



# Extraction and exchange behavior of metal species in therapeutically applied peat characterised by competitive extractants

Luciane P. C. Romão<sup>1</sup>, Ângelo Augusto M J. Silva<sup>2</sup>, Fabiana A. Lobo<sup>2,3</sup>, Danielle Goveia<sup>2,3</sup>, Leonardo F. Fraceto<sup>1</sup>, Julio C. Rocha<sup>3</sup>, Peter Burba<sup>4</sup> and André H. Rosa<sup>1\*</sup>

<sup>1</sup> Universidade Federal de Sergipe, Departamento de Química, 49100-000, São Cristóvão-SE, Brasil

<sup>2</sup> Departamento de Engenharia Ambiental, UNESP, Av. 3 de Março, 511, 18087-180, Sorocaba-SP, Brasil  
Phone: +55 15 32383414

<sup>3</sup> Instituto de Química, UNESP, 14800-900, Araraquara-SP, Brasil;

<sup>4</sup> Institute of Analytical Sciences, Germany  
e-mail: \*ahrosa@sorocaba.unesp.br

## Summary

The binding and availability of metals (Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb) in therapeutically applied peat was characterised by means of a versatile extraction approach. Aqueous extracts of peat were obtained by a standardized batch equilibrium procedure using high-purity water (pH 4.5 and 5.0), 0.01 mol/L ethylenediaminetetraacetic acid and 0.01 mol/L diethylenetriaminepenta-acetic acid solution as metal extractants. Metal determinations were performed by atomic spectrometry methods and dissolved organic matter (DOM) was characterised by UV/VIS. Of the extractants studied Ca, Mg and Mn were the most available metals, in contrast to peat-bound Fe and Al. A pH increase favored the extraction of metals and DOM from peat revealing complex extraction kinetics. Moreover, a competitive exchange between peat-bound metal species and added Cu(II) ions showed that >100 mg of Cu(II) per 50 g wet peat were necessary to exchange the maximum of bound metals.

**Key index words:** balneological peat, metal, extractants, extraction kinetics, dissolved organic matter

## Introduction

Natural peats are continuously formed by complex microbiotic humification processes of plant residues over thousands of years (Marthur and Farnham, 1985). There are about 500 million hectares of peatlands in the world (nearly half of Europe area), which are estimated to contribute about 0.7 billion ( $10^{12}$ ) tons of refractory organic carbon in the environment (Bramryd, 1980). Due their high content of humin, humic acids (HA) and fulvic acid (FA), peats exhibit favorable chemical-physical properties enabling their application in various technical areas, for instance wastewater treatment (Brown *et al.*, 2000), pollution monitoring (Viraraghavan and Rana, 1991), fuel production (Hatcher *et al.*, 1983), enzyme immobilization (Rosa *et al.*, 2000), horticulture growing media, as well as in veterinary and human medicine.

According to some speculations the medical and veterinary effects of peat are probably associated with their high content of humic substances (HS) and humic-bound metal species (Klößing, 1994). As yet, the natural enrichment of metals in balneological peats and their possible (bio)-availability during peat therapy has scarcely been investigated, except for Beer *et al.* (2000) having studied the possible uptake of heavy metals by patients pre-

treated by peat mush baths and peat tampons, respectively. At best, heavy metals in medically applied peats and their aqueous extracts have occasionally been characterised (Burba *et al.*, 2001a). For this purpose, improved metal speciation procedures, differentiating heavy metals in balneological peats as a function of their extractability and availability, respectively, are desired.

Therefore, in the present work the extraction behaviour of dissolved organic matter (DOM) and metal species from therapeutically used peats (Großes Gifhorner Moor, Sassenburg/North Germany) were characterised by means of a versatile analytical approach. In particular, the availability and exchange behaviour of peat-bound metal species towards both competitive chelators (DTPA, EDTA) and metal ions (Ca(II), Cu(II)) were studied. In environmental science, the chelators DTPA and EDTA, respectively, are conventionally used as extractants to assess "(bio)available" metal fractions, for instance in soils and organic-rich sludge samples. The exchangeability of peat-bound metal species towards strongly competitive metal ions can also be supposed to be an insightful exchange mechanism usable to assess available metal fractions, as already shown for heavy metals in humic-rich hydrocolloids competed by Cu(II) ions. On the other side, from Ca(II) ions as operational



competitors can be expected that they only weakly exchange with peat-bound metal species, as they form preferably weak complexes with humic matter.

## Materials and Methods

### Peat samples

The medical peat samples (Vitamoor®, H. Wulfes KG, Sassenburg/Germany) under study stem from "Großes Gifhorner Moor", Sassenburg/North Germany. They were collected in 20 kg portions, each, at 2 different mining sites of this peat deposit. The water content of the samples was assessed by drying sub-samples of approximately 100 g in an oven at 120 °C for 12 hours. The peat samples I and II showed water contents of 77.6 and 79.2 % (m/m), respectively, related to their solid mass. Prior to further investigations the dried peat samples were coarsely ground in an agate mortar (particle sizes obtained: approximately 0.1 to 2 mm) and stored in a closed polyethylene vessel.

### Aqua-regia digestion

To determine total metal concentrations in the dried and ground peat samples, they were conventionally decomposed by *aqua-regia* according to a standardized procedure already proposed for the digestion of aquatic sediments (Sommerfeld and Schwedt, 1996). The standard deviation of peat digestion by aqua regia was assessed from 5 replicates. Metal determinations referring to this were preferably carried by atomic absorption spectrometry. The chemical blanks, assessed by means of two aqua regia digestion runs without any sample, were subtracted from these results.

### Extraction procedures for balneological peats

To characterise the peat, 100 g of the sample (wet) were suspended in 250 mL of high-purity water and some mL of 2 mol/L NaOH solution were added to adjust a pH value of 4.5. Then, the mixture was magnetically stirred (250-300 rotations per minute, room temperature (23-25 °C)). The pH was maintained using a conventional glass electrode. After different periods of time (5, 10, 15, 30, 60 and 120 minutes) small aliquots (about 50 mL, each) of the peat suspension were filtered through a coarse paper filter and, finally, through a 0.45 µm flat membrane to remove particles. The fractions collected this way were characterised by their UV/VIS absorbance *A* at 254 nm and 436 nm, respectively, as well as by metal determinations using atomic spectrometry. Applying an analogous extraction procedure, the influence of an increased pH-value (5.0) and different extractors (0.01 mol/L CaCl<sub>2</sub>, 0.01 mol/L ethylenediaminetetraacetic acid (EDTA) and 0.01 mol/L diethylenetriaminepentaacetic acid (DTPA), respectively, were also studied. Before peat extraction the pH value of the EDTA and DTPA solutions was adjusted to 5.0 by adding 2 mol/L NaOH solution. The standard deviations of the metal extraction procedures applied were assessed from 5 different batch runs extracting 100 g samples of peat II, each (100 g peat II (wet), 250 mL extractant, equilibration for 1 h; metal determinations by ICP-OES and AAS, respectively).

### Metal determinations

Metal concentrations in peat extracts were determined by atomic spectrometry, using conventional atomic absorption spectrometry (AAS) and simultaneous inductively-coupled plasma optical emission spectrometry (ICP-OES), dependent on the analytes and their concentration level in humic-rich solutions (Burba, 1998).

### UV/VIS characterisation of DOM

The DOM in peat extracts was characterised by means of its absorbance *A* at 254 nm and 436 nm measured by a scanning two-beam UV/VIS spectrometer (Varian Cary 1/3, Varian GmbH, Darmstadt/Germany). Pure solutions of the extractants applied served as reference.

### Exchange between peat-bound metals and competitive Cu(II) ions

After a rough estimate of the DOM concentration in a 0.45 µm-filtrated aqueous peat extract by means of its absorbance *A* at 254 nm (50.0 g wet peat and 200.0 mL water, pH 4.5), the Cu(II) concentration in the peat suspension was stepwise increased (by adding 5, 10, 20, 30, 50, 100 and 500 mg Cu(II) dissolved in a commercial copper standard and equilibrated for about 30 minutes after each Cu(II) adding, analogously to Burba *et al.* (2001b). After each time interval an aliquot of the peat sample was filtered and the metals in the filtrates were determined.

## Results

### Kinetical extraction behaviour of metal species and dissolved organic matter in balneological peats

In principle, the extraction of DOM and metal species from typical peats should be assessable by typical equilibrium distribution functions, sufficient equilibration time presupposed. In the case of polydisperse and colloidal DOM, however, kinetic effects might more or less influence the extraction process as already suggested in Burba (2001a). Therefore, the DOM and metal species extraction by water was also studied as a function of time. As a typical example, Fig. 1a and 1b show the influence of the extraction time on both, the yield of remobilized metal species and the absorbance *A*<sub>254 nm</sub> of DOM extracted from peat I at pH 4.5 and 5.0, respectively. As can be seen (see Fig. 1a) the metals studied (Al, Fe, Mn, Sr and Zn) have a similar extraction kinetics but approach their equilibria after slightly different times. The Mn and Zn extraction equilibrated within 15 minutes, while Al and Fe needed about 30 minutes. Sr was practically extracted within 10 minutes. The reason for the significant intermediate concentration maximum of Al, Fe and Zn after 10-15 min is still unknown. Altogether, these results show only rather small differences of the extraction kinetics between the metals studied. In contrast, the Figure 1b reveals that even a small pH raise from 4.5 to 5.0 increases the extraction of Al, Fe and Mn from peat by a factor of 3-8, but hardly that of Sr and Zn. Moreover, the extraction kinetics of the first ones are considerably slowed down requiring about 60 minutes for equilibration. In contrary to this, it can be seen from the time dependence of the UV absorbance *A*<sub>254nm</sub> of



DOM (conventionally used for HS characterisation (Abbt-Braun, 1992)) in both peat extracts (see Figs. 1a and 1b) that the extraction of organic matter does not equilibrate for the first 20 minutes. In addition, the small pH increase from 4.5 to 5.0 strongly raises the UV absorbance  $A_{254\text{nm}}$  of DOM from 0.4 to about 2.5 (assessed as absorbance  $A \cdot \text{cm}^{-1}$ ). But on the other hand, from the relatively small alterations of the absorbance ratio of  $A_{254\text{nm}}/A_{436\text{nm}}$  of DOM during extraction and at equilibrium, as shown in Fig. 2, it can be concluded that, despite the mentioned pH difference, quite similar humic molecules are remobilized from the studied peat matter. At most, the slight decrease of  $A_{254\text{nm}}/A_{436\text{nm}}$  from 9.5 at pH 4.5 to about 7.3 at pH 5.0 might be a hint to small size differences of DOM, in accordance with Aster (1998) having verified that large humic molecules (100-50 kDa) exhibit a decreased specific spectral absorbance at 254 nm in contrast to smaller ones. Probably, at the beginning of DOM extraction from peat at pH 4.5 big humic molecules are predominant in relation to smaller ones. This observation is also in accordance with a recent concept about dynamic humic substances (HS) aggregates in aqueous solution supposedly formed from HS molecules of different sizes (Piccolo, 2000).

#### **Exchange between peat-bound metals species and DTPA, EDTA, Ca(II) and Cu(II) ions, respectively: kinetical factors**

Under natural conditions metal ions bound to humic matter are supposed to form heterogeneous mixtures of macromolecular metal species of varying structure, different conditional stability and availability hardly describable by simple distribution and equilibria functions (Burba, 1998). Consequently, the chemical stability of metals bound to natural peat might significantly be controlled by kinetical factors up to now hardly considered for their characterisation. Under this aspect, the EDTA- and DTPA-availability of metals was also characterised as a function of time, analogously to the conventional use of these chelators for assessing "bio-available" metal fractions in various soils types (Klute, 1986). Typical metal exchange curves, obtained by EDTA and DTPA from medical peat as a function of time, are presented in Fig. 3a and 3b. Both complexants exhibit more or less comparable kinetics of metal and DOM remobilisation, respectively. The remobilisation equilibria for the studied metals are approached within 60 minutes, except Al and Fe needing up to 2 hours. As already discussed EDTA and DTPA solution have a similarly strong capability to remobilize major fractions of heavy metals from peat. Most probably, the chemical extraction mechanisms of both chelators are quite similar. Therefore, EDTA and DTPA might be useful for a simple removal of undesired high metal loads of peat prior to its therapeutic use. The UV absorbance  $A_{254\text{nm}}$  of DOM, co-extracted by EDTA and DTPA solutions, respectively, exhibits significantly higher but similar values compared with those obtained by pure water as extractor (see Figure 1 b). This raise is probably caused by an increasing mobilisation of HS molecules from peat by these complexants.

The availability of colloidal metal species in balneological peat suspensions was not only characterised by exchange

with extreme chelators (EDTA, DTPA) but also with strongly competitive Cu(II) ions, analogously to Burba *et al.* (2001b). Typical metal exchange equilibria between peat-bound metal species (Al, Fe, Mn, and Sr) (peat I) and successively added Cu(II) ions, obtained after an equilibration time of 10 min, each, are shown in Fig. 4. Apparently, an amount of at least 500 mg Cu(II) added to this peat suspension (50 g of wet peat I in 200 mL high-purity water) is required to exchange peat-bound metals up to limiting concentrations  $c_L$ , corresponding to about 21.8 (Al), 3.9 (Fe), 79.0 (Mn), and 81.8 % (Sr) of the respective total metal content. Apparently, Mn and Sr species in this peat sample are largely available for competing Cu(II) ions, but not those of Fe and Al. The latter observation is in agreement to others studies having suggested that Fe and Al are strongly bound in humic-rich hydrocolloids (Stevenson, 1994).

## **Discussion and conclusion**

Exact chemical structures of DOM and metal species in natural peats, suggested to cause therapeutic effects, are still unknown. From the results presented here a number of conclusions, important for further assessing the extraction behaviour of DOM and metals in medical peat, can be drawn: 1) Aqueous extraction of DOM and DOM-bound metal species from peat needs only 15 to 20 min for equilibration, except Al, Fe and DOM at increased pH values, 2) Ca, Mg, and Mn seem to be the most available metals in balneological peats, in contrast to Fe and Al, 3) As expected, the pH value has a strong influence on the extraction behaviour of both DOM and peat-bound metals, 4) DTPA and EDTA exert a strong, well comparable and reproducible extraction capability towards peat-bound heavy metals, similar to the operational DTPA and EDTA availability of metals in soils, 5) In contrast, major fractions of metals in peat are hardly available towards competitive Cu(II) ions, except alkaline earth metal ions and Mn, comparable to metals bound in humic-rich hydrocolloids recently studied.

All in all, the present study, based on a versatile extraction approach, improves the knowledge of metal species and their chemical availability in balneological peats. Additional medical investigations, however, are required to clarify in more detail the positive and negative effects of metals in peat therapy.

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## **Results**

Abbt-Braun, G. (1992). Spectroscopic Characterisation of Humic Substances in the Ultraviolet and Visible Region and by Infrared Spectroscopy. In G. Matthes, F.H. Frimmel, P. Hirsch, H.D. Schulz and E. Usdowski (eds.), *Progress in Hydro-geochemistry*, 29-36. Springer-Verlag, Berlin.

Aster, B. (1998). *Fraktionierung und Charakterisierung von aquatischen Huminstoffen und ihrer Metallspezies: Untersuchungen mittels Mehrstufen-Ultrafiltration, Metallaffinitäts-Chromatographie sowie Austauschreaktionen*. Logos Verlag, Berlin.



- Beer, A.M., Lukanov, J. and Sagorchev P. (2000). The influence of fulvic and ulmic acids from peat, on the spontaneous contractile activity of smooth muscles. *Phytomedicine* **7**, 407-415.
- Bramryd, T. (1980). The Role of Peatlands for the Global Carbon Dioxide Balance. In *Proceedings of 6<sup>th</sup> International Peat Congress*, 9-11.
- Brown, P.A., Gill, S.A. and Allen, S.J. (2000). Metal removal from wastewater using peat. *Water Research* **34**, 3907-3916.
- Burba, P. (1998). Zur Analytik von Huminstoffen und ihren Metallspezies in Gewässern. *Analytiker-Taschenbuch* Bd. **20**, 45-79. Springer-Verlag, Heidelberg.
- Burba, P., Beer, A.M. and Lukanov, J. (2001a). Metal distribution and binding in balneological peats and their aqueous extracts. *Fresenius Journal of Analytical Chemistry* **370**, 419-425.
- Burba P., Van den Bergh, J. and Klockow, D. (2001b). On-site characterisation 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.
- Hatcher, P.G., Breyer, J.A., Dennis, L.N. and Maciel, G.E. (1983). Solid-state <sup>13</sup>C NMR of Sedimentary Humic Substances: New Revelations on their Composition. In R.F. Christman, E.T. Gjessing (eds.), *Aquatic and Terrestrial Humic Materials*, 37-82. Ann Arbor Science, Ann Arbor.
- Klößing, R. (1994). Humic Substances as Potential Therapeutics. In N. Senesi and T. Miano (eds.), *Humic Substances in the Global Environment and Implications on Human Health*, 1245-1257. Elsevier, Amsterdam.
- Klute, A. (1986). *Methods of Soil Analysis, Part 1 – Physical and Mineralogical Methods*, 2<sup>nd</sup> Edition, 310-312. Am. Soc. Agronomy, Inc. Publisher, Madison.
- Marthur, S.P. and Farnham, S. (1985). Geochemistry of Humic Substances in Natural and Cultivated Peatlands. In G.R. Aiken, D.M. McKnight and R.L. Wershaw (eds.), *Humic Substances in Soil, Sediment and Water*, 53-86. Wiley.
- Piccolo, A. (2000). Differences in High Performance Size Exclusion Chromatography Between Humic Substances and Macromolecular Polymers, In E.A. Ghabbour and G. Davies (eds.), *Humic Substances Versatile Components of Plants, Soil and Water*, 111-124. Royal Society of Chemistry, Cornwall.
- Rosa, A.H., Vicente A.A., Rocha J.C. and Trevisan C.H. (2000). A new application of humic substances: activation of supports for invertase immobilization. *Fresenius Journal of Analytical Chemistry* **368**, 730-733.
- Sommerfeld, F. and Schwedt, G. (1996). Comparison of selected elution procedures for the valuation of the mobility of metals from sediment and sewage sludge. *Acta Hydrochimica et Hydrobiologica* **24**, 255-259.
- Stevenson, F.J. (1994). *Humus Chemistry*, 2nd edition. Wiley, 512 pp.
- Viraraghavan, T. and Rana, S.M. (1991). Treatment of septic tank effluent in a peat filter. *International Journal of Environmental Studies* **37**, 213-224.