



Aquatic Humic Substances: Relationship Between Origin and Complexing Capacity

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Abstract

Aiming to determine the relationship between source and complexing capacity, humic substances obtained from three sites (Sorocaba and Itapanhau Brazilian rivers, and Xochimilco Lake in Mexico) were studied. Copper, manganese, zinc and arsenic complexing capacity were determined for the three substances under various pH conditions. Results showed similar complexing capacity for the three elements depending on the chemistry of each one and on the physico-chemical conditions. Speciation diagrams showed that these conditions affect both, the humic substances, and the transition metals and arsenic.

Keywords Aquatic humic substances · Complexation · Transition metals · Arsenic · pH · Redox

Determining chemical interactions among metals, metalloids, nutrients and organic matter is basic for understanding their mobility in aquatic systems. Possible chemical species present in the system are also important for bioaccessibility and toxicity assessment. Organic matter may generate compounds able to react with metals and modify their environmental behavior.

Humic substances (AHS) are the main organic carbon reservoir in an aquatic system (Camargo and Cruz 1999; Chen et al. 2007; Cabaniss 2009; Sachs and Bernhard 2011). These result from the degradation of molecules like proteins, cellulose, tannins, lignins among others, and their subsequent polymerization forming macromolecules containing phenols, carboxylic acids, carbohydrates and heteroatoms (N, O, P, S) (Liu et al. 2010).

Although AHS have common precursory molecules, physico-chemical conditions like concentrations of compounds and substances present, pH and ionic force result on differences on the polymerization interaction points. This

produces different structures of AHS that depend on their formation site and influences their reaction capacity to form complexes (Kononova 1966; Burba et al. 1994; Chakraborty and Chakrabarti 2008).

Carboxylic groups are one of the functional groups present in AHS containing oxygen that react with transition elements to form complex compounds (Rudd et al. 1984; Tipping and Hurley 1992; Tipping et al. 2002; Goveia et al. 2010). Complexing reaction may be considered as acid-base where metals act as Lewis acids (electron acceptors) and ligands as Lewis bases (electron donors).

Complexing capacity depends on physico-chemical parameters like pH. Steric configuration is influenced pH; according to Burba et al. (1994) configuration will be lineal at an alkaline pH and will become spheric with decreasing pH. The pH value will also affect metals speciation.

Their role as micronutrients makes relevant the study of metals in aquatic systems. Metal's chemical form also affects their toxicity. Transition elements may be used to understand the chemical behavior of metals facing AHS elements. In this study elements were selected according to Irving and Williams (1953) series stating that reactivity increases with atomic number being Cu the most reactive and Mn the less in the first transition series. Zinc shows a lower reactivity than Cu but higher than Mn (Su et al. 2012). In addition, the studied elements may proceed from various anthropogenic and natural sources. Copper is employed as fungicide, color fixative, fertilizer and others, it is released by natural and anthropogenic processes (mining). Manganese is employed

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as pesticide, in industrial activities, catalyst and is also released by natural process to the environment. Mining is an important source of Zn; it is widely employed in the metal's industry, and used in several products like batteries, paints, chemicals, etc. The three metals are essential elements for life but became toxic at high concentrations, being thus relevant to determine their interactions in aquatic systems since an excess is dangerous for the environment but a deficit does not permit metabolic processes (Erickson et al. 1996; Gavin et al. 1999; Gaetke and Kuang 2003; Crossgrove and Zheng 2004).

Besides, arsenic is one of the most toxic and widely distributed element that may be present in the environment in high concentrations due to anthropogenic and natural sources, as is the case of Argentina, Bangladesh, India, Chile, Peru, Brasil and Mexico (Silva-Pinto et al. 2010; Armienta and Segovia 2008; Bhattacharya et al. 2007). This makes relevant to propose remediation alternatives to the contamination by this and other elements like Zn and Mn that may pose an environmental threat depending on their concentration.

This study was performed with the aim to compare the complexing capacity with transition metals and a metalloid (As, Cu, Mn and Zn) of AHS obtained from three aquatic systems with different environmental and physico-chemical conditions: two Brazilian rivers (Sorocaba and Itapanhaú) and a Mexican lake (Xochimilco). Physico-chemical characteristics at each site were: pH (Sorocaba River 5, Itapanhaú River 4.5 and Xochimilco Lake 6.8), conductivity (Sorocaba River 135 μScm^{-1} , Itapanhaú River 46 μScm^{-1} and Xochimilco Lake 947 μScm^{-1}) and organic carbon concentration (Sorocaba and Itapanhaú Rivers 18.9 and 15.9 mg l^{-1} respectively, and Xochimilco Lake > 100 mg l^{-1}) (Oliveira et al. 2015; López-López et al. 2006). Minerals rich in Mn and Zn are present in Sao Paulo state where the two rivers are located. The third system in México receives raw and treated waste-water that may contain heavy metals and As, within a volcanic rocks basin with As presence. Metals speciation was also related with AHS complexing capacity.

Materials and Methods

Humic substances were obtained from Sorocaba and Itapanhaú Rivers and Xochimilco Lake. Sorocaba is a river polluted by industry and urban activities. Although a cleaning campaign started some years ago, this has not be totally accomplished. Itapanhaú is a pristine river holding touristic activities and near to a mining zone. Xochimilco is a lake located in Mexico City receiving direct domestic wastes from local dwellers and treated urban wastewater from Mexico City.

Aquatic humic substances were isolated from the water bodies following the method described by Thurman and Malcolm (1981). Solutions containing 100 mg l^{-1} of AHS and 500 mg l^{-1} of Cu, Mn and Zn and 200 mg l^{-1} of As were prepared to from salts CuSO_4 .

$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and As_2O_3 , this latter required to dissolve an alkaline dissolution (NaOH 0.05 N).

Experiments were performed using buffer solutions to have a fixed pH of 4.5, 6, 7.5 and 9, with buffer dissolutions supported in carbonate–bicarbonate–carbonic acid series considering the range common in surficial natural waters. These pH values were selected to simulate natural physico-chemical environmental conditions as a proxy of processes occurring in natural aquatic systems. The solutions containing the AHS and each metal were agitated for an hour. Then they were filtered in a tangential ultrafiltration equipment through 1 kDa membrane and N_2 pressure of 40 psi. These allows to separate the complex produced from the solution. The non-complexed metal remaining in solution was analyzed with an atomic absorption spectrophotometer Perkin Elmer AAnalyst 200. Quality assurance included the use of high purity standards (NIST traceable, accredited to ISO/IEC 17025 Guide 34 for As, Cu and Zn and HYCEL for Mn) for calibration. Under conditions for As $\lambda = 193.7$ nm, EN = 36 and slit 0.7 nm, Cu $\lambda = 324.25$ nm, EN = 78 and slit 2.7/0.8 nm, Mn $\lambda = 279.48$, EN = 65 and slit 1.8/0.6 nm, and Zn $\lambda = 213.86$ nm, EN = 62 and slit 2.7/1.8 nm. Quantitation limits are for As 0.0025 mg l^{-1} , Cu 0.25 mg l^{-1} , Mn 0.10 mg l^{-1} and Cu 0.05 mg l^{-1} . Measures of standards and samples were made by triplicate.

The complexes characterization was performed with infrared (IR) technique, under the Reflectance method ATR in the FTIR SPECTRUM 400 Perkin Elmer equipment. Analytical conditions were: Spectral range 4000–400 cm^{-1} , resolution 4 cm^{-1} and number of sweeps 64.

To determine metal's speciation at the experimental conditions we applied the geochemical program "The Geochemist's Workbench Student Edition".

Complexing capacity was determined from the relation between the complexed amount of metal and the AHS added to the solution, as:

$$CC = \frac{M_{com}}{AHS_{ad}} \quad (1)$$

where *CC* complexing capacity (mmol/g); *M_{com}* Complexed metal concentration (mmol); *AHS_{ad}* concentration of AHS (g).

Results and Discussion

Complexing capacity calculated from experimental results for the studied metals with the AHS obtained from each site, at various pH values is shown in Fig. 1. Copper presented a higher capacity with increasing pH, while Mn had

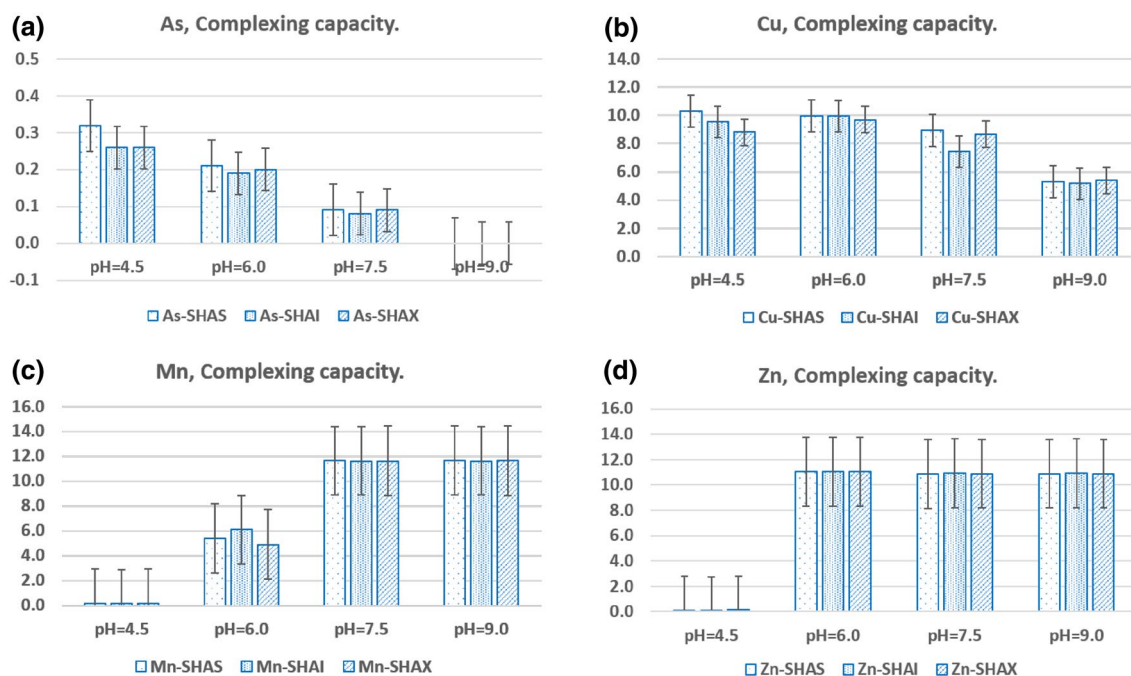


Fig. 1 Complexing capacity of the AHS at various pH values. **a** (As), **b** (Cu), **c** (Mn), **d** (Zn), SHAX (Aquatic Humic Substances Xochimilco) SHAS (Aquatic Humic Substances Sorocaba) SHAI (Aquatic Humic Substances Itapanhaú)

an opposite behaviour. Zinc presented an almost constant complexing capacity at all the pH values but 4.5. At that pH no reaction was observed between Zn and AHS. On the other hand, As (III) had a very low reactivity decreasing with pH until pH 9 where no reaction was observed.

To determine if complexing capacity varied among the AHS from each site we performed a t test at 95% confidence. Results presented in Table 1 indicate the validity of the nule hypothesis. Averages are thus not statistically different and show a similar behavior with each metal and pH non-withstanding their diverse source.

Results shown in Fig. 1 indicate that the formation of Mn complexes is favored at higher pH while that of Cu decreases with pH. On the other hand, formation of As (III) complexes with SHA is not favored in the reaction conditions, while the low variation of Zn with pH (except at pH 4.5) indicates that complex formation only depends on AHS availability.

According to the chemical characteristics of humic substances, due to the pKa values, at a pH below 4.5, carboxylic groups will be in a protonated form, while phenolic groups will protonate at a pH around 7 (Corami et al. 2007). The complexing capacity of AHS will then decrease with decreasing pH (Tipping et al. 2002; Lippold and Lippmann-Pipke 2009; Yang and Van den Berg 2009) since protons will occupy available active sites. Considering complexing as an acid-base reaction it is thus influenced by pH, besides metal’s speciation may also depend on pH (Munier-Lamy et al. 1986).

Table 1 Values obtained from the t test (95% confident level) for each experimental case

pH	SHAS-SHAI	SHAI-SHAX	SHAS-SHAX
As			
9.00	0.00	0.00	0.00
7.50	0.00	0.00	0.00
6.00	0.00	0.00	0.00
4.50	0.00	0.00	0.00
Cu			
9.00	0.36	0.16	0.48
7.50	0.00	0.00	0.03
6.00	0.64	0.00	0.00
4.50	0.39	0.33	0.01
Mn			
9.00	0.05	0.23	0.57
7.50	0.62	0.59	0.06
6.00	0.11	0.06	0.39
4.50	0.00	0.00	0.00
Zn			
9.00	0.04	0.01	0.12
7.50	0.03	0.03	0.37
6.00	0.41	0.73	0.43
4.50	0.00	0.12	0.93

According to various authors (John et al. 1988; Lambert et al. 1995; Elkins and Nelson 2001) complexing proceeds

through a nucleophilic reaction where the molecule active site is negatively charged waiting to react with a cation. However, due to the chemical nature of the salts used in the experiment and to the presence of buffer solutions, theoretical diagrams obtained with GWB program show presence of Cu, Mn and Zn positive ions. These cations may react with active sites of the AHS and form the complexes. On the other hand, arsenic forms oxyanions diminishing thus its reaction capacity with humic substances.

Structure of humic substances constitutes another factor to take into account in complex formation reactions, since a spheric configuration has been reported at an alkaline pH and also at acid pH values and high ionic force. In contrast, a lineal configuration is present at an alkaline pH and low ionic force. Protonation and deprotonation also influences the position of active sites resulting on variations of the reaction mechanisms (Chen et al. 2007; Laglera and Van den Berg 2009).

Complexing capacity values obtained from the application of Eq. (1) at the selected pH values show a more

quantitative complexing of Cu at acid pH than at alkaline pH, while Mn does not react at acid pH but forms complexes at a slightly alkaline pH reaching higher values than those of Cu. Optimum values for complexing reactions are comprised in a pH range between 6 and 7.5. Mn complexing capacity showed similar values at alkaline pH with 11.6 mmol g^{-1} measured at pH 7.5, and 11.6 mmol g^{-1} at pH 9.0 showing an asymptotic behavior. While Cu complexing capacity decreased with pH ($9\text{--}10 \text{ mmol g}^{-1}$ at pH 4.5–5–5.5 mmol g^{-1} at pH 9.0).

Arsenic showed very low complexing capacity values (from non-detected to 0.32 mmol g^{-1}) decreasing with increasing pH. No reaction was observed between Zn and AHS at a pH 4.5 reaching a maximum at pH 6 with 11 mmol g^{-1} , this value was very similar to those at pH 7.5 and 9.0 of 10.9 and 10.8 respectively.

One of the possible reasons of the particular behavior of each metal may be related with their chemical form at each pH. Eh versus pH diagrams were built using the Geochemist Workbench modeling program considering temperature,

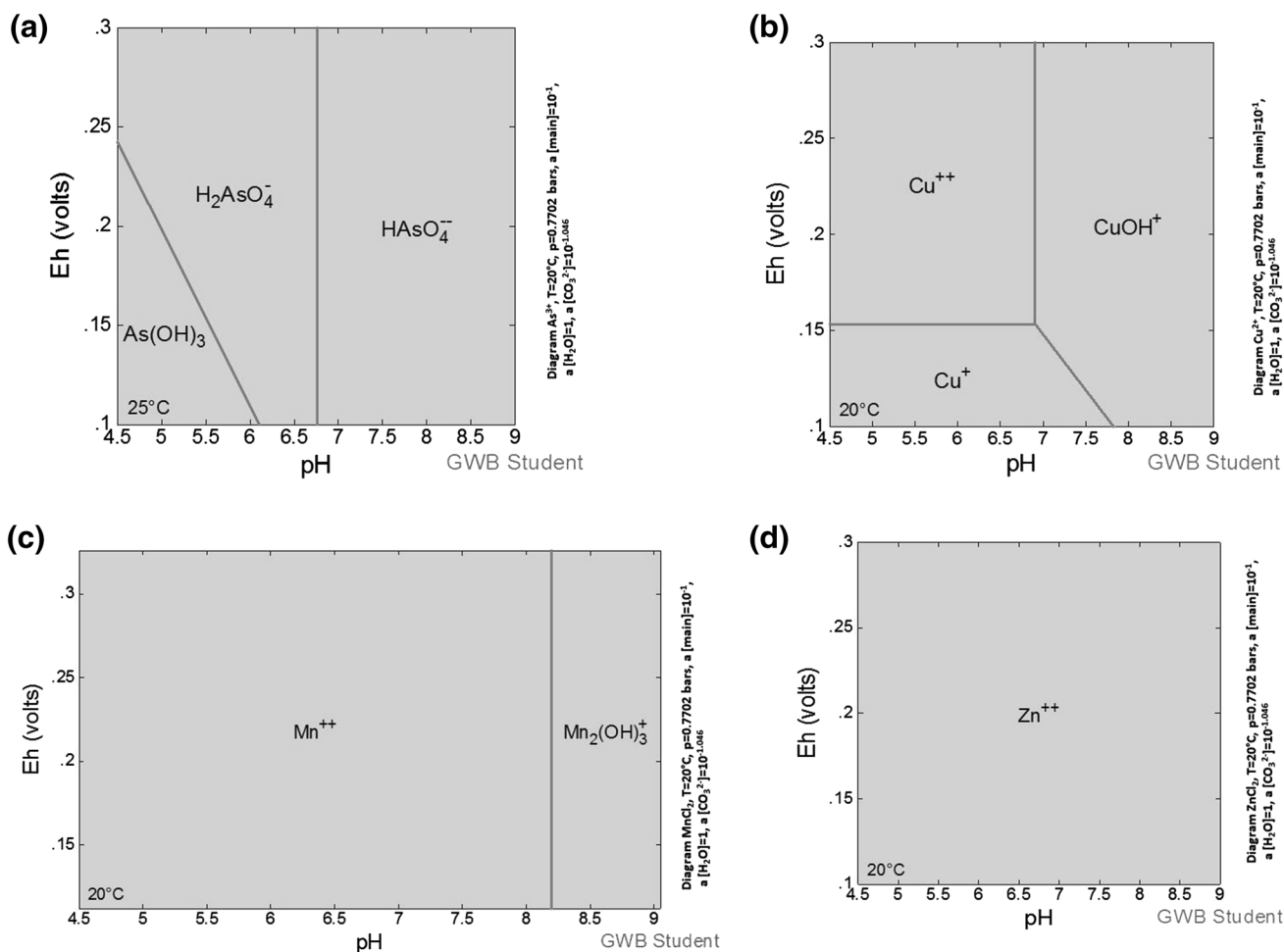


Fig. 2 Speciation diagrams under experimental conditions for **a** Cu, and **b** Mn, **c** As, **d** Zn

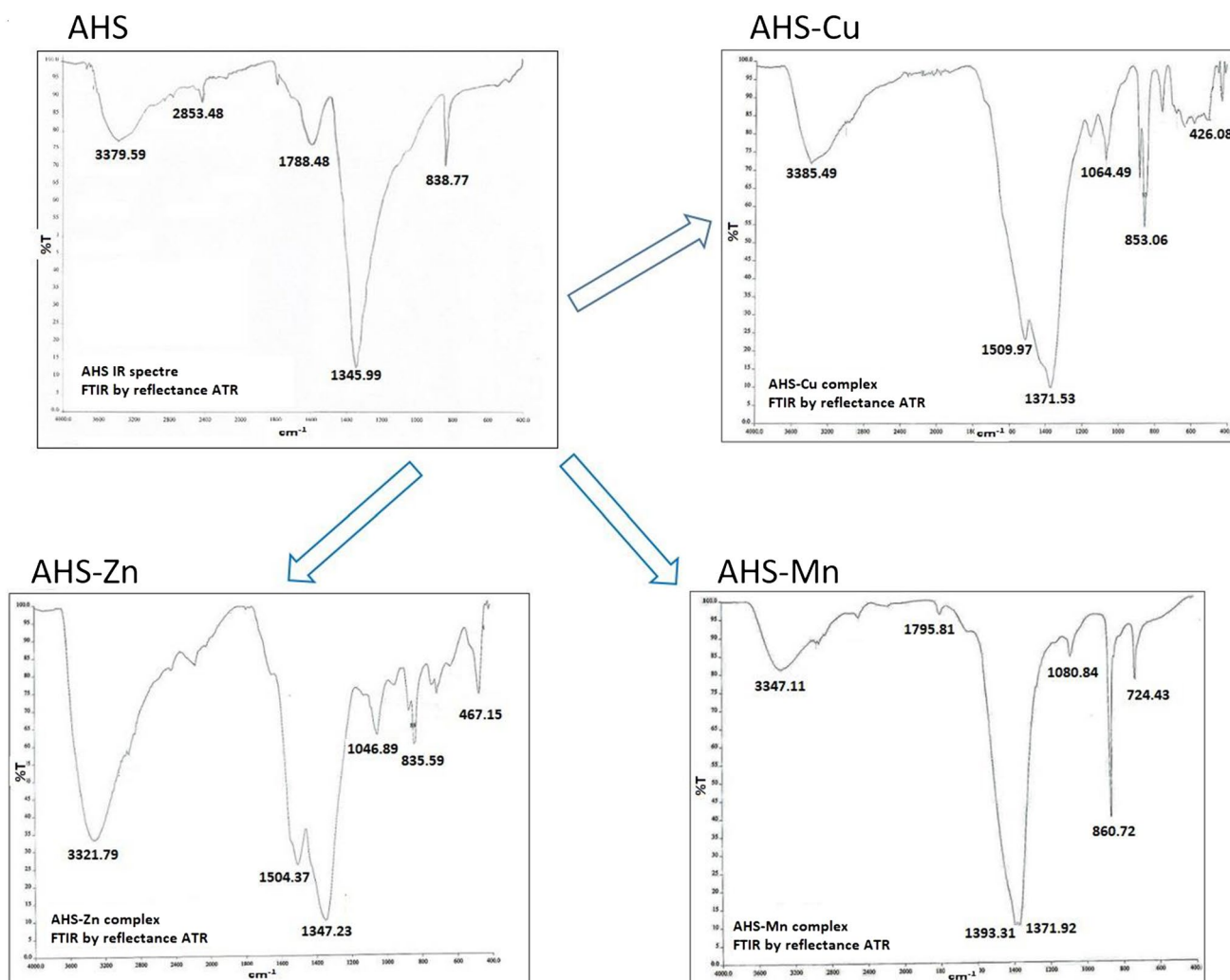


Fig. 3 IR spectra of AHS and complexes formed with Cu, Mn and Zn

pressure, solvent and dissolved ions. The program also makes possible to consider only aquatic species discarding mineral phases.

Diagrams are shown in Fig. 2.

The speciation diagram of Cu shows its presence as cation in the experimental conditions at low pH favoring its reaction with the active sites of humic substances. However, Cu forms hydroxides at high pH and due to their low solubility tend to precipitate decreasing the quantity of the complexing reaction with the AHS. Experiments between Cu and catechol showed a good complexation at a pH close to 8, that was about six times that of Zn (Xue et al. 1995). However, we did not observe that higher complexing capacity of Cu, but the opposite. On the other hand, Lagier et al. (2000) identified a pH of 6 as the optimum value for complex formation. This indicates that Cu has a good affinity to organic matter but complexation reactions are influenced by pH.

Manganese is present mainly as cationic species that may interact with AHS at an alkaline pH. Besides, its presence as hydroxide gives the capacity to form hydrogen bridges with the phenolic groups of humic substances producing more stable complexes. In addition, infrared spectrum of the compound formed 2 weeks after the reaction, showed presence of bands corresponding to MnCO_3 3347.11, 1393.31 and 1371.92 cm^{-1} indicating that the obtained product will precipitate as carbonate if it is maintained in water solution (view Fig. 3).

The infrared spectrum of the produced Cu and Zn complexes showed that they are formed through the link with carboxylic groups, since typical bands at 1700 cm^{-1} are displaced to values close to 1500 cm^{-1} (view Fig. 3).

Values of the complexing capacity and IR spectrum indicate that although reaction of AHS with Mn has a higher quantity with respect to Cu, the Mn complexes formed will tend to precipitate as carbonate.

Regarding the influence of the origin of the AHS on the complexing reactions, statistical tests of the results allowed to approve the nule hypotheses indicating that the complexing capacity does not differ among the studied sites: Xochimilco, Sorocaba and Itapanhaú, as shown in Table 1.

AHS present a similar complexing capacity for Cu, Mn, Zn and As in spite of their origin, only depending on the physico-chemical conditions of the reaction. AHS configuration and metal speciation determined complexation of Cu at acid pH values and of Mn at a slightly alkaline and alkaline pH values. However at a slight basic pH bicarbonate compounds may be formed and finally produce manganese carbonate. Arsenic showed a very low complexing reactivity with the AHS at the experimental conditions. At near neutral conditions Zn and Cu react quantitatively forming stable compounds, and contrary to previous studies with a higher complexing capacity of Zn with respect to Cu. The determined complexing capacity may reflect Cu, Mn and Zn occurrence as free or complexed ion in natural waters and be used to predict their behavior. Experimental results also show the relevance of pH to form a complex or to release the element to the environment. The complexing reactions are a natural way to change toxicity, mobility and bioavailability of metals.

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