Size Distribution, Complexing Capacity, and Stability of Phosphate–Metal–Humic Complexes

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Size distribution, maximum complexing ability, and stability constants for phosphate–metal–humic (PO_4^{3-}–M–HA) complexes involving two trivalent (Fe and Al) and five divalent metal (M) bridges (Zn, Cu, Mn, Ca, and Mg) were investigated at the pH values 4, 6, and 8. Results highlighted the existing competition between metal–humic acid (M–HA) aggregation and the formation of PO_4^{3-}–M–HA complexes. However, the fact that only a very low fraction of complexed metal is involved in PO_4^{3-} fixation seems to be related to the existence of specific electronic and/or steric requirements in the binding site in the metal–humic complex. The importance of the ionic form of phosphate ($H_2PO_4^-$ or HPO_4^{2-}) and the involvement of phenolic and especially carboxylic groups in the phosphate binding are discussed. Finally, the order of stability obtained for PO_4^{3-}–M–HA complexes was similar to that of M–HA complexes. This result suggests that PO_4^{3-}–M–HA might play a significant role in the dynamics of phosphorus in certain soil types.

KEYWORDS: Humic substances; metal–humic complexes; phospho–metal–humic complexes; metal bridges; ultrafiltration; anion-exchange resin

INTRODUCTION

A certain number of studies have suggested that one of the mechanisms employed by humic substances (HS) to increase plant-available soil phosphorus (P) concentration is the formation of soluble phosphate–metal–humic (PO_4^{3-}–M–HA) complexes (*1–5*). These complexes play a beneficial role preventing soil P precipitation (thus avoiding the formation of calcium phosphates in calcareous soils or aluminum and iron phosphates in acidic soils) (*6*).

However, only two recent studies deal with the formation and stability of these complexes. First, Riggle and von Wandruszka (*7*) studied the stability of the complexes formed by different metal–humic complexes and phosphate, using a cobalt-wire phosphate selective electrode at pH 7–8. They found stability constant values in the same order of magnitude as those found for metal complexation by HS. Second, Guardado et al. (*8*) developed a methodological approach for the study of phosphate (PO_4^{3-}) interaction with metal–humic complexes at three different pH values (4, 6, and 8) by means of a bicarbonate-conditioned anion resin. From the study of the stability and the maximum binding ability (MBA) for the phosphate–iron humic complex interaction, it was concluded that only a relatively small proportion of HS-complexed Fe was involved in phosphate fixation (from 10 to 20%, assuming 1:1 Fe/P stoichiometry). Two main causes could explain these results: (a) the formation

of stable HS–Fe–HS aggregates through the formation of Fe bridges, which reduce the concentration of complexed Fe with free coordination bonds available for phosphate fixation, and (b) the existence of a restricted group of Fe complexing sites in HS with the electronic configuration necessary to form stable phosphate–Fe–HS bonds.

The aim of this work is to investigate these two hypotheses. To begin with, the size distribution changes associated with HS metal complexation and phosphate fixation in metal–humic complexes were analyzed to investigate molecular aggregation in these molecular systems. To this end, two different methods, which were expected to show different molecular aggregation behaviors, were employed to obtain PO_4^{3-}–metal–HS complexes. Finally, the relationship between the physicochemical characteristics of metal–humic acid (M–HA) binding sites and their involvement in phosphate fixation were investigated through the analysis of the MBA and the stability of PO_4^{3-}–M–HA complexes involving two trivalent (Fe and Al) and five divalent metal bridges (Zn, Cu, Mn, Ca, and Mg). Metal selection was based on two main criteria: (a) their ability to form humic complexes with different physicochemical properties (*9*), and (b) their potential role in PO_4^{3-} soil dynamics in both acid (Fe, Al, Zn, Cu, and Mn) and calcareous soils (Ca and Mg).

MATERIALS AND METHODS

Humic Acid, Metal–Humic, and Phosphate–Metal–Humic Complex Preparation. The model humic acid (HA) employed in the different experiments was one extracted from leonardite obtained from

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Phosphate–Metal–Humic Complexes

J. Agric. Food Chem., Vol. 55, No. 2, 2007 **409**

Czechia. We selected this humic source for two main reasons: (i) the structural similarities between this HA with different soil humic acids and IHSS standards, and (ii) the availability of this material.

HA was first purified using the International Humic Substances Society (IHSS) methodology without including the HF−hydrochloric acid (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 being stirred at 25 °C in darkness for 48 h, 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 HCl 6 M up 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 washing the humic acids with water to eliminate Cl^- contamination, we freeze-dried them. 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.

M−HA complexes were prepared as described in (*9*). Briefly, to reach a 1.8 mmol metal/g C ratio, Fe(III), Al(III), Zn(II), Cu(II), Mn-(II), Ca(II), and Mg(II) nitrate salts were dissolved in water and slowly dropped into 200 mL of the supernatant containing HA, under vigorous stirring and continuous pH control (*8, 9*) with NaOH. The solution was diluted to 500 mL, stirred for 14 h, and then centrifuged for 15 min at 7000g to separate M−HA complexes in solution from precipitated M−HA complexes and metal hydroxides. When preparing Ca and Mg complexes, free metal in solution was separated using a strongly acidic cation exchanger (Amberlite IR-118, Sigma). Both resin and free metal were eliminated after centrifugation for 15 min at 7000g. Finally, metal and carbon contents in solution were analyzed.

The PO_4^{3-}−M−HA complexes were prepared using two different methods:

Method 1: this synthesis pathway involves two steps. Step one consists of the formation of stable M−HA complexes at pH 8−9, and step two consists of the reaction of PO_4^{3-} with the M−HA complex at different pH values. This method has been used in previous studies (*8*). This method involves the addition of 0.5 mM H_3PO_4 aliquots containing the necessary quantity of PO_4^{3-} to achieve specific metal/P molar ratios, to each M−HA complex at pH 8−9.

Method 2: this synthesis pathway also involves two steps. The first step involves mixing HA with PO_4^{3-} at a specific pH, and the second step consists of the addition of the metal but keeps pH values constant. Thus, in this method a mixture of 0.5 mM H_3PO_4 and HA at the final pH value was followed by the addition of the necessary quantity of dissolved metal nitrate to achieve the same specific metal/P molar ratios as in the first pathway. This latter method is expected to display less molecular aggregation, since PO_4^{3-}−M−HA bonds compete with the formation of HA−M−HA bonds. In fact, other studies describe the use of sodium pyrophosphate to inhibit molecular aggregation in the formation of Fe−HS complexes, probably through the formation of P_2O_7−Fe−HS complexes (*10*).

In both cases, pH was kept at the final values 4, 6, or 8 with 0.5 M HNO_3 or 0.5 M NaOH, and ionic strength values stayed in the 0.01−0.02 M interval. Subsequently, solutions were diluted up to 50 mL with pure water and stirred for 14 h at 22 °C. Once the reactions were completed, the solutions were centrifuged for 15 min at 7000g. Finally, the total C, P, and metal content in solution was analyzed. The concentration in solution of P (as PO_4^{3-}) complexed in M−HA by methods 1 and 2 was measured using the HCO$_3$–R methodology described below.

Analysis of PO_4^{3-} Fixed in Metal−HA Complexes. The analysis of fixed PO_4^{3-} was carried out as described in (*8*). Briefly, the Amberjet 4400 anion OH$^-$ exchange resin (Sigma) conditioned in its HCO$_3^-$ form (HCO$_3$–R) to reach 1.8 mmolc g^{-1} of exchange capacity and 30 μmol PO_4^{3-} g^{-1} of PO_4^{3-} fixation capacity was used in the experiments. Once conditioned in 1 M NaHCO$_3$, 0.9 g of HCO$_3$–R was added to 10 mL of the reaction solution containing a total P content of 50 mg L^{-1} and pH values of 4, 6, and 8. After 30 min, the solutions were filtered and PO_4^{3-} content in solution was analyzed. The PO_4^{3-} fixed by M−HA was calculated by subtracting the P in solution in the reactions from the free P in solution in the blank. This blank (PO_4^{3-} and HCO$_3$–R only, with the same pH and ionic strength conditions as the reactions) indicates the amount of free PO_4^{3-} that is not fixed by HCO$_3$–R in these ionic strength conditions. All analytical determinations were carried out with three replications.

Size Distribution Analysis. The influence of metal complexation and phosphate fixation in M−HA complexes on the size distribution of the humic systems was evaluated using ultrafiltration for the PO_4^{3-}−M−HA complexes involving Fe and Zn and obtained using methods 1 and 2. PO_4^{3-}−M−HA complexes were pretreated with HCO$_3$–R to eliminate free PO_4^{3-}. Samples of the different systems used in the study were diluted to 750 mg L^{-1} of organic carbon and fractionated using a UF cell (Amicon 8200) under inert atmosphere (N$_2$). Five polyether-sulfone membranes (Millipore) of 0.22 μm, 100, 50, 10, and 5 kDa molecular weight cutoffs (MWCO) were used. First, the different molecular systems were filtered through the membrane with the largest pore size (0.22 μm). The process was halted once the volume of the retained solution had been reduced to 5−10% of the initial solution volume. The filtrate was introduced in the UF cell and filtered through another membrane of lower MWCO. This operation was repeated for each membrane. The mass percentage corresponding to each MWCO range was evaluated by measuring the difference in P, Fe, or Zn and total organic carbon in each fraction.

Study of the Stability of PO_4^{3-}−M−HA Complexes. The pH-dependent stability of PO_4^{3-}−M−HA complexes in solution was estimated using an apparent stability constant (K) calculated by the Scatchard method (*8, 11*):

$$(P_{TC}/MBA)/P_{TF} = K - (P_{TC}/MBA)K$$

or

$$\theta/P_{TF} = K - \theta K$$

where P_{TF} is the total free P at the equilibrium, P_{TC} is the total fixed P, and θ is the sites bound/MBA (P_{TC}/MBA) ratio, where K was obtained from the value of the slope in θ/PTF versus θ plot.

The MBA value was estimated by extrapolation of the straight-line segment corresponding to binding at high PO_4^{3-} ion saturation in a P_{TC} versus P_{TC}/P_{TF} plot.

To obtain P_{TC} and P_{TF} values, the reaction of aliquots of the M−HA complex (method 1) (or the equivalent concentrations of HA and metallic nitrate for method 2) with different P (as PO_4^{3-}) concentrations (in the range of 2.5−20 mg L^{-1} of P) were carried out as described above for the preparation of PO_4^{3-}−M−HA complexes (*8*). The analytical determinations were carried out with three replications. MBA and log K values were obtained by linear regression analysis.

An example of log K determination is presented in **Figures 1** and **2**.

Elemental Analysis. The concentration of P (as PO_4^{3-}) in solution for the determination of MBA values was analyzed using the green malachite method (*12*). All other P analyses were performed by ICP-OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL). The C content in solution was analyzed using elemental analysis (LECO CHN 2000 and Shimadzu TOC-5050), and the metal content in solution was determined using ICP-OES spectrometry.

Statistical Analysis. The statistical studies were carried out with the software SPSS v. 13 and Excel 2003. Comparisons between all MBA and log K values were performed by the direct application of the $p < 0.05$ confidence interval (CI) definition (*13*):

$$\bar{x} - 1.96 \cdot SE \leq \mu \leq \bar{x} + 1.96 \cdot SE$$

where SE is the standard error value of the linear regression and $\bar{x}$ is
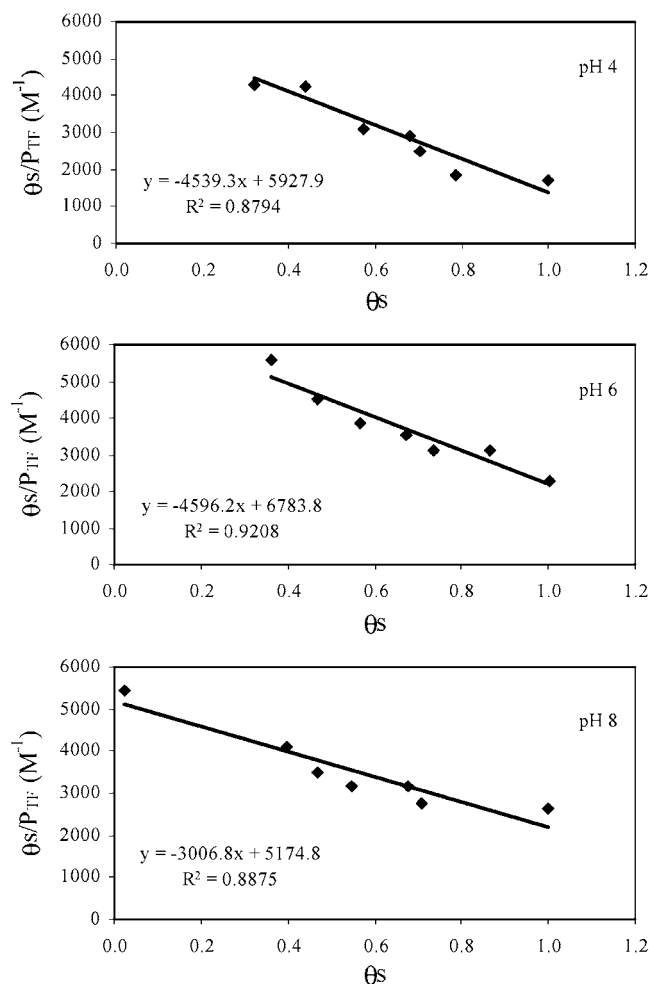
Figure 1. Calculation of the apparent stability constant for the fixation of phosphate in Ca–humic complexes at different pH (method 1) using the Scatchard model.

Figure 2. Calculation of the apparent stability constant for the fixation of phosphate in Cu–humic complexes at different pH (method 2) using the Scatchard model.
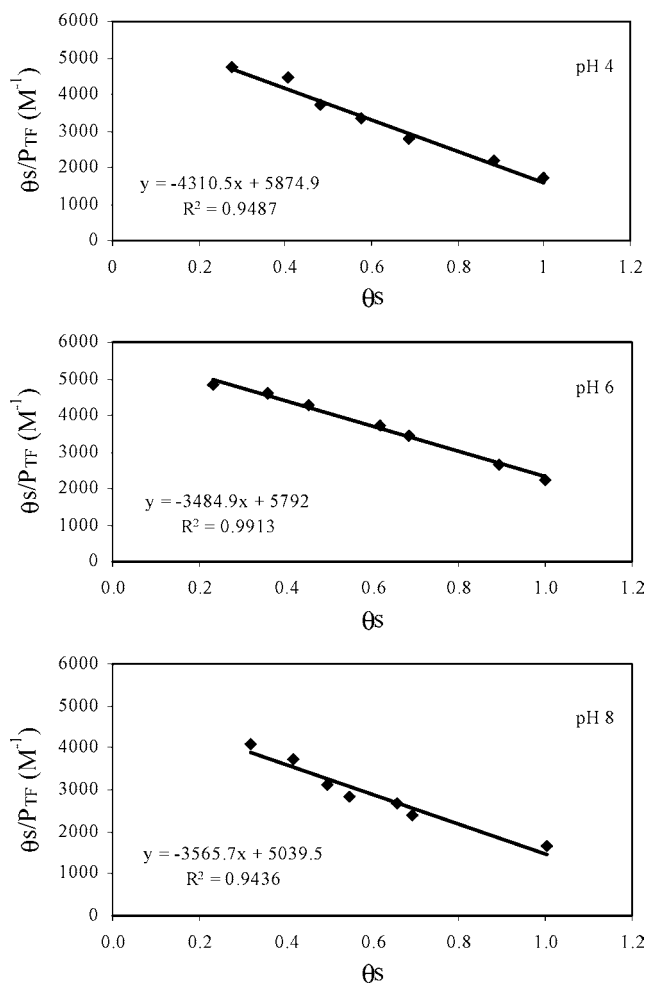
the mean value. Therefore, when a mean value was found inside the CI of another, there were no significant differences between them.

RESULTS AND DISCUSSION

Influence of Molecular Aggregation in the Formation of $PO_4^{3-}-M-HA$ Complexes. Figures 3 and **4** show that the HA size distribution varied significantly as a function of pH. At pH 4, 80% of the total C was concentrated in the 0.22 μm to 100 kDa fraction, whereas at pH 6, 60% was found in the 100−50 kDa fraction, indicating that acid pH enhanced aggregation (pH 6) and precipitation (pH 4) through the formation of intermolecular links such as hydrogen bonds or hydrophobic interactions (*11*). On the contrary, basic pH promoted intermolecular disaggregation, probably due to electrostatic repulsion between ionized acid functional groups (*11*). In fact, at pH 8, the major HA fraction corresponded to the 10−5 kDa size interval (60% of the total C).

At pH 8−9 and 6, the formation of Fe−HA and Zn−HA complexes (**Figures 4**) was accompanied by a clear molecular aggregation that was reflected in the increase in the C corresponding to that of the 0.22 μm to 100 kDa fraction (around 60% of total C). At pH 4, however, a slight dissagregation accompanied the formation of both complexes (**Figures 3** and **4**).

Concerning the formation of $PO_4^{3-}-M-HA$ complexes, both methods 1 and 2 were associated with a disaggregation effect for all studied pH values, except in the case of the $PO_4^{3-}-$

Zn−HA complex at pH 6 (**Figures 3** and **4**). These results suggest that, in both forms of synthesis, the formation of stable $PO_4^{3-}-M-HA$ bonds was associated with a disruption of a certain number of HA−M−HA bonds. These results are consistent with the conclusions of other studies carried out using pyrophosphate instead of phosphate (*10*).

In principle, the lack of significant differences between the molecular size distributions corresponding to $PO_4^{3-}-M-HA$ complexes obtained using methods 1 and 2 indicates that molecular aggregation is not the main cause to explain the very low concentration of HA complexed metal that seems to be involved in PO_4^{3-} fixation (*8*). Other factors, probably related to the physicochemical characteristics of M−HA binding sites, must be involved in the whole process.

Influence of the Physicochemical Characteristics of M−HA Binding Sites on the Formation of Stable $PO_4^{3-}-M-HA$ Complexes. *The Variation of MBA Values as a Function of Metal Bridge, pH, and Synthesis Method.* The MBA values for the total concentration of binding sites (MBA$_T$) are presented in **Table 1**; those for the highest stability sites (MBA$_H$) are presented in **Table 2**.
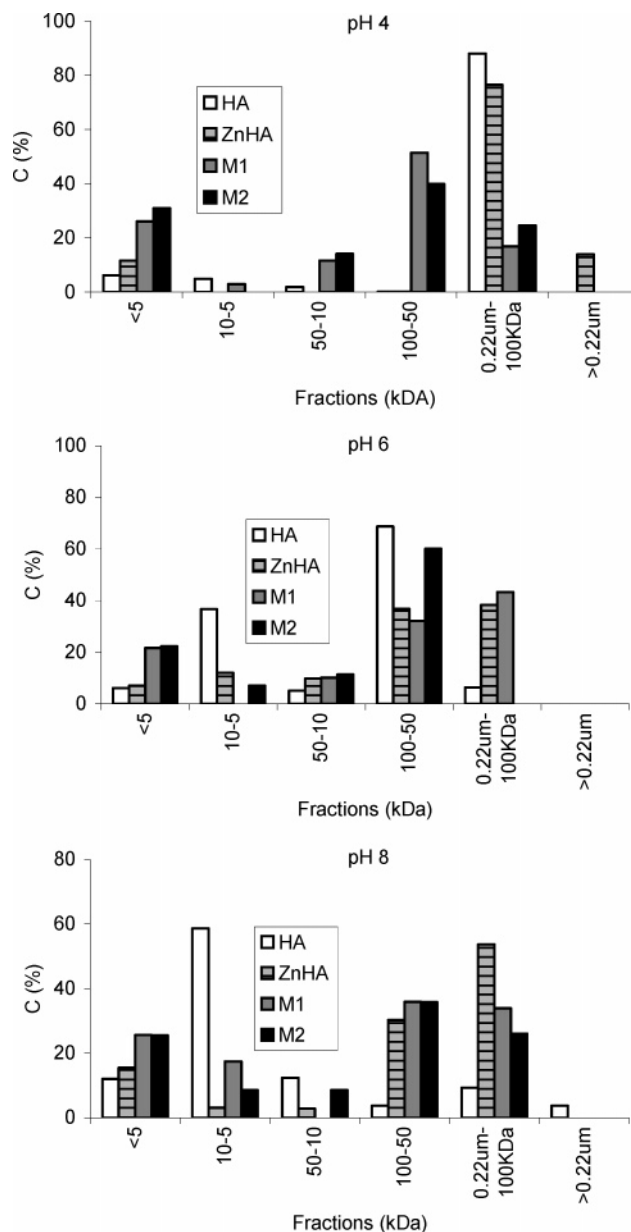
Data in **Table 1** show that MBA$_T$ values for PO_4^{3-} fixation were low when compared with the total HA complexed metal. Thus, assuming the stoichiometry PO_4^{3-}/M, 1:1 in $PO_4^{3-}-M-HA$ complexes, only 15% of the total complexed metal would be involved in PO_4^{3-} fixation. These results are consistent with previous studies on $PO_4^{3-}-Fe-HA$ complex formations (*8*).

Phosphate–Metal–Humic Complexes

J. Agric. Food Chem., Vol. 55, No. 2, 2007 **411**
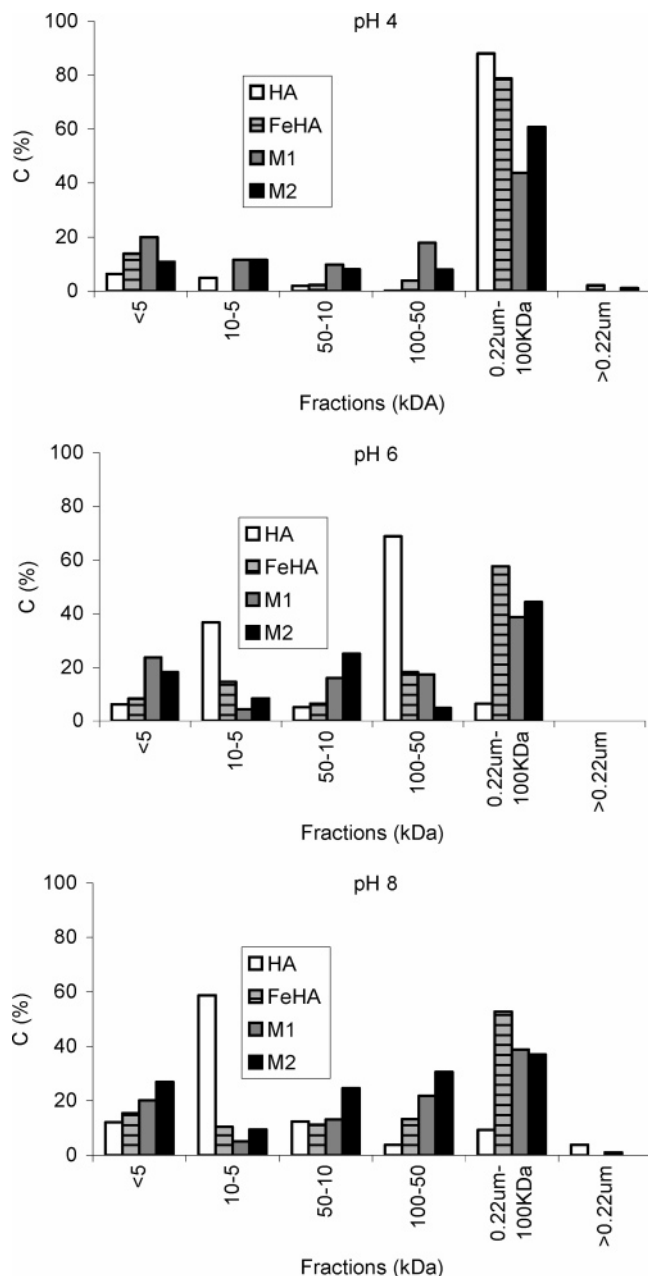
Figure 3. C distribution as a function of size in the HA, Fe–HA, and PO_4^{3-}–Fe–HA complexes obtained using methods 1 (M1) and 2 (M2).

Figure 4. C distribution as a function of size in the HA, Zn–HA, and PO_4^{3-}–Zn–HA complexes obtained using methods 1 (M1) and 2 (M2).

As for the influence of metal and pH value, both MBA_T and MBA_H values indicated the existence of three groups of $PO_4^{3-}-$M$-$HA complexes, regardless of the type of synthesis involved. Thus, the highest MBA values were obtained for complexes involving Fe and Al (among them, the order depended on the pH value). The second group was formed by complexes involving Cu, Zn, and Mn. Finally, the third group included complexes involving Ca and Mg. Therefore, the MBA general order would be: Fe, Al > Cu, Zn, Mn > Ca, Mg.

To better understand how MBA values are affected by the different synthesis methods, we must bear in mind that method 1 involves the formation of M$-$HA complexes at basic pH (8$-$9). In these conditions, the majority of acid groups (both carboxylic and phenolic groups) would be ionized and available to take part in phosphate fixation. On the contrary, method 2 involves pH-dependent acid group participation due to the formation of M$-$HA complexes at different pH values (4, 6, and 8). Thus, at pH 4 and 6, the main ionized functional groups

Table 1. Maximum Binding Ability for Phosphate (MBA_T) in PO_4^{3-}–M–HA Complexes Obtained Using Methods 1 (M1) and 2 (M2)

	M1				M2		
	$MBA_T/[HA]^a$				$MBA_T/[HA]^a$		
pH	4	6	8	pH	4	6	8
Fe	0.192	0.179	0.140	Fe	0.261	0.259	0.251
Al	0.181	0.219	0.192	Al	0.138	0.184	0.178
Ca	0.061	0.073	0.078	Ca	0.033	0.046	0.048
Mg	0.032	0.040	0.040	Mg	0.038	0.072	0.080
Cu	0.083	0.101	0.085	Cu	0.040	0.055	0.111
Mn	0.093	0.120	0.131	Mn	0.056	0.052	0.137
Zn	0.109	0.160	0.128	Zn	0.075	0.100	0.212

^{a} MBA values refer to the HA quantity and are expressed as mmol PO_4^{3-} g^{-1} of HA.

would be the carboxylic groups, whereas at pH 8, both carboxylic and phenolic groups would be ionized.

Regarding MBA_T values, differences between methods 1 and 2 varied as a function of pH. Generally, at pH 4 and 6, MBA_T

Table 2. Maximum Binding Ability for Phosphate Corresponding to the Binding Sites of Higher Stability (MBA$_H$) in PO$_4^{3-}$–M–HA Complexes Obtained Using Methods 1 (M1) and 2 (M2)

	M1				M2		
	MBA$_H$/[HA][a]				MBA$_H$/[HA][a]		
pH	4	6	8	pH	4	6	8
Fe	0.052	0.046	0.028	Fe	0.060	0.078	0.083
Al	0.044	0.059	0.031	Al	0.053	0.091	0.124
Ca	0.014	0.018	0.023	Ca	0.024	0.021	0.040
Mg	0.016	0.025	0.015	Mg	0.017	0.027	0.034
Cu	0.025	0.033	0.020	Cu	0.031	0.050	0.063
Mn	0.023	0.012	0.033	Mn	0.022	0.042	0.088
Zn	0.026	0.040	0.028	Zn	0.039	0.050	0.043

[a] MBA values refer to the HA quantity and are expressed as mmol PO$_4^{3-}$ g^{-1} of HA.

Table 3. Apparent Stability Constant Values for the Binding Sites of Higher Stability (log K_H) for PO$_4^{3-}$–M–HA Complexes Obtained Using Methods 1 (M1) and 2 (M2)

	M1				M2		
	log K_H				log K_H		
pH	4	6	8	pH	4	6	8
Fe	3.27	3.98	4.09	Fe	4.13	3.55	4.07
Al	4.03	4.06	3.92	Al	3.33	4.05	4.10
Ca	3.66	3.66	3.48	Ca	3.47	3.65	3.07
Mg	3.51	3.61	3.56	Mg	3.17	3.46	3.43
Cu	3.79	3.65	3.55	Cu	3.63	3.54	3.36
Mn	3.02	4.05	3.94	Mn	3.05	3.05	3.11
Zn	3.78	3.36	3.57	Zn	3.53	3.69	3.69

values corresponding to method 1 were significantly higher than those related to method 2 (p < 0.05), except in the case of PO$_4^{3-}$–Fe–HA complexes. This general trend might be the consequence of different metal binding sites available for PO$_4^{3-}$ fixation at acid pH: in method 1, all acid functional groups (carboxylic and phenolic) were ionized and available for metal complexation in the formation of M–HA, whereas in method 2, only carboxylic groups would be totally available. This is also supported by the fact that, for method 2, MBA$_T$ values at pH 8 were significantly higher than those corresponding to pH 4 and 6.

The MBA$_T$ values corresponding to method 2 at pH 8 were significantly higher than those corresponding to method 1. This behavior is not necessarily due to different functional group participation since M–HA complexes were obtained at basic pH in both methods. It could be related to competition between the formation of HA–M–HA aggregates and PO$_4^{3-}$–M–HA complexes during the reaction. Even when no clear differences were found in method 1 and method 2 aggregation patterns, differences in the steric availability of binding sites associated with both methods could be significant. Thus, the synthesis pathway involved in method 2 may favor the formation of PO$_4^{3-}$–M–HA bonds in detriment of HA–M–HA bonds.

As indicated above, MBA$_T$ values for the PO$_4^{3-}$–Fe–HA complexes obtained by method 2 were significantly higher that those for method 1 (**Table 1**). Likewise, for these complexes, method 2 showed no pH dependence, whereas in method 1 MBA$_T$ values at acid pH were significantly higher that those for basic pH (**Table 1**). These results indicate that the main functional groups involved in PO$_4^{3-}$ fixation in Fe–HA complexes were available at acid pH, suggesting that carboxylic groups play an important role. These results were in line with other results obtained in studies of Fe complexation by different humic substances (*14*).

Finally, in method 1, MBA$_T$ values corresponding to pH 6 and 8 were significantly higher than those for pH 4. This result could be the consequence of the different PO$_4^{3-}$ ionic species present as a function of pH (H$_2$PO$_4^-$ at acid pH and HPO$_4^{2-}$ at basic pH).

With respect to MBA$_H$ values, differences between methods 1 and 2 were of less importance (**Table 2**). When present, MBA$_H$ values for method 2 were higher than those for method 1, indicating that the HA–M–HA//PO$_4^{3-}$–M–HA competition may be influencing the whole process. Only Zn, Cu, and Ca PO$_4^{3-}$–M–HA complexes at pH 4, Al, Cu, and Zn PO$_4^{3-}$–M–HA complexes at pH 6, and Fe, Al, and Mn PO$_4^{3-}$–M–HA complexes at pH 8 escaped the general tendency. Likewise,

the fact that the differences in MBA$_H$ values associated with each M–HA complex as a function of pH were not significant (method 2) or showed higher values at acid pH (method 1) indicates that the functional groups involved in PO$_4^{3-}$ fixation in the interaction with highest stability were already available at acid pH (presumably carboxylic groups).

Another factor that could also influence MBA values as a function of pH, principally in method 2, is related to the ionic species that the different metals can present in solution depending on pH. Thus, Al, Fe, and Cu can undergo oxo-polymerization, whereas Ca, Mg, and Zn are probably present in the complex in monoatomic form.

Variation of the Stability of PO$_4^{3-}$ Fixation in Higher Stability Binding Sites of M–HA Complexes. Apparent stability constant values (log K_H) corresponding to higher stability binding sites of PO$_4^{3-}$–M–HA complexes are displayed in **Table 3**. Few differences were found between methods, and if there were any, they indicated higher stability associated with complexes obtained by method 1. That was the case of the following systems: complexes involving Cu, Zn, Ca, and Mg at pH 4, complexes involving Fe and Mn at pH 6, and those with Mn and Ca at pH 8.

Although in other studies (*14*) different M–HS complexes presented their maximum stability in the pH interval of 8–9, little pH dependence was detected in the case of PO$_4^{3-}$ binding. In fact, the study of the effect of pH on the stability of the studied PO$_4^{3-}$–M–HA complexes showed that only method 1 complexes involving Al and Mn displayed apparent stability constants significantly higher at pH 6–8 than at pH 4.

With regard to the metal bridge influence, it was deeply pH-dependent. Thus, at pH 4, both method 1 and method 2 complexes presented the following general order of stability: Fe ≥ Cu, Zn > Ca, Mg, Al, Mn. However, at pH 6 and 8, this order varied depending on the method used. Thus, for method 1 complexes at pH 6 the order of stability was Fe, Al, Mn > Cu, Zn, Ca, Mg, whereas for method 2 it was Al > Fe, Cu, Zn, Ca, Mg > Mn. In the case of pH 8, the order was Al > Fe, Mn > Cu, Zn, Ca, Mg for method 1 and Fe, Al, Zn > Cu, Mn, Ca, Mg for method 2.

It is also noteworthy that the order of magnitude of the apparent stability constants for PO$_4^{3-}$–M–HA complexes was very close to that corresponding to M–HA complexes obtained by fluorescence spectroscopy following the Ryan and Weber method (data not shown). This result was consistent with the results obtained by Riggle and von Wandruszka (*7*), although they found higher stability values, probably because of different P concentration ranges used in the experiments.

Given that M–HA complexes play an important role in soil-complexed metal dynamics (*9*, *14–16*), this work suggests that PO$_4^{3-}$–M–HA complexes might also affect soil P dynamics.

Phosphate–Metal–Humic Complexes

J. Agric. Food Chem., Vol. 55, No. 2, 2007 **413**

Further investigations are needed, however, to better illustrate the effect of $PO_4^{3-}-M-HA$ complexes on P dynamics in both the soil and the rhizosphere.

ABBREVIATIONS USED

HS, humic substances; HA, humic acid; M–HA, metal–humic complexes; $PO_4^{3-}-M-HA$, phosphate–metal–humic complexes; MBA, maximum binding ability; MWCO, molecular weight cutoffs.

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