

Influence of the Extractant on the Complexing Capacity of Humic Substances from Peat for Macro and Micronutrients Using Continuous Flow: Agricultural Application and Environmental Impacts

Wander G. Botero,^{*,a} Luciana C. Oliveira,^b Alexandre D. M. Cavagis,^b
Andre H. Rosa,^c Julio C. Rocha^d and Ademir Santos^d

^aFederal University of Alagoas (UFAL), CP 61, 57309-005 Arapiraca-AL, Brazil

^bFederal University of São Carlos (UFSCar), 18052-780 Sorocaba-SP, Brazil

^cDepartment of Environmental Engineering, São Paulo State University (UNESP),
18087-180 Sorocaba-SP, Brazil

^dDepartment of Analytical Chemistry, Institute of Chemistry, São Paulo State University (UNESP),
CP 355, 14801-970 Araraquara-SP, Brazil

O objetivo do presente trabalho foi otimizar o procedimento para extração de substâncias húmicas de turfa, utilizando fluxo contínuo, com diferentes extratores (NaOH, KOH e Na₄P₂O₇). A influência do extrator na capacidade de complexação das substâncias húmicas por macro e micronutrientes também foi investigada. Os resultados mostraram que a extração por fluxo contínuo é eficaz, reduzindo o tempo necessário para o passo de extração e, além disso, indicando que as substâncias húmicas apresentaram elevada afinidade para os nutrientes essenciais Ca²⁺ e Mg²⁺. Além disso, as maiores capacidades de complexação foram obtidas quando utilizou o extrator Na₄P₂O₇, mostrando não só a eficiência desse composto como agente de extração, mas também a sua aplicação promissora no processo, uma vez que a sua elevada capacidade de complexação permite disponibilizar os nutrientes complexados, bem como diminuir a quantidade de estes nutrientes que podem ser lixiviados, contribuindo, assim, para evitar contaminação do meio ambiente.

The aim of the present work was to optimize a procedure for extraction of humic substances (HS) from peat, using continuous flow with different extractants (NaOH, KOH and Na₄P₂O₇). The influence of extractant on the complexing capacity of humic substances for macro and micronutrients was also investigated. Our results showed that the extraction by flow is efficient, reducing the time required in the extraction step and, furthermore, indicating that the extracted HS have a high affinity for the essential nutrients Ca and Mg. Moreover, an enhanced complexation was achieved by using Na₄P₂O₇, demonstrating not only the efficiency of this compound as extractant, but also its promising application in the process, since its high complexing capacity allows to make available the complexed nutrients as well as diminishing the amount of these nutrients that could be lixiviated, thus contributing to prevent environmental contaminations.

Keywords: humic substances, peat, complexation capacity, continuous flow extraction

Introduction

Peat are natural sinkholes of macro and micronutrients and carbon of the soil. The accumulation of these species in the peat is due to processes involving biochemical reactions and complexation with organic matter.¹ The fraction of this organic matter, with undefined chemical

structure, is called "humic substances" (HS) and the study of their characteristics as complexing agents is of great environmental interest, because they act directly on the bioavailability of different species in the environment.²

One of the first issues, when studying HS, is the extraction of humic material, taking into account that the extraction conditions may change their physicochemical properties. Thus, it is fundamentally important to extract them with minimal change into its original structure.³

*e-mail: wander_iq@yahoo.com.br

Considering the extraction of humic substances from soil, two classes of extractants are designated:

- moderate extractants;
- alkaline extractants.

The moderate extractants represent a class of less drastic extractants, and the major are: sodium pyrophosphate, organic complexing agents in aqueous medium, such as acetylacetone and 8-hydroxyquinoline, formic acid, acid mixture and organic solvents of various types.³⁻⁵ These extractants seem to decrease the occurrence of structural changes into the organic material, in spite being less effective than alkaline extractants.

Due to the high extracting power, NaOH, Na₂CO₃ and KOH solutions have been the most used in the extraction of soil organic matter, however, different extractants, such as Na₄P₂O₇ and their mixtures with NaOH, have also been used.⁶⁻¹¹ Despite the higher efficiency, the use of alkaline solution for extracting HS can change the organic matter by hydrolysis or self-oxidation.^{5,12} Nevertheless, the most used procedure in such extractions is still based on the use of alkaline solution (NaOH), enabling comparisons among different works.^{9,11,13}

Nowadays, fertilizers commercialized as “organics” and/or “natural”, e.g., peat or humic substances extracted from peat, are enriched with macro and micronutrients which, according to the manufacturers, are released to the plant as it needs. This is a relatively new market, with great economic potential and, therefore, very attractive. However, there is still no proper regulation for this sector, mainly because there is no scientific data about the releasing processes of those nutrients by HS. Having in view a better yield and lower environmental impact, studies are necessary to analyze the interactions (for example, the complexation capacity) between metal species (essential macro and micronutrients) and humic substances.¹⁴

Several chemical models seek to evaluate the modeling of the interactions between the HS and metal species.^{15,16} The NICA-Donnan model is a combination of the non-ideal competitive adsorption (NICA) isotherm description of binding to a heterogeneous material, coupled with a Donnan electrostatic sub-model describing the electrostatic interactions between ions and the humic material. NICA-Donnan has been shown in several studies to be a versatile model capable of describing observed humic ion-binding behavior well over a wide range of conditions.^{17,18}

Therefore, the objective of this study was the optimization of the extraction process by continuous flow of humic substances from peat for commercial purposes, using different extractants (NaOH 0.1 mol L⁻¹, Na₄P₂O₇ 0.1 mol L⁻¹ and KOH 0.1 mol L⁻¹) as well as the evaluation

of the influence of the extraction on the complexation of nutrients by HS. In this way, these results may provide subsidies for the application of HS enriched with nutrients in agricultural soils, reducing environmental impacts.

Experimental

Sample collection and preparation

The peat samples have been collected around the Mogi River region, near to Ribeirão Preto city, São Paulo State, Brazil. Figure 1 shows the sampling procedure, at a depth of 50 cm, so that single samples A₁-A₅ formed the composite sample A.

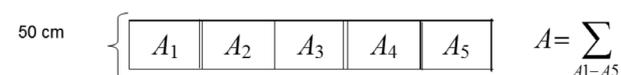


Figure 1. Diagram of the procedure used to collect peat samples.

The samples were, then, transferred to wood trays and, after air drying, passed in a plastic sieve with a 2 mm mesh.

Extraction of humic substances

Continuous flow

The extraction of HS by continuous flow was carried out by transferring a 5.00 g sample of peat into the extraction flask (EF), under magnetic stirring; the extractant solution (50.00 mL) (R₁) was added at a flow rate of 1.0 mL min⁻¹ through the tube 1 (T₁). Nitrogen was injected into the EF at a flow rate of 100 mL min⁻¹ to minimize the material oxidation. The extracted HS were suctioned to the tube 2 (T₂) at a flow rate of 1.0 mL min⁻¹, passing through the filter (F₁) in order to retain the insoluble part, called ‘humins’ and the supernatant was then dried in a greenhouse with air exchange for subsequent determination of the yield (Figure 2). In the extraction of HS by continuous flow, the addition of the extracting agent occurs at a rate of 1.0 mL min⁻¹, thus the overall extraction time decreases, being performed within 2 h. The extractants used were: NaOH, KOH and Na₄P₂O₇, at the concentration of 0.1 mol L⁻¹.

Batch

The extraction of humic substances by batch was made through methodology, recommended by IHSS.¹³ The extractants solutions: NaOH, KOH and Na₄P₂O₇ (0.1 mol L⁻¹) were mixed with 5.0 g of peat in the ratio of 1:10 (m/v) and the mixture was left under mechanical stirring for 4 hours in the Jar Test equipment - JT 102, under a nitrogen atmosphere. Afterwards, the supernatant

Table 1. Comparative extraction yields of humic substances of peat samples using continuous flow system and batch with different extractants

Extractant	Batch	Continuous flow
	Yield / %	
NaOH 0.1 mol L ⁻¹	50	68
KOH 0.1 mol L ⁻¹	35	42
Na ₄ P ₂ O ₇ 0.1 mol L ⁻¹	42	54

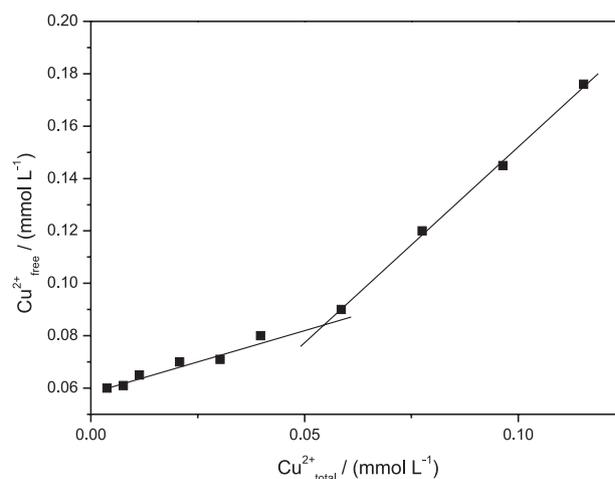
Considering the different extractants, the highest yields for both extraction systems were obtained with NaOH 0.1 mol L⁻¹. Among the different extractants, Na₄P₂O₇ has been shown to be a strategic extractant, aiming agricultural applications of humic material, because the extraction in this case may occur simultaneously with the enrichment of the HS with phosphorus, an essential macronutrient. The literature has been suggesting the use of a mixture of NaOH and Na₄P₂O₇ as extractant, due to the little modifications in the chemical structure of extracted humic material.^{9,10} However, it is important to emphasize that there should be a control on the amount of extractant added, especially of Na₄P₂O₇ and the phosphorus amount retained in HS, because an excess of phosphorus might cause high environmental impact. Thus, parameters for controlling the amount of phosphorus released are of great environmental concern and the determination of the complexing ability of HS with macronutrients has shown to be a promising parameter to this purpose.

Determination of the conditional complexing capacity of HS of peat extracted by flow with different extractants for macro and micronutrients.

For the commercialization of humic substances as organic fertilizer, beyond the extraction step, the enrichment of the extracted material with macro and micronutrients is a common practice, providing to consumers a better product from the agronomic viewpoint. However, little is known about the ability of extracted HS to complex those nutrients. Therefore, the complexing capacity (CC) data are useful, giving the maximum concentrations of nutrients that HS can complex, preventing waste as well as possible environmental contaminations due to the excess of nutrients lixiviated into springs, which could lead to eutrophication.

The determination of the conditional complexing ability of HS at pH 5.5, extracted from peat samples by flow for macronutrients (Ca²⁺ and Mg²⁺) and micronutrients (Zn²⁺, Co²⁺, Mn²⁺, Cu²⁺ and Mo²⁺) was made by titration, using the tangential ultrafiltration system. During titration, firstly occurs the saturation of the stronger binding ligands and, after that, the saturation of the weaker. Thus, a typical

curve used in the determination of the complexing ability of HS with those nutrients is obtained by plotting the concentration of the free nutrient against the total nutrient concentration (mmol L⁻¹) (Figure 3). The curve shows an inflection point and the complexing ability is obtained by the intersection of the two linear sections of the graphic (Figure 3).

**Figure 3.** Graph for determination of the conditional complexing capacity of extracted humic substances from peat samples with NaOH 0.1 mol L⁻¹ by Cu²⁺ ions at pH ≈ 5.5 and [HS] = 100 mg L⁻¹.

The CC values determined for the HS bring important quantitative data, such as the complexed amount of each metal as well as information about the affinity of the HS for different metal species. Thereby, according to the Table 2, the following increasing order of affinity between the studied metal ions and the humic substances from peat, extracted with different extractants, can be established:

HS extracted with NaOH 0.1 mol L⁻¹: Mo²⁺ < Co²⁺ < Cu²⁺ < Zn²⁺ < Mn²⁺ < Mg²⁺ < Ca²⁺

HS extracted with KOH 0.1 mol L⁻¹: Mo²⁺ < Cu²⁺ < Co²⁺ < Zn²⁺ < Mn²⁺ < Mg²⁺ < Ca²⁺

HS extracted with Na₄P₂O₇ 0.1 mol L⁻¹: Mo²⁺ < Cu²⁺ < Co²⁺ < Zn²⁺ < Mn²⁺ < Mg²⁺ < Ca²⁺

Analyzing the conditional complexing capacity of humic substances extracted from peat samples with different extractants, it was observed that the greater affinity is related to magnesium and calcium. This higher affinity, in turn, may be related to the great amount of oxygenated groups present in the HS samples. The elements Mo²⁺, Co²⁺ and Cu²⁺ showed low affinity for humic substances, independently of the extractant used.

The results from Table 2 also show that the studied elements (Ca²⁺, Mg²⁺ and Mn²⁺) presented higher conditional complexing capacity for the humic substances

Table 2. Conditional complexing ability of humic substances at pH 5.5, extracted by flow from peat samples, using NaOH 0.1 mol L⁻¹, KOH 0.1 mol L⁻¹ and Na₄P₂O₇ 0.1 mol L⁻¹

Samples	Conditional complexing ability / (mmol metal g ⁻¹ HS)						
	Ca	Mg	Zn	Co	Mn	Cu	Mo
HS extracted with NaOH 0.1 mol L ⁻¹	0.97±0.02	0.65±0.01	0.50±0.01	0.39±0.03	0.55±0.01	0.45±0.01	0.20±0.03
HS extracted with KOH 0.1 mol L ⁻¹	0.88±0.01	0.63±0.01	0.51±0.01	0.48±0.02	0.53±0.01	0.43±0.02	0.14±0.03
HS extracted with Na ₄ P ₂ O ₇ 0.1 mol L ⁻¹	1.07±0.02	0.70±0.02	0.53±0.01	0.43±0.01	0.57±0.01	0.41±0.01	0.22±0.02

extracted with sodium pyrophosphate, when compared to other extractants. Furthermore, several studies show that humic substances extracted with sodium pyrophosphate have higher concentrations of oxidized molecules.^{23,24} According to Gieguzynska *et al.*,²⁴ Ca²⁺ and Mg²⁺ are able to establish bonds between the local charges of negative surface and anionic humic macromolecules.

Studies proposed by Milne *et al.*¹⁶ using the NICA-Donnan model showed that in interactions between fulvic acid (FA) and calcium ions, the metal ions are almost entirely connected with the first distribution sites (carboxylic groups present in the structure of HS), with a greater contribution to the sites of the second distribution (smaller groups present in the structure of HS) for metal-FA. Thus, it might be inferred that the greater affinity of Ca²⁺ and Mg²⁺ for HS extracted with pyrophosphate may be related to the large amount of oxygenated groups present in the HS structures.

The interactions between the metal species and HS strongly depend on the metal ion and pH. At pH 5.5 (experiments in this work) the contribution of electrostatic interactions will be through the Donnan effect.¹⁶

In determination of the complexing ability of HS occurs the saturation of binding sites. In this case, the observed differences in the conditional complexing capacity shows the differences between the HS materials come from the extraction procedures and there are differences in the total number of sites available for binding metals.

The highest conditional complexing ability of the extracted HS for the macronutrients calcium and magnesium was observed with all extractants, indicating a high affinity of HS for Ca²⁺ and Mg²⁺. Moreover, the complexation of these macronutrients by HS is also favored when using sodium pyrophosphate as extractant. Studies proposed by Rosa *et al.*,²² showed that the use of different extractants influences directly the performance of the HS, however, they might not act, depending on the functional groups present in its structure. Accordingly, the greater affinity of HS extracted with pyrophosphate for nutrients is due to possible molecular rearrangements occurred during the extraction process. These results show that the use of sodium pyrophosphate as humic extractant is interesting, since it has high affinity for macro and micronutrients, compared to other extractants. Thus, the

HS extracted with sodium pyrophosphate, can be enriched with nutrients, presenting interesting characteristics for commercial purposes.

Once applied to the soil, these HS can provide the complexed nutrients and, thanks to a high complexing capacity, reduce the amount of these nutrients that could be leached. Therefore, our results provide a manner for an appropriate enrichment of HS extracted from peat for commercial purposes, preventing environmental impacts as well as adding commercial value to the product.

Conclusions

The continuous flow showed to be adequate and efficient, reducing the time required for extraction and providing higher yields when compared with the batch procedure, saving work in the steps of filtration, centrifugation, pH adjustment and handling of samples.

The conditional complexing capacities showed that, independently of the extractant, the HS presented higher affinity for Ca and Mg, indicating that, even if a change in the chemical structure of humic molecules occurs during the extraction, it does not affect the complexing capacity. However, the molecular rearrangement during the extraction process, using sodium pyrophosphate, favored complexation with essential macronutrients. Thus, the use of sodium pyrophosphate as extractant, despite not presenting the highest yield, increases the complexation capacity of humic material with essential macronutrients, demonstrating to be very promising in the extraction of HS, aiming their agricultural application.

Acknowledgement

The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support and fellowship research.

References

1. Yoon, S. J.; Yáñez, C.; Bruns, M. A.; Villegas, N. M.; Martínez, C. E.; *Geochim. Cosmochim. Acta.* **2012**, *84*, 165.

2. Oliveira, L. C.; Sargentini Junior, É.; Rosa, A. H.; Rocha, J. C.; Simões, M. L.; Martin Neto, L.; Silva, W. T. L.; Serudo, R. L.; *J. Braz. Chem. Soc.* **2007**, *18*, 860.
3. Hayes, M. H. B.; *Soil Sci. Soc. Am. J.* **2006**, *70*, 986.
4. Hayes, M. H. B.; Swift, R. S.; Wardlea, R. E.; Brown, J. K.; *Geoderma* **1975**, *13*, 231.
5. Stevenson, F. J.; *Humus Chemistry*, 4th ed.; Wiley: New York, 1994.
6. Posner, A. M.; *J. Soil Sci.* **1966**, *17*, 65.
7. Achard, F. K.; *Crell's Chem. Ann.* **1986**, *2*, 391.
8. Hayes, M. H. B.; Clapp, C. E.; *Soil Sci.* **2001**, *166*, 723.
9. Zacccone, C.; Cacoza, C.; D'orazio, V.; Plaza, C.; Cheburkin, A.; Miano, T. M.; *Talanta* **2007**, *73*, 820.
10. Zacccone, C.; Soler-Rovira, P.; Plaza, C.; Cacoza, C.; Miano, T. M.; *J. Hazard. Mater.* **2009**, *167*, 987.
11. Huey, L. J.; Ahmed, O. H.; Muhamad, N.; Majid, A.; *Am. J. Appl. Sci.* **2010**, *7*, 920.
12. Rosa, A. H.; Goveia, D.; Bellin, I. C.; Lessa, S. S.; Dias Filho, N. L.; Padilha, P. M.; *Anal. Bioanal. Chem.* **2006**, *386*, 2153.
13. Dobbss, L. B.; Canellas, L. P.; Alleoni, L. R. F.; Rezende, C. E.; Fontes, M. P. F.; Velloso, A. C. X.; *Rev. Bras. Ciênc. Solo* **2008**, *32*, 985.
14. Botero, W. G.; Oliveira, L. C.; Rocha, J. C.; Rosa, A. H.; Santos, A.; *J. Hazard. Mat.* **2010**, *177*, 307.
15. Tipping, E.; *Aquat. Geochem.* **1998**, *4*, 3.
16. Milne, C. J.; Kinniburgh, D. G.; Van Riemsdijk, W. H.; Tipping, E.; *Environ. Sci. Technol.* **2003**, *37*, 958.
17. Christensen, J. B.; Tipping, E.; Kinniburgh, D. G.; Grøn, C.; Christensen, T. H.; *Environ. Sci. Technol.* **1998**, *32*, 3346.
18. Pinheiro, J. P.; Mota, A. M.; Benedetti, M. F.; *Environ. Sci. Technol.* **1999**, *33*, 3398.
19. Town, R. M.; Powell, H. K. J.; *Anal. Chim. Acta* **1992**, *256*, 81.
20. Burba, P.; Van Den Bergh, J.; Klockow, D.; *Fresenius J. Anal. Chem.* **2001**, *371*, 660.
21. Santos, A.; Botero, W. G.; Bellin, I. C.; Oliveira, L. C.; Rocha, J. C.; Mendonça, A. G. R.; Godinho, A. F.; *J. Braz. Chem. Soc.* **2007**, *18*, 824.
22. Rosa, A. H.; Vicente, A. A.; Rocha, J. C.; Trevisan, H. C.; *Fres. J. Anal. Chem.* **2000**, *368*, 730.
23. Lavorenti, A.; Peixoto, M. F. S. P.; Burauel, P.; Wais, A.; Fuhr, F.; *Revista de Ecotoxicologia e Meio Ambiente* **1997**, *7*, 77.
24. Gieguzynska, E.; Amine-Khodja, A.; Trubetskoj, O. A.; Trubetskaya, O. E.; Guyot, G.; Halle, A. T.; Golebiowska, D.; Richard, C.; *Chemosphere* **2009**, *75*, 1082.

Submitted: June 6, 2013

Published online: October 16, 2013

FAPESP has sponsored the publication of this article.