

Organic Complexed Superphosphates (CSP): Physicochemical Characterization and Agronomical Properties

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ABSTRACT: A new type of superphosphate (organic complexed superphosphate (CSP)) has been developed by the introduction of organic chelating agents, preferably a humic acid (HA), into the chemical reaction of single superphosphate (SSP) production. This modification yielded a product containing monocalcium phosphate complexed by the chelating organic agent through Ca bridges. Theoretically, the presence of these monocalcium–phosphate–humic complexes (MPHC) inhibits phosphate fixation in soil, thus increasing P fertilizer efficiency. This study investigated the structural and functional features of CSP fertilizers produced employing diverse HA with different structural features. To this end were used complementary analytical techniques: solid-phase ³¹P NMR, ¹³C NMR, laser-confocal microscopy, X-ray diffraction, and molecular modeling. Finally, the agronomical efficiency of four CSP have been compared with that of SSP as P sources for wheat plants grown in both alkaline and acidic soils in greenhouse pot trials under controlled conditions. The results obtained from the diverse analytical studies showed the formation of MPHC in CSP. Plant–soil studies showed that CSP products were more efficient than SSP in providing available phosphate for wheat plants cultivated in various soils with different physicochemical features. This fact is probably associated with the ability of CSP complexes to inhibit phosphate fixation in soil.

KEYWORDS: *organic complexed superphosphate (CSP), monocalcium–phosphate–humic complexes, wheat, phosphate fixation*

■ INTRODUCTION

Single superphosphate (SSP) is a common and widespread water-soluble phosphorus (P) fertilizer, despite its low agronomic efficiency, mainly related to an intense P fixation–precipitation in soil, and eventual leaching depending on soil features.^{1–3} In this context, the aim of obtaining chemically modified SSP with lower P fixation rates in soils and higher agronomical efficiency with respect to ordinary SSP becomes an important issue. A number of studies have shown that the association or mixture of soluble phosphate with organic matter or humic substances was, normally, accompanied by significant improvements in P plant-available soil concentration.^{4–8} However, these studies also showed that the concentration of active humic substances that is necessary to apply to obtain reliable results has to be relatively high, thus causing additional problems for farmers related to economical costs, physical management, and field application.

In this study, we describe the chemical characterization of a special superphosphate, called complexed superphosphate (CSP), obtained by reaction of a mineral acid (phosphoric and/or sulfuric acid) and rock phosphate in the presence of specific amounts of diverse humic acids. Our hypothesis is that this chemical reaction yields the formation of monocalcium phosphate molecules complexed by oxygen-related binding sites in humic molecules. These P–Ca–humic complexes might increase the agronomical efficiency of the SSP, by both decreasing P fixation in soils and increasing P plant availability. The possible formation of phosphate–metal–humic complexes

in soil solution was proposed by Gerke et al.⁹ as a hypothesis to explain P protection against soil fixation and precipitation. Recently, a number of studies have shown that these types of P–humic related complexes can be formed in liquid phase under specific experimental conditions.^{9–13} Likewise, these complexes have been physicochemically characterized in liquid solution.^{9–13} These studies showed that the stability and solubility of P–metal–Humic complexes were similar to those of metal–humic complexes.^{11,13} This fact is relevant because it indicates that these P–humic related complexes may play important functional roles in ecosystems as metal–humic complexes do. Likewise, fluorescence and infrared (FTIR) studies showed that the interaction between metal–humic complexes and phosphate involved the formation of metal bridges between the two anion ligands.¹¹

On the other hand, in a previous work, it has been reported that prepared and isolated double metal phosphate humic complexes (PMAH), with, potentially, characteristics similar to those probably involved in CSP production, modified the dynamic of P in soils, both preventing P sorption (and therefore increasing P solubility in soil solution) and increasing P root uptake by diverse plant species cultivated both in soils and hydroponically.^{14,15}

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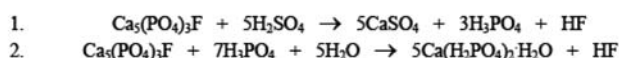
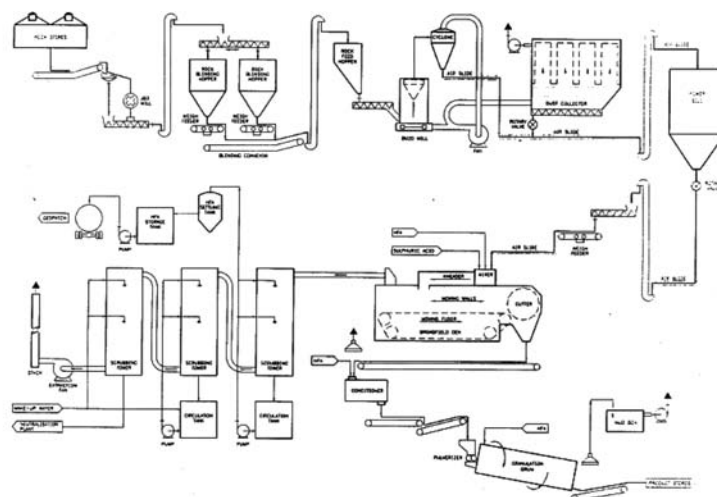


Figure 1. Scheme of the manufacturing process and the main chemical reactions involved in SSP industrial production.

In this study, we investigated both the formation and physicochemical characterization of monocalcium–phosphate–humic complexes in CSP, as well as the influence of the presence of these complexes on CSP-agronomical efficiency in comparison with that of SSP. According to this framework, the following steps were involved in the experimental design: (i) We studied the theoretical chemical viability and stability of monocalcium–phosphate–humic complex formation by studying the complexing process between monocalcium phosphate and an organic ligand model, salicylate, which represents the binding site in humic molecule. The theoretical study was carried out using molecular modeling (density functional theory (DFT)/*ab initio* method). (ii) We studied the manufacturing process of CSP using a specific humic acid (HA1). We investigated the formation of the monocalcium phosphate–HA1 complex in this CSP type (CSP1) using different complementary analytical techniques: ^{31}P NMR (solid phase), confocal microscopy, and X-ray diffraction spectrometry. The functional organic carbon distribution of HA1 was characterized using solid-phase ^{13}C NMR. (iii) To investigate the influence of the structural features of the humic acid in both CSP synthesis and CSP efficiency as P fertilizer, we studied the manufacture of CSP using three new humic acid samples in addition to HA1. Finally, we tested the efficiency of these different types of CSP as P fertilizers, in comparison with that of SSP, for wheat plants cultivated in pots on both acidic and alkaline (calcareous) soils. These experiments were carried out under glasshouse conditions.

MATERIALS AND METHODS

Theoretical Assessment of the Chemical and Structural Viability of Monocalcium–Humic Complexes. To investigate the theoretical chemical viability of the MPHIC, we studied the electronic and structural features of this complex type using quantum chemical methods. The molecular modeling of several penta- and hexacoordinated complexes of Ca(II) with phosphate as monocalcium phosphate and salicylate ligands as humic binding site model has been performed with the Gaussian03 computational program.¹⁶ The B3LYP method, which belongs to the family of computational methods based on

Table 1. Analysis of the Alkaline–Calcareous Soil and Acid Soils

parameter	alkaline soil	acidic soil with high organic matter	acidic soil with low organic matter
conductivity (dS m ⁻¹)	2.34	0.14	0.02
pH	7.60	5.30	5.60
NaHCO ₃ -P (mg kg ⁻¹)	0.05	0.65	4.02
CBD-P (mg kg ⁻¹)	44.00	118	
NaOH-P (mg kg ⁻¹)	0.00	140	169
HCl-P (mg kg ⁻¹)	165	0.00	
residual P (mg kg ⁻¹)	98.00	165	
K (mmol kg ⁻¹)	4.80	2.30	1.20
N (g kg ⁻¹)	0.10	0.52	0.22
Mg (mmol kg ⁻¹)	22.10	19.60	0.01
Ca (mmol kg ⁻¹)	305	23.00	4.35
Na (mmol kg ⁻¹)	8.50	2.50	0.01
Fe (mmol kg ⁻¹)	0.10	1.09	0.43
Mn (mmol kg ⁻¹)	0.02	0.37	0.35
Cu (mmol kg ⁻¹)	0.01	0.02	0.00
Zn (mmol kg ⁻¹)	0.00	0.04	0.00
organic matter (g kg ⁻¹)	0.60	68.60	10.30
active lime (g CaCO ₃ kg ⁻¹)	214	0.60	5.50
sand (g kg ⁻¹)	377	541	150
silt (g kg ⁻¹)	337	359	440
clay (g kg ⁻¹)	286	100	410

DFT¹⁷ in combination with the 6-31G (d,p) basis set, was employed in the calculations.¹⁷ The first coordination spheres were completed with water molecules, whereas the remaining solvent environment was simulated using the polarizable continuum model (PCM).¹⁸ The optimized geometries of the complexes were characterized as energy minima in the potential energy surface by means of the vibrational frequency analysis. Vibrational frequency analysis was also employed to get the values of the molecular entropies, which, together with the molecular electronic energies, provided an estimation of the relative Gibbs free energies.

Table 2. Chemical Composition and Solubility of SSP and CSP1^a

	Ca (%)		Mg (%)		P (%)		S (%)		Fe (mg kg ⁻¹)	
	SP	CSP1	SP	CSP1	SP	CSP1	SP	CSP1	SP	CSP1
citrate	15.7 ± 0.5	16.4 ± 0.4	0.25 ± 0.02	0.30 ± 0.01	6.72 ± 0.12	7.20 ± 0.2	13.4 ± 0.2	14.8 ± 0.3	813 ± 20	862 ± 32
water	12.4 ± 0.3	13.6 ± 0.4	0.25 ± 0.02	0.30 ± 0.04	6.32 ± 0.31	7.45 ± 0.1	12.7 ± 0.5	14.5 ± 0.6	94.0 ± 5.2	224 ± 15
citrate/total	96.2 ± 1.3	96.5 ± 1.0	85.5 ± 1.1	90.2 ± 0.8	85.0 ± 0.7	89.9 ± 0.8	92.8 ± 1.5	95.9 ± 0.8	95.1 ± 0.3	76.9 ± 0.9
water/total	76.3 ± 0.3	80.2 ± 0.7	85.3 ± 1.3	90.1 ± 0.9	80.6 ± 0.3	93.7 ± 1.2	88.1 ± 1.2	94.4 ± 0.6	11.2 ± 0.5	20.5 ± 0.3

^aResults are the average and standard error of three independent determinations.

SSP and CSP Manufacturing Process. A classical flow-sheet for the manufacture of SSP is described in Figure 1, along with the main reactions involved in the process. In general, SSP manufacture involves the reaction between diluted sulfuric acid and milled rock phosphate. The process includes a first step in which sulfuric acid and rock phosphate are mixed continuously with strong agitation (cone mixer). The product formed in the reaction (mainly liquid) falls on a conveyor belt, where the reaction completes and the product becomes solid in texture. At the end of the conveyor belt there is a disintegration system that produces SSP powder (Figure 1). The main water-soluble phosphate that is produced in the process is monocalcium phosphate. However, a small amount of bicalcium phosphate is also produced. The main stoichiometry between rock phosphate and diluted sulfuric acid (75% w/w) used in the industrial process is 100 g of milled rock phosphate (80% < 100 μm) and 76.56 g of H₂SO₄ (75% w/w). This stoichiometry can change as a function of rock phosphate reactivity and grinding (particle size) and type (geometry and size) of mixing reactor.

The temperature that is reached in the reactor is within the range of 120–130 °C, depending on the concentration (dilution in water) of sulfuric acid and/or rock phosphate particular size.

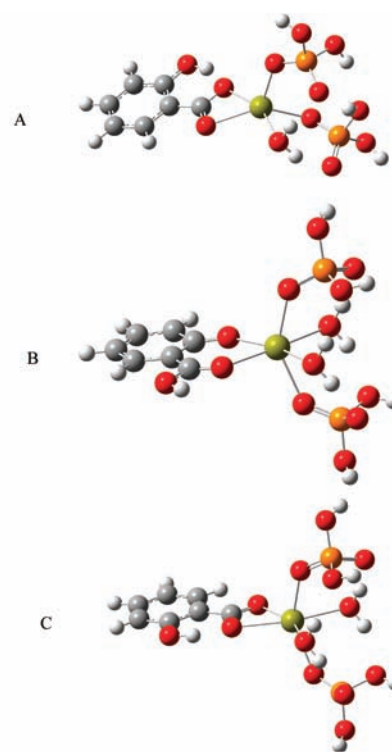
The rock phosphate used in the experiments was obtained from Algeria (Lodosa Factory-Roullier Group). The concentration of P₂O₅ was 30%, and the degree of grinding was 80% < 100 μm (Lodosa Factory-Roullier Group).

To investigate the more adequate process for the preparation of CSP, we first studied the inclusion of a specific concentration of the organic chelating agent (HA1 in the first step of studies) in the reactor, simultaneously with rock phosphate and diluted sulfuric acid. To add the humic acid fraction to the reaction process, we dissolved HA1 in an alkaline (KOH)–water solution (pH 8.0). The proportion between rock phosphate and diluted sulfuric acid in the CSP process was 100 g of milled rock phosphate (80% < 100 μm) and 76.56 g of H₂SO₄ (75% w/w). To investigate the influence of HA concentration in the process using laser confocal microscopy and X-ray diffraction spectroscopy, we prepared two CSP1-type products containing final carbon concentrations of 0.1 and 0.6%. The whole process, also extended to other complementary chemical or manufacturing related conditions, is protected under patent (WO 2011080496; FR2954939).¹⁹

In a second step we carried out the same study as previously described for HA1 but using three new humic acids (HA2, HA3, and HA4). As in the case of HA1, humic acids were applied dissolved in alkaline–water solutions (with KOH, pH 8). In all cases, the manufacture of CSP involved the same raw materials (rock phosphate and diluted sulfuric acid) and 0.1% of organic carbon from different humic materials: HA2, HA3, and HA4. These CSP reactions yielded CSP2, CSP3, and CSP4, respectively. The value of 0.1% of organic carbon is referred to the final CSP product.

The products obtained from the manufacture process were left for 10 days at room temperature to complete the reaction (final curing) (There were no differences between 10 and 25 days of curing concerning SSP and CSP analysis, under our experimental conditions.) Finally, the products were milled to get a homogeneous powder for chemical and agronomical characterization described below (100% < 100 μm).

The industrial process was further validated in a semi-industrial pilot plant of CERA-laboratory (Roullier Group, Dinard, France) (production = 3 ton/h) and in the Industrial Fertilizer Factory of Rio Grande (Brazil) (Timac Agro Brazil, Roullier Group) (production = 20 ton/h).



	G _{res} (kcal/mol)
A) Ca-penta	0.00
B) Ca-hexa-1	2.20
C) Ca-hexa-2	6.91

Figure 2. Molecular modeling of the monocalcium–phosphate–humic complex formed in the manufacturing reaction involved in CSP production and Gibbs energies of the most stable conformations (white atoms are hydrogen; gray atoms are carbon; yellow atoms are calcium; and red atoms are oxygen).

Chemical Analysis of SSP and CSP. The chemical analysis of total P, water-soluble P, neutral ammonium citrate P, and free water concentration was carried out using the Official Methods of Analysis of the European Community.²⁰

Analytical Study of the Formation of the Monocalcium–Phosphate–Humic Complex in CSP. To investigate the formation of the monocalcium–phosphate (Ca (H₂PO₄)₂)–humic complex during CSP reaction, we studied more in depth the product corresponding to HA1 (CSP1) employing several complementary techniques: solid-phase ³¹P NMR, X-ray diffraction, and laser confocal microscopy. The physical mixture between SSP and HA1, at the same proportion as that of CSP reaction, was used as a control.

Solid-Phase ³¹P NMR Analysis. The ³¹P NMR spectra of SSP and CSP1–4 and the physical mixture SSP–HA1 were obtained at room temperature using a Varian Inova spectrometer, with a static field of 17.6 T. Chemical shifts were calibrated with Na₂PO₄ at –150 ppm as an external standard. The experiments were performed using a decoupling MAS acquisition pulse. The relaxation delay was 4 s. FID were acquired with 32K complex points. All ³¹P spectra were treated with MestreNova software.

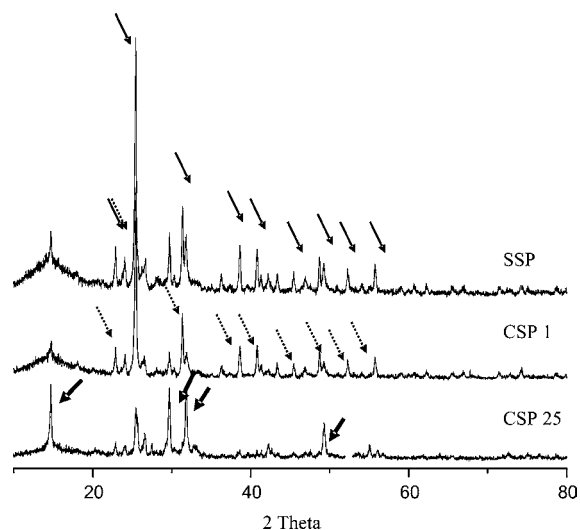


Figure 3. X-ray analysis of SSP, CSP1, and CSP25: relevant peaks of SSP (thin arrows); spectrum modification in CSP1 (0.1% of HA1) (dotted arrows) and in CSP25 (0.6% of HA1) (thick arrows).

X-ray Diffraction (XRD) Analysis. The mineralogical phases contained in SSP, the physical mixture of SSP and HA1, and CSP1 were analyzed by XRD using a Bruker D8 Advance diffractometer (Karlsruhe, Germany), according to the diffraction powder method, with a Cu $K\alpha_1$ radiation and 0.02° 2θ increment and 1 s step^{-1} sweep from 2° to 90° 2θ . The results were compared with the ICDD database.

Laser Confocal Microscopy. Laser confocal microscopy is an optical imaging technique used to increase micrograph contrast in specimens that are thicker than the focal plane. The SSP, CSP1 (considering two concentrations of HA1 in the reaction: 0.1 and 0.6% of organic carbon in final CSP1s), and a physical mixture of HA1 and SSP (in the same proportion as that of CSP1 containing 0.1% of organic carbon) were analyzed using laser confocal microscopy by detecting the fluorescence emitted in the range of 675–685 nm after excitation with light of 633 nm. The analysis of the image corresponding to each product allowed the study of the differences among the distribution and aggregation of the humic material in each type of CSP fertilizer.

Chemical Characterization of Humic Acids. HA1 was obtained from leonardite (Czech Republic), HA2 from leonardite (China), HA3 from peat (Galicia, Spain), and HA4 from humified lignin. The extraction, isolation, and purification processes were carried out as described in Garcia-Mina et al.⁵

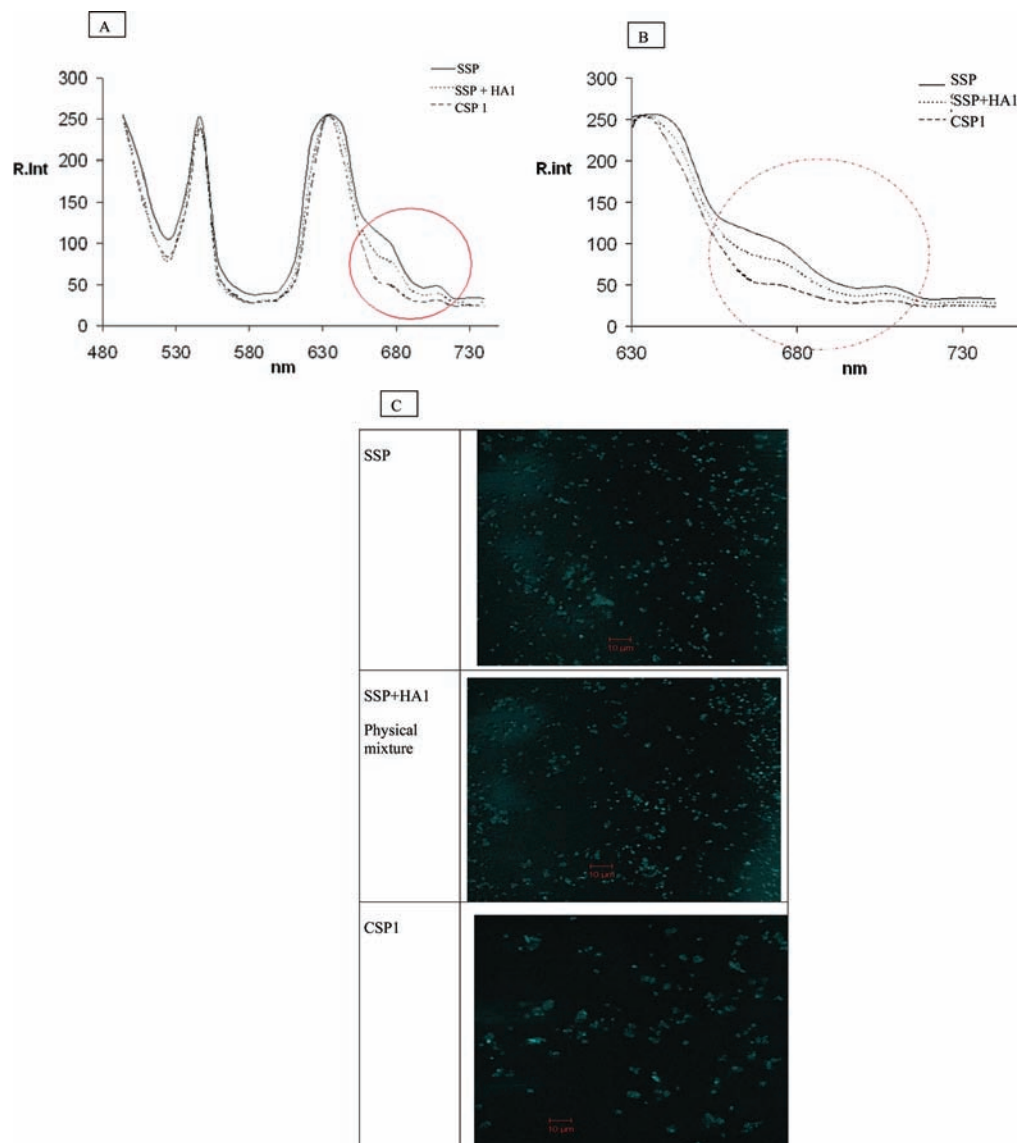


Figure 4. (A) Fluorescence spectra of SSP, the physical mixture (SSP + HA1), and CSP1 obtained from laser confocal microscopy analysis; (B) zoom of Figure 3A; (C) laser confocal microscopy images.

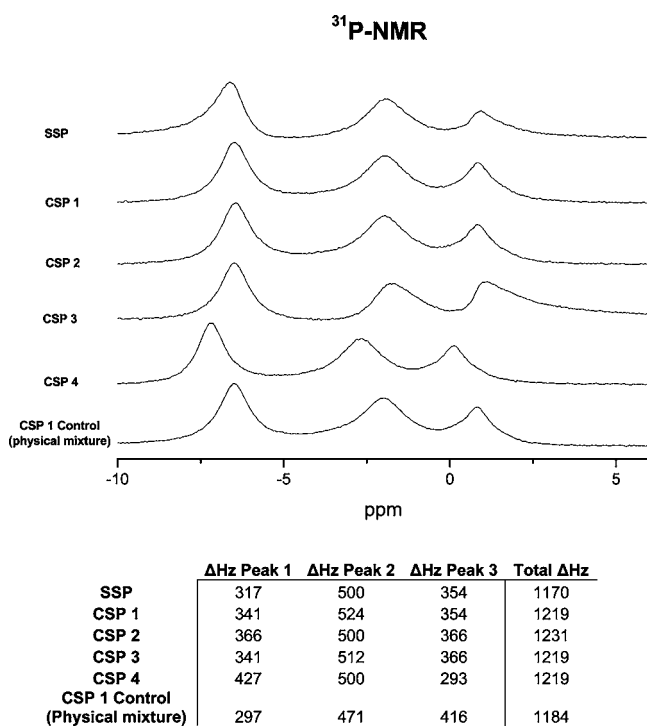


Figure 5. SSP, CSP1–4, and CSP1 control (physical mixture SSP + HA1) solid-phase ³¹P NMR spectra and width values (half-peak height) for the three main peaks.

Elemental analysis of the different humic acids (HA1–4) used in the development of four types of CSP fertilizers (CSP1, CSP2, CSP3, and CSP4) was carried out using a LECO CHN 2000. The C-functional group distribution was analyzed employing ¹³C NMR spectrometry. Solid-state ¹³C NMR spectra were obtained with a Bruker Avance AV-400WB (Bruker Corp., Billerica, MA) spectrometer (9.4 T) at 100.47 MHz, using the cross-polarization magic angle spinning technique, with a rotation speed of 12 kHz, a 90° pulse width, a 30 ms acquisition time, and a 4.0 s delay.

Plant–Soil Studies. Soil Characteristics. Three soils from Navarra (Spain) with low available phosphorus content were selected for plant–soil experiments: two acid soils with different concentration of organic matter and an alkaline–calcareous soil. Soil samples were air-dried and sieved at 2 mm and analyzed completely according to official analysis methods.^{21–24} With regard to P fractionation, the concentration of P corresponding to each P fraction in the soils was measured following the scheme of Harrell and Wang.²¹ This method considers five P fractions: NaHCO₃-P (equivalent to Olsen-P); citrate–bicarbonate–dithionite-P (CBD-P); NaOH-P; HCl-P; and residual P. Both the NaHCO₃OP and CBD-P fractions may be considered as representative of potentially available P for plants and microorganisms. NaOH-P is considered to be representative of P fixed in organic matter (slow release source of available P for plants), and, finally, HCl-P and residual P are fractions of P that can be considered as nonavailable.

The main physicochemical features of the three soils were the following.

The alkaline–calcareous soil (Table 1) is a soil with very low available P, very low N, and normal content of Mg, K, and Na. It shows high amounts of Ca and CaCO₃ and a similar content in clay, sand, and silt. It contains some residual and CBD-P and a relatively large percentage of HCl-P. This is probably due to the very high content of Ca in the soil, which implies the formation of calcium phosphates with very low water solubility. This fraction is extracted by HCl. This fact indicates that in this soil phosphate precipitation is probably the main factor governing P immobilization.

With regard to the acid soil with high organic matter content (Table 1), it is an acid soil with very low plant-available P, very low N, and normal content of Mg, Fe, Zn, and Mn. It shows negligible content of Ca and CaCO₃, very high content in sand, high content in silt, and low clay content. It contains some residual and CBD-P and a relatively large percentage of NaOH-P (Table 1). This is probably due to the very high content of organic matter. This fact indicates that in this soil, the adsorption on mineral surfaces and organic matter could be the main factor governing P immobilization.

Finally, the acid soil with low organic matter content (Table 1) is a soil with low plant-available P, very low nutrient content (N, K, Mg, Cu, Na, Zn, and Ca) except for Fe, and very low content of organic matter. It shows a very high content in clay and silt and low sand content. Thus, it constitutes a very poor soil.

Plant Material and Plant Growth Conditions. Wheat plants were grown in alkaline and acid soils, in a glasshouse with a temperature range of 24/18 °C day/night and a relative humidity of 40–60% for 45 days. Briefly, 50 g of perlite and 500 g of soil were mixed in a mixer device for 5 s at the maximum power with SSP or CSPs at the optimized dose of 30 mg P/kg soil (this dose was calculated from a previous dose–response study employing several concentrations of P added to the different soils: 5–100 mg P/kg soil).

Ten plants of wheat (*Triticum aestivum* L.) cv. 'Fiel' germinated in a chamber (22 °C and 80% humidity for 4 days) were planted to a depth of 1 cm in each pot. Then the pots were filled to field capacity with a urea and KCl solution (NK solution) to obtain 200 mg/kg soil N and 200 mg/kg of soil K. The treatments were a control (without P added), SSP, and the four CSPs. Each treatment was carried out in triplicate with 10 wheat plants per pot. During the experiment, the pots were watered to field capacity by weighing the pots every 4 days and irrigated with the NK solution every 15 days to reconstitute nutrients. Finally, after 45 days of growth, the pots were harvested and plant yield and P concentration in shoots were determined. 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 (6.5 mL) and H₂O₂ (2 mL) and digested in a microwave oven (Milestone-Ethos) to determine P by ICP-OES spectrometry (Thermo Scientific; iCAB 6000 series). Dry matter was calculated by the difference between the shoot dry matter corresponding to fertilizer application and the shoot dry matter corresponding to the control without added P.

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

RESULTS AND DISCUSSION

Differences between the Physicochemical Properties of SSP and CSP. Chemical Analysis. The most important differences between the chemical analysis of SSP and the CSP made employing HA1 (CSP1) were related to both water and neutral ammonium citrate solubility of mineral elements (Table 2). Thus, CSP1 presented an increase in the water and ammonium citrate solubility of P, Ca, Mg, Fe, and S (Table 2). These results were obtained in three independent experiments. In principle, this increase might be related to the dispersant effect of the humic acid present in the reaction, which may affect organic particle aggregation in the ground rock phosphate matrix. In this sense, the complexing action of the humic acid may also favor the inclusion of some metals and sulfate anions (in this case through the oxygen atoms) in the macrostructure of monocalcium–phosphate–humic complexes.²⁵

Molecular Modeling. To explore the theoretical chemical viability of stable structures of complexes between monocalcium phosphate (Ca(H₂PO₄)₂) and a model-binding site of humic acids, we investigated the thermodynamical properties of these types of complexes using DFT quantum chemical methods. The thermodynamically stable structures for penta- and

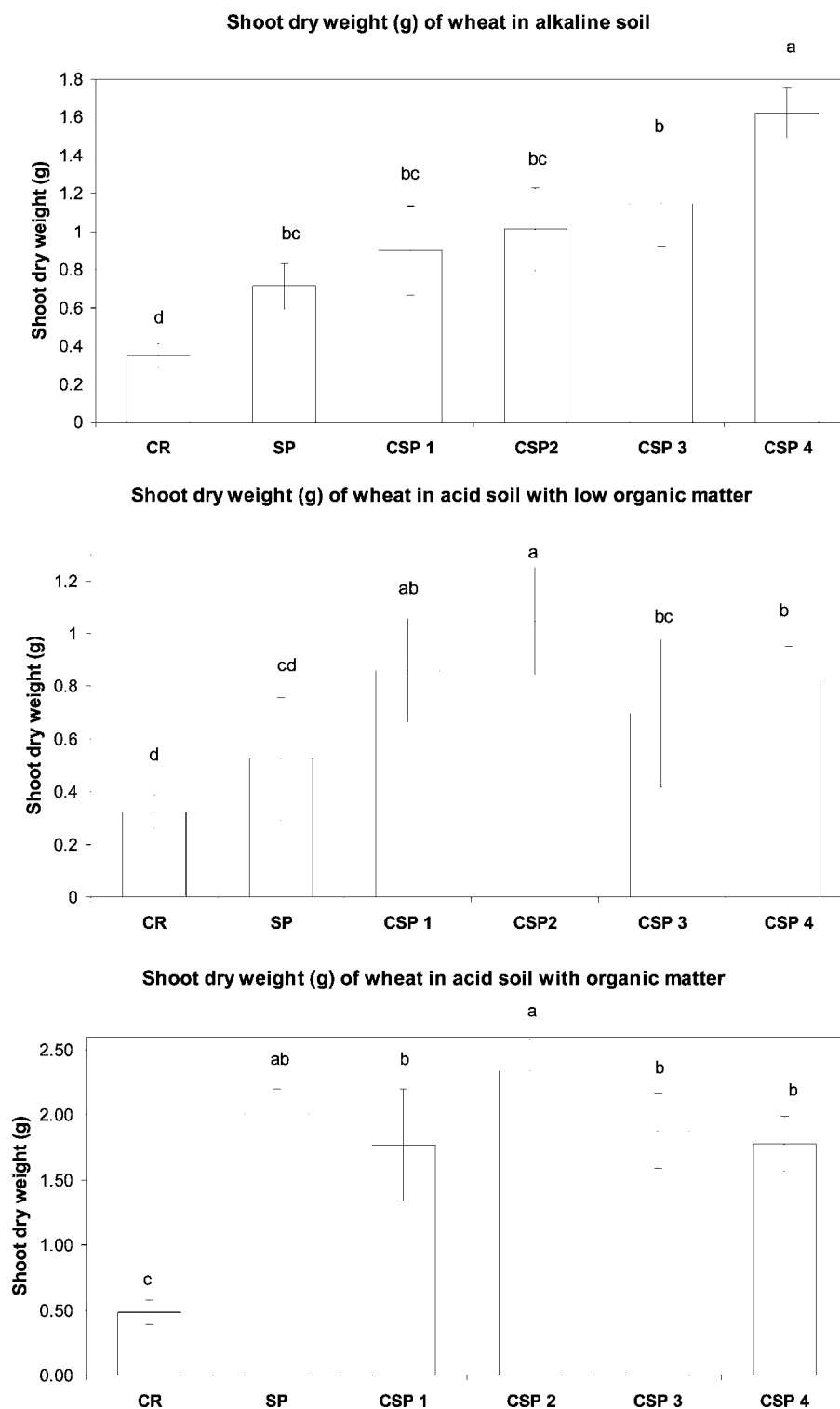


Figure 6. Results of shoot dry weight production for wheat plants cultivated in the different soil types and fertilized with several P fertilizers (different letters correspond to a significant difference for $p < 0.05$ in the posthoc Fisher test for statistical analysis of intermean significant differences).

hexacoordinated complexes obtained from this study are shown in Figure 2. The calculated Gibbs free energies for the complex formation in water are also compared in Figure 2. The lower value of Gibbs energy corresponded to the pentacoordinated complex, thus indicating that this complex is the most probable (it is the most stable) structure involved in the formation of these complexes during the reaction of the CSP.

XRD Analysis. The comparative spectra of the X-ray analysis are summarized in Figure 3. The results obtained did not show any significant difference between the spectrum corresponding to CSP1 and that corresponding to the physical mixture equivalent to CSP1 (data not shown). With regard to SSP and CSP1 spectra, the results showed some differences between SSP and CSP1. The solid arrows on the SSP spectrum indicate

the characteristic peaks of anhydrous CaSO_4 (anhydrite crystal system: orthorhombic). On the CSP1 spectrum, the intensity of those peaks was reduced when the reaction (or the physical mixture) occurred in the presence of 0.1% of organic C from HA1, and this effect was much more visible when the reaction took place in the presence of 0.6% of organic carbon from HA1 (CSP2S in Figure 3). This result suggests the presence of chemical modifications in the crystalline forms of the main salts in SSP with the introduction of the HA1, probably by the formation of new chemical links. The thick arrows on the CSP1 spectrum correspond to the formation of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (gypsum crystal system: monoclinic). This result indicated a potential increase in the solubility of the partially hydrated calcium sulfate obtained in the reaction for CSP production. This fact was confirmed by the increase of sulfur and calcium water solubility described in Table 2. However, all of these effects observed in XRD spectra seem to be associated with the simple presence of HA1, either in the CSP reaction or in the physical mixture SSP–HA1. This fact indicated that this analytical technique is not adequate to study the formation of monocalcium–phosphate–humic complexes.

Laser Confocal Microscopy. Laser confocal microscopy and fluorescence emission in the wavelength range of 670–685 nm after excitation with light of 663 nm make it possible to study the changes in the distribution and aggregation of the humic acid in the superphosphate matrix after the manufacture of CSP1. Fluorescence in this range of wavelength mainly corresponds to condensed aromatics moieties in humic acid and/or to an increase in the intermolecular aggregation of humic molecules. The fluorescence emission spectrum of each product (SSP, CSP1, and the physical mixture equivalent to CSP1) showed a decrease in intensity (quenching) for CSP1 with respect to SSP in this wavelength range (670–685 nm) (Figure 4). The physical mixture equivalent to CSP1 showed a slight quenching effect in this wavelength range that was lower than that of CSP1 (Figure 4A,B). The presence of a variation (quenching or increase) in the intensity of the peak reflects the chemical interaction between the fluorophore groups in HA1 (phenols, carboxylic groups in aromatic structures, etc.) and some cation in the mineral matrix.²⁶ In this context, the result obtained for CSP1 indicated the presence of chemical interactions between some functional groups in HA1 and the mineral matrix (probably Ca). These results were in line with those obtained using fluorescence imaging analysis of the image obtained from the laser confocal microscopy analysis of P fertilizers (Figure 4C). These images showed higher size fluorescence spots in the CSP1 matrix than those observed in SSP matrix and the physical mixture equivalent to CSP1 (SSP + HA1) (Figure 4). This fact indicated the presence of higher aggregation of humic structures in CSP1, thus suggesting the formation of organomineral complexes, which normally causes humic molecular aggregation due to the formation of inter- and intramolecular metallic bridges.

Solid-Phase ^{31}P NMR Analysis. SSP and CSP1, as well as the samples corresponding to CSP2, CSP3, and CSP4, were analyzed using solid-phase ^{31}P NMR. The solid-phase ^{31}P NMR spectra of SSP and CSP1–4 showed three characteristic peaks at -6.40 , -1.91 , and 0.99 ppm (Figure 5). When these spectra were compared with standards of monocalcium phosphate, dicalcium phosphate, and tricalcium phosphate, we were able to assign the first and second peaks to monocalcium phosphate and the third peak to tricalcium phosphate (data not shown). The presence of dicalcium phosphate was negligible in all

Table 3. Shoot Phosphorus Concentration of Wheat Plants Cultivated in Different Soils at Harvest^a

	P concentration in wheat shoots ($\mu\text{g P/g}$ dry shoot)		
	alkaline soil	acid soil with low organic matter	acid soil with high organic matter
control	1155 b	852 b	1203 b
SSP	1279 b	1004 b	1249 b
CSP1	1423 a	1433 a	1474 a
CSP2	1341 ab	1389 a	1389 ab
CSP3	1422 a	1383 a	1480 a
CSP4	1240 b	1480 a	1337 ab

^aDifferent letters correspond to a significant difference for $p < 0.05$ in the posthoc Fisher test for statistical analysis of intermean significant differences.

samples. In the solid matrix, the formation of MPHIC was reflected in an increase in the width of the first peak corresponding to monocalcium phosphate (Figure 5). This fact was in line with the results obtained by Riggle and von Wandruszka¹² from ^{31}P NMR analysis of phospho–metal–humic acid complexes via metal anchors. However, the physical mixture equivalent to CSP1 did not show any difference in the width of the first peak with respect to SSP in the ^{31}P NMR spectrum. It was noteworthy that the magnitude of this increase in the width of the first peak changed significantly depending on the humic acid used in the reaction. This fact could be related to the number of binding sites in HA samples and/or the degree of stability of the complex.¹² Anyway, these results proved the formation of monocalcium–phosphate–HA complexes in CSP samples.

Agronomical Test: Effect of SSP and CSP1–4 on the Growth and P Uptake of Wheat Plants Cultivated in Diverse Soil Types. The results concerning plant growth (shoot dry matter) and P shoot concentration are summarized in Figure 6 and Table 3.

Shoot dry weights of plants grown in the acid soil with organic matter were higher than those of plants grown in the alkaline–calcareous and acid–low organic matter soils. This fact is, in principle, quite logical as the presence of high contents of organic matter in the soil is normally associated with high potential soil fertility.

With regard to the effects linked to P fertilizer applications, the results obtained show that CSP treatments, ion average, presented higher performance than SSP in relation to either shoot dry weight or P shoot concentration for the three soils used in the study. Concerning the different CSPs, the results showed that their agronomical efficiency depended on the type of soil used in the experiment. As a consequence, these results indicated that, although CSP strategy seems to be efficient to enhance plant yield and (or) potential plant P availability compared to SSP, it is necessary to select the humic material to be used in CSP production to optimize CSP agronomical efficiency. Thus, for the alkaline soil, CSP3 and CSP4 were the most efficient treatments, involving significantly higher plant yields than the other tested P fertilizers. However, with regard to the acid soils, CSP2 yielded the highest shoot dry weight. The other CSPs (CSP1 and CSP2 in the alkaline soil and CSP1, CSP3, and CSP4 in the acid soils) did not present significant differences in shoot dry weight yields compared with SSP. Nevertheless, higher P shoot concentrations than obtained for SSP were also observed in these cases. This fact suggested that this increase in P shoot concentrations observed for all

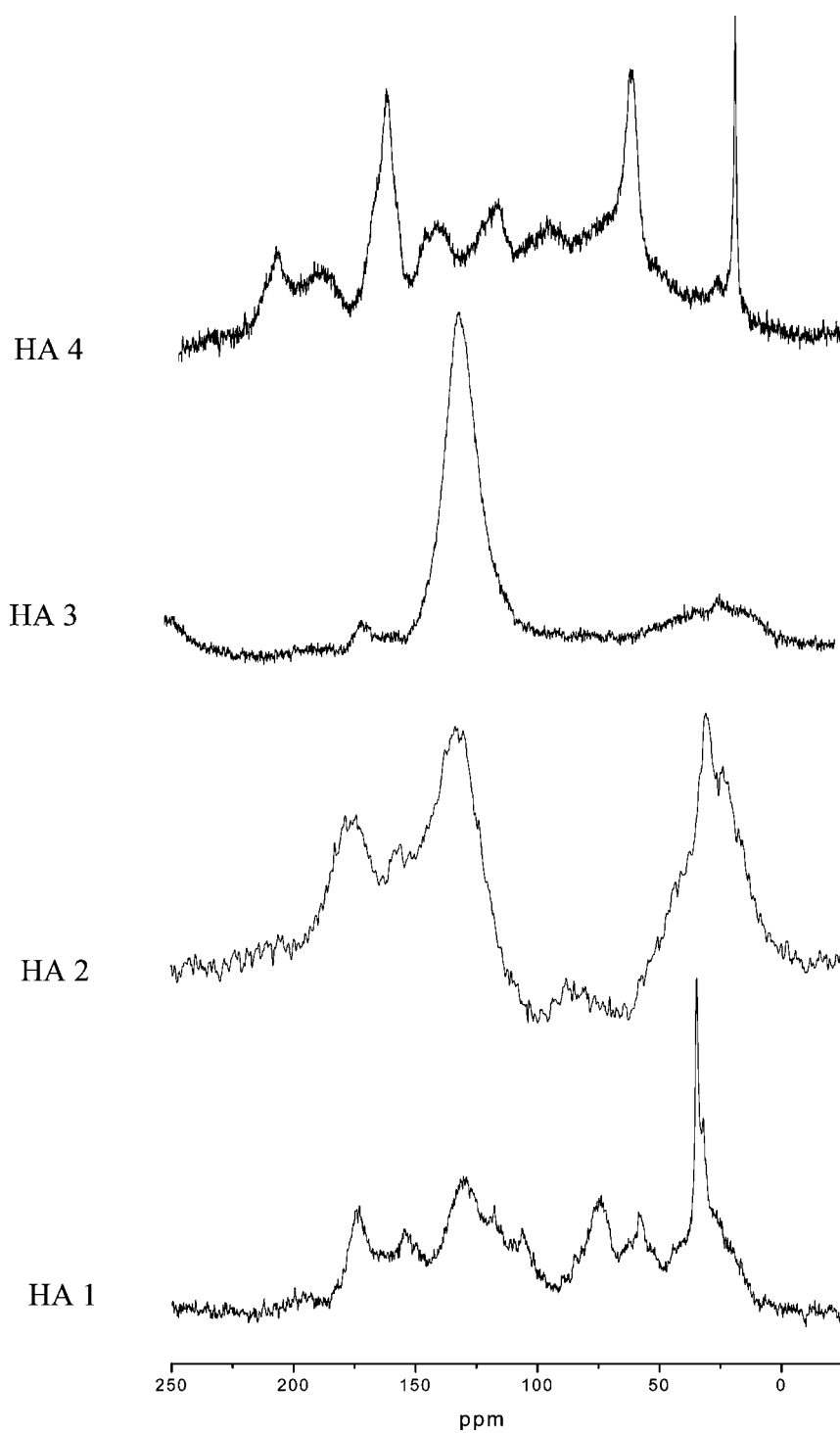


Figure 7. ^{13}C NMR spectra for the different humic acids employed in the synthesis of CSP1–4 (HA1–4).

Table 4. Relative Abundances of Different Carbon Types for the Different Humic Acids (HA1–4) Employed in the Production of CSP1–4, Measured by ^{13}C NMR

	region					
	alkyl C 0–45 ppm	O-alkyl C 45–110 ppm	aromatic C 110–160 ppm	phenolic C 140–160 ppm	carboxylic C 160–190 ppm	carbonylic C 190–230 ppm
HA1	21.2	32.01	23.7	9.11	10.8	3.18
HA2	27.4	12.6	33.7	12.8	10.1	3.4
HA3	13.8	3.2	74.1	5.4	3.2	0.3
HA4	5.26	41.2	10.9	20	11.9	10.7

CSP treatments, including those that did not present differences with respect to SSP, would probably trigger higher yields than that of SSP over time. Likewise, the results concerning P shoot concentrations indicated that CSP treatments were associated with higher soil plant P availability, probably as a result of the presence of MPHIC that prevented P from soil fixation. This conclusion was supported by complementary results obtained in P fertilizer–soil incubation experiments, related to potentially plant-available P fraction in soil (data not shown).

Taken together, these results indicated that CSP strategy was very efficient to prepare SP-type fertilizers with enhanced potential P availability for plants and plant nutritional properties.

Finally, to study the relationships between the structural features of the different humic acids employed to prepare CSP samples and the effect of CSP products on plant growth and P plant uptake, elemental analysis and ^{13}C NMR spectra for HA1–4 were carried out. Elemental compositions of HA1–4 were as follows: 40.10% of C, 4.72% of H, and 2.17% of N for HA1; 41.57% of C, 3.50% of H, and 1.21% of N for HA2; 44.47% of C, 2.61% of H, and 1.20% of N for HA3; and 42.17% of C, 3.21% of H, and 1.52% of N for HA4.

The ^{13}C NMR spectra were divided into chemical shift regions assigned to the following classes of chemical groups: alkyl C (0–45 ppm), O-alkyl (45–110 ppm), aromatic C (110–160 ppm), phenolic C (140–160 ppm), carboxyl C (160–190 ppm), and carbonyl C (190–230 ppm), respectively (Figure 7; Table 4). The relative intensities of these regions were determined by the integration of the corresponding peak areas.^{27–29} The high percentage of functional groups (phenolic, carboxylic, and carbonylic C) in HA4 and HA3 involved an important potential chemical reactivity for this organic material in the formation of phosphate–Ca–humic complexes. On the other hand, HA3 presented the highest aromatic content, which is normally associated with high hydrophobicity. This fact might also affect calcium–phosphate–humic acid interaction during CSP reaction. In this context, when we studied the above-mentioned agronomical effects of each CSP with respect to those of the other CSPs and SSP, we observed some HA structure–CSP agronomical effect relationships. Thus, the high presence of oxygen-containing functional groups and aromatic C in HA4 and HA3, respectively, could be involved in their high fertilizer efficiency in the alkaline soil (Table 3). This effect might derive from a high stability of MPHIC in these CSP matrices that, in turn, enhanced P protection against absorption and/or precipitation in soil. In this sense, Pearson's correlation study of data showed a negative correlation between aliphatic C content and shoot dry weight production in the alkaline soil (Table 5). With regard to the results for acid soils, the humic acid employed to prepare the most efficient CSP (CSP2; HA2) presented the highest alkyl C concentration, along with a significant aromatic degree, which was lower than the aliphatic degree, and high phenol C. These results suggested that the presence of a ramified structure (mainly aliphatic), containing isolated phenol–carboxylic sites, in the HA general structure affected specific MPHIC features that might be involved in CSP agronomical efficiency for these soil types. However, this hypothesis was not reflected in the Pearson's correlation data study (Table 5), probably because this study does not consider qualitative structural features, such as the functional group distribution in the whole humic structure. Finally, it was also noteworthy that, in general, correlation coefficient values were higher for the acid soil with low organic matter than for the acid

Table 5. Pearson's Correlation ($p < 0.1$) among Organic Carbon Types for Each HA and Wheat Shoot Dry Weight Obtained from the Pot Study Using the Different CSPs

	shoot dry weight		
	alkaline soil	acid soil with low organic matter	acid soil with high organic matter
alkyl C	−0.97	0.45	0.54
O-alkyl C	0.59	0.29	−0.44
aromatic C	−0.29	−0.66	0.08
phenolic C	0.69	0.54	0.05
carboxylic C	0.59	0.63	0.39
carbonylic C	0.80	0.35	−0.17

soil with high organic matter. This fact indicated that humic acid features and reactivity seem to be agronomically more relevant in soils with low potential fertility.

Conclusions. The results obtained in this study demonstrated the efficiency of CSP-based fertilizers to enhance available P for plants cultivated in P-deficient soils. This fact had positive consequences increasing both plant growth and P plant uptake. This enhancement in agronomical efficiency associated with CSP-based fertilizers is possibly related to the formation of stable monocalcium–phosphate–humic complexes during CSP preparation.

However, the results presented here also show that the physicochemical features of the humic acids involved in CSP reaction affected CSP agronomical efficiency depending on the properties of the soil used for crop production.

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ABBREVIATIONS USED

SP, superphosphate; SSP, single superphosphate; CSP, complexed superphosphate; MPHIC, monocalcium–phosphate–humic complexes; PMAH, phosphorus metal humic complexes; HA, humic acid; NaOH, sodium hydroxide; CB, citrate bicarbonate extraction; CBD, citrate bicarbonate dithionite extraction.

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