was reflected in a significant reduction of ammonia volatilization and nitrate leaching.⁶

Previous studies using scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS) showed the

coexistence of C, P, Mg, Ca, and Zn in RCF matrix particles.⁶ These results indicated the possible formation of humic–multi-metal–phosphates.⁶ Likewise, previous assays in hydroponics demonstrated the capacity of plants to mobilize and take up P from this RCF matrix through root exudation of specific organic polycarboxylic acids.⁷ In this context the aims of this study are (i) to confirm and characterize the organo–metal–phosphates present in the RCF matrix using solid-state ³¹P NMR and (ii) to assess the efficiency of the RCF matrix as a source of available P for plants grown in various types of P-deficient soils. To this end, we studied growth and P shoot content in wheat plants grown in a P-deficient alkaline or acid soil supplied with P in the form of various mineral fertilizers including P-RCF (RCF), the water-insoluble fraction of P-RCF (WI), simple superphosphate (SSP), and dicalcium phosphate (DCP), all at variable rates. In addition, we used sequential P fractionation to examine interactions between the P fertilizers with the soils.

MATERIALS AND METHODS

Structural Characterization of RCF Using Solid-State ³¹P NMR Spectroscopy. To carry out the structural study of RCF samples using solid-state ³¹P NMR, the following compounds were prepared:

(1) The RCF matrix consisting in the reaction of phosphoric acid (68%) (Panreac, Spain) with magnesium oxide (72% MgO) (Magna S.A.,

Received: June 30, 2010

Revised: December 10, 2010

Accepted: December 13, 2010

Published: January 21, 2011

Spain) and zinc carbonate (52.14% of Zn) (Panreac) in the presence of a humic acid extracted and purified from leonardite (58.47% C, 1.98 mmol g⁻¹ HA of carboxylic groups and 1.18 mmol g⁻¹ HA of phenolic groups; 1.46% N, 2.69% H, 25.76% O, 0.01% P, 0.99% S, 1.02% Fe, and 0.95% Al) at the following proportion: 50.3% of phosphoric acid (68% w/w), 27.6% of magnesium oxide, 11.05% of zinc carbonate, and 11.05% of humic acid.

(2) The magnesium zinc phosphate (MZP) consisting in the same reaction as that of the RCF matrix but in the absence of humic acid.

(3) The magnesium phosphate (MgP) consisting in the reaction of phosphoric acid (68%) with magnesium oxide (72% MgO) at the following proportion: 29.3% of MgO and 70.7% of phosphoric acid (68%).

(4) The zinc phosphate (ZnP) consisting in the reaction of phosphoric acid (68%) with zinc carbonate (52.14% of Zn) at the following proportion: 56.6% of ZnCO₃ and 43.4% of phosphoric acid (68%).

To complete the study one sample of pure crystallized magnesium phosphate (Panreac Spain) and another sample of pure crystallized zinc phosphate (Panreac) were also analyzed.

The ³¹P NMR spectra of the above-described compounds were obtained at room temperature using a Bruker DRX-500 spectrometer, with a static field of 11.7 T. A ¹H-BBI probe with Z gradient was used. Chemical shifts were calibrated with liquid H₃PO₄ as an external standard. The experiments were performed without sample rotation using a ³¹P pulse at 90° under H1 decoupling on a Waltz16 scheme with a field of 3.13 kHz conditions. The relaxation delay was 3 s, the number of scans was 256, and FID was acquired with 32K complex points. All ³¹P spectra were treated with MestreNova software.

Study of the Efficiency of RCF Matrix as P Source for Wheat Plants Cultivated in Diverse P-Deficient Soils. *Soil.* The study was conducted on two different soils, namely, an alkaline soil from Zariquiegui (Navarra) and an acid soil from Eugui (Navarra). Samples from the subsurface (>2 m) layer of both soils were collected, air-dried, and ground to pass through a 2-mm sieve before analysis.

Soil Analyses. Soil analysis included total C and N (LECO C/N analyzer); P extracted sequentially by sodium bicarbonate;¹¹ sodium hydroxide, citrate–bicarbonate–dithionite, and HCl in this order; and residual P;¹² K, Mg, Ca, and Na previously extracted with acetate;¹³ and Fe, Cu, Zn, and Mn, extracted with diethylenetriaminepentaacetic acid (DTPA) at pH 7.¹⁴ All metals were quantified by induced coupled plasma optical emission spectrometry (ICP-OES). Organic matter was determined by dichromate oxidation¹⁵ and calcium carbonate by precipitation with oxalate and subsequent titration with potassium permanganate. Soil pH and electrical conductivity were measured in 1:2.5 soil/water suspensions. Particle size analysis was done by immersing a pycnometer in each soil solution following dilution with sodium hexametaphosphate as dispersant (Table 1).

Phosphorus Fertilizers. The P sources studied included the following:

(a) *Simple Superphosphate (SSP).* This is a controlled water-soluble source of P containing 86.2 g of P kg⁻¹, 86.3% of which is soluble in water and 82.9% in ammonium citrate at pH 7.

(b) *Complete RCF (RCF).* The RCF matrix consists of metal humic phosphates (MHPs), where magnesium (129.4 g kg⁻¹) and zinc (68.9 g kg⁻¹) act as binding metals and humic acids extracted from leonardite (51.1 g organic C kg⁻¹) as chelating agents. The matrix contains 97.5 g of P kg⁻¹, 41% of which is soluble in water (starter fraction) and 96% in ammonium citrate (representing rhizospheric acids) (RCF fraction). This fertilizer is obtained by reacting phosphoric acid with magnesium oxide and zinc oxide in the presence of humic acids in specific proportions.^{5,6}

(c) *WI.* This is the water-insoluble fraction of RCF obtained by washing RCF with deionized water to remove water-soluble P. In this work, we used 8 g of RCF, which was stirred in 1 L of deionized water for 1 h, and the water was then removed by suction through a Büchner

Table 1. Selected Chemical and Physical Properties for Alkaline and Acidic Soils

property	alkaline soil	acidic soil
conductivity (dS m ⁻¹)	2.34	0.14
pH	7.60	5.30
NaHCO ₃ -P (mg kg ⁻¹)	0.05	0.65
CBD-P (mg kg ⁻¹)	44.0	118
NaOH-P (mg kg ⁻¹)	0.00	140
HCl-P (mg kg ⁻¹)	165	0.00
residual P (mg kg ⁻¹)	98.0	165
K (mmol kg ⁻¹)	4.80	2.30
N (g kg ⁻¹)	0.10	0.52
Mg (mmol kg ⁻¹)	11.1	9.80
Ca (mmol kg ⁻¹)	153	11.5
Na (mmol kg ⁻¹)	8.50	2.50
Fe (mmol kg ⁻¹)	0.10	1.09
Mn (mmol kg ⁻¹)	0.01	0.19
Cu (mmol kg ⁻¹)	0.00	0.01
Zn (mmol kg ⁻¹)	0.00	0.02
organic matter (g kg ⁻¹)	0.60	68.6
active lime (g of CaCO ₃ kg ⁻¹)	214	0.60
sand (g kg ⁻¹)	377	541
silt (g kg ⁻¹)	337	359
clay (g kg ⁻¹)	286	100

funnel. The residue remaining on the filter paper was washed with two 80 mL portions of deionized water and dried in an oven for 24 h. As a result, all P was present in the RCF fraction, which contained 74 g of P kg⁻¹, 105.8 g of Mg kg⁻¹, 65.3 g of Zn kg⁻¹, and 61.1 g of organic C kg⁻¹.

(d) *Dicalcium Phosphate (DCP), Panreac (121226.1210).* This is a controlled water-insoluble, citrate-soluble source of P containing 225.5 g of P kg⁻¹, 0.02% of which is soluble in water and 98% in ammonium citrate at pH 7.

Fertilizer–Soil Interactions Studies. *Incubation of the Soils with the Fertilizers.* Amounts of 150 g of soil were placed in 300 mL plastic pots, supplied with the different fertilizers (SSP, DCP, RCF, and WI), and mixed. A fertilizer rate of 250 mg of P kg⁻¹ soil was used in all instances. Also, a control treatment involving the addition of no phosphorus was also performed. In such a way, soil P fractions of each fertilizer extracted in the sequential fractionation were obtained from the difference between the extracted P of the control and the extracted P of each source of P added to the soil. The treated soil samples were homogenized and supplied with type I deionized water to bring the soils to field capacity, which was previously determined by moistening a soil column and allowing it to drain freely. Pots were closed and allowed to stand at ambient temperature in the dark for 90 days. Triplicate samples were taken after 20, 30, 60, and 90 days and dried for analysis.

Sequential Analysis of P Fractions. Phosphorus was sequentially fractionated using two different methods depending on the particular soil pH. The method of Harrell and Wang,¹² which is henceforward referred to as the “standard P fractionation method”, was used with both types of soil (alkaline and acid). Briefly, application of the method involved the following five extractants: (1) 0.5 M sodium bicarbonate, NaHCO₃-P; (2) 0.1 M sodium hydroxide washed twice with 1 M NaCl, NaOH-P; (3) 0.3 M sodium citrate and 1 M sodium bicarbonate, CB-P; (4) 0.3 M sodium citrate, 1 M sodium bicarbonate, and sodium dithionite washed twice with saturated NaCl, CBD-P; (5) 1 M hydrochloric acid, HCl-P.

The acid soil was additionally subjected to an alternative sequential P fractionation method due to the low correlation of results from the

standard P fractionation with the soil–plant system study (data not shown). The new method provides the P fractions soluble in water, anion-exchange resins, sodium citrate (pH 5.0), the citrate–bicarbonate–dithionite mixture, hydrochloric acid, and sodium hydroxide. This method, which is referred to here as the “alternative P fractionation method”, was implemented in the experimental setup used for standard fractionation. Samples were withdrawn for analysis after 10, 20, and 30 days. The method involved the following six steps: (1) 30 mL of deionized water, water-P; (2) anion-exchange resin nylon bag in chloride form (20–50 mesh Dowex 1X4),¹⁶ resin-P; (3) 0.3 M sodium citrate acidified to pH 5.0 with 1 M HCl, C-P; (4) 0.3 M sodium citrate, 1 M sodium bicarbonate, and sodium dithionite washed twice with saturated NaCl, CBD-P; (5) 1 M hydrochloric acid: HCl-P; (6) 0.1 M sodium hydroxide washed twice with 1 M NaCl, NaOH-P.

P-extracted fractions of both methods were finally determined by ICP-OES.

Plant Experiments. Each soil–plant system (wheat–alkaline soil and wheat–acid soil) was subjected to two complementary experiments:

Experiment 1. This experiment was carried out in a greenhouse under controlled temperature and lighting conditions. A 24/18 °C day/night temperature regimen and a relative humidity of 40–60% were used.

Triplicate samples of 500 g of each type of soil were blended with one of the three fertilizers (superphosphate, dicalcium phosphate, or RCF) in a Thermomix at maximum power (450 W) for 5 s prior to placement in plastic pots. The P addition rates used were 5, 15, 30, 50, and 100 mg of P kg^{−1} of soil. A control treatment with fertilizer containing NK but no phosphorus was also applied. Each soil–fertilizer mixture was carefully blended with 50 g of perlite and supplied with 10 seeds of wheat (*Triticum aestivum* L. cv. ‘Fiel’), previously germinated in a chamber, planted within the topmost centimeter of soil. Finally, N at a concentration of 200 mg kg^{−1} of soil and K at 200 mg kg^{−1} of soil were added as urea plus potassium chloride to complete fertilization. The relative amounts of Mg and Zn introduced by RCF were adjusted in the other treatments by adding MgSO₄·7H₂O and ZnSO₄·7H₂O. Pots were brought to field capacity on a daily basis. Also, an appropriate volume of NK solution was applied at 15-day intervals to replenish nutrients.

Pots were harvested 2 months after planting, and once analyzed for fresh matter, shoots were dried in an oven at 40 °C for 3 days to determine dry matter. Next, the dry shoots were homogenized in a mill and subsamples attacked with HNO₃ and H₂O₂ and digested in a microwave oven, to determine P by ICP-OES.

The plant P extraction was calculated by multiplying the previously calculated P concentration by shoot dry weight. Finally, P uptake and use efficiency by plants were estimated via two coefficients that were determined as described elsewhere.^{17–19} Thus, the P uptake efficiency index for each treatment was determined as the ratio of fertilizer-supplied P absorbed by plant shoots (Pup) to the total amount of P (Pap):

$$\text{PupE} = \text{Pup}/\text{Pap}$$

Pup (mg of P) was calculated as the difference in shoot-absorbed P between treated and control samples (i.e., samples to which no external P was supplied).

The P use efficiency coefficient for each treatment was calculated as the ratio of the amount of dry matter obtained from the fertilizer to the total amount of P supplied:

$$\text{PusE} = \text{DM}/\text{Pap}$$

DM denotes dry matter yield obtained from the fertilizer (mg) and was calculated as the difference in shoot dry matter between treated and control samples.

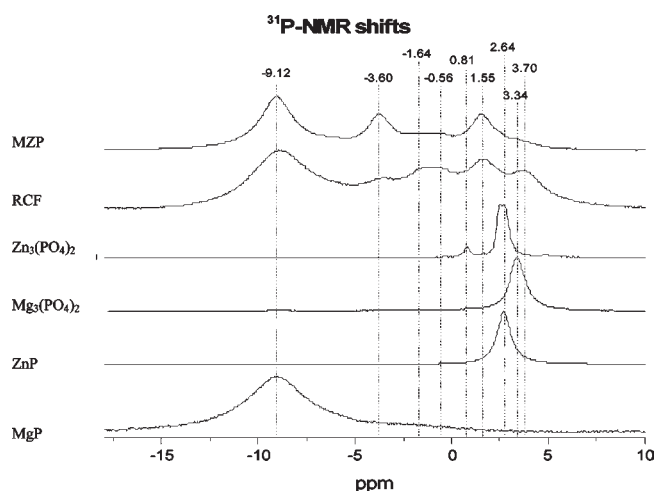


Figure 1. ³¹P NMR spectroscopy of RCF matrix and possible components of its structure.

All of the data were obtained by subtracting control values from the results observed with each P fertilizer applied to appreciate the effect due to each source of added P.

Experiment 2. Experiment 1 was replicated by using the P rates that resulted in the greatest differences among treatments in it. This experiment included WI, and each treatment was applied in quintuplicate. The target parameters were the same as in experiment 1.

Statistical Analyses. The experimental results were subjected to multiple pairwise comparisons between treatments, using Fisher's least significant difference (LSD) method with the overall α level set at 0.05.

RESULTS AND DISCUSSION

Structural Characterization of RCF. The ³¹P NMR spectra corresponding to the different compounds analyzed are presented in Figure 1.

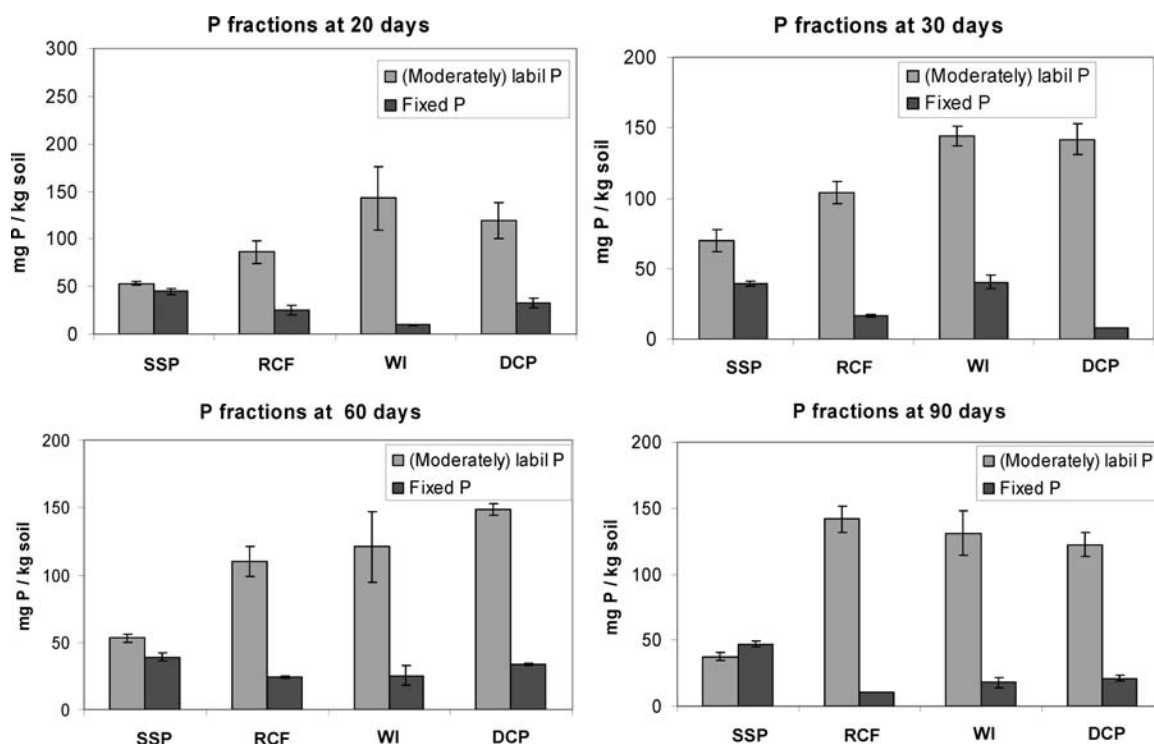
The MZP reaction corresponding to the RCF reaction in the absence of the humic acid presented the following characteristic peaks: −9.12, −3.60, −1.64, −0.56, 1.55, 3.34, and 3.7. It is noticeable that the peaks at −0.56 and −3.60 ppm are the same as those presented by bicalcium phosphate in a previous study (data not shown) and can be associated with double-phosphate structures. If we compare this spectrum with those of MgP and ZnP, it becomes clear that the −9.12 peak corresponds to the MgP reaction. It is noteworthy that this peak does not appear in the spectrum of pure crystallized magnesium phosphate. This result suggests that in the MgP reaction magnesium phosphate probably contains other elements that are also present in magnesium oxide sample such as Ca. However, the main peak for the ZnP reaction at 2.64 (which coincides with that of pure crystallized Zn phosphate) disappears in the MZP reaction, thus suggesting the incorporation of Zn in phosphates different from pure zinc phosphate, probably those involving Mg (Ca) (double MgZn phosphate). In this sense, the other peaks in the MZP reaction (−3.60, −1.64, −0.56, 1.55, 3.34, and 3.7), which do not appear in MgP and ZnP, could correspond to those phosphates involving Mg (Ca) and Zn (double-triple phosphates) in different proportions. Thus, the spectrum of the MZP reaction indicates the presence of magnesium (calcium) phosphates and magnesium (calcium) zinc phosphates.

When the humic acid was included in the MZP reaction to form the RCF matrix, several significant changes in the spectrum

Table 2. Increment of Phosphorus Fractions with Different P Fertilizers by Sequential Fractionation at 20, 30, 60, and 90 Days for Alkaline Soil^a

treatment	NaHCO ₃ -P (mg kg ⁻¹)	NaOH-P (mg kg ⁻¹)	CB-P (mg kg ⁻¹)	CBD-P (mg kg ⁻¹)	HCl-P (mg kg ⁻¹)
20 Days					
SSP	42 ± 1 (42)	0 ± 0 (0)	12 ± 1 (12)	6 ± 1 (6)	39 ± 2 (39)
RCF	42 ± 2 (38)	18 ± 4 (12)	27 ± 6 (24)	13 ± 4 (12)	12 ± 1 (11)
DCP	71 ± 6 (47)	7 ± 2 (5)	41 ± 11 (27)	22 ± 5 (14)	11 ± 0 (7)
WI	69 ± 13 (45)	39 ± 13 (26)	35 ± 7 (23)	9 ± 1 (6)	0 ± 0 (0)
30 Days					
SSP	35 ± 6 (32)	14 ± 1 (13)	21 ± 1 (19)	10 ± 1 (9)	29 ± 1 (27)
RCF	61 ± 4 (51)	18 ± 3 (15)	24 ± 1 (20)	17 ± 1 (14)	0 ± 0 (0)
DCP	109 ± 4 (73)	16 ± 6 (11)	16 ± 1 (11)	8 ± 0 (5)	0 ± 0 (0)
WI	68 ± 3 (37)	31 ± 2 (17)	46 ± 2 (25)	26 ± 4 (14)	15 ± 1 (8)
60 Days					
SSP	18 ± 2 (20)	13 ± 1 (14)	21 ± 0 (23)	9 ± 0 (10)	30 ± 3 (33)
RCF	58 ± 2 (43)	21 ± 6 (16)	31 ± 3 (23)	9 ± 0 (7)	15 ± 1 (11)
DCP	95 ± 1 (52)	23 ± 2 (13)	30 ± 1 (16)	10 ± 0 (5)	24 ± 1 (13)
WI	61 ± 11 (41)	35 ± 11 (24)	25 ± 4 (17)	10 ± 3 (7)	16 ± 4 (11)
90 Days					
SSP	23 ± 2 (27)	9 ± 1 (11)	5 ± 0 (6)	11 ± 1 (13)	36 ± 1 (43)
RCF	68 ± 7 (44)	34 ± 2 (22)	40 ± 1 (26)	9 ± 0 (6)	2 ± 0 (1)
DCP	105 ± 7 (73)	10 ± 1 (7)	7 ± 1 (5)	8 ± 1 (6)	13 ± 1 (9)
WI	63 ± 10 (42)	43 ± 3 (29)	25 ± 4 (17)	10 ± 4 (17)	8 ± 1 (5)

^aData are means of three replicates ± standard deviation; numbers in parentheses represent the percentage of total P. The rest until 100% corresponds to residual P. SSP, simple superphosphate; RCF, rhizosphere-controlled fertilizer; DCP, dicalcium phosphate; WI, water-insoluble RCF.

**Figure 2.** Increment of P pools with each treatment in alkaline soil at 20, 30, 60, and 90 days, evaluated by the standard P sequential fractionation method (SSP, simple superphosphate; RCF, rhizosphere-controlled fertilizer; DCP, dicalcium phosphate; WI, water-insoluble RCF).

were observed. Thus, the peaks at -3.60 and 1.55 present lower intensity, whereas those corresponding to -1.64 , -0.56 , 3.34 , and 3.7 increase in intensity very significantly. These results

indicate that the chelating groups of the humic acid affect the coordination sphere of P through metal complexation. The increase of peak intensity at -0.56 ppm could be related to a

stronger double phosphate between Zn, binding also organic matter, because Zn is better bound to organic matter than Mg in organic complexes. Likewise, these results also suggest that the presence of humic acid favors the formation of specific multi-metal phosphates. In consequence, these results confirm the formation of metal–humic–phosphates (MHP) with different stoichiometries. Finally, these results are in line with those derived from X-ray diffractometry. SEM and EDS are described in Erro et al.⁶

Fertilizer–Soil Interactions in Relation to Fertilizer–Plant–Soil Interactions. *Alkaline Soil.* The accuracy of the determination of the different fractions obtained using ICP–OES P was tested in previous assays (data not shown) and in the literature.²⁰

Bicarbonate-P, which provides a close estimate of plant-available P, was the dominant P fraction (residual P excluded); in fact, it accounted for 47–73% of total P extracted with DCP, 38–51% with WI and RCF, and 20–42% with SSP (Table 2). Citrate–bicarbonate-P was the second major fraction in RCF, sodium hydroxide-P was the second major fraction in WI except at 30 days when citrate–bicarbonate-P prevailed, and citrate–bicarbonate-P along with sodium hydroxide-P was the second major fraction in DCP. In SSP, however, HCl-P was more abundant than citrate–bicarbonate-P; thus, the former accounted for 30–40% of the total amount of P extracted. By contrast, HCl-P and citrate–bicarbonate–dithionite-P were, in general, the smallest fractions with DCP, WI, and RCF, in which they accounted for <14% of total P (Table 2). These results are suggestive of strong retrogradation with SSP-P relative to RCF-P, WI-P, and DCP-P. Perhaps the local accumulation in soil of soluble Ca and P present in SSP fertilizer may speed the P fixation process in soils with the subsequent reduction of available P. With RCF and WI, the protective effect of P against retrogradation may have been a result of the low proportion of water-soluble P in these products and the presence of the humic acid, which may have altered P precipitation patterns.²¹ The amount of P extracted was pH-dependent. Thus, most P obtained from RCF and WI was provided by the alkaline extractants (Table 2). This can be ascribed to the increased solubility of MHPs in these alkaline extractants by the effect of the presence of the humic moiety.^{6,22} Thus, the more labile compounds of this type may be extracted by bicarbonate and the rest by sodium hydroxide in the second step, as previously found by Schoenau et al.,²³ Delgado et al.,²⁴ Nwoke et al.,²⁵ McDowell et al.,²⁶ and Ribeiro et al.²⁷

The combination of the first three extracts was negatively correlated with that of the last two (Figure 2). This suggests that these two groups of extracts may be representative of two P pools differing in potential plant availability, namely, labile or moderately labile P (potentially plant available P) and fixed or long-term available P (low-soluble precipitates or strongly absorbed P). This classification of extracts in terms of potential plant availability of P is supported by previous results of Guo et al.,²⁸ who found the dynamics of the labile P pool to be governed by the balance among the three fractions. The results of Figure 2, which shows the variation of the P pools with time, indicate that RCF, WI, and DCP contain more labile or moderately labile P than does SSP and also that the opposite holds for fixed P. At the beginning of soil incubation, DCP contained more labile or moderately labile P than did RCF; the difference, however, decreased with time. No similar initial difference with DCP was observed in WI, which suggests that the P fraction soluble in citrate but insoluble in water exerts a marked influence on the

Table 3. Increment of Plant P Extraction, Shoot P Concentration, Shoot Dry Matter, and P Use and P Uptake Efficiency Coefficients after Cropping with Each Treatment in Alkaline Soil at Five Doses of P: Experiment 1 with Alkaline Soil^a

treatment	doses (mg of P kg ⁻¹ of soil)				
	5	15	30	50	100
Plant P Extraction (mg)					
SSP	0.16 b	0.85 c	1.55 c	3.31 c	4.23 c
RCF	0.54 a	1.37 b	2.54 b	4.62 b	8.04 b
DCP	0.41 ab	1.73 a	3.00 a	5.96 a	10.11 a
Shoot P (μg of P g⁻¹ of Shoot Dry Weight)					
SSP	490 b	566 b	752 b	965 c	1306 c
RCF	806 a	774 a	951 a	1266 b	2481 b
DCP	837 a	812 a	1016 a	1701 a	2973 a
Shoot Dry Matter (g)					
SSP	0.30 b	1.50 c	2.06 b	3.43 a	3.24 a
RCF	0.67 a	1.77 b	2.67 a	3.65 a	3.58 a
DCP	0.49 ab	2.13 a	2.95 a	3.50 a	3.40 a
PusE					
SSP	120 b	200 c	137 b	137 a	65 a
RCF	268 a	236 b	178 a	146 a	72 a
DCP	196 ab	284 a	197 a	140 a	68 a
PupE					
SSP	0.06 b	0.11 c	0.10 c	0.13 c	0.09 c
RCF	0.22 a	0.18 b	0.17 b	0.18 b	0.16 b
DCP	0.16 ab	0.22 a	0.20 a	0.24 a	0.20 a

^a SSP, simple superphosphate; RCF, rhizosphere-controlled fertilizer; DCP, dicalcium phosphate; WI, water-insoluble RCF.

potential P plant availability of the fertilizer in this type of soil. Worth special note in this respect was the increased significance of bicarbonate-P (labile P) in this pool for DCP. By contrast, RCF and, especially, WI contained more moderately labile P (i.e., P extracted by NaOH and CB). The presence of more labile P (especially in the CB-P fraction) and less HCl-P in RCF and WI than in SSP may also have resulted from that of the humic moiety.^{24,29,30} The large labile P (NaHCO₃-P) fraction contained in DCP was unexpected because this product is insoluble in water and very sparsely soluble in bicarbonate (results not shown). This result suggests that DCP probably undergoes some transformation gradually facilitating its solubilization in this type of soil. On the basis of the foregoing, RCF, WI, and DCP are potentially more efficient P sources than is SSP.

The main conclusions drawn from the study of fertilizer–soil interactions were confirmed by those for the soil–plant system. Thus, there were significant differences in plant P extraction and dry matter yield (Tables 3 and 4) between treatments. In fact, RCF, WI, and DCP resulted in higher P uptake by plants and better yields than did SSP. These differences also reflected in PupE and PusE (Tables 3 and 4), which were consistent with the results of the fertilizer–soil interaction study (i.e., with an increased efficiency in the former three fertilizers). In fact, the fertilizers containing increased amounts of labile and moderately labile P soil resulted in also increased P plant uptake and growth. This was reflected in strong correlations of the P fractions extracted by bicarbonate, sodium hydroxide, and citrate–bicarbonate

Table 4. Increment of Plant P Extraction, Shoot P Concentration, Shoot Dry Matter, and P Uptake and P Use Efficiency Coefficients after Cropping with Each Treatment in Alkaline Soil at Two Doses of P: Experiment 2 with Alkaline Soil^a

treatment	doses (mg of P kg ⁻¹ of soil)	
	5	30
Plant P Extraction (mg)		
SSP	0.11 c	1.76 b
RCF	0.35 b	2.09 b
DCP	0.54 a	2.85 a
WI	0.33 b	3.17 a
Shoot P (μg of P g⁻¹ of Shoot Dry Weight)		
SSP	901 b	964 b
RCF	736 c	986 b
DCP	1512 a	1285 a
WI	673 c	1205 a
Shoot Dry Matter (g)		
SSP	0.12 b	1.82 c
RCF	0.47 a	2.12 bc
DCP	0.35 a	2.22 b
WI	0.49 a	2.63 a
PupE		
SSP	0.04 c	0.12 b
RCF	0.14 b	0.14 b
DCP	0.21 a	0.19 a
WI	0.13 b	0.21 a
PusE		
SSP	49.6 b	122 c
RCF	188 a	141 bc
DCP	142 a	142 b
WI	194 a	143 a

^a SSP, simple superphosphate; RCF, rhizosphere-controlled fertilizer; DCP, dicalcium phosphate; WI, water-insoluble RCF.

with the amount of P absorbed by plants after 60 days (i.e., at harvest). In principle, these results are also consistent with those of Hagin,¹ who concluded that citrate-soluble phosphates might be more efficient than water-soluble phosphates in alkaline soils. However, our results depart slightly from those reported by Mullins and Sikora,³¹ who found the need for a water-soluble phosphate fraction in fertilizers to increase with increasing alkalinity of the soil.

An increase in plant P extraction was generally accompanied by a concomitant increase in shoot dry matter yield except at the highest P fertilizer rates (50 and 100 mg of P kg⁻¹, Tables 3 and 4). This may have been a result of the absence of P limiting conditions at such P rates. The plants treated with DCP had increased plant P extraction relative to those treated with RCF (Tables 3 and 4). Whereas these differences were significant, those in dry matter yield at 30 and 50 mg of P kg⁻¹ soil were not. This suggests that the plants receiving RCF absorbed only the amount of P strictly required to fulfill their needs and thus controlled P uptake by themselves. This result is consistent with others previously obtained in hydroponically grown plants.⁷ The previous assumption is further supported by the results for WI.

Table 5. Increment of Plant P Extraction, Shoot P Concentration, Shoot Dry Matter, and Soil P per Pot after Cropping with Each Treatment in Acidic Soil at Five Doses of P: Experiment 1 with Acid Soil^a

treatment	doses (mg of P kg ⁻¹ of soil)				
	5	15	30	50	100
Plant P Extraction (mg)					
SSP	0.02 ab	0.04 b	0.23 b	0.99 a	2.83 b
RCF	0.07 a	0.10 a	0.38 a	1.32 a	4.42 a
DCP	0.02 b	0.06 ab	0.11 c	0.39 b	1.79 c
Shoot P (μg of P g⁻¹ of Shoot Dry Weight)					
SSP	553 a	976 a	777 a	1102 a	546 c
RCF	416 b	498 c	606 b	551 c	650 b
DCP	326 c	740 b	684 ab	980 ab	792 a
Shoot Dry Matter (g)					
SSP	0.04 b	0.04 b	0.29 b	0.90 b	5.18 b
RCF	0.16 a	0.21 a	0.63 a	2.39 a	6.80 a
DCP	0.07 b	0.08 ab	0.16 b	0.39 b	2.27 c
PupE					
SSP	0.01 ab	0.01 a	0.02 b	0.04 a	0.06 b
RCF	0.03 a	0.01 a	0.03 a	0.05 a	0.09 a
DCP	0.01 b	0.01 a	0.01 c	0.02 b	0.04 c
PusE					
SSP	8.67 b	2.89 b	9.67 b	17.9 b	51.7 b
RCF	32.0 a	14.0 a	21.0 a	47.8 a	68.0 a
DCP	13.3 b	5.33 ab	5.22 b	7.87 b	22.7 c

^a SSP, simple superphosphate; RCF, rhizosphere-controlled fertilizer; DCP, dicalcium phosphate; WI, water-insoluble RCF.

Thus, WI-treated plants differed in plant P extraction from DCP plants at the lower P rates, but not at the higher ones, where the former plants exhibited even higher shoot dry matter yields (Tables 3 and 4). This is consistent with the results of the incubation study, which showed the content in moderately labile P of RCF-treated plants to exceed that of DCP-treated plants. Therefore, moderately labile (citrate extracted) P must be more efficient than labile (bicarbonate extracted) P in fulfilling plant requirements. These results are reflected in PupE and PusE. Thus, the PupE values for RCF and WI were higher than those for SSP, but lower than those for DCP (Tables 3 and 4). On the other hand, PusE was essentially identical for RCF, WI, and DCP (Tables 3 and 4). This suggests that the citrate-soluble P fraction in MHPs constitutes a more efficient fertilizer than does water-soluble P in SSP or even citrate-soluble P in DCP.

Acid Soil. Results of plant growth and plant P extraction showed significant differences in shoot P extraction and dry matter yield between treatments (Tables 5 and 6). RCF was the most efficient P source for plants and provided the best yields as a result. This reflected in differences in PupE and PusE between P sources (Tables 5 and 6). Thus, WI exhibited PupE and PusE values similar to those for SSP, but lower than those for RCF (Tables 5 and 6). This result indicates that the presence of a water-soluble P fraction in the fertilizer may be very important with a view to fulfilling the P nutritional requirements of plants in acid soils, as found in previous studies.³² This conclusion is supported by the results for DCP, which was rather inefficient as a P source for wheat plants in this type of soil (Tables 5 and 6). In

Table 6. Increment of Plant P Extraction, Shoot P Concentration, Shoot Dry Matter, and P Uptake and P Use Efficiency Coefficients after Cropping with Each Treatment in Acidic Soil at Two Doses of P: Experiment 2 with Acid Soil^a

treatment	doses (mg of P kg ⁻¹ of soil)	
	50	100
Plant P Extraction (mg)		
SSP	1.22 b	2.44 a
RCF	2.77 a	3.12 a
DCP	0.41 c	1.57 b
WI	1.41 b	3.39 a
Shoot P (μg of P g⁻¹ of Shoot Dry Weight)		
SSP	914 b	734 a
RCF	1291 a	798 a
DCP	898 b	819 a
WI	870 b	1059 a
Shoot Dry Matter (g)		
SSP	1.33 b	3.32 b
RCF	2.15 a	3.91 a
DCP	0.45 c	191 c
WI	1.62 b	3.20 b
PupE		
SSP	0.05 b	0.05 a
RCF	0.11 a	0.06 a
DCP	0.02 c	0.03 b
WI	0.06 b	0.07 a
PusE		
SSP	26.6 b	33.2 b
RCF	43.0 a	39.1 a
DCP	9.05 c	19.1 c
WI	32.5 b	32.0 b

^a SSP, simple superphosphate; RCF, rhizosphere-controlled fertilizer; DCP, dicalcium phosphate; WI, water-insoluble RCF.

fact, this fertilizer provided the worst results with regard to plant P extraction and yield, especially at the higher P application rates. The increased content in organic matter of this soil may have boosted plant development and/or facilitated P retention, thus minimizing differences between treatments at low fertilizer rates. As a consequence, differences in plant growth between treatments were appreciable only in the presence of large amounts of applied P (50 or 100 mg of P kg⁻¹ of soil). These results suggest that, under these soil conditions, plants are less efficient in mobilizing P from DCP, even though the acid environment should facilitate solubilization of P. This is consistent with the previous results of Brennan and Bolland,³² who found DCP to be less efficient than superphosphate in acid soils. Therefore, RCF-based fertilizers are highly efficient, even more so than superphosphate, in both alkaline and acid soils. On the other hand, DCP acts efficiently only in alkaline soils. These conclusions are supported by the P uptake and use efficiency coefficients shown in Tables 5 and 6.

The fertilizer efficiency results obtained by using the standard P fractionation method departed markedly from those provided by the plant–soil interaction study (data not shown). Therefore, the standard P fractionation method is unsuitable for assessing

Table 7. pH Values of Soils and Soil-Extractant Suspensions Used in the Standard Sequential Analysis

sample	pH	
	soil/solvent 1:50 bicarbonate	soil/solvent 2:1 deionized water
acid soil	8.65	4.80
calcareous soil	8.70	7.60

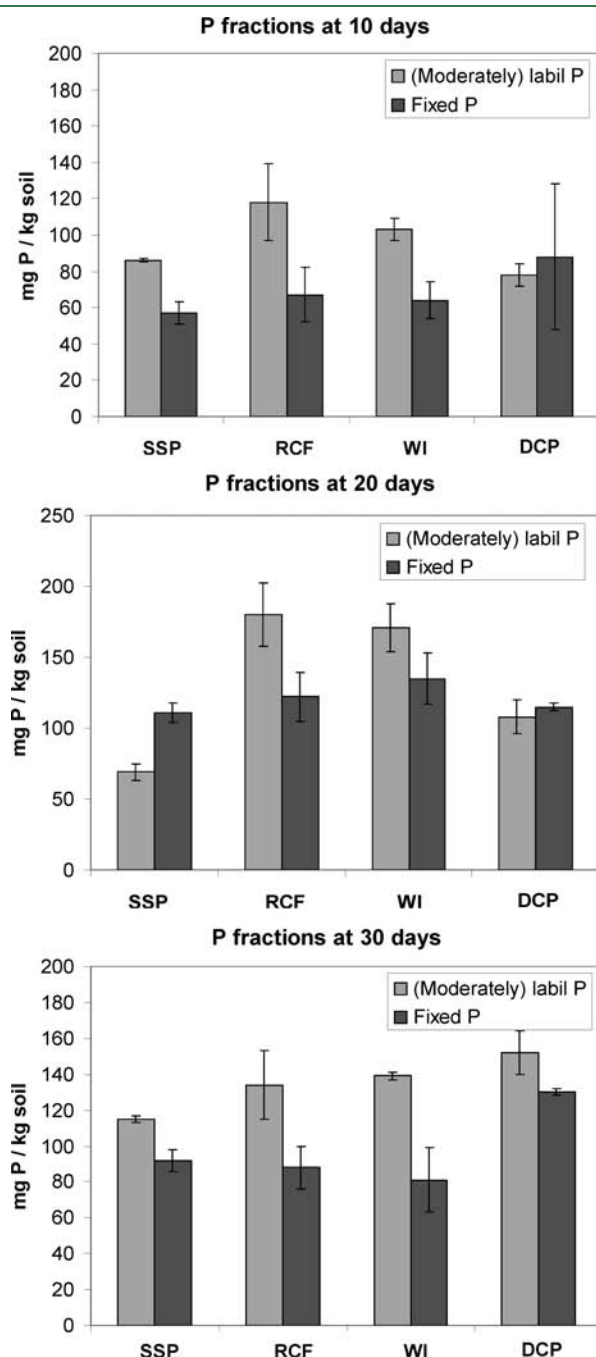


Figure 3. Increment of P pools with each treatment in acidic soil at 10, 20, and 30 days following the alternative P fractionation method (SSP, simple superphosphate; RCF, rhizosphere-controlled fertilizer; DCP, dicalcium phosphate; WI, water-insoluble RCF).

Table 8. Increment of Phosphorus Fractions with Different P Fertilizers by Alternative Sequential Fractionation at 10, 20, and 30 Days for Acidic Soil^a

treatment	water-P (mg kg ⁻¹)	resin-P (mg kg ⁻¹)	C-P (mg kg ⁻¹)	CBD-P (mg kg ⁻¹)	HCl-P (mg kg ⁻¹)	NaOH-P (mg kg ⁻¹)
10 Days						
SSP	2 ± 0 (1)	31 ± 1 (22)	53 ± 0 (37)	48 ± 6 (34)	9 ± 0 (6)	0 ± 0 (0)
RCF	6 ± 1 (3)	38 ± 2 (21)	74 ± 18 (40)	60 ± 15 (32)	7 ± 0 (4)	0 ± 0 (0)
WI	6 ± 0 (4)	42 ± 2 (25)	55 ± 4 (33)	56 ± 9 (34)	8 ± 1 (5)	0 ± 0 (0)
DCP	7 ± 2 (4)	35 ± 2 (21)	36 ± 2 (22)	69 ± 40 (42)	19 ± 0 (11)	0 ± 0 (0)
20 Days						
SSP	2 ± 0 (1)	23 ± 1 (13)	44 ± 5 (24)	99 ± 2 (55)	8 ± 3 (4)	4 ± 2 (2)
RCF	7 ± 0 (2)	36 ± 2 (12)	137 ± 20 (45)	107 ± 15 (35)	11 ± 1 (4)	4 ± 1 (1)
WI	7 ± 1 (2)	48 ± 2 (16)	116 ± 14 (38)	117 ± 14 (38)	13 ± 3 (4)	5 ± 1 (2)
DCP	7 ± 1 (3)	35 ± 4 (16)	66 ± 7 (30)	89 ± 2 (40)	25 ± 0 (11)	1 ± 1 (0)
30 Days						
SSP	2 ± 1 (1)	19 ± 0 (11)	94 ± 1 (32)	80 ± 2 (48)	12 ± 4 (7)	0 ± 0 (0)
RCF	3 ± 1 (1)	29 ± 2 (13)	102 ± 16 (46)	70 ± 7 (32)	18 ± 5 (8)	0 ± 0 (0)
WI	5 ± 1 (2)	31 ± 1 (14)	103 ± 0 (47)	71 ± 15 (32)	10 ± 3 (5)	0 ± 0 (0)
DCP	4 ± 1 (1)	32 ± 1 (11)	116 ± 10 (41)	106 ± 0 (38)	24 ± 2 (9)	0 ± 0 (0)

^aData are means of three replicates ± standard deviation; numbers in parentheses represent the percentage of total P extracted. The rest until 100% corresponds to residual P. SSP, simple superphosphate; RCF, rhizosphere-controlled fertilizer; DCP, dicalcium phosphate; WI, water-insoluble RCF.

the fraction of potentially plant-available P in, at least, the studied acid soil; in fact, using the extractants prescribed for calcareous soils¹² leads to a spurious interpretation of extracted fractions. This is hardly surprising because the chemical conditions associated with the extractants used to determine labile P (bicarbonate, sodium hydroxide, and citrate–bicarbonate) are not representative of those governing P uptake by plant roots in an acid soil. Thus, the highly alkaline pH of such extractants (Table 7) bears no relationship to the natural conditions in acid soils.

In fact, some authors have shown the Olsen extraction method, which was developed for calcareous soils, to be less suitable for estimating plant available P in acid soils.^{16,33,34} This suggests that, when these extractants alter the chemical or physical characteristics of the soil, the extracts reflect the nature of the extractants rather than the actual availability of P in the soil. This assumption is consistent with the large amounts of P extracted by bicarbonate and sodium hydroxide from the acid soil by the effect of organic matter also extracted by these solvents, lowering the soil pH (data not shown). This led us to use an alternative P fractionation method to simulate the conditions in acid soil more closely. The alternative procedure includes two additional steps that afford direct evaluation of plant available P (water-P and resin-P) and plant-mobilizable P (citrate at pH 5, C-P). The alternative procedure might therefore be more accurate than the standard method; the results, however, are still inconclusive (Figure 3). In fact, although no significant differences in directly plant-available P between DCP and the other treatments were observed, plant-mobilizable P (C-P) was closely correlated with plant P uptake and growth at the beginning of soil incubation (10 days, Table 8). This may account for the differences in efficiency between the P fertilizers and suggests that the assessment method used should consider the particular growth stage at which plants require P most for growth and development. However, these are only preliminary results, and further work is needed to assess the suitability of this alternative sequential P fractionation procedure as an analytical tool for studying plant-available P pools.

The differences in efficiency between DCP and WI suggest that, although both fertilizers are soluble in ammonium citrate (pH 7), there must be some difference in their solubility pattern under acid conditions, a difference associated with phosphate structure. Thus, the humic moiety in MHPs may prevent the formation of highly insoluble phosphates in the presence of Fe(III) and Al(III).

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ACKNOWLEDGMENT

We thank Antonio Losada and Paul Miller for critical review of the English quality of the manuscript.

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