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Stability, solubility and maximum metal binding capacity in metal–humic complexes involving humic substances extracted from peat and organic compost

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Abstract

The aim of this work is to investigate the influence of pH and the metal:humic substances (HS) ratio on HS complexing capacity and the stability and solubility of metal–HS complexes in solution. We selected four HS with different physico-chemical properties and studied their interaction with Cu(II), Zn(II) and Fe(II) at different pH and metal:HS ratios. The selected HS were a humic acid and a whole humic extract (containing the humic and fulvic acids) extracted from black peat, and a fulvic acid and a whole humic extract extracted from a compost of grape solid wastes.

Our results showed that HS complexing capacity significantly varied as a function of pH, thus indicating the influence of both functional group ionisation and molecular conformation on this property. As was expected, total acidity affected the complexing capacity of the selected HS.

The results related to stability and complexing capacity indicated the possible presence of two binding patterns, one at acid-neutral pH probably involving carboxylates, and another at alkaline pH probably involving carboxylates and phenolic groups. The relationship between these binding patterns and the strength of the binding process varied according to the complexed metal.

Complex solubility was greatly affected by the ratio between the concentration of free ionised functional groups and the molecular weight in the HS studied.

1. Introduction

A number of studies published in the literature have proven that HS play very important roles in many different processes, both biological and chemical, occurring in natural ecosystems related to plant development as well as to the dynamics of metal and xenobiotics in soils and aquatic environments (Piccolo, 1996). Thus, many studies have reported that HS, or their organic complexes with certain metals, can enhance or diminish the uptake of several mineral nutrients by plants cultivated in hydroponics or soil, depending on the experimental conditions (Chen and Aviad, 1990; Chen, 1996; Garcia-Mina et al., 2004). In principle, these apparently contradictory results could be adequately explained if the dynamics in solution of metal–HS complexes,

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especially their stability and solubility, was explicitly considered (Garcia-Mina et al., 2004). Thus, it can be assumed that those metal–humic complexes that are not soluble or that are too stable to be hydrolysed during the plant metal uptake process are not really available to plants, whilst those that are soluble and whose stability allows them to remain in solution without negatively affecting the plant capacity to assimilate the complexed metal are really available to plants. Unfortunately, there are few studies in the literature that describe the physicochemical properties of the metal–humic complexes used in experiments involving plant–soil systems. Garcia-Mina et al. (2004) observed that the capacity of certain metal–humic complexes to increase the metal uptake by plants cultivated in different soil types depended, amongst other factors, on the fraction of soil plant available metal. Thus, in the case of alfalfa plants cultivated in an acid soil with a high concentration of potentially available iron (Fe), the application of HS or metal–HS complexes led to a significant decrease in Fe plant uptake, whereas the use of different metal–HS complexes in soils presenting potential metal deficiencies was associated with significant enhancements in metal plant uptake (Garcia-Mina et al., 2004). These results considered along with those related to the stability and solubility of the complexes revealed that metal plant uptake was principally influenced by the solubility (mobility) of the complexes provided that complex stability is strong enough to keep the complex in solution (Garcia-Mina et al., 2004). These results were in line with those reported by other authors, in which the important role of metal–HS complex diffusion in soil in relation to metal bioavailability was also well documented (Kumar and Prasad, 1988; Bar-Ness and Chen, 1991; Pandeya et al., 1998). In consequence a more complete understanding of the stability and solubility of metal–humic complexes is of great value in order to foresee the potential ability of these complexes to supply or regulate the metal uptake of plants cultivated in either soil or nutrient solution. Likewise, the study of the influence of HS properties on the stability and solubility of the metal–HS complexes would also be of great interest.

In this context, the aim of this work is to investigate the variation of the stability, solubility and the maximum metal binding capacity (MBA) as a function of pH (pH range of 5–12) and the metal: HS ratio, in molecular systems formed by metal-organic complexes including HS with different genesis and physico-chemical characteristics.

2. Material and methods

2.1. Extraction and physicochemical characterisation of the selected HS used in the study

Four different HS were used in the experiments: a humic acid (HP) and an unfractionated humic extract (HFP) both obtained from an acid black peat (Galicia, Spain); and a fulvic acid (FC) and an unfractionated humic extract (HFC) both obtained from a compost of solid wastes from wineries. The metals considered in the experiments were Cu(II), Zn(II) and Fe(II).

The different HS were extracted from the parent organic material using the methodology described by Stevenson (1994) with some operational modifications. The unfractionated humic extracts (HFP and HFC) were obtained as is described below.

In short, 10 g of non-dried organic material were weighed in a 250 ml flask to which 0.1 M NaOH was added until all the air had been displaced. After 48 h stirring at 25 °C in darkness, the supernatant containing the unfractionated humic extract was separated from the solid fraction by centrifugation at 7650*g* for 30 min. Subsequently the supernatant was treated with the necessary amount of cation exchange resin (Amberlite IRA – $118H^+$) until pH values were in the 3.5–4 range. The resin was separated by filtration (Whatman No. 42 filter paper), and the supernatant containing the unfractionated humic extract was freeze-dried.

The humic acid (HP) was obtained by acidifying an aliquot of the alkaline extract containing the humic and fulvic acids obtained from peat with hydrochloric acid (HCl) 6 M up to a pH of 1.5. After 12 h, the acidified sample was centrifuged at 7650*g* for 30 min in order to separate the precipitated humic acid from the supernatant containing the fulvic acids and other acid-soluble organic compounds. After washing humic acids with water to eliminate Cl^- contamination, these were freeze-dried. The elimination of Cl^- in the sample was evaluated using the $AgNO_3$ test.

The fulvic acid (FC) was obtained by using a methodology similar to that above described for humic acids, in which the fulvic acids contained in the alkaline extract obtained from grape compost were separated from the other organic substances also present in the supernatant after acidification, by selective absorption in XAD-8 resin. Finally, the fraction corresponding to the fulvic acids was eluted from the resin column with sodium hydroxide

0.1 M, desalted with a cation exchange resin (Amberlite IRA – 118H$^+$), and freeze-dried.

The main chemical properties – elemental analysis (Elementary analysis. Carlo Erba EA7), E4/E6 (Chen et al., 1977), functional analysis (Potentiometric titration, Takamatsu and Yoshida, 1978), and approximate molecular weight (MW) (Chin et al., 1994) – of the HS studied are presented in Table 1.

2.2. Study of the stability and solubility of the metal humic complexes

In order to study the influence of the metal:HS reaction stoichiometry on the stability and solubility of the complexes at different pH values, the reaction between a constant concentration of the HS (90 mg l^{-1}) and different concentrations of each micronutrient (Cu(II), Zn(II), and Fe(II)) was carried out at the following pH values: 5, 6, 7, 8, 9, 10, 11 and 12. The ionic strength (I) (3 mM) was chosen according to its main value in soil solution from soils under irrigation (Elprince, 1986). pH and I were kept constant in all the reactions. The metal:HS (w:w) ratios in the reaction were: 0.10:2, 0.25:2, 0.40:2, 0.55:2, 0.70:2, 0.85:2, 1.00:2, 1.50:2, and 2:2.

All the reactions were carried out in darkness, with constant stirring, at a temperature of 25 °C, for a period of 24 h. Metals (Cu(II), Fe(II) and Zn(II)) were added very slowly as sulphate solutions, adjusting pH values with 0.1 M NaOH.

In order to measure the concentrations in solution of complexed metal, free metal and HS, at the end of the reactions, the following methodology was used.

Once the reaction had finished, the precipitated fraction containing metal hydroxides and insoluble humic complexes was separated by centrifugation at 10,000*g* for 30 min. Ultrafiltration was used to separate complexed and uncomplexed metal in the solution obtained after centrifugation (Garcia-Mina et al., 2004). A 1000 Da ultrafiltration cell (Filtron, polyethersulfon type) was used and ultrafiltration plots were first obtained with known concentrations of each metal in similar conditions of pH and I (Garcia-Mina et al., 2004). In all cases, the retention coefficient of the ultrafiltration cell for metal–HS complexes and HS was 1 (full retention).

From the supernatant, the concentration of HS in solution was determined using oxidation with 0.01 N $KMnO_4$ in an alkaline medium (Primo Yúfera and Carrasco, 1987). Metal concentration in solution, before and after ultrafiltration, was analysed using atomic absorption spectrometry. Values of HS in solution, total complexed metal and total free metal (total metal – total complexed metal) selected for stability studies were obtained from reactions in which there were no precipitation of HS.

The relative solubility of humic complexes as a function of pH and metal:HS reaction stoichiometry was determined by measuring the concentration of the humic system that remained in solution after the reaction, taking into account that the different HS studied were totally soluble, at the concentration used, in the pH range of the study. The results are presented using contour plots obtained by means of a quadratic function calculated utilizing the software Statistica v. 6.0.

The pH-dependent stability of the humic complexes in solution was estimated using an apparent (conditional) stability constant (K) calculated by the Scatchard method (Stevenson, 1994):

$$\theta/M_{FT} = K - \theta K$$

M_{FT} being the total free metal in the equilibrium; M_{CT} the total complexed metal; and θ the sites bound/maximum metal binding capacity (MBA)

Table 1
Physicochemical properties of the different humic materials

Humic systems	Elemental analysis (g 100 g^{-1})				Functional analysis (mmol g^{-1})				Approximate (MW Da)	E4/E6
	C	H	O	N	SA[a]	WA[b]	VWA[c]	Total		
HFP	56.2	4.9	37.0	1.9	0.78	2.35	2.74	5.90	2533	5.52
HP	60.1	5.4	32.7	1.8	1.45	2.37	2.95	6.80	2686	5.24
HFC	48.2	3.7	43.5	4.6	1.38	2.43	2.95	6.76	2716	6.35
FC	38.9	4.2	53.4	3.5	2.34	3.23	2.53	8.10	1447	9.20

[a] Strong acidity, probably involving a fraction of carboxylic groups.

[b] Weak acidity, probably involving a fraction of carboxylic groups.

[c] Very weak acidity, probably involving a fraction of carboxylic groups and phenolic groups.

(M_{CT}/MBA) ratio, where K (K_1 for low metal ion saturation and K_2 for high metal ion saturation) was obtained from the plot θ/M_{FT} vs. θ.

The MBA values were estimated by extrapolating the straight-line segment corresponding to binding at high metal ion saturation in a M_{CT} vs. M_{CT}/M_{FT} plot. Results corresponding to metal:HS reaction ratios without precipitation of HS were only considered in the calculation of K.

3. Results and discussion

3.1. Physicochemical characteristics of the HS studied

As can be observed in Table 1, the different HS studied presented two groups with well-differentiated physicochemical properties. As expected, whereas the unfractionated humic system derived from peat (HFP) presented E4/E6 values and contents of the different elements close to those typical of humic acids, the unfractionated humic system obtained from the compost of solid grape wastes (HFC) presented values in these parameters close to those of fulvic acids (Stevenson, 1994). On the other hand, HP as well as FC presented compositions and E4/E6 values characteristic of humic acids and fulvic acids, respectively (Stevenson, 1994). These qualitative differences were also reflected in the content of acidic groups, either carboxylic or phenolic, in the different humic systems (Table 1). It is noteworthy that the grape compost extracted HS presented an N content higher than that of peat extracted HS.

Regarding the approximate MW, the results were in line with those expected for humic and fulvic acids, with clear differences between the MW of FC (1447 Da) and those of the other humic systems (around 2600 Da).

In consequence, we have two humic groups, one closer to humic acids (HP and HFP) and another closer to fulvic acids (FC and HFC), with different molecular properties principally in relation to MW, N composition, and total acidity (Table 1).

3.2. Determination of MBA distribution as a function of pH for the different HS

The results obtained concerning MBA values for the different selected HS in the 5–12 pH range, and for the three metals (micronutrients) studied, are presented in Fig. 1. The MBA values covered a range between 1 and 6.6 mmol g^{-1}, depending on the pH, the HS studied and the metal considered, which, in turn, signified the involvement of between 10% and 98% of the total acidic groups present in the different HS. This order of MBA values is in line with those obtained by other authors using other analytical techniques for metal speciation such as fluorescence quenching (Plaza et al., 2005), ion selective electrodes (ISE) (Gamble et al., 1980; Gondar et al., 2000; Antonelli et al., 2001), ion-exchange resins (Ardakani and Stevenson, 1972) or dialysis (Zunino and Martin, 1977; Truitt and Weber, 1981; Rainville and Weber, 1982). However, they are higher than those obtained using polarographic methods (Baron et al., 1990; Antelo et al., 2000). This fact associated with the use of polarographic methodologies, which has also been observed by other authors (Baron et al., 1990), seems to be related to the hydrolysis on the electrode of certain fractions of metal–humic complexes corresponding to lower stability (Baron et al., 1990).

As general trends, we observed a clear increase in MBA values in line with pH alkalisation, within the pH range of 6–9 (or 10). This result, also observed by other authors in other humic systems (Gondar et al., 2000; Antonelli et al., 2001), could be related to the progressive ionisation of phenolic groups in addition to carboxylic groups, both functional groups probably involved in metal complexation (Livens, 1991; Senesi, 1992). A clear decrease in the range of pH between 9 (or 10) and 12 was also observed, which might be related to the hydrolysis of metal–humic complexes due to their lower stability in this pH range, as well as to changes in the inorganic species of the different metals. In some cases, a certain decrease in the pH range between 5 and 7 was also noticed that could be reflecting the involvement of protonated amino groups in metal complexation at acidic pH (Senesi, 1992) (Fig. 1).

On the other hand, some differences were observed concerning the maximum MBA values according to the origin of the HS studied. Thus, peat derived HS (HP and HFP) presented this value around pH 10, while grape compost derived HS (FC and HFC) presented this value around pH 9. This result may be related to the different acidity of the phenol groups involved in metal complexation, more acidic in nature in fulvic-like systems such as FC and HFC (Stevenson, 1994). However, the possible involvement of other complexing groups cannot be neglected.

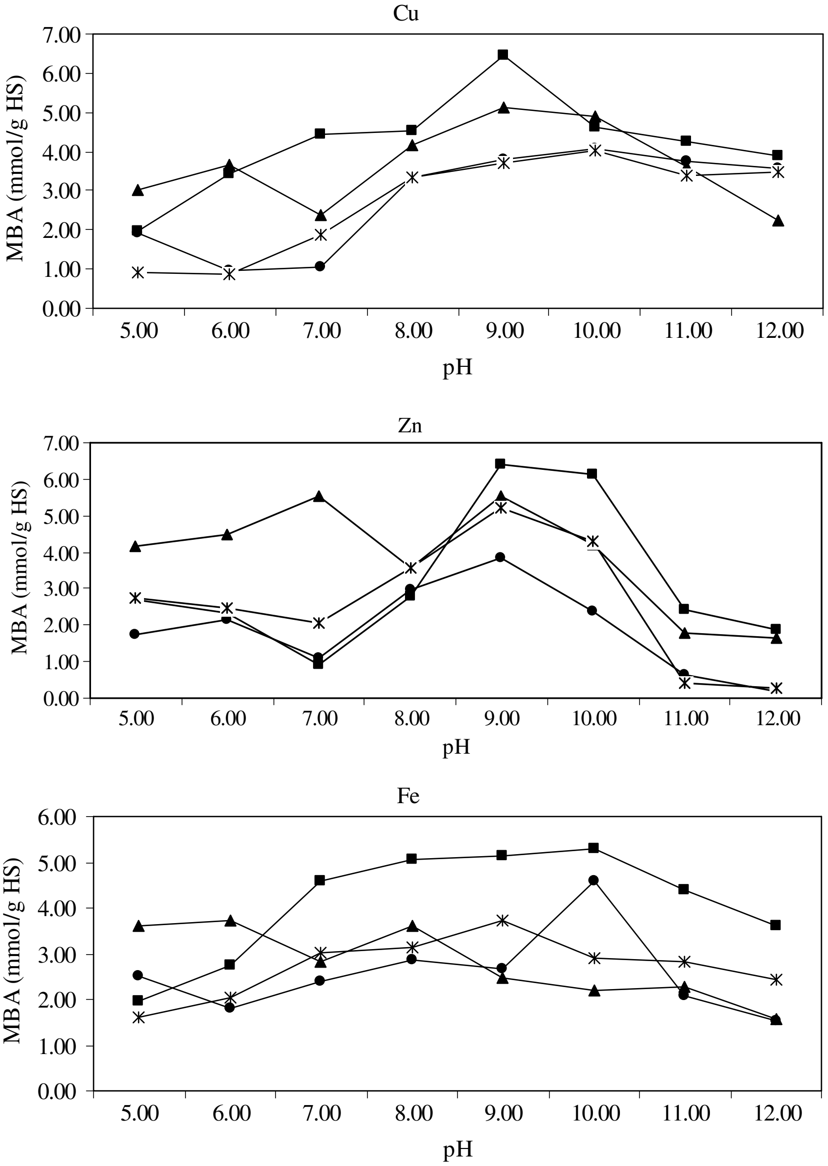

Fig. 1. Maximum binding ability (MBA) distribution as a function of pH for the different HS studied: HP (-■-), HFP (-✱-), HFC (-●-), FC (-▲-).

Regarding MBA variations associated with the complexation of each metal, Cu complexes presented MBA values that were higher for grape compost derived HS than for peat derived HS (Fig. 1). In the case of FC, this result could be explained by the different (higher) total acidity (Table 1). However, the result related to HFC that presented a total acidity similar to that of HP but the highest MBA distribution, suggests that other factors, different from the total acidity, may be involved in this process, such as the effects of the molecular conformation on the integrity of binding sites (García-Mina, 2004). This fact is specially supported by the results obtained for FC and HFC, since FC was associated with MBA values lower than those of HFC, which, in turn, presented lower total acidity than FC.

This possible influence of the macromolecular character (conformational changes) on the chemical identity of the binding sites was also found in the Zn–HS complexes, since the binding pattern of HFC and HF presented the same differences as in the case of Cu–HS complexes. In the case of Zn–HS complexes (Fig. 1), the increment of MBA in the pH range of 7–9/10 is clearer, thus indicating the important role that phenolic groups could play in the complexation process. In this sense, the results associated with Zn complexation seem to present two binding patterns, one in the range of acid pH, that could involve protonated amino groups and carboxylates, and another in the range of alkaline pH probably involving phenolic groups and carboxylates. These results are in line with those obtained by other authors in spectroscopic studies of processes involving Cu and Zn complexation by HS (Livens, 1991; Senesi, 1992; Frenkel et al., 2000) as will be further discussed below.

In the case of Fe–HS complexes, however, the results related to those HS containing humic acids (HP, HFP, HFC) indicated the possible existence of only one binding pattern that presented maximum MBA values in the pH range of 8–9/10. This fact also suggests the involvement of principally carboxylates and phenolic groups in the binding process. The case of the fulvic system (FC) presented, however, noticeable differences with respect to the other HS studied. Thus, FC presented two MBA maxima, one in the range of acidic pH and another in the range of alkaline pH (pH 8). These results suggest, as in the case of Zn–HS complexes, the possible presence of two binding patterns depending on pH: one at acid pH that could principally involve carboxylates, and another in the alkaline range that probably involves carboxylates and acidic phenols. In this sense, the fact that, in general, MBA values for Fe–HS complexes were lower than those associated with Cu–, and Zn–HS complexes could indicate that a more restricted type of binding sites are involved in Fe complexation.

Regarding the influence of HS properties on MBA, our results indicate that total acidity, logically, plays an important role, although the influence of the macromolecular character of the different HS, reflected in the possible influence of the conformational changes on the identity and integrity of the binding sites, cannot be ruled out.

3.3. *Study of the apparent (conditional) stability constant (log K) distribution as a function of pH for the different HS*

The distributions of the apparent stability constant for both low saturation binding sites (log K_1) and high saturation binding sites (log K_2), as a function of pH are presented in Figs. 2 (for Cu–HS complexes), 3 (for Zn–HS complexes) and 4 (for Fe–HS complexes). As can be observed in these figures the order of the maximum value of log K_1 (around 3–4 depending on pH and the HS studied) was in line with that obtained by other authors in diverse metal–HS systems, principally for HS of terrestrial origin (Stevenson, 1994). In this sense, aquatic HS

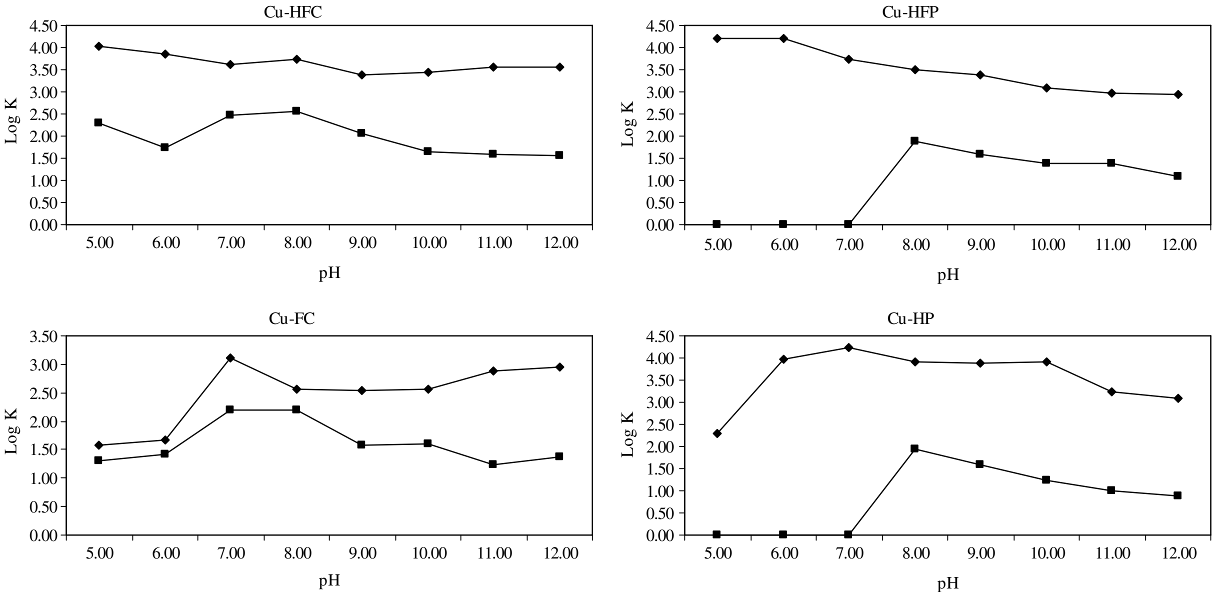

Fig. 2. Conditional stability (log K) distribution as a function of pH for the different Cu–HS complexes: log K_1 corresponding to low occupation of binding sites (-◆-) and log K_2 corresponding to high occupation of binding sites (-■-).

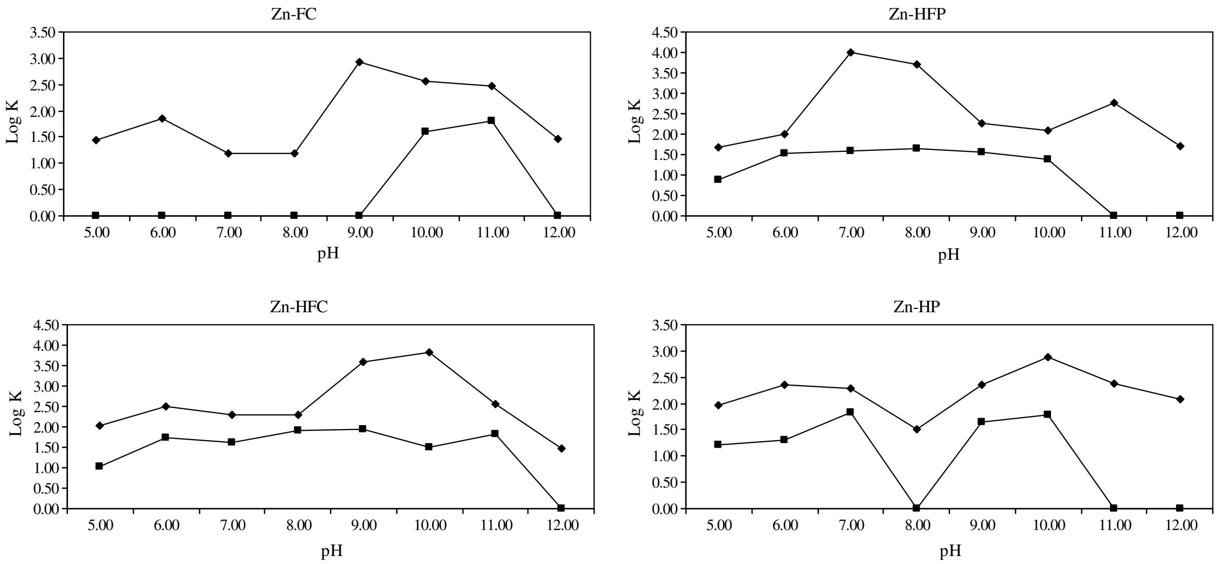

Fig. 3. Conditional stability (log K) distribution as a function of pH for the different Zn–HS complexes: log K_1 corresponding to low occupation of binding sites (-◆-) and log K_2 corresponding to high occupation of binding sites (-■-).

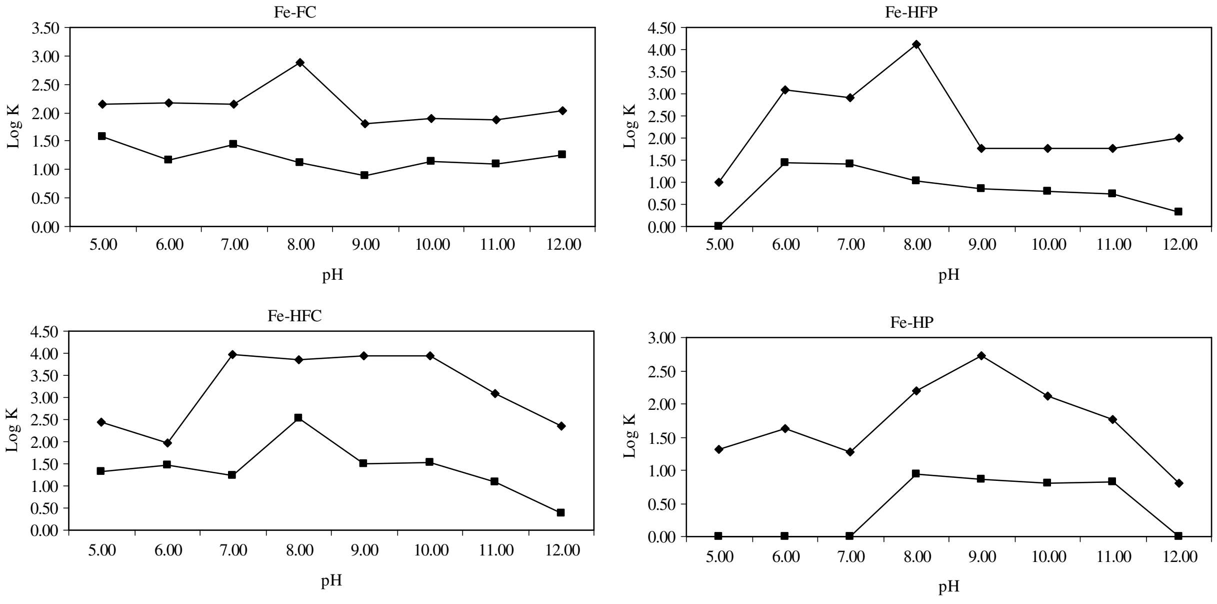

Fig. 4. Conditional stability (log K) distribution as a function of pH for the different Fe–HS complexes: log K_1 corresponding to low occupation of binding sites (-◆-), and log K_2 corresponding to high occupation of binding sites (-■-).

tended to be associated with higher values of log K (around 6–8) (McKnight et al., 1983; Nifant'eva et al., 2001). We have not found studies on the interaction with metals of HS obtained from compost of solid grape wastes but other studies using HS extracted from digested urban wastes presented log K values for Cu binding (also calculated using ultrafiltration as in our study) between 4.3 and 4.7 depending on pH (Gould and Genetelli, 1978).

As for Cu–HS complexes, the results obtained indicated that log K_1 presented a general decrease in line with the alkalisation of pH. Similar results were obtained by other authors but in shorter pH intervals (Cao et al., 1995; Gondar et al., 2000). These results indicate the presence of one specific interaction in the complexation process, especially significant at acid-neutral pH, whose involvement in the binding process became gradually less significant as the pH became more basic. Some authors have related this fact to the involvement of protonated amino groups in the process (Senesi, 1992; Frenkel et al., 2000). In this sense, the unfractionated HS (HFP and HFC) presented maximum values of log K_1 around pH 5, whereas the humic acid (HP) and the fulvic acid (FC), the isolated HS studied, presented this maximum at pH 7. These results indicate that the relative importance of carboxylates and the fraction of more acidic phenolic groups in the strength of the complexation process might be more significant for the isolated HS. Likewise, these differences between the isolated HS and the unfractionated HS could reflect the influence of the conformational structural arrangement on the identity and stability of the binding sites, since the process of sample preparation very significantly affected molecular conformation (García-Mina, 2004).

Also particularly noteworthy is the absence of correlation between the MBA distribution and log K_1 distribution (maximum stability at acid-neutral pH and maximum MBA at alkaline pH). This result reflects the great heterogeneity in the nature of the binding sites present in the HS studied as well as the fact that, possibly, the functional groups involved in metal complexation in this pH range could play a more important role concerning the stability of the binding sites, than phenol groups, which, in turn, significantly affected the total concentration of binding sites.

As to log K_2, it is noteworthy that in the case of peat extracted HS the stability was significant only in the pH range corresponding to alkaline pH, thus suggesting the importance in this case of the involvement of phenol groups, in addition to carboxylates, in the structural arrangement of this class of binding sites. In the case of grape compost extracted HS the distribution of log K_2 was very similar to that of log K_1 indicating that the binding sites involved in both types of interactions could be qualitatively similar to some extent.

Regarding the influence of HS properties on Cu–HS interaction, it was clear that HS containing humic acids (HFC, HFP, and HP) presented higher values of log K than the fulvic acid studied (FC).

The results concerning Zn–HS complexes were different from the above-mentioned results related to Cu–HS complexes. Thus, Zn–HS complexes including HP, HFC and FC showed maximum stability (log K_1) in the range of alkaline pH (9–10). It is clear that in this case the binding sites involved at basic pH (probably including carboxylates and phenolic groups) are associated with a stronger interaction. The complexes including HFP, however, presented maximum stability at pH 7. This result that corresponded to very low MBA (Fig. 1) suggests that a specific fraction of binding sites, probably involving carboxyl groups, is in this case related to the maximum strength. In general, the results related with log K_2 were in line with those reported for log K_1.

Regarding the influence of HS properties on the stability of Zn–HS complexes, we found no clear relationships between the different physicochemical characteristics of the HS studied and the strength of the binding process. It is noteworthy that the whole HS (HFP and HFC) presented a maximum of stability higher than that of the isolated HS (HP and FC). This result, which could not be related to any of the physicochemical properties studied, might be indicative of the influence of the molecular conformation in solution on both the constitution of binding sites and the strength of the interaction (García-Mina, 2004).

As for Fe–HS complexes, the results obtained indicated a clear increase in the stability (log K_1) in line with the alkalisation of pH in the pH range of 5–(8/9/10) depending on the HS studied. Thus, HFC maintained maximum stability in the pH interval of 7–10, whereas FC and HFP presented the maximum at pH 8 and HP at pH 9. In all cases, however, the possible involvement of carboxylates and specific fractions of phenolic groups in the binding sites responsible for the maximum strength in the complexation process seem to be clear.

Log K_2 distribution varied according to the HS studied (Fig. 4). Thus, whereas HFC and HP presented log K_2 distributions similar in shape to those of log K_1, FC and HFP presented a maximum at acid pH with a progressive decrease in line with the alkalisation of pH.

As in the case of Zn–HS complexes, the unfractionated HS (HFP and HFC) presented a maximum of stability higher than that of the isolated HS (HP and FC).

Regarding the order of stability as a function of the complexed metal, our results indicated an order of stability of Cu > Fe ⩾ Zn for all HS studied, except in the case of HP that presented the order Cu > Zn ⩾ Fe. In principle, these results are in line with the order of stability found by many authors working with very different HS (Stevenson, 1994).

In conclusion, we found no clear relationships between the strength of the binding process and the studied physicochemical properties of the selected HS: elemental composition, functional analysis or MW. However, some relationships between stability and molecular conformation in solution could exist, since the unfractionated HS (HFP and HFC), which probably presented a molecular conformation closer to that corresponding to natural conditions, tended to present an order of stability higher than that of the isolated HS (HP and FC), which may present in solution more artificial conformations due to the effect of the isolation process. Likewise, in the case of Cu–HS complexes the humic systems containing humic acids tended to form more stable complexes than the fulvic system. In fact, in general the fulvic system (FC) tended to form metal complexes with lower stability. There are in the literature a great number of studies focusing on the description of metal binding by specific HS as well as on the physicochemical modelling of the process (Tipping, 2002). However, we have not found in the literature, many studies dealing with the relationships between metal complexation by HS and the physicochemical properties of HS. Mantoura et al. (1978) observed that the order of stability for metal–humic complexes formed from HS with different origin was: HS sea sediment > fulvic acid sea sediment > HS natural waters > soil humic acid > soil fulvic acid. These results indicated that: (i) aquatic HS tended to form more stable complexes than terrestrial HS; (ii) fulvic acids tended to form metal complexes with a stability lower than that of humic acids, and (iii) unfractionated HS tended to form metal complexes with a stability higher than that of isolated humic or fulvic acids. As can be observed our results concerning the order of complex stability for the different HS, and principally in relation to the unfractionated HS (HFP and HFC) and isolated HS (HP and FC), are in line with the above-mentioned results of Mantoura et al. (1978). More recently, Stevenson and Chen (1991) compared the stability of Cu–HS complexes obtained using HS with different origins and molecular properties. Their results indicated the following order of stability: soil humic acid > peat humic acid > lignite humic acid > soil fulvic acid > melanine. There were no clear relationships between the HS molecular properties studied by these authors and the strength of the interaction, although also in this case, as in our study, the complexes formed by fulvic acids showed a stability lower than that of the complexes formed by humic acids.

Matsuda and Ito (1970) studied the relationships between humification degree and the strength of Zn complexation by humic and fulvic acids of different origins. They reported a certain correlation between the two parameters in the case of humic acids, observing an increase in the stability in line with the increase in the degree of humification, but not in the case of fulvic acids. Likewise in the majority of the soils studied humic acids formed more stable Zn complexes than fulvic acids. In our case, if we assume that peat extracted HS are expected to be more humified than grape compost extracted HS, we observed no clear relationships between the assumed humification degree of HS and metal complexation stability. Regarding the identity of the binding sites, our experimental design only permits us to propose the possible presence of certain functional groups in the structure of the binding sites. These hypotheses should be evaluated in complementary studies using spectroscopic techniques. In general, as has been discussed above, our results, either related to MBA or complex stability, indicate the possible presence of two different binding patterns depending on pH. One in the range of acid pH, probably principally involving carboxylates, and another in the range of basic pH probably involving carboxylates and phenolic groups. In the case of the Cu–SH complexes studied, the binding pattern intervening at acid pH appears to be related to the maximum stability, whereas in the case of the Fe–, and Zn–HS complexes studied, the binding pattern intervening at basic pH seems to be related to the maximum stability. A number of studies suggested the direct involvement of specific structural arrangements, such as phthalic-like and salcylate-like structures, in metal complexation by HS (Senesi, 1992). More studies, however, probably involving the use of a multi-technique approach, are needed to elucidate the chemical identity of the main binding sites involved in metal complexation by HS.

3.4. Study of the solubility of the metal–HS complexes studied

The results concerning the solubility of the complexes formed by the different metals with HP as a

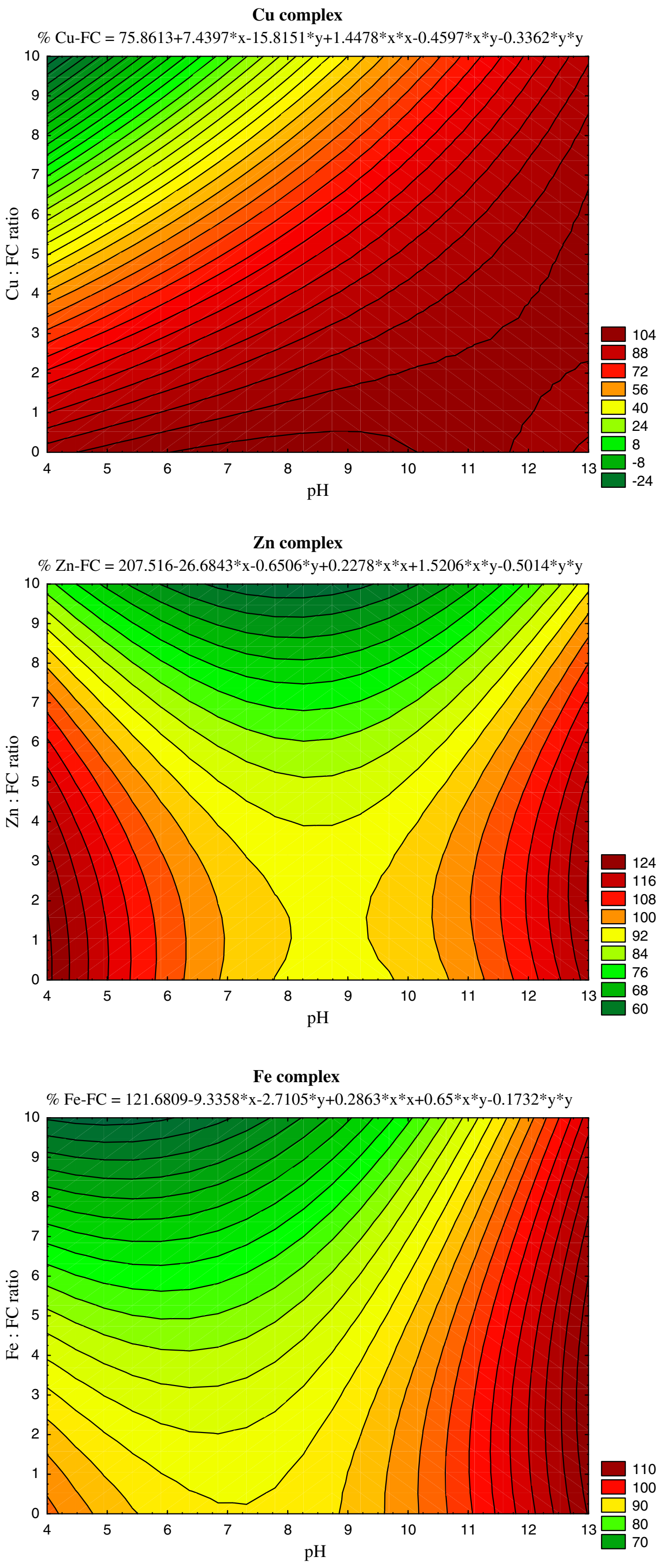

Fig. 5. Percentage of metal–FC complexes that remains in solution (solubility) as a function of pH and the metal (mg): FC (mg) ratio: 1 (0.10:2), 2 (0.25:2), 3 (0.40:2), 4 (0.55:2), 5 (0.70:2), 6 (0.85:2), 7 (1.00:2), 8 (1.50:2) and 9 (2:2).

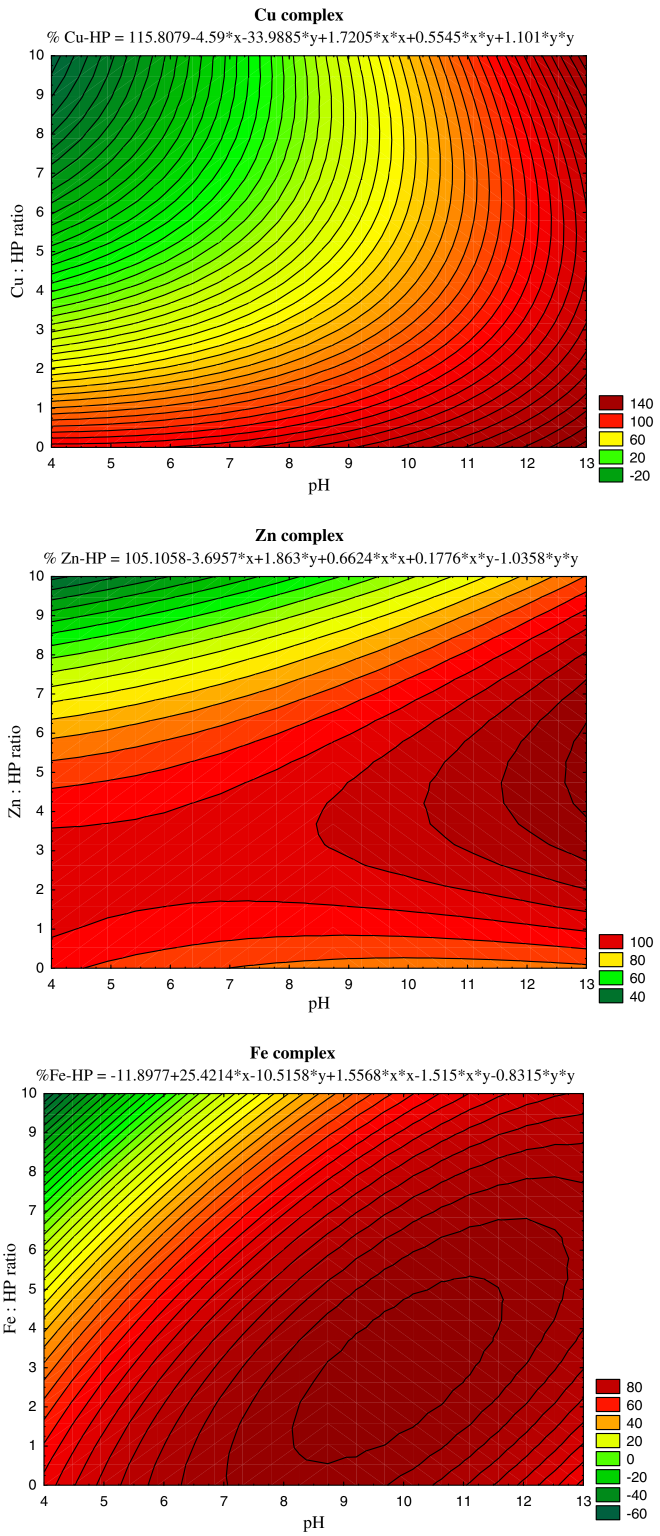

Fig. 6. Percentage of metal–HP complexes that remains in solution (solubility) as a function of pH and the metal (mg): FC (mg) ratio: 1 (0.10:2), 2 (0.25:2), 3 (0.40:2), 4 (0.55:2), 5 (0.70:2), 6 (0.85:2), 7 (1.00:2), 8 (1.50:2) and 9 (2:2).

function of pH and the metal:HP ratio are presented in Fig. 5, whereas those results related to the interaction between the different metals with FC are presented in Fig. 6. The results regarding the interaction of the different metals with HFP or HFC were similar to those related to HP and FC.

As general trends we observed the following main patterns:

- The solubility of the different metal–HS complexes increased in line with pH alkalisation. This fact was especially clear in the case of Cu–HS complexes and Fe–HS complexes. This result may be explained by the variations in both the molecular conformation and the ionisation of the acidic functional groups with pH (Swift, 1989).
- The solubility of the complexes decreased in line with the increase in the metal:HS ratio. In this sense, for a same pH, those complexes corresponding to higher metal:HS ratio presented lower solubility. These results, also observed by other authors in studies on the flocculation of HS with different metals (Stevenson, 1994; Spark et al., 1997), could be related to the gradual reduction of the free ionised functional groups, in line with the increase in complexation, associated with the increase in the metal–HS ratio.
- In general, for the same pH value and metal:HS ratio, metal–HS complexes including the HS with more fulvic character (FC and HFC) presented more solubility than those complexes including the HS with more humic character (HP and HFP). This result might be related to the proportion between the ionised functional groups and the MW. Thus, FC that presented the highest total functionality and the lowest MW presented the highest solubility.
- In general, the order of solubility of the different metal complexes as a function of the complexed metal was Zn–HS > Fe–HS > Cu–HS. As can be observed, this order of solubility is inversely related to the order of stability described above, at least in the case of the unfractionated HS studied.

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