Investigations on the Thermodynamic Stability and Availability of Nutrients for Plants by Humic Substances Extracted from Peat Samples

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Abstract: This work evaluated the complexation capacity, exchange constants and availability of micronutrients for plants and humic substances extracted from peat samples. Samples of humic substances extracted from two tropical peats (HS-P1 and HS-P2) were enriched with the micronutrients Cu(II), Co(II), Fe(II), Mn(II), Ni(II) and Zn(II) and the parameters for formation of the complexes (HS-N) were evaluated at different pH. The Scatchard model was used to calculate the maximum complexation capacity and the nutrient availability was studied using exchange capacity experiments based on ultrafiltration procedure. The optimum pH for complexation was 4.5 and the order of affinity was: Fe(II) > Cu(II) > Co(II) > Mn(II) = Ni(II) > Zn(II). The maximum complexation capacity reached 56.8 mg·g⁻¹ Fe of HS-P1 (the highest) and 1.7 mg·g⁻¹ Zn of HS-P2 (the slightest). The exchange experiments showed that HS-P-Fe complexes were formed preferentially. The least stable complex was formed with Zn, which was therefore, more easily available. The results contribute to understand the behavior and availability of some nutrients in soils.

Key words: Peat humic substances, complexation, nutrient availability, exchange capacity, soil.

1. Introduction

Humic substances have undefined chemical structures and can vary widely depending on the characteristics of the environment in which they are formed [1, 2]. The most widely recognized functions of HS in the environment are the complexation and precipitation of cationic species, such as metals and the adsorption of organic compounds [3-6]. These capabilities are due to the functional groups present in the chemical structure of HS, such as carboxylic groups, phenols and ketones, which are responsible for the interactions [7-9]. The interaction of HS with metal species results in the formation of HS-Metal complexes, hence, influence speciation transport and availability of inorganic species in the environment [10, 11].

The beneficial effects of HS in soils include heat retention (due to its dark color), which contribute in

seed germination and water retention, which prevents soil erosion. Also HS can be used as a soil conditioner that increases the stability of aggregates, hence, reduces risks of erosion by runoff and decreasing water infiltration rates, which is beneficial in terms of crop productivity [1, 12].

Successful and sustainable food production depends on the quality of the soil, which needs to contain the organic matter and nutrients required for plant growth [13]. HS enriched with micronutrients responsible by plant growth present themselves as an attractive alternative for increasing agricultural productivity. There are some published studies have only evaluated the complexation capacity of elements present as contaminants without considering the natural competition that occurs between elements in the soil [14-16]. The appropriate soil management requires adequate knowledge of the interactions that involve humic substances, which play a major role in the transport, complexation and bioavailability of

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nutrients in the soil. Although this is of fundamental importance in soil chemistry, the literature is fairly sparse in terms of detailed analyses of these processes. Then, the present study investigates complexation and exchange capacity using humic substances extracted from peat samples (HS-P), with the main micronutrients (Cu(II), Co(II), Fe(II), Mn(II), Ni(II) and Zn(II)) required for photosynthesis and plant growth.

2. Material and Methods

2.1 Reagents and Equipment

The stock micronutrient standard solution ($100.0 \text{ mg}\cdot\text{L}^{-1}$) was prepared from the following salts: $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, $\text{CoSO}_4\cdot 7\text{H}_2\text{O}$, $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$, $\text{MnSO}_4\cdot 5\text{H}_2\text{O}$, $\text{NiSO}_4\cdot 6\text{H}_2\text{O}$ and $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$. The solution was acidified with HNO_3 and dilute solutions of HCl and NaOH were used to adjust the pH. The solutions were prepared and/or diluted with deionized water (18 M Ω , Milli-QPlus and Millipore) and all materials were decontaminated overnight in 10% (w/v) HNO_3 . The micronutrients were quantified by inductively coupled plasma optical emission spectroscopy (ICP-OES), using an Agilent Model 720 instrument equipped with a “seaspray” nebulizer. The instrumental conditions were: RF power of 1.10 kW, argon plasma flow of $15.0 \text{ L}\cdot\text{min}^{-1}$ and spray pressure of 200 kPa. Calibration standards were prepared from a $100.0 \text{ mg}\cdot\text{L}^{-1}$ stock solution (SpecSol). The detection limits, calculated from the standard deviation of the readings of ten analytical blanks, were ($\mu\text{g}\cdot\text{L}^{-1}$): 3.2 (Cu), 2.4 (Co), 2.3 (Fe), 3.0 (Mn), 1.4 (Ni) and 2.5 (Zn).

2.2 Peat Sampling and Humic Substances Extraction

The peat samples were collected from two peatlands at Sergipe State, Brazil on February 2010, at the 0-20 cm layer, with 5 samples obtained at each sampling point. Both peatlands are distance themselves about 50 km and the differences are little being one with more sand than other. The peat samples were stored in polyethylene bags, air-dried,

trituated and homogenized in a porcelain mortar and sieved (2 mm mesh). The humic substances (HS) from the two peatlands (identified here as HS-P1 and HS-P2) were then extracted using the alkaline extraction procedure suggested by the International Humic Substances Society. A peat mass of 300 g was placed in a beaker, which was added 3.0 L of $0.1 \text{ mol}\cdot\text{L}^{-1}$ NaOH (1:10 ratio, m/v). The beaker was agitated under a nitrogen atmosphere for 4 h at room temperature (25°C), using a horizontal shaker which is followed by centrifugation at 2,500 rpm. The humic substances (contained in the supernatant) were concentrated using a rotary evaporator and dried in a ventilated oven at 40°C .

2.3 Humic Substances Characterization

The two humic substances samples were characterized by elemental and spectroscopic analyses. The elemental analysis employed a Flash EA1112 analyzer (Finnigan Thermo). The carbon, hydrogen, and nitrogen are determined by the combustion method and the oxygen by the pyrolysis method. The spectroscopic analysis was performed by ^{13}C NMR with cross-polarization (CP) and magic angle spinning (MAS), using an Avance III 400 MHz spectrometer (Bruker), with a rotation of 5 kHz, a contact time of 2 ms and a relaxation time of 5 s and 11,000 scans. The ^{13}C NMR spectrum was analyzed according to the chemical shifts of carbon in different spectral regions [17].

2.4 Determination of Complexation Capacity (CC)

The equilibration time was determined in order to establish the ideal times for the complexation of Cu, Co, Fe, Mn, Ni and Zn with the humic substances. A volume of 10 mL of the stock solution containing $100.0 \text{ mg}\cdot\text{L}^{-1}$ of each nutrient was placed in an Erlenmeyer flask together with 90 mL of $100.0 \text{ mg}\cdot\text{L}^{-1}$ (w/v) humic substances (HS-P1 or HS-P2). The flasks were kept under agitation at ambient temperature (25°C) and the ionic strength was maintained to 0.1

$\text{mol}\cdot\text{L}^{-1}$ with NaNO_3 . The analytical procedure proposed by Burba et al. [18] was employed, which is based on a tangential ultrafiltration system equipped with a 1 kDa porosity cellulose acetate membrane (Millipore) (Fig. 1). This membrane prevented the passage of HS and HS-N complexes with a molecular size greater than 1 kDa, hence, only allowing the passage of micronutrients that were not complexed with the HS (or that were free in solution). At predetermined times (10, 20, 30, 40, 60, 90, 120, 240 and 1,440 min), aliquots (2 mL) of the filtrate were collected for determination of the micronutrient concentrations. The experiments were performed in triplicate.

The complexation capacity (CC) of the humic substances with the micronutrients was evaluated at four different pH values (2.5, 4.5, 6.0 and 8.0) in order to identify the conditions that provided the highest degree of complexation. A volume of 100 mL of the $100.0\text{ mg}\cdot\text{L}^{-1}$ HS-P1 or HS-P2 sample solutions were added to Erlenmeyer flasks kept under continuous stirring and with the pH values already adjusted. The ionic strength was maintained to $0.1\text{ mol}\cdot\text{L}^{-1}$ with NaNO_3 . Aliquots of 2 mL were then collected from the solutions containing only humic substances (at time zero, prior to addition of the micronutrients). The micronutrients Cu(II), Co(II), Fe(II), Mn(II), Ni(II) and Zn(II) were then added using successive aliquots of $100.0\text{ mg}\cdot\text{L}^{-1}$ stock solution, every 20 minutes (equilibration time), to give final concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2.0, 3.0, 4.0, 5.0, 6.0 and $8.0\text{ mg}\cdot\text{L}^{-1}$. After every addition, aliquots of the filtrates (2 mL) were collected for subsequent determination of the micronutrients. The pH was corrected for each aliquot added of the micronutrient stock solution. The experiments were performed in triplicate.

2.5 Determination of Exchange Capacity (EC)

The exchange capacity of each micronutrient was determined in the presence of the other micronutrients.

Fig. 1 ^{13}C NMR spectra for the HS-P1 and HS-P2 samples (conditions: CPMAS ^{13}C NMR with 5 kHz rotation, 2 ms contact time, 5 s relaxation time and 11,000 scans).

Each exchange micronutrient was added to a solution of previously complexed humic substances. The exchange experiments were conducted using Erlenmeyer flasks containing 100 mL of the $100.0\text{ mg}\cdot\text{L}^{-1}$ HS-P1 or HS-P2 sample solutions which was previously complexed with the micronutrients at the highest concentrations found in the CC experiments. The pH was adjusted to the optimum value found in the CC experiments (pH 4.5). An aliquot of the filtrate (2 mL) was collected prior to addition of the exchange micronutrient and this point was taken as zero. Aliquots of each exchange micronutrient (copper, cobalt, iron, manganese, nickel and zinc from an individual stock solution of $100.0\text{ mg}\cdot\text{L}^{-1}$) were successively added every 20 min to give concentrations of 0.2, 0.6, 1.0, 1.4, 1.8 and $3.0\text{ mg}\cdot\text{L}^{-1}$.

4. Results

4.1 Structural Characterization

The elemental composition (carbon, hydrogen, oxygen and nitrogen) results are presented in Table 1, together with the atomic ratios. The H/C and O/C atomic ratios for the HS-P1 sample were lower than for HS-P2. The H/C atomic ratio provides information on the degree of saturation of carbon in an organic molecule, with lower ratios being indicative of greater aromaticity of the HS. Besides, the O/C ratio is

Table 1 Elemental composition (% C, H, O and N) of the HS-P1 and HS-P2 samples and atomic ratios.

	C	H	O	N	H/C	O/C	C/N
HS-P1	43.4	3.7	31.5	1.5	1.00	0.54	36.0
HS-P2	37.2	3.6	17.5	1.8	1.20	0.55	25.8

indicative of the carbohydrate content, with a lower ratio reflecting lower carbohydrate content. Therefore, the aromaticity of the HS-P1 sample was higher than that of HS-P2. The C/N ratio provides an indication of the origin of organic matter in natural environments. Values below 20 are indicative of humification processes favored by microbial activity, while values greater than 20 indicate the predominance of plants in the humification process [19]. For both HS samples, the humification process was associated with the presence of plants.

Nuclear magnetic resonance analysis (^{13}C NMR) enables the relative percentages of the different types of carbon present in the structures of the samples to be estimated, so, possible structures of the humic substances can be proposed. Fig. 1 shows ^{13}C NMR spectra for the HS-P1 and HS-P2 samples and provides the values of the relative percentages referring to the different carbon groups present which is obtained from integration of areas in different regions of the spectra. Shifts in the 0-45 ppm region were assigned to alkyl carbons and shifts of methoxylated carbons were found in the 45-60 ppm region. Other features included shifts at 60-110 ppm (alkyl carbon), 110-140 ppm (aromatic carbon), 140-160 ppm (phenolic compounds), 160-185 ppm (carboxylic carbon) and 185-245 ppm (carbonyl carbon) [20, 21]. More specific carbon shifts have been reported previously, such as those at 60-95 ppm (related to O-alkyl carbons) and at 95-110 ppm (related to di-alkyl carbon) [22]. It can be seen from the relative percentages of carbon groups that the shifts related to aromatic, phenolic and carboxylic carbons were more pronounced for the HS-P1 sample. While, the HS-P2 sample showed more pronounced shifts related to aliphatic carbons. This implies that the structure of HS-P1 was more aromatic when

compared to HS-P2. This is in agreement with the elemental analysis (Table 1). Since the HS-P1 sample presented a chemical structure that was richer in aromatic phenolic and carboxylic groups, compared to the HS-P2 sample, it would be expected to show different behavior in terms of the complexation process, micronutrient enrichment and nutrient bioavailability.

4.2 Complexation Capacity (CC)

The equilibration time was established by measuring the concentrations of micronutrients free in solution as a function of time after their addition. Equilibrium between the humic substances and the micronutrients (HS-N) was reached in 20 min, after which no changes were observed in the micronutrient concentrations (Fig. 2).

The concentrations of the micronutrients free in solution were plotted against the concentrations complexed to the HS at different pH values. The graphs obtained resembled the adsorption isotherm curves generally obtained from highly adsorbent materials [23]. The behavior obtained from the complexation capacity of HS-P1 and HS-P2 enriched with the micronutrients was studied in four different pH levels. In most cases, exponential curve profiles indicated that the complexation of micronutrients proceeded progressively up to the point where the active complexation sites began to be saturated with the micronutrients. After complete saturation, the maximum complexation capacity could be determined from the inflection point of the curve [24]. For both samples (HS-P1 and HS-P2) and all micronutrients, the best complexation capacity was obtained at pH 4.5.

Once the best complexation conditions have been established, the CC can be calculated using mathematical models [25-28]. The Scatchard model,

Fig. 2 Equilibrium time for the complexation of micronutrient: Cobalt (-), Copper (-■-), Iron (-●-), Manganese (-▼-), Nickel (-▲-) and Zinc (-◆-) with peat humic substances samples HS-P1 and HS-P2. (conditions: humic sample concentration 100.0 mg L⁻¹).

which has been used in several previous studies [29-30], considers the complexation process described by Eq. (1) and the stability constant of the HS-N complex can be obtained using Eq. (2).

$$Kc = \frac{[ML]}{[M][L]} \quad (2)$$

Where, [M] is the concentration of micronutrient free in solution (mg·L⁻¹), [L] is the concentration of ligand (HS), [ML] is the concentration of complexed micronutrient and Kc is the stability constant of the HS-N complex.

Eq. (3) describes the Scatchard model, which represents the relationship between the concentration of the complexed micronutrient (plotted graphically on the x-axis) and the ratio between the concentrations of the complexed micronutrient and the micronutrient free in solution (y-axis) (Fig. 2). For 1:1 stoichiometry, assuming one binding site, a straight line is obtained, with the value of Kc being obtained from the slope and the value of L from the linear coefficient divided by Kc. A graph showing two separate lines is indicative of two or more binding sites, the values of Kc1 and Kc2 are obtained from the angular coefficients of each line, and the values of L1 and L2

are obtained from the linear coefficient divided by Kc1 and Kc2 respectively. The complexation capacity can then be calculated according to Eq. (4).

$$\frac{[ML]}{[M]} = L \times Kc - Kc \times [ML] \quad (3)$$

$$CC = \frac{L}{P} \quad (4)$$

Where, [ML] is the concentration of the complexed micronutrient, [M] is the concentration of the free micronutrient, L is the concentration of the binding sites, Kc is the stability constant of the HS-N complex, CC is the maximum complexation capacity and P is the concentration of the ligand (g·L⁻¹).

Table 2 shows the values of CC calculated for the micronutrients using samples HS-P1 and HS-P2. Sample HS-P1 was able to complex higher concentrations of the nutrients, which could be explained by the structural differences identified from the ¹³C NMR and CHN analyses. The structure of HS-P1 was more aromatic, containing phenolic and carboxylic groups that increased the complexation capacity. For both HS samples, the order of affinity was: Fe(II) > Cu(II) > Co(II) > Mn(II) = Ni(II) > Zn(II). This order of affinity can be explained by Pearson's theory, as well as by the Irving-Williams

Table 2 Complexation capacities of the peat humic substances (HS-P1 and HS-P2) for Cu(II), Co(II), Fe(II), Mn(II), Ni(II), and Zn(II).

	HS-P1 mg·g ⁻¹ of HS	HS-P2 mg·g ⁻¹ of HS
Cu(II)	56.8	37.2
Co(II)	47.5	23.6
Fe(II)	59.8	57.4
Mn(II)	35.3	21.7
Ni(II)	32.7	19.4
Zn(II)	3.1	1.7

series, which considers the stability of the complexes formed with metal ions and takes into account the ionic potential of each metallic element.

4.3 Exchange Capacity (EC)

Once formed, it was important to determine the stability of the HS-N complex in order to evaluate nutrient bioavailability. The stability is normally quite high due to strong interaction of the anionic groups with the metal cations. However, under certain circumstances, the complex can be destabilized as in the presence of another metallic element with higher affinity or due to changes in redox and pH conditions. The exchange capacity provides information about possible changes in the stability of the initial complexes that could occur under different conditions. The exchange constant can be calculated using mathematical models similar to those used for the CC. The simplistic Scatchard mathematical model can be expressed as in Eq. (5):

Where, [M] is the concentration of the micronutrient, [X] is the concentration of the exchange micronutrient added, [HS-N] is the concentration of the micronutrient M complexed to the HS and [HS-X] is the concentration of the added exchange micronutrient X complexed to the HS. The exchange constant, K_e , is given by Eq. (6):

$$K_e = \frac{[\text{HS} - \text{X}][\text{M}]}{[\text{HS} - \text{M}][\text{X}]} \quad (6)$$

The value of K_e can be obtained from the angular

coefficient for the linearized plot of [M]/[HS-M] against [X]/[HS-X].

According to Eq. (6), K_e is inversely proportional to the stability of the HS-N complex. In other words, K_e values greater than 1 indicate that exchange of the initially complexed micronutrient by the subsequently added micronutrient is favored.

The K_e values obtained showed that in the exchange experiments with Cu(II), the complexes of HS with the micronutrients Co(II), Mn(II), Ni(II) and Zn(II) were rather unstable. So, these micronutrients could be easily released (exchanged) to the medium, while, Cu(II) became complexed with the HS. The same behavior was observed for the exchange micronutrient Fe, since it could exchange with Cu, showing that the HS-Fe complex was more stable than any other complex. The two samples of HS showed differences in terms of the degree of exchange, which was generally higher for HS-P1, especially for Cu and Fe.

5. Discussion

Understand the complexation processes involved in the environment is not easy due to the complexity of the composition of all substances present in each environmental compartment. In the soil systems, the processes are influenced by several factors like the solution soil pH, temperature, solar radiation, content of OM, the micro fauna and the concentration of nutrients and others substances with toxic potential [31]. Peat is extremely rich in organic matter and humic substances (HS) is the organic fraction extracted. The structural characteristics of HS are intrinsic to each region, since these are formed by the decomposition of plant and animal residues, which strongly influenced by some factors such as climate, topography and vegetation of the site. Thus, it is important to characterize each HS sample to better understand their composition and therefore, to understand the processes that the HS are involved and operated. The HS extracted from Brazilian peat were

characterized by techniques as elemental analysis and nuclear magnetic resonance of ^{13}C . According to elemental analysis, using the atomic ratios between C, H, O and N, differences can be seen in the two HS samples analyzed. The HS-P1 sample showed a greater content of aromatic C than the HS-P2 sample and therefore, it is expected that the complexation of these samples with the micronutrients will be different. Values of H/C and O/C observed in this study were very similar to those found by Gondar et al. [32] for fulvic and humic acid samples extracted from Spanish peat, where aromaticity was more evident in the humic acids. Fernandes et al. [33] observed values of H/C lower than obtained here for humic substances extracted from highly humified Brazilian peat. This is in agreement with the greater degree of unsaturation (aromaticity) that is observed during advanced stages of decomposition. From this finding, it can be concluded that the HS-P1 sample possibly is in the most advanced stage of decomposition. The results of the NMR analysis confirm the data of elemental analysis, which is evident the shifts from aromatic, carboxylic and phenolic compounds in the HS-P1 sample, while the shifts from HS-P2 sample refer to aliphatic C is more pronounced.

Micronutrients are defined as substances essential to plant development at low concentrations. The main ones that can be cited are copper, molybdenum, iron, boron, manganese chloride, nickel, zinc and cobalt. In this study, it was decided to evaluate the micronutrients that are in cationic form in a wide pH range, such as copper, cobalt, iron, manganese, nickel and zinc. The availability of plant nutrients is influenced by the organic matter content in the soil. Thus, the complexation reactions that involve HS and micronutrients enable to know the dynamics and transport of these nutrients. The process of complexation is influenced by environmental factors such as pH and concentration of organic matter and nutrient. The pH of complexation evaluated in this study was chosen based on the pH observed in

Brazilian soils, which can be acid and basic depending on the region in the country. The best pH for complexation of micronutrients with the HS was observed at 4.5. At pH 2.5, the functional groups present in humic substances are likely to be protonated, which hinders the complexation of cationic species due to charge repulsion. In addition, at this pH, micronutrient solubility is high (at the concentrations used). However, at pH 4.5, 6.0 and 8.0, the functional groups present in the HS structure tend to be ionized and are therefore, available for the complexation of cationic species in order to neutralize the electrostatic charges. At pH between 3 and 6, the carboxylic and phenolic groups (major constituents of humic substances) are preferably ionized and therefore, capable of complexing the micronutrients to equilibrate the charges [34]. At pH 6.0, elements such as Fe and Mn can be precipitated and/or hydrolyzed (at the concentrations evaluated), while at pH 8.0, there is further precipitation, with the elements Cu and Zn becoming less soluble in solution. These considerations provide an explanation for the observation that the best complexation was achieved at pH 4.5, since the elements were available in solution and the carboxylic and phenolic groups were preferentially ionized. Garcia-Mina et al. [30] found that the maximum complexation capacity of Cu, Zn, and Fe in HS samples extracted from peat occurred at pH 6-9 due to the greater ionization of the acidic groups present in the HS structure.

From the viewpoint of soil applications, there could be a possibility of soil acidification if the HS-N complexes were applied at pH 4.5. Moreover, López et al. [35] showed that the formation of metal-organic complexes leads to an increase in acidity of soil solution. Nonetheless, acidification can be easily controlled using lime, and silicon has also been shown to provide effective support for the plant [36]. Furthermore, any drawbacks could be compensated by the ability to use a single product containing the micronutrients that are most important to plants as

well as the organic material needed to promote healthy growth.

The Scatchard model was applied to the results of complexation at pH 4.5 to calculate the maximum complexation capacity (CC). And the CC which was obtained confirmed that the HS-P1 sample, which has a chemical structure rich in aromatic, carboxylic and phenolic compounds, interacts with a greater amount of nutrient when compared with the HS-P2 sample. This shows that the knowledge of the chemical structure is important for understanding the processes of complexation and consequently, the availability of the nutrient complexed. Moreover, it can be verified an affinity order for the micronutrients: $\text{Fe(II)} > \text{Cu(II)} > \text{Co(II)} > \text{Mn(II)} = \text{Ni(II)} > \text{Zn(II)}$. According to Pearson [37], all the micronutrients evaluated (with valency of II) are regarded as unreliable or are not even classified as hard or soft acids. However, some studies have shown the ability of the HS to influence the formation of species such as Fe(II) and Fe(III) under certain experimental conditions including solar or artificial irradiation [38, 39]. In addition, Fe(III) is classified as a hard acid and therefore, able to interact with hard bases such as hydroxyl and carboxylic groups. Since humic substances contain phenolic (-OH) and carboxylic (-COOH) groups, they are considered to be hard bases, which explains the stronger interaction with Fe. In the case of the other micronutrients, for which the valency remains unchanged, the Irving-Williams series is applicable: $\text{Mn(II)} < \text{Co(II)} < \text{Ni(II)} < \text{Cu(II)} > \text{Zn(II)}$, where Cu is the element that forms the most stable complexes [40]. Schnitzer and Kerndorff [41] evaluated the sorption of eleven metals on humic acid and observed a similar behavior being the Fe most sorbed while the Mn was least sorbed. They also verified that the sorption increase with the pH, but some elements like Fe and Cu could form hydroxides at pH 5.8. The results more consistent obtained by them were verified at pH 4.7, which similar to the author's results.

The stability of the complexes can be changed

depending on the environment in which they are exposed. For example, different soil types have different concentrations of nutrients and metal species as aluminum or other contaminants such as lead and cadmium. These substances can act in the complex in case of them which are present in high concentrations or have a greater affinity with the HS. Thus, the need to evaluate the behavior of the nutrients previously complexed to HS against to others substances, allows to know the dynamics of them in the environment. The exchange capacity experiments showed that the complexes previously formed with micronutrients are altered in the presence of other micronutrients. The HS-Fe complex was the most stable since in the presence of this element (iron), the exchange constants were favored. Araujo et al. [42] assessed the capability for exchange between the elements Al, Fe, Cu and Co. He found that the HS-Al and HS-Fe complexes were more stable than HS-Cu and HS-Co. This behavior was attributed to the valency of the ions. Whereby, trivalent ions such as Al(III) and Fe(III) have less dependency on stability as a function of molecular size. In the case of Zn, in all the experiments it was observed that there was a release of this element in the presence of others. This could be advantageous in certain cultivations like maize, while Zn is a limiting micronutrient for optimal development [43]. The application of HS enriched with a mixture of micronutrients should favor the release of zinc, which would then be available to assist plant growth.

To resume, the complexation processes evaluated in this work showed the pH obtained for optimum complexation was 4.5 and the order of affinity observed was: $\text{Fe(II)} > \text{Cu(II)} > \text{Co(II)} > \text{Mn(II)} = \text{Ni(II)} > \text{Zn(II)}$. The maximum complexation capacity reached $56.8 \text{ mg}\cdot\text{g}^{-1}$ Fe of HS-P1 (the highest) and $1.7 \text{ mg}\cdot\text{g}^{-1}$ Zn of HS-P2 (the slightest). The difference observed between the two peat humic substances samples were assigned to chemical structure of each sample. The exchange experiments showed the nutrient availability in presence of another

micronutrient. The HS-P-Fe complexes were formed preferentially. The HS-P-Zn complexes were the least stable. The results support the understanding of the processes of nutrients availability and thus, could help to comprehend how the micronutrients are distributed in the soil.

6. Conclusions

The present study investigated the complexation and exchange capacity of humic substances extracted from peat samples (HS-P1 and HS-P2), with the main micronutrients (Cu(II), Co(II), Fe(II), Mn(II), Ni(II) and Zn(II)) required for photosynthesis and plant growth. The optimum pH for complexation was 4.5 and the order of affinity was: Fe(II) > Cu(II) > Co(II) > Mn(II) = Ni(II) > Zn(II). The maximum complexation capacity reached 56.8 mg·g⁻¹ Fe of HS-P1 (the highest) and 1.7 mg·g⁻¹ Zn of HS-P2 (the slightest). The exchange experiments showed that HS-P-Fe complexes were formed preferentially. The least stable complex was formed with Zn, which was therefore, more easily available. The results contribute to understand the behavior and availability of some nutrients in soils.

Acknowledgements

This research was supported by the following agencies: Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP), Conselho Nacional Desenvolvimento Científico Tecnológico (CNPq), and Coordenacao Aperfeicoamento Pessoal Nível Superior (CAPES). The authors are also grateful to Dr. Tiago Venancio (Universidade Estadual Sao Carlos) for assistance with the NMR ¹³C analysis. The authors dedicate this work for Professor Julio Cesar Rocha for his retirement.

References

- [1] Piccolo, A., Pietramellara, G., and Mbagwu, J. 1997. "Use of Humic Substances as Soil Conditioners to Increase Aggregate Stability." *Geoderma* 75: 267-277.
- [2] Tan, K. H. 2014. *Humic Matter in Soil and the Environment: Principles and Controversies*. Boca Ranton: CRC Press.
- [3] Kulikova, N., and Perminova, I. 2002. "Binding of Atrazine to Humic Substances from Soil, Peat and Coal Related to Their Structure." *Environmental Science & Technology* 36: 3720-3724.
- [4] Davies, G., and Ghabbour, E. 2003. *Humic Substances: Nature's Most Versatile Materials*. New York: Taylor & Francis.
- [5] Chien, S. W. C., Chen, C. Y., Chang, J. H., Chen, S. H., Wang, M. C., and Mannepalli, M. R. 2010. "Sorption of Toluene by Humic Acids Derived from Lake Sediment and Mountain Soil at Different pH." *Journal Hazardous Materials* 177: 1068-1076.
- [6] Chai, X. L., Liu, G. X., Zhao, X., Hao, Y. X., and Zhao, Y. C. 2012. "Complexion between Mercury and Humic Substances from Different Landfill Stabilization Processes and Its Implication for the Environment." *Journal Hazardous Materials* 209: 59-66.
- [7] Ghabbour, E. A., Davies, G., and Chemistry, R. 2001. *Humic Substances: Structures, Models and Functions*. London: Royal Society of Chemistry.
- [8] Segnini, A., Posadas, A., Quiroz, R., Milori, D., Saab, S., and Neto, L. et al. 2010. "Spectroscopic Assessment of Soil Organic Matter in Wetlands from the High Andes." *Soil Science Society of America Journal* 74: 2246-2253.
- [9] Segnini, A., De-Souza, A. A., Novotny, E. H., Milori, D. M. B. P., Da-Silva, W. T. L., and Bonagamba, T. J. et al. 2013. "Characterization of Peatland Soils from the High Andes through C-13 Nuclear Magnetic Resonance Spectroscopy." *Soil Science Society of America Journal* 77: 673-679.
- [10] Senesi, N. 1992. *Biogeochemistry of Trace*. Boca Raton: Lewis Publishers.
- [11] Hernandez-Soriano, M., and Jimenez-Lopez, J. 2012. "Effects of Soil Water Content and Organic Matter Addition on the Speciation and Bioavailability of Heavy Metals." *Science of the Total Environment* 423: 55-61.
- [12] Cunha, T., Bassoi, L., Simoes, M., Martin-Neto, L., Petrere, V., and Ribeiro, P. 2009. "Humic Acids in a Fertirrigated Soil in the Sao Francisco River Valley, Brazil." *Revista Brasileira De Ciencia Do Solo* 33: 1583-1592.
- [13] Lal, R., and Lal, L. 2005. *Encyclopedia of Soil Science*. Boca Ranton: CRC Press Inc..
- [14] Gondar, D., Lopez, R., Fiol, S., Antelo, J., and Arce, F. 2006. "Cadmium, Lead, and Copper Binding to Humic Acid and Fulvic Acid Extracted from an Ombrotrophic Peat Bog." *Geoderma* 135: 196-203.
- [15] Yang, R., and Van Den Berg, C. 2009. "Metal Complexation by Humic Substances in Seawater." *Environmental Science and Technology* 43: 7192-7197.

- [16] Pantano, G., Campanha, M., Moreira, A., and Bisinoti, M. 2014. "Occurrence of Cu and Cr in the Sedimentary Humic Substances and Pore Water from a Typical Sugar Cane Cultivation Area in Sao Paulo, Brazil." *Journal of Soils and Sediments* 14: 377-384.
- [17] Knicker, H., Nikolova, R., Dick, D. P., and Dalmolin, R. S. D. 2012. "Alteration of Quality and Stability of Organic Matter in Grassland Soils of Southern Brazil Highlands after Ceasing Biannual Burning." *Geoderma* 181: 11-21.
- [18] Burba, P., Van Den Bergh, J., and Klockow, D. 2001. "On-Site Characterization of Humic-Rich Hydrocolloids and Their Metal Loading by Means of Mobile Size-Fractionation and Exchange Techniques." *Fresenius Journal of Analytical Chemistry* 371: 660-669.
- [19] Sanches, S. M., Campos, S. X. D., and Vieira, E. M. 2007. "Caracterizacao Das Fracoes Das Substancias Humicas De Diferentes Tamanhos Moleculares." *Ecletica Quimica* 32: 49-56.
- [20] Becker, E. D. 2000. *High Resolution NMR: Theory and Chemical Applications*. San Diego: Academic Press.
- [21] Knicker, H., Totsche, K. U., Almendros, G., and Gonzalez-Vila, F. J. 2005. "Condensation Degree of Burnt Peat and Plant Residues and the Reliability of Solid-State VACP MAS C-13 NMR Spectra Obtained from Pyrogenic Humic Material." *Organic Geochemistry* 36: 1359-1377.
- [22] Novotny, E. H., and Martin-Neto, L. 2002. "Effects of Humidity and Metal Ions on the Free Radicals Analysis of Peat Humus." *Geoderma* 106: 305-317.
- [23] Limousin, G., Gaudet, J. P., Charlet, L., Szenknect, S., Barthes, V., and Krimissa, M. 2007. "Sorption Isotherms: A review on Physical Bases, Modeling and Measurement." *Applied Geochemistry* 22: 249-275.
- [24] Einax, J., and Kunze, C. 1996. "Complexation Capacity of Aquatic Systems in Dependence on Different Ligands and Heavy Metals—Electroanalytical Investigations and Statistical Evaluation." *Analytical and Bioanalytical Chemistry* 354: 895-9.
- [25] Scatchard, G. 1949. "The Attractions of Proteins for Small Molecules and Ions" *Annals of the New York Academy of Sciences* 51: 660-672.
- [26] Van Den Berg, C. M. G., and Kramer, J. R. 1979. "Determination of Complexing Capacities of Ligands in Natural-Waters and Conditional Stability-Constants of the Copper-Complexes by Means of Manganese-Dioxide." *Analytica Chimica Acta* 106: 113-120.
- [27] Ruzic, I. 1982. "Theoretical Aspects of the Direct Titration of Natural-Waters and Its Information Yield for Trace-Metal Speciation." *Analytica Chimica Acta* 140: 99-113.
- [28] Pantano, G., Tadini, A., Bisinoti, M., and Moreira, A. 2014. "Seasonal Variability of a Conditional Stability Constant and the Characterization of Sedimentary Humic Substances from Typical Agricultural and Urban Areas." *Journal of Soils and Sediments* 14: 385-393.
- [29] Romao, L. P. C., Castro, G. R., Rosa, A. H., Rocha, J. C., Padilha, P. M., and Silva, H. C. 2003. "Tangential-Flow Ultrafiltration: A Versatile Methodology for Determination of Complexation Parameters in Refractory Organic Matter from Brazilian Water and Soil Samples." *Analytical and Bioanalytical Chemistry* 375: 1097-1100.
- [30] Garcia-Mina, J. 2006. "Stability, Solubility and Maximum Metal Binding Capacity in Metal-Humic Complexes Involving Humic Substances Extracted from Peat and Organic Compost." *Organic Geochemistry* 37: 1960-1972.
- [31] Tabatabai, A., and Sparks, D. L. 2005. *Chemical Processes in Soils*. Madison: Soil Science Society of America.
- [32] Gondar, D., Lopez, R., Fiol, S., Antelo, J., and Arce, F. 2005. "Characterization and Acid-Base Properties of Fulvic and Humic Acids Isolated from two Horizons of an Ombrotrophic Peat Bog." *Geoderma* 126: 367-374.
- [33] Fernandes, A. N., Giovanela, M., Esteves, V. I., and Sierra, M. M. D. 2010. "Elemental and Spectral Properties of Peat and Soil Samples and Their Respective Humic Substances." *Journal of Molecular Structure* 971: 33-38.
- [34] Tipping, E. 2002. *Cation Binding by Humic Substances*. Cambridge: Cambridge University Press.
- [35] Lopez, R., Gondar, D., Antelo, J., Fiol, S., and Arce, F. 2012. "Study of the Acid-Base Properties of a Peat Soil and Its Humin and Humic Acid Fractions." *European Journal of Soil Science* 63: 487-494.
- [36] Luz, P. H. D., Faria, L. D., De-Macedo, F. B., Herling, V. R., Sanches, A. B., and Rodrigues, R. C. 2011. "Effect of Silicate Fertilization on Soil and on Palisade Grass Plants under Grazing Intensities." *Revista Brasileira De Zootecnia* 40: 1458-1465.
- [37] Pearson, R. G. 1963. "Hard and Soft Acids and Bases." *Journal of America Chemical Society* 85: 3533.
- [38] Voelker, B. M., Morel, F. M. M., and Sulzberger, B. 1997. "Iron Redox Cycling in Surface Waters: Effects of Humic Substances and Light." *Environmental Science and Technology* 31: 1004-1011.
- [39] Garg, S., Jiang, C., Miller, C. J., Rose, A. L., and Waite, T. D. 2013. "Iron Redox Transformations in Continuously Photolyzed Acidic Solutions Containing Natural Organic Matter: Kinetic and Mechanistic Insights." *Environmental Science and Technology* 47: 9190-9197.
- [40] Irving, H., and Williams, R. J. P. 1953. "The Stability of Transition-Metal Complexes." *Journal of the Chemical*

Society 1953: 3192-3210.

- [41] Kerndorff, H., and Schnitzer, M. 1980. "Sorption of Metals on Humic-Acid." *Geochimica Cosmochimica Acta* 44: 1701-1708.

- [42] Araujo, A. B., Rosa, A. H., Rocha, J. C., and Romao, L. P. C. 2002. "Metal Distribution and Characterization of Exchange Constants between Metal Species and Aquatic

Humic Fractions with Different Molecular Sizes." *Química Nova* 25: 1103-1107.

- [43] Nacke, H., Goncalves, A. C., Schwantes, D., Nava, I. A., Strey, L., and Coelho, G. F. 2013. "Availability of Heavy Metals (Cd, Pb and Cr) in Agriculture from Commercial Fertilizers." *Archives of Environmental Contamination and Toxicology* 64: 537-544.