

Development and Agronomical Validation of New Fertilizer Compositions of High Bioavailability and Reduced Potential Nutrient Losses

JAVIER ERRO,[†] OSCAR URRUTIA,[§] SARA SAN FRANCISCO,[§] AND
 JOSE M. GARCIA-MINA^{*,†,§}

Department of Chemistry and Soil Chemistry, University of Navarra, 31080 Pamplona, Spain, and
 R&D Department, Inabonos-Roullier Group, Poligono Arazuri-Orcoyen, 31160 Orcoyen, Spain

To optimize the economical cost of each unit of fertilizer applied and to reduce the environmental contamination caused by nutrient losses, the development of highly efficient granulated fertilizers is of great importance. This study proposes a strategy that consists of developing specific fertilizers having nutrient release patterns that are dependent on plant activity in the rhizosphere. This type of fertilizer is named “rhizosphere-controlled fertilizer” (RCF fertilizer). This fertilizer is based on the introduction of an organomineral matrix composed of metal [Mg (Ca is also possible), Zn (Fe and other metals are also possible)]–humic phosphates. The presence of this matrix modifies the nutrient release pattern of the fertilizer. In this way there are two main nutrient fractions: (i) a water-soluble fraction or “starter” fraction and (ii) a “rhizosphere-controlled” fraction insoluble in water but soluble by the action of the rhizospheric acids released by plants and microorganisms. This study shows the chemical and structural characterization of the organomineral matrix, as well as its efficiency in slowing the nutrient release rate of the RCF fertilizer, principally with respect to P and N. It is demonstrated how these properties of the matrix were also reflected in the significant reduction in both ammonia volatilization and N leaching in a pot system consisting of wheat plants cultivated in a calcareous soil and fertilized with a RCF fertilizer.

KEYWORDS: Metal–humic phosphate; rhizosphere-controlled fertilizers; humic substances; micromorphological identification; X-ray diffraction; scanning electron microscope; nutrient release pattern; nutrient bioavailability; ammonia volatilization; nutrient leaching

INTRODUCTION

The development of highly efficient granulated fertilizers, containing the principal mineral nutrients involved in plant nutrition, is of great interest for at least two complementary reasons: (i) the need to optimize the economical cost of each fertilizer unit by increasing the ratio between yield and the fertilizer unit applied and (ii) the need to decrease environmental contamination deriving from nutrient leaching and nitrogen (N) volatilization.

In principle, an effective way to control nutrient losses would be to adapt the nutrient release from the fertilizer into the soil solution to plant nutritional needs and activities. However, whereas this approach is feasible in drip-irrigated plants (fertigation), it is much more complicated in plants growing without localized irrigation.

Equally, two complementary operational practices are normally used to adapt fertilizer nutrient release to plant activity

in nonirrigated crops: (i) to fractionate fertilizer application during the plant cycle and (ii) to use slow-release fertilizers (principally coated fertilizers). There arise, however, certain problems associated with these practices. For instance, the fractionation of fertilizer application involves additional economical costs, whereas the nutrient release pattern of conventional slow-release fertilizers is not specifically adapted to plant nutritional needs. In fact, the nutrient release dynamics of coated fertilizers principally depends on certain environmental conditions such as soil moisture, soil temperature, and soil composition (*1*).

A possible alternative strategy would be to develop special fertilizers having nutrient release patterns that depend on the plant activity expressed in the rhizosphere through specific chemical signals [“rhizosphere-controlled fertilizers” (RCF)]. It is well-known that plants have special mechanisms in the roots that are activated as a function of their physiological status (*2*). These mechanisms involve specific root actions that affect the chemical and biological environment of the rhizosphere (*3*). Among these actions both proton release (rhizosphere acidification) (*4, 5*) and the release of certain organic acids with

* Author to whom correspondence should be addressed [telephone (34)948324550; fax (34)948324032; e-mail jgmina@inabonos.com].

[†] University of Navarra.

[§] Poligono Arazuri-Orcoyen.

complexing ability have been well studied (6). A number of studies have shown that both plants and microorganisms have the capacity of releasing different types of organic acids to the rhizosphere as a normal expression of their activity (7). This capacity is especially enhanced under conditions of nutrient deficiency (8, 9). These compounds include organic acids with low molecular weight, such as citric acid or oxalic acid, and special acidic compounds named siderophores (released by microorganisms) and phytosiderophores (released by graminaceous plants) (10). The main function of these organic acids seems to be the mobilization of nutrients with metallic character through the formation of stable complexes (11). Likewise, this complexing capacity has also positive effects on certain anions that can be blocked in the soil by the formation of water-insoluble salts with metallic elements (12). This is the case of certain phosphates, such as calcium phosphate or iron phosphate (13).

In this context, a possible approach to the development of RCF fertilizers might be to obtain a fertilizer having a solubility in soil solution that is principally governed by interaction between RCF particles and the organic acids present in the rhizosphere. Consequently, a design of effective RCF could involve the development of fertilizers containing two main nutrient fractions. First, the "starter" fraction has the function of assuring adequate onset of the plant cycle: in the case of germinated seeds the root implantation in the soil and the creation of the rhizosphere, and in the case of perennial plants the adequate start of the productive cycle. This fraction would be composed of those nutrients soluble in water. Second, the "rhizosphere-controlled" fraction has the function of covering plant nutritional needs for the rest of the plant cycle, while minimizing nutrient losses. This fraction would be formed by those nutrients that are released by the action of rhizospheric organic acids and may consist of an organic or inorganic matrix formed by water-insoluble compounds that can be solubilized by the action of rhizospheric organic acids. This matrix could constitute a kind of structural network in which the nutrients not directly included in the matrix composition can be inserted. In fact, a number of studies have demonstrated the efficiency of certain inorganic salts as nutrient carriers probably due to their solubility in the rhizospheric organic acids. This is the case of Vivianite, an iron phosphate that has proven to be effective to correct iron chlorosis (14).

There exist other fertilizers that depend on the soil microbial activity to release the nutrients. That is the case of certain urea polymers (i.e., urea-formaldehyde) or the combination of nitrogen fertilizers with inhibitors of ammonium nitrification and/or urea hydrolysis in soils (15). These fertilizers, however, are not significantly affected by the rhizospheric organic acids (15).

In this work we describe (i) the manufacture, chemical-structural characterization, and solubility of a potential RCF matrix formed by the reaction of phosphoric acid (or an acid source of phosphate) with magnesium oxide (or carbonate) (calcium oxide or calcium carbonate are also suitable) and iron (or zinc) oxide (or carbonate) in the presence of a humic acid (16). This reaction leads to the formation of chemical species involving phosphates, Mg, Zn, and humic substances (MHP compounds) (16). Also described are (ii) the manufacture of a complete NPK fertilizer containing MHP (RCF fertilizer), (iii) the nutrient release pattern of the RCF fertilizer, and (iv) the efficiency of this RCF fertilizer to provide available nutrients for wheat plants cultivated in a calcareous soil while minimizing nitrogen leaching and ammonium volatilization.

MATERIALS AND METHODS

Preparation and Physicochemical Characterization of the RCF Matrix. The RCF matrix proposed in this study consists of metallic phosphates prepared in the presence of a humic acid: metallic humic-phosphates (MHP) (16).

Preparation of MHP. The different types of MHP were obtained by reaction of phosphoric acid (68% w/w) (49.3% P₂O₅) with magnesium oxide (72% MgO) and zinc carbonate (52.14% Zn) in the presence of different concentrations of a humic acid extracted from leonardite (HA). The relative proportions of the different ingredients in the reaction in the absence of HA was 56.6% of phosphoric acid (68% w/w), 31% of magnesium oxide, and 12.4% of zinc carbonate. This is the sample named MP1.

The relative proportions in percentage of HA with respect to MP1 in MHP samples were 0, 0.6, 3, 6, 9, and 11. The different MHP samples obtained were named MP1 (no HA), MHP-HA1 (0.6% HA; 99.4% MP1), MHP-HA2 (3% HA; 97% MP1), MHP-HA3 (6% HA; 94% MP1), MHP-HA4 (9% HA; 91% MP1), and MHP-HA5 (11% HA; 89% MP1). The reaction was carried out at room temperature and atmospheric pressure and with continuous stirring as described in ref 16.

HA was first purified using the International Humic Substances Society (IHSS) methodology without including the HF-HCl mixture treatment. In short, 10 g of nondried leonardite was weighed in a 250 mL flask to which 0.1 M NaOH was added until all the air had been displaced. After 48 h of stirring at 25 °C in darkness, the supernatant containing the unfractionated humic extract was separated from the solid fraction by centrifugation at 7650g for 30 min. The HA was obtained by acidifying an aliquot of the alkaline extract containing the humic and fulvic acids obtained from leonardite, with 6 M hydrochloric acid (HCl) down to a pH of 1.5. After 12 h, the acidified sample was centrifuged at 7650g for 30 min to separate the precipitated humic acid from the supernatant containing the fulvic acids and other acid-soluble organic compounds. After humic acids had been washed with water to eliminate Cl⁻ contamination, these were freeze-dried. The main composition of the purified HA was 58.47% C, 1.46% N, 2.69% H, 25.76% O, 0.01% P, 0.99% S, 1.02% Fe, and 0.95% Al. The contents of C, H, and N in HA were obtained using elemental analysis (LECO CHN 2000), whereas the contents of P, S, Fe, and Al were obtained by ICP-OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL). The content of O was calculated by difference. The main acidic functional group concentration, obtained using potentiometric analysis, was 1.98 mmol g⁻¹ HA of carboxylic groups and 1.18 mmol g⁻¹ HA of phenolic groups.

To investigate the new water-soluble and water-insoluble chemical species formed in the reaction, samples of MHP-HA5 weighing 8 g were stirred with 1 L of deionized water for 1 h. The water was then removed under suction in a Büchner-type funnel. The residue remaining in the filter paper was washed with two 80 mL portions of deionized water and dried in an oven for 24 h. These samples were named WWS-MHPHA5.

All of the different products were homogenized using a mill and a mortar before analysis.

Chemical Analysis of the Different Nutrient Fractions in MHP. The concentrations of total (HCl 35%), water-soluble and soluble in citric acid (2%), and neutral ammonium citrate (0.96 M; 65 °C temperature) of phosphorus (P), zinc (Zn), and magnesium (Mg) were analyzed following the official methods of analysis (17). The fraction of MHP insoluble in water but potentially soluble in the rhizospheric acids was evaluated by studying the fraction soluble in neutral ammonium citrate.

Structural Characterization of MHP. The structural characterization of MHP samples was carried out using X-ray diffractometry, scanning electron microscopy (SEM), and energy dispersion spectroscopy (EDS).

The samples were scanned using a Bruker D8 X-ray powder diffractometer at a starting point of 2.0 2-theta (degree) and an end point of 80.0 2-theta (degree) with a step size of 0.020 2-theta (degree) and a dwell time of 1 s and using Cu radiation at 40 kV and 30 mA. To detect the new water-soluble and water-insoluble chemical species formed in the MHP reaction, the X-ray powder diffraction patterns corresponding to MP1, magnesium phosphate, magnesium oxide, zinc

Table 1. Chemical Analysis of the Different MHPs Synthesized Using European Official Methods

	MP 1			MHP-AH1			MHP-AH2			MHP-AH3			MHP-AH4			MHP-AH5		
	Mg (%)	P (%)	Zn (%)	Mg (%)	P (%)	Zn (%)	Mg (%)	P (%)	Zn (%)	Mg (%)	P (%)	Zn (%)	Mg (%)	P (%)	Zn (%)	Mg (%)	P (%)	Zn (%)
total ^a	13.97	14.04	7.30	13.98	10.59	7.35	13.55	10.37	7.25	13.26	9.92	7.02	12.60	9.38	6.73	12.94	9.75	6.89
citric ^b	12.55	13.58	6.78	12.84	10.43	7.22	12.43	10.39	7.14	12.03	9.94	6.89	11.78	9.53	6.71	11.46	9.58	6.60
citrate ^c	12.74	9.69	6.79	12.79	10.54	7.45	11.85	9.83	6.88	12.16	9.82	6.86	11.49	9.36	6.61	11.32	9.32	6.60
water ^d	1.95	4.08	0.05	1.68	3.70	0.04	1.73	3.67	0.06	1.57	3.59	0.08	1.49	3.45	0.07	1.92	3.98	0.44
	extracted/total ratio			extracted/total ratio			extracted/total ratio			extracted/total ratio			extracted/total ratio			extracted/total ratio		
	Mg	P	Zn	Mg	P	Zn	Mg	P	Zn	Mg	P	Zn	Mg	P	Zn	Mg	P	Zn
citric	0.90	0.97	0.93	0.92	0.98	0.98	0.92	1.00	0.99	0.91	1.00	0.98	0.93	1.02	1.00	0.89	0.98	0.96
citrate	0.91	0.69	0.93	0.91	1.00	1.01	0.87	0.95	0.95	0.92	0.99	0.98	0.91	1.00	0.98	0.87	0.96	0.96
water	0.14	0.29	0.01	0.12	0.35	0.01	0.13	0.35	0.01	0.12	0.36	0.01	0.12	0.37	0.01	0.15	0.41	0.06

^a Soluble in HCl 35%. ^b Soluble in citric acid 2%. ^c Soluble in neutral ammonium citrate 0.96 M. ^d Soluble in water.

carbonate, zinc phosphate, MHP-HA5, and WWS-MHPHA5 were systematically compared (Supporting Information).

For the EDS study the samples were scanned in a Philips model XL30CP scanning electron microscope (accelerating voltage of 15 kV) using an EDAX Phoenix energy disperse spectroscopy analyzer. Qualitative elemental composition analysis was carried out on selected areas, which were also photographed at 3000 \times .

The samples were also scanned in a Zeiss model DSM-940A scanning electron microscopy (accelerating voltage of 15 kV) with a DISS Point Electronic GmbH image acquisition and processing system. The samples were placed on an adhesive connected by silver and glue to an aluminum support with an earth wire. Before being scanned, the samples were shaded with gold during 2 min at 15 mA in a vacuum atmosphere using an Emitek K550 sputter-coater. Finally, they were photographed at 3000 \times .

Study of Phosphorus Solubility in MHP Samples in Water and Sodium Citrate over Time. The solubility of MHP samples in water and sodium citrate (0.96 M) at pH 5.0, 7.0, and 8.5 was studied at different extraction times. The ammonium citrate solvent was replaced by sodium citrate to avoid the precipitation of magnesium ammonium phosphate.

The procedure is described briefly: 0.4 g of each MHP sample was weighed in 50 mL plastic bottles and diluted to 40 mL with each extraction reagent. The mixture was shaken in a Heidolph reax 2 model mixer overhead at the minimum speed, and at defined times aliquots of the solution were taken with a 1 mL syringe. These times were 5, 10, 15, 30, 50, 80, 120, 240, 360, and 480 min. Subsequently, the aliquot was filtered with 0.45 μ m polypropylene filters, and 200 μ L was diluted to 10 mL with sodium citrate at pH 4 to avoid humic acid and phosphates precipitation. The extractions were carried out at 25 $^{\circ}$ C.

The concentration of phosphorus in solution was analyzed using an ICP-OES spectrometry (Termo Elemental Co. Iris Intrepid II XDL). The study was carried out in duplicate.

Preparation of a NPK-RCF Fertilizer Containing MHP. A granulated complete fertilizer was manufactured in a pilot plant using MHP-HA5 as RCF matrix as described in ref 16 (16). The NPK-RCF fertilizer had the following nutrient equilibrium: 19% N, 9% P₂O₅, 11% K₂O. The product contained 4% MgO and 2% Zn. Potassium chloride and urea were chosen to provide K and N. Therefore, the product contained only ureic nitrogen. The MHP-HA5 matrix was the source of P, Mg, and Zn. The content of MHP-HA5 in the RCF-NPK fertilizer was fixed at 30% according to previous studies (16). The size of the granules was 95% between 2 and 4 mm.

Nutrient Release Pattern in Water of the NPK-RCF Fertilizer.

To evaluate the effect of the RCF matrix (MHP-HA5) on the pattern of the nutrient release in water of the NPK-RCF fertilizer, samples of the NPK-RCF fertilizer and the corresponding NPK fertilizer without the RCF matrix (in this case the source of P was simple superphosphate) were studied using the methodology described by Cartagena and Diaz Lopez (15) to evaluate slow-release fertilizers with some modifications. Briefly, 1 g of the samples was placed in a Büchner-type funnel of 3.5 cm of diameter with a 2 μ m filter paper. Ten deionized water applications of 10 mL were passed through the product, added without creating turbulences, and filtered by gravity. The filtrates were weighed,

diluted, and analyzed for N, P, and K contents. N content was analyzed through the determination of ammonium prior to urea hydrolysis using urease (18). P and K were directly analyzed using ICP-OES spectrometry.

Nutrient Solubility in Sodium Citrate of the NPK-RCF Fertilizer.

A complementary study on the solubility of the different nutrients present in the NPK-RCF fertilizer in sodium citrate (pH 7) was also carried out, following the above-described methodology for the study of the solubility of the RCF matrix (MHP) in sodium citrate over time.

Efficiency of the RCF-MHP Process To Reduce Nitrogen Losses.

Ammonia Volatilization Study. The degree of ammonia volatilization associated with urea and the urea included in the NPK-RCF fertilizer was studied using an in vitro system as described in ref 18. Soil incubations were carried out in 250 mL bottles containing 100 g of a calcareous soil with 20% of sand (2–3 mm) and a fertilizer amount equivalent to 0.0874 g of nitrogen.

In short, moistened air swept out the volatilized ammonium into a 100 mL vessel with 80 mL of 1% boric acid that trapped the ammonium. This solution was eventually titulated with 0.01 N HCl using a pH-meter.

The treatments employed were a control treatment that consisted of granulated urea and the RCF treatment containing urea in RCF-NPK 19–9–11 granules. Both the granules of urea and NPK-RCF had a size between 2 and 3 mm in diameter. The treatments were applied on the soil surface and on the soil surface plus organic matter (0.7 g of wheat straw), in duplicate. Wheat straw was previously cut in pieces of 0.5 cm in size. The organic matter (wheat straw) was added to increase microbial activity and favor urea hydrolysis. The soil used had the following composition: pH 8.14; electrical conductivity at 25 $^{\circ}$ C, 0.21 dS m⁻¹; organic matter content, 12.8 g kg⁻¹; 1.4 g kg⁻¹ N; 11.22 mg kg⁻¹ P; 0.18 cmol_c kg⁻¹ K; 1.28 cmol_c kg⁻¹ Mg; 25.96 cmol_c kg⁻¹ Ca; 0.95 cmol_c kg⁻¹ Na; 2.92 mg kg⁻¹ Fe; 0.30 mg kg⁻¹ Cu; 0.22 mg kg⁻¹ Zn; 2.84 mg kg⁻¹ Mn; CaCO₃, 473 g kg⁻¹ %; sand, 272.6 g kg⁻¹; silt, 292.8 g kg⁻¹; and clay, 434.6 g kg⁻¹.

Multiple pairwise comparisons among treatments were made using Fisher's least significant difference (LSD) method, with the overall α -level set at 0.05 and 0.1.

Soil-Plant Study. To investigate the effect of a RCF type fertilizer on plant development and N losses, the growth and N leaching in a plant-soil system formed by wheat plants (*Triticum aestivum* cv. Farak) cultivated in an alkaline and calcareous soil were studied. We compared the effects of the RCF-NPK 19–9–11 fertilizer with that of a corresponding standard fertilizer with the same chemical composition for K (KCl) and N (urea). The P in the standard fertilizer was introduced as simple superphosphate.

The plants were cultivated in pots of 6.6 L and 30 cm height (seven plants per pot and six pots per treatment). The culture substrate used consisted of a mixture (50:50 w/w) of the soil used in volatilization studies with sand (diameter = 0.5–1.5 mm). The leachate corresponding to each pot was collected twice per week.

The treatments were a control without fertilization; T1, which received the standard NPK fertilization consisting of the application of 8–15–15 NPK at sowing and urea before tillering; and T2, which

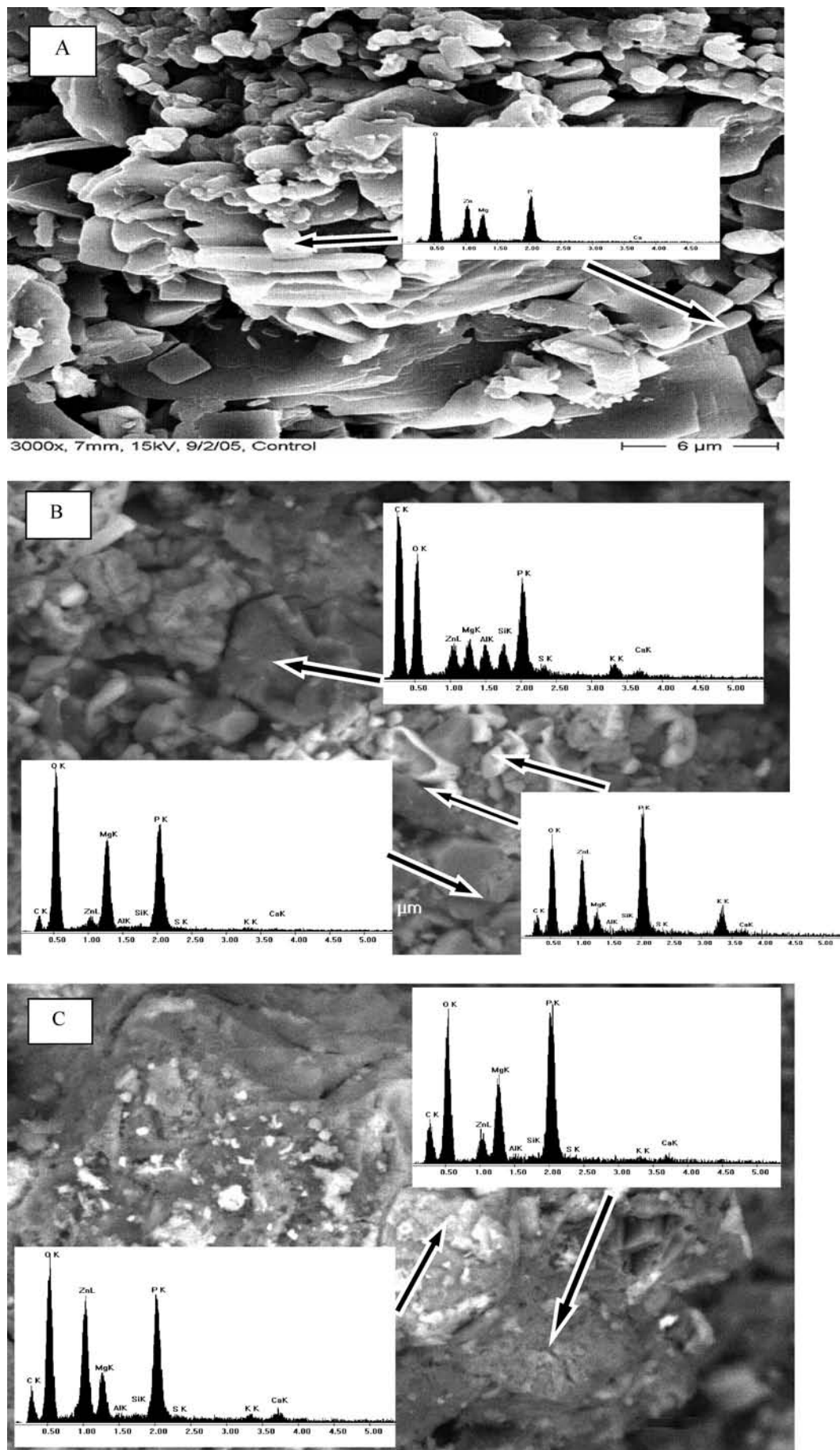


Figure 1. Scanning electron microscopy of MP1 (A), MHP-HA5 (B), and WWS-MHPHA5 (C).

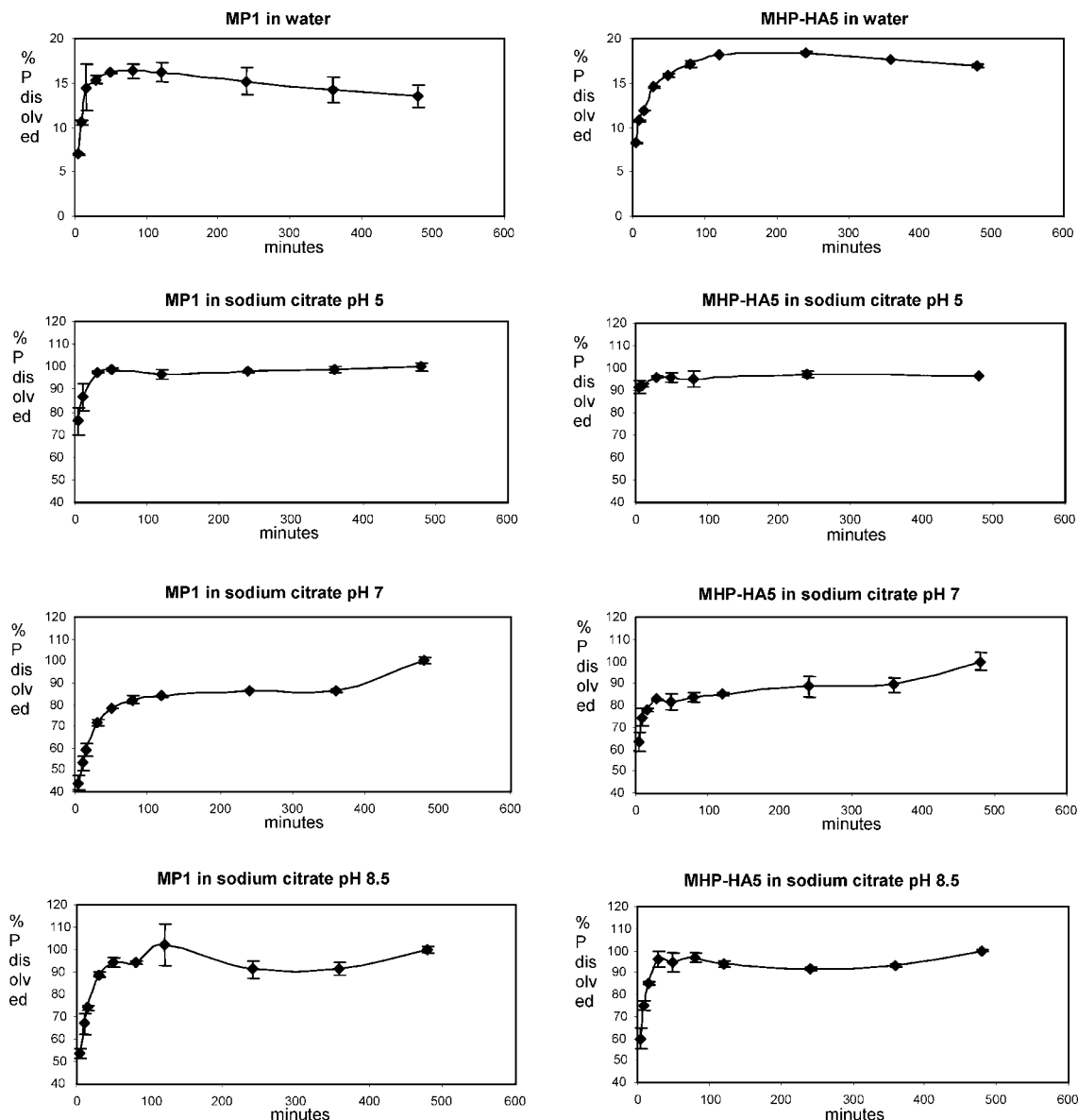


Figure 2. MP1 and MHP-HA5 solubility kinetics in water and sodium citrate at different pH values.

received the RCF-NPK fertilizer at sowing. The dose of nitrogen per pot was equivalent to 180 kg ha^{-1} . All treatments received the equivalent to 52.2 kg ha^{-1} of P_2O_5 and 52.5 kg ha^{-1} of K_2O .

After 60 days, plants were harvested and the shoot, grain, and root dry matters were obtained. The shoot content of the main mineral nutrients was also evaluated.

The total N content in the leachate was analyzed using micro-Kjeldahl methodology. The concentration of the different nutrients in the shoot was analyzed using ICP-OES, except in the case of total N, which was analyzed using elemental analysis (LECO CHN-2000).

Multiple pairwise comparisons among treatments of the different parameters studied were made using Fisher's least significant difference (LSD) method, with the overall α -level set at 0.05.

RESULTS AND DISCUSSION

Chemical and Structural Features of MHP (RCF Matrix).

As for the physical characteristics of the reaction for the preparation of MHP, when the different ingredients were mixed, the mixture was first fluid, but became solid in 2–3 min. The temperature reached values around $109 \text{ }^\circ\text{C}$. In the presence of HA the dynamics of the reaction did not change very significantly, but the texture of the solid formed was smoother and softer, thus suggesting the formation of new compounds

involving HA. In fact, this effect of HA was proportional to HA concentration.

Regarding the solubility of the different nutrients in the extraction reagents used, in general the proportion of the different elements soluble in citric acid and ammonium citrate was near 100% (Table 1). Only in the absence of HA (MP1) was the percentage of P soluble in ammonium citrate lower (around 70%). However, the presence of HA was associated with a significant increase in this parameter (around 100%) (Table 1) (the results obtained when sodium citrate was used were similar; data not shown). In the case of the water-soluble fraction, the proportion of P soluble in water was around 30% in the absence of HA (MP1) (Table 1) with a slight increment being observed in the presence of HA (Table 1). However, in the case of Mg and Zn the proportion of the water-soluble fraction was very low. This fact suggests that the P water-soluble fraction may be principally related to the presence of free phosphoric acid (in fact, the final pH of the mixture was around 3) and that this fraction may be controlled by modifying the proportion of phosphoric acid used in the reaction with respect to the other ingredients, principally magnesium oxide and zinc carbonate.

In principle, these results indicate the suitability of the MHP process to prepare the RCF matrix, because we obtain a principal fraction (around 70%) insoluble in water but soluble in ammonium citrate (this fraction, the RCF matrix, is considered to be the fraction soluble in the rhizospheric acids) and a minor fraction (around 30%) soluble in water (this fraction is included in the starter fraction). Moreover, the water-soluble (starter) fraction can be controlled by modifying the phosphoric acid/ (magnesium oxide + zinc carbonate) ratio in the reaction mixture.

As for the structural composition of MHP, the results obtained from the X-ray diffractograms suggest that the process of preparation of MHP principally leads to the formation of new water-insoluble chemical species (Supporting Information Figure SI 1). These results are in agreement with those concerning nutrient solubility, discussed above, that indicated that the P water-soluble fraction seemed to be principally related to free phosphoric acid. As far as the nature of these new chemical species is concerned, our results suggest that they may involve phosphate, Mg, Zn, and HA. In this way, they could be formed of double phosphates of Mg and Zn associated with HA through metal bridges.

This hypothesis was confirmed by the results obtained in the EDS study. Thus, as can be observed in **Figure 1A**, the reaction in the absence of HA (MP1 samples) led to the formation of phosphates containing Mg and Zn. The proportion of Mg and Zn in the phosphate varied depending on the crystal particle analyzed (data not shown). When the reaction was carried out in the presence of HA (MHP-HA5 samples), a clear formation of phosphate–humic complexes of Mg and Zn was observed (**Figure 1B**), which was reflected in the presence of significant relative concentrations of C, P, O, Zn, and Mg in the crystal particles analyzed. The disappearance of phosphate–humic complexes with high C content in the sample corresponding to the water-insoluble fraction of MHP-HA5 (WWS-MHPHA5) (**Figure 1C**) reveals that water solubility increases in line with HA concentration in the complexes. This result confirms that obtained in the study of the variation in the P water-soluble fraction as a function of HA concentration (**Table 1**).

In summary this study indicates that the process of preparation of MHP is associated with the formation of new chemical species consisting of phosphate–metal (Mg, Zn)–humic complexes having water solubility that is directly related to the relative concentration of the humic system in these complexes. In any case the relative proportion of the water-soluble fraction of these complexes is of little significance when compared with that corresponding to water-insoluble compounds.

As for the nature of these phosphate–metal–humic complexes, previous studies carried out in our laboratory indicated that these compounds are principally formed by metal bridges between the humic molecules and phosphate (19, 20). These studies also revealed that the level of stability of this type of complex is similar to that of the corresponding humic–metal complexes (19, 20).

Degree of Solubility of MHP (RCF Matrix) in Water and Sodium Citrate over Time. The results concerning the solubility of P over time in water and sodium citrate of MHP samples obtained in the presence (MHP-HA5) and in the absence of HA (MP1) are presented in **Figure 2**. In the study using sodium citrate, we employed three different pH values (5, 7, and 8.5) to match the main pH values of acidic, neutral, and alkaline soils.

In general, MP1 samples presented the maximum of solubility in water (around 16%) after 50 min of extraction. The presence

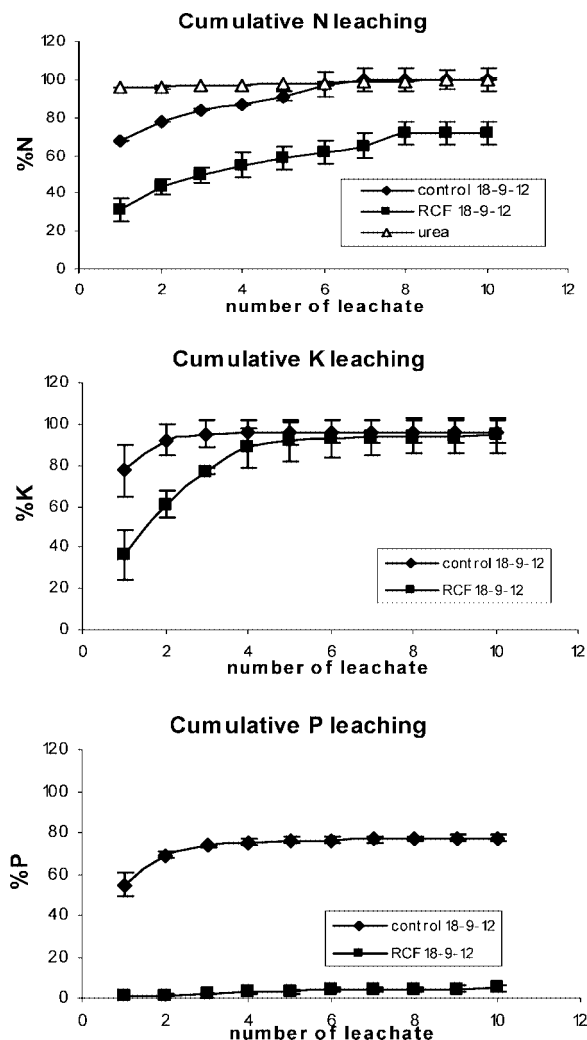


Figure 3. Cumulative water solubility of N, P, and K for different granulated fertilizers: control NPK (19–9–11) fertilizer, NPK-RCF (19–9–11) fertilizer, and urea.

of HA in MHP (MHP-HA5) caused a slight increase in water solubility, although the extraction rate was slower than in the case of MP1.

The solubility study in sodium citrate showed slightly different results depending on pH value. Thus, at pH 5, both MP1 and MHP-HA5 presented a rapid extraction of P (around 100% of the total P) but, in this case, the extraction rate corresponding to MHP-HA5 was faster than that of MP1. A similar result was obtained at pH 8.5. At pH 7, however, P extraction was slower, reaching a first maximum around 85% after 30 (MHP-HA5) and 50 (MP1) min of extraction, which increased up to 100% after 500 min of extraction.

These results confirm the results obtained in the analysis of the main P fractions in MHP (**Table 1**). The fact that in this study P water solubility is around 16–18%, whereas in the application of the official method of analysis it was around 30–40%, may be explained by the different times of contact of the product with the solvent in both methods.

On the other hand, this study also confirms the suitability of MHP as RCF matrix, because it presents a rather low water solubility, whereas the solubility in sodium citrate was rapid and totally independent of pH value.

Application of the RCF Matrix (MHP) in the Preparation of a NPK-RCF Granulated Fertilizer: Effect on Fertilizer Properties. *Effect of RCF Matrix on the Release Pattern of*

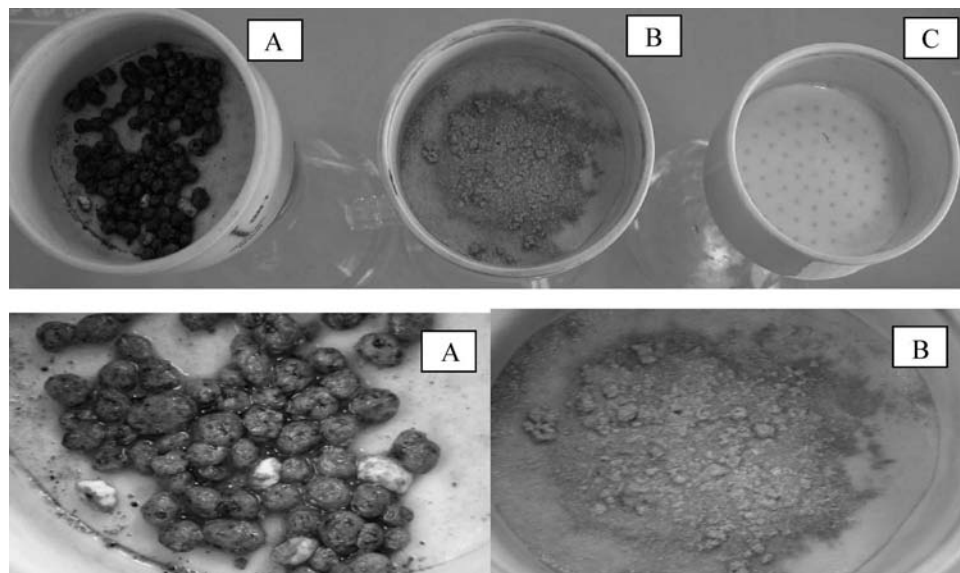


Figure 4. Final texture of the different granulated fertilizers after 10 water washings of the treatments: NPK-RCF 19-9-11 (A), control NPK 19-9-11 (B), and urea (C).

Nutrients in RCF-NPK Fertilizer. In **Figure 3** we present the results concerning the nutrient release pattern in water corresponding to a NPK-RCF fertilizer (RCF 19-9-11) containing MHP (MHP-HA5) as RCF matrix, the equivalent NPK fertilizer (19-9-11), and urea.

In the case of N, whereas both the NPK fertilizer and urea presented a rapid release of N (100% for urea and 70% for NPK fertilizer in the first leachate; and 100% for NPK fertilizer in the sixth leachate), the RCF-NPK fertilizer presented a slower release that was reflected in a degree of N release of 30% in the first leachate that increased to a stable value of 60% in the eighth leachate. These results show both the efficiency of the RCF matrix to decrease the release rate of the water-soluble fraction of N present in RCF-NPK fertilizer and the capacity of the RCF matrix to retain a significant fraction (around 40%) of N that is potentially soluble in rhizospheric acids. This retention of urea molecules in the RCF matrix could be related to both the formation of a kind of coating with MHP and the capacity of HA to form stable complexes with urea through the formation of H-bonds.

In the case of K, the slow-release effect of RCF matrix is significant but less important than in the case of N (**Figure 3**). This result was expected due to the monovalent character of K and its high mobility in water.

Finally, in the case of P, RCF-NPK fertilizer presented a slow-release rate of this nutrient, whereas the equivalent NPK fertilizer presented a rapid release of the P fraction soluble in water (monocalcium phosphate). This result is in agreement with the presence of superphosphate in the standard fertilizer.

These different results were also reflected in the physical appearance of each fertilizer after water washing (**Figure 4**). Whereas the RCF-NPK fertilizer maintained the granular skeleton, both the equivalent NPK fertilizer and urea were totally disaggregated. The mineral residue observed in NPK fertilizer corresponded to the calcium sulfate and dicalcium phosphate present in superphosphate.

However, the interaction of the NPK-RCF fertilizer with sodium citrate (0.96 M; pH 7) solubilized the different nutrients present in the fertilizer (P, N, and K) (**Figure 5**). This result indicates the potential solubility of the NPK-RCK fertilizer in the rhizospheric organic acids.

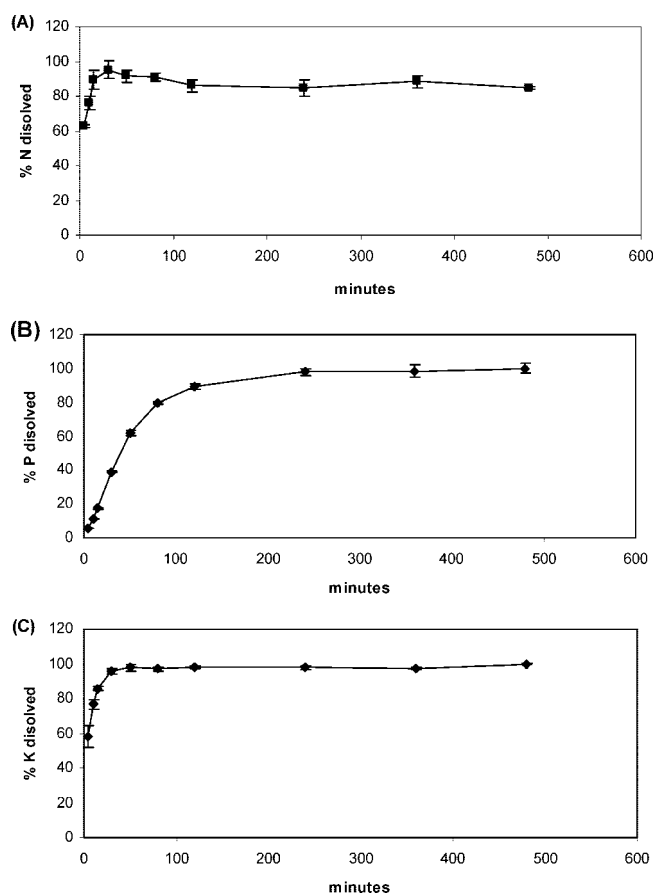


Figure 5. Solubility of the nitrogen (A), phosphorus (B), and potassium (C) contained in NPK-RCF (19-9-11) in sodium citrate (pH 7) over time.

In summary, these studies show the capacity of the RCF matrix (MHP) to significantly affect the release rate of the nutrients contained in the RCF-NPK fertilizer in water, principally in the case of N and P. These results have been obtained including 30% of the RCF matrix in the fertilizer. Other studies showed that we could alter the nutrient release pattern by modifying the proportion of RCF matrix in the composition of the RCF-NPK fertilizer (data not shown) (16). Likewise, the

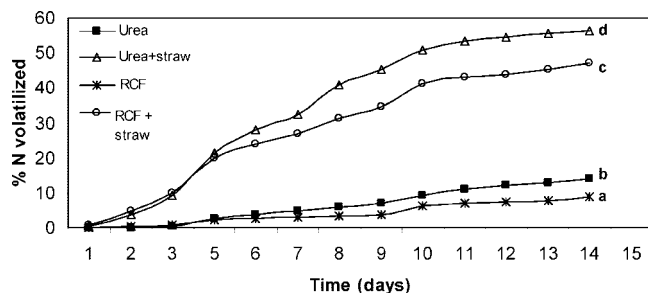


Figure 6. Percentage of the total nitrogen that is volatilized as ammonia, corresponding to urea and the NPK-RCF fertilizer (19–9–11) in soil and soil plus wheat straw (0.7%). Treatments followed by different letters were significantly different (Fisher LSD test) at $p < 0.05$.

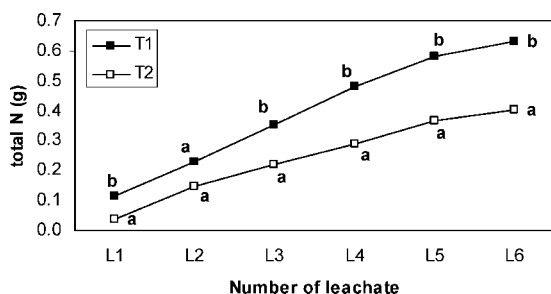


Figure 7. Cumulative nitrogen leaching for the different fertilizer treatments, T1 (standard fertilizer 8–15–15 plus urea) and T2 (NPK-RCF 19–9–11). Treatments followed by different letters were significantly different (Fisher LSD test) at $p < 0.05$.

results obtained from the interaction of the NPK-RCF fertilizer with sodium citrate also indicate the potential release of the different nutrients present in the fertilizer as a consequence of the action of the rhizospheric acids.

Effect of RCF Matrix on the Nitrogen Losses Associated with the Application of a RCF-NPK Fertilizer in a Soil–Plant System. To evaluate if the specific nutrient release pattern of the RCF-NPK fertilizer studied is also reflected in an effective control of nutrient losses in a plant–soil system, we investigated the ammonia volatilization and N leaching associated with a pot system consisting of wheat plants cultivated in an alkaline and calcareous soil.

As for ammonia volatilization, the results obtained (Figure 6) indicate that under conditions of both high (in the presence of wheat straw in soil surface) and low urease activity, the treatment with the RCF-NPK fertilizer was associated with a significant reduction of ammonia volatilization. This result is probably related to the presence in RCF-NPK fertilizer of a fraction of ureic-N that is retained in the RCF matrix (around 40%; Figure 3).

In relation to N leaching, the treatment with the RCF-NPK fertilizer was also related to a significant reduction in the total N leached associated with pot irrigation (Figure 7). This fact was also associated with a general increase of growth parameters in those plants treated with the RCF-NPK fertilizer with respect to those fed with the equivalent NPK fertilizer, although these increments were not significant at $p < 0.05$ (Table 2). In principle, this effect on plant growth may also involve the action of the humic fraction present in the fertilizer, because many studies have shown the capacity of humic substances to improve plant growth and mineral nutrition (21, 22). Regarding shoot nutrient concentration, there were no significant differences between the plants fed with the RCF-NPK fertilizer and those receiving the equivalent NPK fertilizer (data not shown).

Table 2. Effect of the Different Fertilizer Treatments (Control 0, without Fertilization; T1, 8–15–15 plus Urea; T2, NPK-RCF 19–9–11) on the Dry Matter Production of Shoot and Root and on the Number of Grains of Wheat Plants^a

treatment	dry matter (g/pot)		
	shoot av	root av	grain no. av
control 0	1.39b	0.45b	44 b
T1	3.01a	0.68a	65.5a
T2	4.13a	0.7 a	81.2a

^a Treatments followed by different letters were significantly different (Fisher LSD test) at $p < 0.05$.

In summary, this study shows the suitability of the use of phosphate–metal (Mg, Zn)–humic complexes (MHP compounds) as RCF matrix in RCF-type fertilizers. Thus, MHP contains a significant fraction that is insoluble in water, but soluble in ammonium (sodium) citrate at different pH values and, potentially, in rhizospheric acids. This fact explains the significant effect of the presence of MHP in a granulated fertilizer, modifying its nutrient release pattern, principally in relation to N and P. These effects were also reflected in the significant reduction in both ammonia volatilization and N leaching associated with an application of a RCF-NPK fertilizer in wheat plants cultivated in a calcareous soil.

ABBREVIATIONS USED

MP, metal phosphates; MHP-HA, metal–humic–phosphates; WWS-MHPHA, without water-soluble metal–humic–phosphates; RCF, rhizosphere control fertilizer; SEM, scanning electron microscope; N, nitrogen; K, potassium; P, phosphorus; P₂O₅, phosphorus oxide; K₂O, potassium oxide; HCl, hydrochloric acid; Fe, iron.

Supporting Information Available: X-ray diffractograms of the RCF matrix are shown in this section (Figure SI 1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

LITERATURE CITED

- Brennan, R. F.; Bolland, M. D. A. Effectiveness of dicalcium phosphate compared with superphosphate for wheat grown on acidic sandy soils. *J. Plant Nutr.* **2005**, *28*, 725–736.
- Gardner, W. K.; Barber, D. A.; Parberry, D. G. The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. *Plant Soil* **1983**, *70*, 107–114.
- Marschner, H.; Römheld, V.; Cakmak, I. Root-induced changes of nutrient availability in the rhizosphere. *J. Plant Nutr.* **1987**, *10*, 9–16.
- Yan, L.; Guohua, M.; Fanjun, Ch.; Jianhua, Z.; Fusuo, Z. Rhizosphere effect and root growth of two maize (*Zea mays* L.) genotypes with contrasting P efficiency at low P availability. *Plant Sci.* **2004**, *167*, 217–223.
- Petersen, W.; Böttger, M. Contribution of organic acids to the acidification of the rhizosphere of maize seedlings. *Plant Soil* **1991**, *132*, 159–163.
- Gaume, A.; Mächler, F.; De León, C.; Narro, L.; Frossard, E. Low-P tolerance by maize (*Zea mays* L.) genotypes: significance of root growth, and organic acids and acid phosphatase root exudation. *Plant Soil* **2001**, *228*, 253–264.
- Neumann, G.; Römheld, V. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. *Plant Soil* **1999**, *211*, 121–130.

- (8) Jones, D. L.; Darrah, P. R. Role of root derived organic-acids in the mobilization of nutrients from the rhizosphere. *Plant Soil* **1994**, *166*, 247–257.
- (9) Jones, D. L. Organic acids in the rhizosphere—a critical review. *Plant Soil* **1998**, *205*, 25–44.
- (10) Crowley, D. Function of siderophores in the plant rhizosphere. In *The Rhizosphere*; Dekker: New York, 2001; pp 223–261.
- (11) Ström, L.; Owen, A. G.; Godbold, D. L.; Jones, D. L. Organic acid mediated P mobilization in the rhizosphere and uptake by maize roots. *Soil Biol. Biochem.* **2002**, *34*, 703–710.
- (12) Mengel, K. Agronomic measures for better utilization of soils and fertilizer phosphates. *Eur. J. Agron.* **1997**, *7*, 221–233.
- (13) Dinkelaker, B.; Römheld, V.; Marschner, H. Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). *Plant Cell Environ.* **1989**, *12*, 285–292.
- (14) Rosado, R.; Del Campillo, M. C.; Martínez, M. A.; Barrón, V.; Torrent, J. Long-term effectiveness of vivianite in reducing iron chlorosis in olive trees. *Plant Soil* **2002**, *241*, 139–144.
- (15) *Fertilizantes de liberación lenta*; Jiménez Gómez, S., Ed.; Mundi-Prensa: Madrid, Spain, 1992; 146 pp.
- (16) Garcia-Mina, J. M. Fertilizing composition and process for the obtainment thereof. EP Patent 1,612,200, January 4, 2006.
- (17) *Métodos Oficiales de Análisis. Tomo III*; Ministerio de agricultura, pesca y alimentación; Secretaría General Técnica Ministerio de Agricultura, Pesca y Alimentación: Madrid, Spain, 1994; pp 565–578.
- (18) Zhengping, W.; Van Cleemput, O.; Demeyer, P.; Baert, L. Effect of urease inhibitors on urea hydrolysis and ammonia volatilization. *Biol. Fert. Soils* **1991**, *11*, 43–47.
- (19) Guardado, I.; Urrutia, O.; García-Mina, J. M. Methodological approach of the study of the formation and physicochemical properties of phosphate–metal–humic complexes in solution. *J. Agric. Food Chem.* **2005**, *53*, 8673–8678.
- (20) Guardado, I.; Urrutia, O.; García-Mina, J. M. Size distribution, complexing capacity and stability of phosphate–metal–humic complexes. *J. Agric. Food Chem.* **2007**, *55*, 408–413.
- (21) García-Mina, J. M.; Antol, M. C.; Sanchez-Díaz, M. Metal–humic complexes and plant micronutrient uptake: a study based on different plant species cultivated in diverse soil types. *Plant Soil* **2004**, *258*, 57–68.
- (22) Chen, Y.; De Nobili, M.; Aviad, T. Stimulatory effects of humic substances on plant growth. In *Soil Organic Matter in Sustainable Agriculture*; CRC Press: Boca Raton, FL, 2004; pp 103–130.

Received for review March 22, 2007. Revised manuscript received July 20, 2007. Accepted July 27, 2007. This research was funded by the Roullier Group and the government of Navarra.

JF0708490