Review Article

Physico-chemical characterization of humic-metal-phosphate complexes and their potential application to the manufacture of new types of phosphate-based fertilizers

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Abstract

The aim of this review is to describe the main physicochemical characteristics of diverse types of humic-metal-phosphate acid complexes. The effects of these complexes on phosphorus (P) fixation in soils with different pH values and physicochemical features and on plant phosphorus uptake are also discussed. Humic-metal-phosphate complexes have apparent stability constants in the same range as those of metal-humic complexes, in solutions with diverse pH and ionic-strength values. Likewise, the molecular-size distribution of humic-metal-phosphate complexes as a function of pH is similar to that of potassium or sodium humates and metal-humic complexes. Humic-metal-phosphate complexes are able to decrease phosphate fixation in soils and increase plant growth and phosphate uptake. Phosphorus fertilizers containing humic-metal-phosphate complexes proved to be efficient to improve plant growth and P uptake with respect to conventional fertilizers such as single superphosphate. The values of parameters related to plant phosphorus-utilization efficiency (PUt E) suggest that the regulation of root acquisition of phosphate from these complexes could involve the interregulation of a system for the optimization of metabolic P utilization in the shoot and another system involving stress responses of roots under phosphorus deficiency.

Key words: fertilizer / phosphorus / humic substances / humic complexes / soil fertility / soil organic matter

1 Introduction

It is known that the availability of phosphorus (P) for diverse crops cultivated in various soil types is significantly influenced by the physicochemical features of each soil. Alkaline-calcareous soils favors phosphate precipitation by consecutive formation of monocalcium—bicalcium—tricalcium phosphates, while acid soils may facilitate P precipitation by the formation of Fe^{III} or Al^{III} phosphates, and adsorption on clays and Fe or Al oxides (*Rowell,* 1994). So the general efficiency, on average, of P mineral fertilizers as a source of available P for crops cultivated in these soil types is quite low, normally lower than 40% of total water-soluble P added to the soil (*Marschner*, 2012). It is clear that these percentages are also affected by the diverse efficiency of each crop to solubilize/mobilize P from the rhizosphere through the activation of specific morphological and physiological stress responses of roots (*Marschner*, 2012). The effective symbiosis of plant roots with some types of microorganisms, such as the mycorrhizas, also plays an important role in P plant uptake and further assimilation (*Marschner*, 2012).

In this context, many studies have shown that the association of composted or sedimentary organic matter with P fertilizers normally increases the efficiency of these fertilizers as P source for crops cultivated in P-fixing soils (*Broadbent*, 1986). This positive effect of organic-matter amendments, on P availability for plants, has also been observed when these types of products were added to the soil without previous mixing with P fertilizers (*Broadbent*, 1986). However, the doses that are necessary to apply in order to have a sustainable effect are rather high (more than 10 t ha^{-1}; *Chen* et al., 2004). On the other hand, it is generally accepted that the beneficial effect of organic amendments on P bioavailavility in soil is directly associated with the presence of humic substances (HS) in these materials (*Delgado* et al., 2002; *Chen* et al., 2004).

The mechanisms proposed to explain these positive effects of organic matter on soil-P availability for plants are diverse and, probably, complementary to each other. These mechanisms include: (1) the competition between organic anions contained in the organic matter (humic, fulvic, and simpler organic anions, such as citric or oxalic anions) with inorganic phosphate for adsorption sites of clays and metal oxides (*Stauton* and *Leprince*, 1996) (2) the effect of HS modifying

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the precipitation pattern of insoluble P salts with calcium in calcareous soils, or iron and aluminum in acid soils (*Grossl* and *Inskeep*, 1991) (3) the ability of HS to complex metals, which might both inhibit the formation of metal-P water-insoluble salts and solubilize P from insoluble P salts (*Lobartini* et al., 1998), and (4) the interaction of organic matter (or specific fractions of organic matter) with microorganisms in the rhizosphere that can favor P protection from soil fixation and P solubilization from water-insoluble P compounds (*e.g.,* mycorrhiza, plant growth–promoting rhizobacteria; *Pinton* et al., 2001).

Gerke (1992), *Gerke* and *Meyer* (1995), and *Gerke* et al. (1995) observed that the level of P in the soil solution was associated with a the presence in the soil solution of HS, Fe^{III}, and Al^{III}. The first explanation of this fact might be that HS protected P from the interaction with Fe^{III} or Al^{III} in soil solution through the complexation of these cations (*Chen* et al., 2004). However, *Gerke* (1992) and *Gerke* et al. (1995) proposed that these results might also be explained in terms of the interaction (complexation) of P with specific ligand sites in the HS structure through the formation of metal bridges involving Fe or Al (Fig. 1).
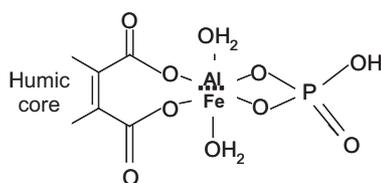

Figure 1: Proposed structure by *Gerke* et al. (1995) for humic-Al or Fe phosphate complex.

According to Gerke's hypothesis, these compounds would be stable and soluble in soil solution. Likewise, complexed P would also be available for plant-root uptake through the interaction of natural organic ligands secreted by roots, such as citric or oxalic anions, with the metal involved in humic-P-metal bridges. This would be associated with the release of P from HS complex, which may be taken up by plant roots. This hypothesis is attractive because the authors proposed that the role of these types of P-metal-HS complexes could be very relevant in order to explain the beneficial action of HS on P bioavailability in soil. Numerous studies have indicated the presence of P-metal-humic complexes in natural environments (*Gerke*, 2010). Thus, the use of molecular-size-filtration (ultrafiltration) technique (GPC) has shown the simultaneous accumulation of Fe^{III} (or Al^{III}) and P in those fractions corresponding to organic colloids with high apparent molecular size, both in lakes and freshwater samples (*Gerke,* 2010).

Other authors, working with GPC and isotopic techniques, showed the formation of a type of complex including humic colloids, the metal (Fe or Al) and P may be involved (*Jones* et al., 1988, 1993; *De Haan* et al., 1990). On the other hand, although the detection of this type of chemical association (humic-metal-P complexes) in soil solution is difficult due to the very low concentration in this environment, several studies indicated that humic-metal-P complexes might play an important role in P bioavailability (*Gerke*, 2010). These studies revealed that the integrity of humic-metal-P complexes in solution was affected by pH values in soil solution: acidic pH favored the release from the complex of both Fe^{III} and P to the soil solution (*Gerke* and *Meyer*, 1995). Finally, several studies carried out at laboratory level also reported data that strongly suggested the real formation of humic (fulvic)-Fe^{III}-P complexes. These studies showed the different mobility and solubility of this type of complex depending on pH, humic or fulvic sample, and stoichiometry (*Sinha*, 1971; *Levesque* and *Schnitzer*, 1967).

The main aim of this review is to highlight new results supporting the chemical viability of HS-metal-P (HMP) complexes and their properties concerning P dynamics in soils and P bioavailability. In addition, the practical utilization of P-metal-HS complexes in the development of new types of P fertilizers is discussed.

2 Study of the main physico-chemical features of HMP complexes in solution

As described above, a number of studies involving several humic and fulvic substances, metals and P reported the formation of HMP in solution, as well as their presence in lakes, freshwaters, and soil solution (*Sinha*, 1971; *Levesque* and *Schnitzer*, 1967; *Gerke,* 1992, 2010; *Jones* et al., 1993; *Lobartini* et al., 1994, 1998; *Petrovic* et al., 1996; *Delgado* et al., 2002; *Leytem* and *Westerman*, 2003). More recently, *Guardado* et al. (2004, 2005) studied some of the main physicochemical properties of HMP complexes: maximum binding affinity (MBA), apparent stability as a function of pH, and molecular-size distribution. The authors compared two different separation techniques to discriminate the P fraction that is complexed and noncomplexed by a Fe-humic acid (HA) complex: ultrafiltration and the interaction with a bicarbonate-stabilized anionic resin. Results obtained with both techniques were reasonably consistent with each other and they indicated that a significant part of P was fixed in Fe-HA complex (*Guardado* et al., 2004, 2005). On the other hand, *Riggle* and *von Wandruszka* (2005), using another experimental approach (a Co-based P-selective electrode), calculated apparent stability constants of diverse HMP complexes. In general, the values of stability constants obtained by *Riggle* and *von Wandruszka* (2005) were higher than those obtained by *Guardado* et al. (2005). These differences may be explained in terms of the different range of MBA values employed in these studies. The MBA range obtained using ion-selective electrodes is normally lower (it corresponds to complexation sites with higher stability) than that obtained in studies using filtration, precipitation or resin competition, which also include binding sites with low stability (*Tipping*, 2002). This hypothesis was confirmed by *Guardado* et al. (2007) using diverse metal-HA complexes and MBA ranges. The use of the resin-exchange technique allowed the determination of two fractions of metal-HA complexed P with different apparent stabilities (*Guardado* et al., 2007). It was noteworthy that apparent stability-constant values for HMP complexes were in line with those for metal-HA complexes. This
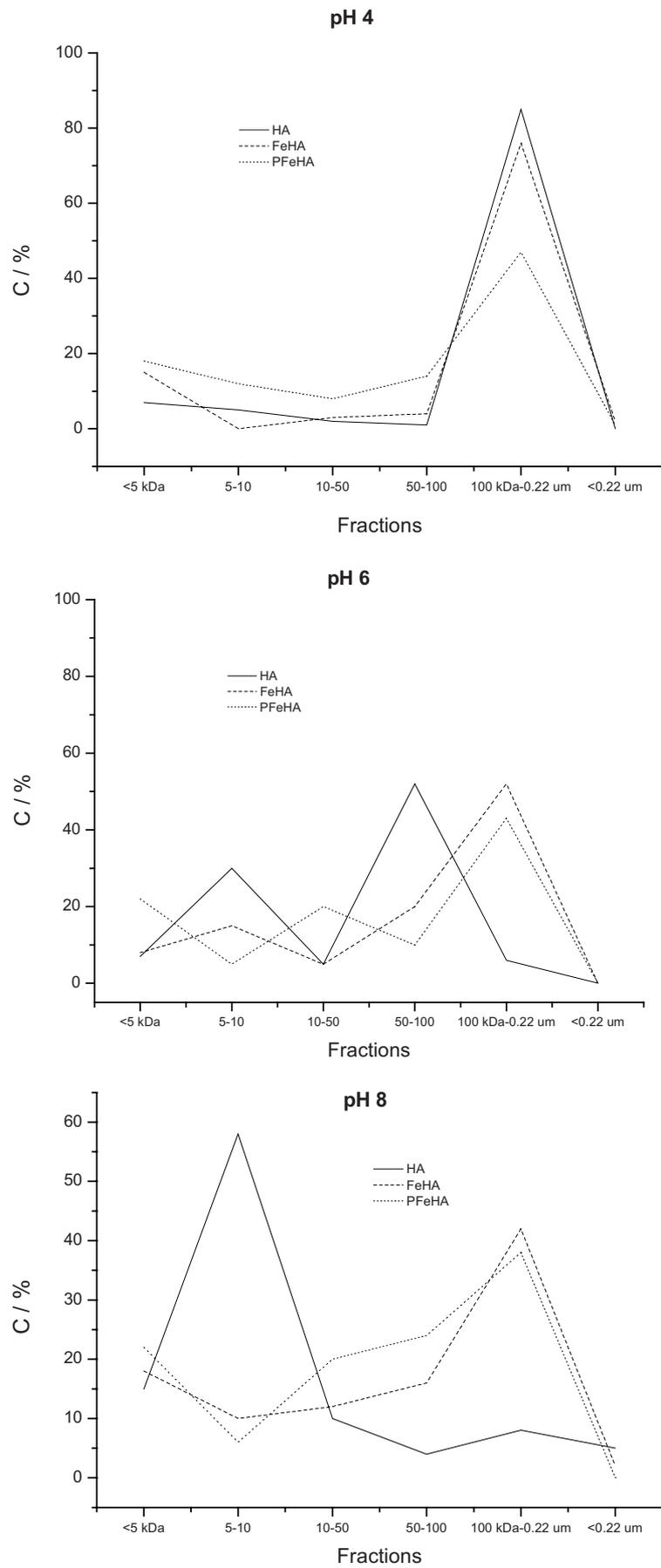
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pH 4

pH 6

pH 8

Figure 2: Size distribution at different pH for humic acid (HA), iron-humic acid complex (FeHA), and phospho-iron-humic acid complex (PFeHA). Data from *Guardado* et al. (2007).

Table 1: Apparent stability constants and maximum phosphate-binding ability for PFeHA and PAlHA complexes. The values are the average of two alternative synthesis methods. Data from *Guardado* et al., 2007).

	PFeHA			PAlHA		
	pH			pH		
	4	6	8	4	6	8
$MBA_T/[HA]$[a]	0.227	0.219	0.196	0.160	0.202	0.185
$MBA_H/[HA]$	0.056	0.062	0.056	0.049	0.075	0.078
log K_H	3.70	3.77	4.08	3.68	4.06	4.01

[a] $MBA_T/[HA]$, Maximum Binding Ability for Phosphate in PMHA Complexes (M = Fe,Al)
$MBA_H/[HA]$, Maximum Binding Ability for Phosphate corresponding to the Binding sites of Higher Stability in PMHA Complexes
log K_H, Apparent Stability constant Values for the Binding Sites of Higher Stability in PMHA Complexes

fact indicates that these new chemical species (HMP) could be stable in soil solution.

From the MBA values, the authors concluded that the relative proportion of HA-complexed metal involved in P complexation is relatively low. This fact indicated the presence of some electrochemical and/or sterical requirements in the metal-HA binding site for P complexation (*Guardado* et al., 2004, 2005, 2007). Complementary studies showed that sterical aspects seemed to be less relevant than electronic ones (*Guardado* et al., 2007). When the authors employed two different methods to obtain HMP complexes in solution, which might affect the sterical or spatial availability of Fe-HA ligand binding sites for P complexation, they did not obtain significant differences between the two methods concerning size distribution, MBA, or apparent stability constants (*Guardado* et al., 2007; Fig. 2; Tab. 1). A further study using molecular modeling revealed that this type of multiligand complexation requires specific electronic features at the binding site: The higher the stability of a specific metal-HA binding site, the lower the possibility that this binding site is involved in the stable complexation of P (*Guardado* et al., 2008).

In addition to these findings, the complementary study of HMP chemical features employing fluorescence and infrared spectrometry (FTIR), confirmed the formation of new chemical bonds between HA, metal, and P (*Guardado* et al., 2008). These results were in line with those obtained by *Riggle* and *von Wandruszka* (2007) in the study of the effect of HMP formation on the ^{31}P-NMR spectrum. These authors observed that the presence of new ligands in the sphere of coordination of P associated with the presence of metal-HA complexes, led to an increase in the width of the peak corresponding to P. These authors proposed that this effect might derive from a restriction in the movement of phosphorus-nucleus due to the presence of these new complexing ligands.

Taken together, all these studies demonstrate that the main physicochemical features of HMP complexes are in the range of those metal-humic complexes. This fact support the data reported in previous studies proposing an important role of these complexes in the dynamics of P in natural ecosystems, either aquatic or terrestrial (*Gerke*, 1992, 2010). The protection of P fixation in soil and the increase in P bioavailability would be included in this role of PHM complexes (*Gerke*,

2010). Therefore, the possibility of obtaining these complexes opens a new experimental approach to prepare P-based fertilizers with, potentially, higher P efficiency for plants cultivated in P-fixing soils. However, this hypothesis requires investigating more in depth whether these complexes are able to both protect P from soil fixation and provide available P for plants.

3 The ability of HMP to both inhibit P fixation in different soil types and to provide available P for diverse plant species

Recent studies have investigated the above-mentioned questions (*Urrutia* et al., 2013). Regarding the ability of HMP complexes to inhibit P fixation, P adsorption isotherms using different HMP, diammonium phosphate (DAP), and DAP plus HA, show that these types of complexes (P-Fe-HA, P-Al-HA, and P-Ca-HA) were able to significantly reduce the degree of P fixation in soils with diverse physicochemical features and pH values (Tab. 2), which may involve both the inhibition of P fixation and P desorption fixed at the soil surface.

On the other hand, regarding the availability of HMP-complexed P for plant uptake, a number of experiments show that diverse plant species were able to acquire P from HMP, at least at the same level as that obtained with conventional P sources (monopotassium phosphate, KH_2PO_4; *Urrutia* et al., 2013). The authors observed that, although plants receiving HMP developed similar or higher dry matter than control plants fed with KH_2PO_4, the concentration of P in the leaf of HMP-treated plants was significantly lower than that of control plants fed with KH_2PO_4. This fact indicates that the physiological utilization efficiency of plants for PHM-P was higher than for KH_2PO_4-P (Tab. 3). This hypothesis is also supported by the fact that the nutrient-uptake efficiency (the ratio between the nutrient taken up by the plant and the nutrient available in the rhizosphere) is higher in those plants receiving KH_2PO_4-P. Phosphorus included in HMP is not free but complexed. Therefore, plant roots have to compete with HMP to obtain HMP-complexed P. This fact might involve the activation of stress responses under P deficiency, with the investment of additional energy. In order to reduce this energetic demand plants might activate specific mechanisms to optimize the physiological and metabolic utilization of P present in the shoot. This functional cycle could lead to a physiological status involving a cross-talk regulation of two interrelated

Table 2: Percentage of added P (%) that is adsorbed in two different soils: an alkaline calcareous soil (A) and an acidic soil (B). The treatments are: DAP (diammonium phosphate), HA + DAP (diammonium phosphate plus humic acid at the same concentration as in PMHA complexes), PFeHA (phospho-iron-humic acid complex, PAlHA (phospho-aluminum-humic acid complex), PCaHA (phospho-calcium-humic acid complex. Data from *Urrutia* et al., 2013).

a)

	mM P					
Doses	0.18	0.36	0.54	0.72	0.89	1.07
DAP	18 a	32 a	38 a	43 a	50 a	54 a
HA+DAP	17 a	28 b	36 a	42 a	51 a	56 a
PFeHA	10 c	10 d	10 c	10 c	8.0 c	2.0 c
PAlHA	12 b	16 c	21 b	28 b	33 b	41 b

	mM P					
Doses	0.06	0.13	0.18	0.24	0.30	0.35
DAP	6.5 a	13 a	17 a	23 a	27 a	28 a
HA+DAP	6.0 a	12 a	16 a	20 b	24 b	26 b
PCaHA	5.5 a	9.0 b	12 b	15 c	18 c	20 c

LSD Fisher test ($p < 0.05$)

b)

	mM P					
Doses	0.18	0.36	0.54	0.72	0.89	1.07
DAP	18 a	33 a	42 a	57 a	62 a	72 a
HA+DAP	14 b	23 b	37 b	43 b	54 b	61 b
PFeHA	2.2 d	2.3 d	3.2 d	3.7 cd	3.8 d	4.2 d
PAlHA	5.0 c	4.9 c	16 c	20 c	24 c	30 c

	mM P					
Doses	0.06	0.13	0.18	0.24	0.30	0.35
DAP	7.5 a	13 a	18 a	25 a	29 a	32 a
HA+DAP	6.3 b	9.7 b	15 a	18 b	20 b	24 b
PCaHA	5.5 c	7.0 c	11 c	15 c	19 b	21 c

LSD Fisher test ($p < 0.05$)

systems: the system involved in the optimization of P metabolic utilization in the shoot and that involved in P root uptake under P deficiency (Fig. 3). This hypothesis may explain why

Table 3: Shoot dry weight, shoot P concentration (%), and P-utilization efficiency (PUt E) for two different crops. PFeHA: phospho-Fe-humic acid complex, KP: mono potassium phosphate. Data from *Urrutia* et al., 2013).

Wheat

	shoot dry weight / mg	P / %	PUt E[a]
PFeHA	119 a	0.412 b	0.029
KP	120 a	0.868 a	0.014

Chickpea

	shoot dry weight / mg	P / %	PUt E
PFeHA	1963 a	0.294 b	0.668
KP	1733 a	0.516 a	0.336

[a] P-utilization efficiency (PUt E): (shoot dry weight / mg) / (P concentration in shoot / mg kg^{-1})
LSD Fisher test ($p < 0.05$)

plants receiving P from PHA have the same shoot dry weight as those fed with P from monopotassium phosphate but having a lower P concentration in the leaf. Consequently, while P-uptake efficiency is higher for plants fed with monopotassium phosphate, P-utilization efficiency is higher in plants fed with HMP complexes. Obviously, this P-dependent shoot-to-root communication is, probably, governed by molecular events involving specific genes and molecular signals (*Franco-Zorrilla* et al., 2004, 2005, 2008).

4 Potential usefulness of HMP complexes to prepare P-based fertilizers with higher efficiency than that of conventional water-soluble P fertilizers

The results discussed above suggest that mineral fertilizers containing HMP may allow higher P efficiency of plants than those based on water-soluble P. Based on this hypothesis we have worked on two complementary approaches:

(1) The preparation of fertilizers containing HMP complexes with a large proportion of water-insoluble complexed P
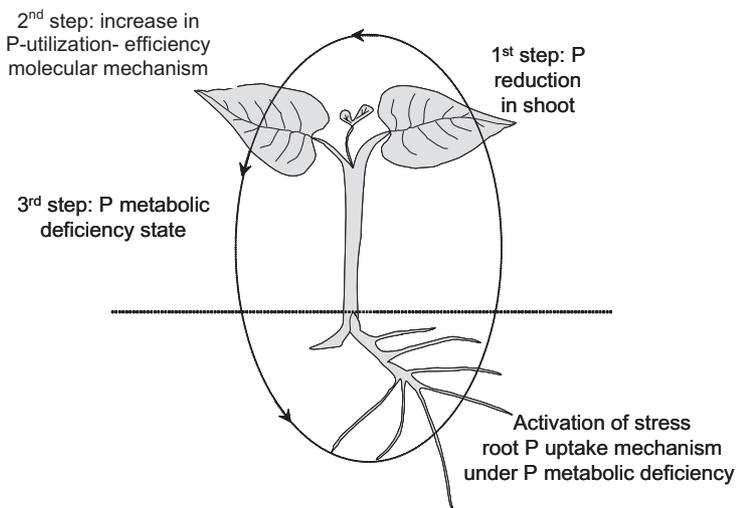
Figure 3: Proposed relationships and coregulation between stress root responses to P deficiency and plant metabolic P-utilization efficiency.

that can be solubilized by the organic anions released by plant roots as an expression of P metabolic need (*Erro* et al., 2007, 2011). We call these fertilizers "rhizosphere-controlled fertilizers" (RCF). These types of fertilizers are produced through the simultaneous reaction of rock phosphate with phosphoric acid in the presence of, at least, two metals, and a specific proportion of an HA (*Erro* et al., 2007). This reaction leads to the formation of complexes between HA and P through metal bridges involving one or two metals (*Erro* et al., 2007). The formation of HMP involving two metals leads to the formation of water-insoluble HMP complexes, while the presence of only one metal in the complex is associated with a fraction of HMP complexes that are partially soluble in water (*Erro* et al., 2007). For instance, the reaction involving Mg and Zn oxides or carbonates favored the formation of a significant concentration of water-insoluble HMP complexes

containing both Mg and Zn (around 70%), while the water-soluble fraction involving HMP complexes containing either Mg or Zn was minor (Fig. 4). The concentration of humic acid also influenced the water solubility of HMP complexes (*Erro* et al., 2007).

(2) The preparation of P fertilizers based on a specific modification of superphosphate (SSP) manufacture technique that leads to the formation of monocalcium phosphate-HA complexes (*Erro* et al., 2012). In this case, this type of HMP complexes is water-soluble because it only involves one cation. This product is called "complexed superphosphate" (CSP). The formation of monocalcium phosphate-HA complexes was checked studying the width of the P peak in the HPM-^{31}P NMR spectrum in comparison with that for SSP (*Riggle* and *von Wandruszka*, 2007). The chemical stability of this type of
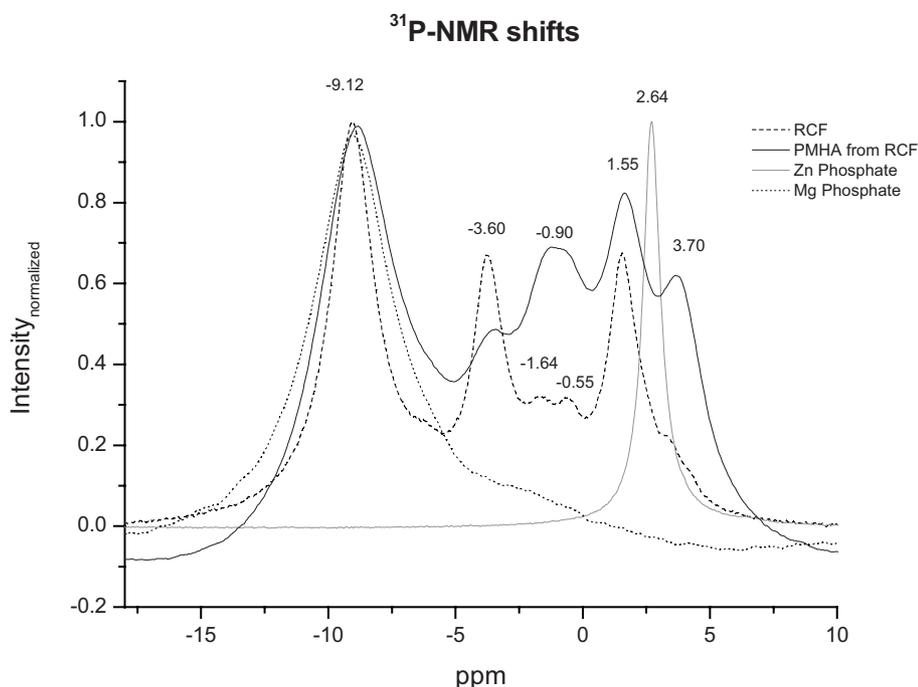

Figure 4: ^{31}P-NMR shifts for principal peaks involving Zn and Mg PMHA complexes. Zn phosphate peak disappears, Mg phosphate broadening changes, peak at 3.70 ppm from RCF disappears, peaks at –3.60 and 1.55 ppm change its intensity, and a new peak at –0.90 ppm appears in PMHA from RCF. Data from *Erro* et al. (2011).

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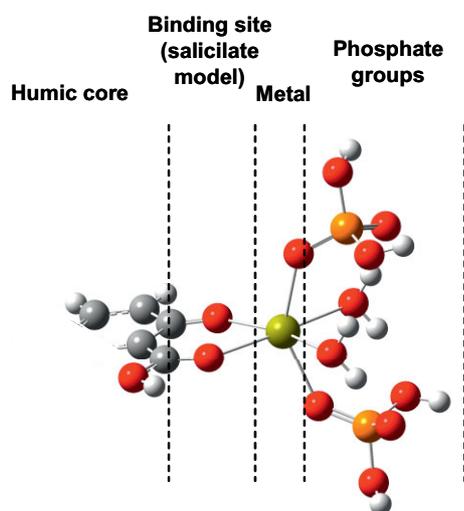
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Figure 5: DFT-ab initio quantum-chemical modeling of humic acid–monocalcium phosphate complex. Data from *Erro* et al. (2012).

complex was proved using molecular modeling (Fig. 5; *Erro* et al., 2012; *Baigorri* et al., 2013).

Regarding the efficiency of RCF as P fertilizers, a number of pot studies show that this type of fertilizer was able to improve P nutrition in wheat plants cultivated in both alkaline and acid soil in comparison with SSP (*Erro* et al., 2011). This effect was also slightly better than that of bicalcium phosphate (a P salt used as positive control that is not water-soluble but is soluble in neutral ammonium citrate) in alkaline soil. This fact was principally reflected by the metabolic P-utilization efficiency for the diverse fertilizers (*Erro* et al., 2011). These experiments also showed that the presence of a fraction of water-soluble P in RCF did not cause significant improvements with respect to treatments receiving P only from the HMP water-insoluble fraction in this soil type (alkaline-calcareous soil; *Erro* et al., 2011).

Surprisingly, in the acid soil, RCF fertilizers were much more efficient as P source for wheat than both bicalcium phosphate and SSP (*Erro* et al., 2011). However, in this type of soil, the presence of the water-soluble P fraction in RCF, consisting of water-soluble HMP complexes, played a relevant role in the whole efficacy of RCF (*Erro* et al., 2011). These results, taken together with those obtained in nutrient solution with HMP (Tab. 2), indicate that the protection of P from fixation could play an important role. Soil-fertilizer incubation studies show that RCF were able to keep higher pools of potentially plant-available soil P than conventional water-soluble fertilizers such as SSP (*Erro* et al., 2011).

In order to investigate the role of stress responses under P deficiency during P uptake from RCF, we studied the concentration and type of organic anions secreted by plant roots into the rhizosphere by plants fed with HMP-P (*Erro* et al., 2009a, b, 2010). The results obtained show that the efficiency of RCF to provide P for plants was clearly related to the plant ability to release organic anions into the rhizosphere. This fact, previously proposed by *Gerke* et al. (1995), indicates

the involvement of, at least, some stress responses under P deficiency in P uptake from RCF (*Erro* et al. 2009a, b, 2010). Along with this role of root exudates in P acquisition from HMP complexes, other complementary mechanisms might also be involved such as rhizosphere acidification and metal reduction in the rhizosphere (for instance, Fe^{III} to Fe^{II}) or root-surface increase. These findings were corroborated in field trials involving different crops and soil types (*San Francisco* et al., 2009).

As for the efficiency of CSP as P fertilizers, the incubation of CSP-P with different soil types, both acid and alkaline-calcareous soils, show that the fraction of plant-available P for CSP was significantly higher than that for SSP (*Storino*, 2010; *Giovanini* et al., 2013). The results included significant increases in P uptake of soil-cultivated wheat plants in pot experiments, principally during the early stages of plant development (*Storino*, 2010; *Erro* et al., 2012). As in the case of RCF, the parameter defining the P-utilization efficiency was significantly higher for plants fed with CSP than for control plants receiving SSP (*Trinchera* et al., 2009; *Erro* et al., 2012). These results obtained in pot experiments, were confirmed in field trials with soya and corn, in Iguazu (Paraguay—Brazil; *Zabini*, 2011).

5 Conclusions

The results obtained with HMP and HMP-based fertilizers (RCF and CSP) strongly support the important potential role of HMP complexes in P bioavailability in soils (*Gerke*, 1992, 2010; *Gerke* et al., 1995). The study of physicochemical features shows that HMP have a significant apparent stability and solubility for diverse pH values. The studies also show that HMP are able to both protect complexed P from fixation in soil, and provide available P for plants. The mechanism of root acquisition of complexed P from HMP is probably more complex than that involved in the uptake of water-soluble free P. These mechanisms may involve the cross-talk regulation of a system focused on the optimization of P-utilization efficiency in the shoot and the system involved in stress responses under P deficiency. This fact has also been observed in the case of other nutrients such as iron (*Bacaicoa* and *Garcia-Mina*, 2009; *Bacaicoa* et al., 2011; *Garcia-Mina* et al., 2013).

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