Peat humic substances enriched with nutrients for agricultural applications: Competition between nutrients and non-essential metals present in tropical soils

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ABSTRACT

Improved agricultural productivity, and reduction of environmental impacts, require studies of the interactions between different soil components. Fertilizers marketed as "organic" or "natural", such as peats or humic substances (HS) extracted from peats, are enriched with macro and micronutrients that, according to the manufacturers, are released to the plant in accordance with its needs. This work investigates the complexation capacity of HS for macro and micronutrient metal species, considering the competition, for HS complexation sites, between non-essential metals (aluminium and lead), present in the soil, and the nutrients. Humic substances were found to possess strong affinities for Pb(II) and Al(III), forming stable complexes, with concomitant release of complexed nutrients. Although HS are already used commercially as organic fertilizers, further studies of methods of HS enrichment, aimed at avoiding losses, are highly desirable from environmental and economic perspectives.

1. Introduction

Soils are extremely important environmental matrices that result from interactions between the lithosphere, hydrosphere, atmosphere and biosphere, with the nature of the chemical reactions occurring being dependent on the conditions of the medium. Peat is one type of soil that is of particular interest due to its wide range of possible applications, including as fertilizers in agriculture, because of its high organic matter content [1].

Soil organic matter is produced from the breakdown of animal and plant remains, and the chemically complex fraction that results from extensive decomposition is termed humic substances (HS). These belong to a class of compounds lacking any generic identity, and containing many functional groups in their structures, which gives them an exceptional capacity for complexation with substances including metal ions [2]. As a consequence, humic substances have broad environmental application.

Fertilizers marketed as "organic" or "natural", such as peats or humic substances extracted from peats, are enriched with macro and micronutrients that, according to the manufacturers, are released to the plant in accordance with its needs. This is a relatively new market that has economic potential, and is consequently highly attractive. However, the sector remains inadequately regulated, mainly because of a lack of scientific data concerning the mechanisms of nutrient release from the HS, through which essential elements are made available to the rhizosphere. Improvement of agricultural productivity, and reduction of environmental impacts, requires studies of the interactions (such as complexation) between macro and micronutrient metal species, and humic substances. Furthermore, since soils naturally contain metal species that are non-essential to plants, studies of the competition for HS complexation sites between these species, and macro and micronutrients, should provide the fundamental knowledge required for application of nutrient-enriched HS to agricultural soils.

The objective of this work was to extract humic substances from peats, characterize them using techniques including elemental analysis, electron paramagnetic resonance and UV–vis spectrometry, and study the complexation capacity of the HS for macro and micronutrient metal species, as well as the competition for HS complexation sites between metals (aluminium and lead) present in the soil, and the nutrients.

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2. Experimental

2.1. Chemicals and reagents

All reagents used were of high-purity grade unless otherwise stated. Working solutions of acids were prepared by diluting 30% hydrochloric acid (Suprapur, Merck AG) and 65% nitric acid (Merck AG, analytical grade, pre-purified by sub-boiling distillation) with high-purity water (Milli-Q system, Millipore). A synthetic standard (ICP multi-element standard solution IV, Merck AG) was used for calibrations.

2.2. Extraction of humic substances

Peat samples were collected in the Mogi river region of the municipality of Ribeirão Preto, São Paulo State, Brazil. The humic substances were extracted following the procedure recommended by the International Humic Substances Society.

2.3. Purification of humic substances

Humic substances extracted from the peat samples were transferred into dialysis tubes, and dialyzed against water in order to eliminate soluble salts. The external water was replaced three times after 3–5 h, and then every 12 h until no Cl^- could be detected. The HS extracts were subsequently evaporated under vacuum and dried at 55 °C in a re-circulating air oven.

2.4. Characterization of the humic substances

2.4.1. Elemental analysis

The elemental composition (C, H, O, N and S) of the HS was determined using a Thermo Finnigan Flash EA 1112 elemental analyzer.

2.4.2. Electron paramagnetic resonance (EPR)

EPR spectra were acquired at ambient temperature using a Bruker EMX spectrometer, operating in the X band ($\sim$9 GHz). For quantitative analysis, quartz tubes were filled with the HS samples, recording the masses for later data normalization. The $I \times (\Delta H_{PP})^2$ approximation was used to obtain the peak areas, where I is the signal intensity and ΔH_{PP} the width of the peak to peak signal. The semiquinone-type free radical was quantified by the secondary standard method using a ruby crystal (Al_2O_3) containing 0.5 wt.% of Cr(III). Its g factor is 1.263, so it does not interfere with the semiquinone free radical, whose g value lies at around 2.003 [3].

2.4.3. Spectroscopy in the visible region (E_4/E_6)

The E_4/E_6 ratio was determined by dissolving 2.0 mg of peat HS in 10 mL of a solution of 0.05 mol L^{-1} $NaHCO_3$, followed by measurement of absorbance at 465 and 665 nm using a Hitachi U2000 spectrometer [4].

2.5. Interaction of humic substances and metals

2.5.1. Determination of the complexation capacity of peat HS for micro and macronutrients, and for non-essential metals present in tropical soils

The analytical procedure proposed by Burba et al. [5] was used to determine the complexation capacity between HS and either non-essential metals (Al and Pb) or macro and micronutrients (Co, Cu, Mn, Mo, Ni, Zn, Ca and Mg) (Fig. 1). This procedure employed a tangential ultrafiltration system (Sartorius Ultrasart X), equipped with a 1 kDa membrane (Gelman Pall-Filtron OMEGA) that restricted passage of HS and HS/metal complexes having a molecular size greater than 1 kDa. Free metal ions that were not complexed with
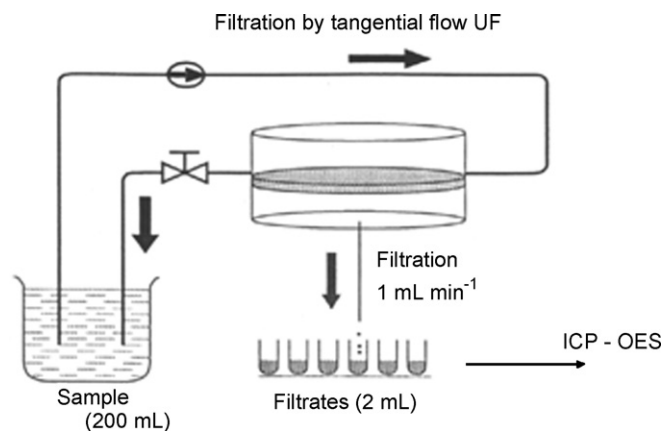

Fig. 1. Schematic of the analytical procedure used to determine the complexation capacity of humic substances extracted from peat samples for nutrient or non-essential metals. Conditions: ultrafiltration system (Sartorius Ultrasart X) equipped with 47 mm diameter, and 1 kDa polyethersulfone membrane (Gelman Pall-Filtron OMEGA).

the HS, or that were exchanged by them, passed through the membrane.

2.5.2. Competition between non-essential metals present in tropical soils, and macro and micronutrients in enriched humic substances

A 10 mL portion of the multi-element macro and micronutrient standard solution (containing 20 mg L^{-1} of each metal) was added to 40 mL of 0.5 mg mL^{-1} HS solution. The amount of the metals used was based on the estimated complexation capacity of the HS. The pH was then adjusted to 5.0 using 0.1 mol L^{-1} NaOH, the volume made up to 200 mL with water, and the mixture left under agitation at ambient temperature ($\sim$28 °C) for 24 h. The ultrafiltration system was operated for around 5 min in order to condition the membrane, then the first aliquot ($\sim$2 mL), corresponding to time zero, was filtered before addition of the non-essential metal solution. This aliquot contained a small quantity of metals corresponding to the free (uncomplexed) fraction, plus the HS-linked fraction possessing a molecular size smaller than 1 kDa. Quantities of the non-essential metals standard solution (500 mg L^{-1}) were added to the solution of HS and nutrients to give final concentrations of 0.25, 0.50, 1.0, 2.0, 5.0, 10.0 and 20.0 mg L^{-1}. After each addition, the mixture was agitated for 30 min to achieve exchange equilibrium. Aliquots ($\sim$2 mL) were collected, and the concentrations of free metal species determined using inductively coupled plasma atomic emission spectrometry (ICP-OES).

3. Results and discussion

3.1. Characterization of humic substances

Atomic ratios obtained using elemental analyses can be used to provide information concerning the structural characteristics of humic substances. The H/C ratio is related to the degree of aromaticity, where the smaller the ratio, the greater the aromaticity. In other words, an increase in the hydrogen content is indicative of a greater number of aliphatic carbons (CH_2) than unsaturated carbons (CH_2=CH_2). The value of the H/C ratio obtained here was similar to values reported in the literature (Table 1) [6–11].

The O/C atomic ratio reflects the carbohydrate content of the HS, with high values indicative of lower humification, due to lower carbohydrate levels. The sample studied showed the presence of a smaller number of oxygenated groups, compared with literature values (Table 1) [12].

Table 1
Atomic ratios (H/C, O/C) in different samples of HS extracted from peat or soils originating from different regions, in the present study and as reported in the literature.

Sample	H/C	O/C	Reference
Humic acid from tropical soils	1.29–1.45	0.47–0.75	Canellas et al. [6]
Humic acid from peat	1.05–1.30	0.40–0.65	Zaconne et al. [7]
Humic acid from tropical soils	1.21	0.63	Gonzáles-Pérez et al. [8]
Humic substances from tropical soils	0.20–0.71	0.80–3.23	Rosa et al. [9]
Humic acid from peat	1.0–1.6	0.36–0.52	Li and Chiou [10]
Humic substances from peat	0.79	0.67	Lu et al. [11]
This study	1.16	1.24	–

The concentration of semiquinone-type free radicals provides a good indication of the extent of humification [8,9,13,14]. Here the average free radical content of the HS samples was $2.6 \times 10^{18} \pm 0.5 \, \text{spin g}^{-1}$ of carbon, which is comparable to previous measurements of radical concentrations in HS extracted from highly humified tropical soils [9].

The E_4/E_6 ratio is an important indicator of the degree of condensation of the humic macromolecule, and is generally associated with its aromaticity. Higher ratios are indicative of a structure having lower aromaticity [15]. According to the literature, ratios lower than 4 are indicative of a greater presence of condensed aromatic structures, while values greater than 4 indicate a lack of such structures [16]. A mean value of 3.47 for the humic substances extracted from the Rio Mogi peat samples was therefore suggestive of a high degree of humification.

3.2. Complexation capacity of peat HS for macro and micronutrients, and for non-essential metals present in tropical agricultural soils

Aluminium is the most important non-essential metal present in tropical soils, existing as fragments of aluminosilicate rocks, secondary minerals, hydroxides, aluminium salts and in solution. The amount of available aluminium is pH dependent [17]. In excess, the metal can affect root development and reduce growth rates [18].

The concentration of lead in soils is extremely variable, with higher values usually found in regions that are densely populated or near to industrial areas. Fadigas et al. [19] proposed reference values for natural concentrations of potentially toxic metals, including lead, with permissible levels being dependent on various factors, the most important being soil composition. Additions of organic matter, liming, and phosphate application all act to reduce levels of available lead, and consequently rates of its uptake by plants. Release of lead in soils increases as a function of acidity, and is greater for soils with low organic matter contents [17].

Essential mineral elements are classified as macro or micronutrients, and can be provided by the soil itself or by means of fertilizers and plant supplements. Macronutrients include calcium and magnesium, and micronutrients include cobalt, copper, iron, manganese, molybdenum and zinc [18]. The nickel is essential trace metals only required to urease enzyme [20]. It is important to note that, in excess, these nutrients may present toxicity, and that direct application to soils without previous interaction studies may lead to environmental damage and/or reduced crop yields.

Humic substance complexation capacity (CC) reflects the maximum affinity of the HS ligand sites for a given metal species. Fig. 2 illustrates a typical complexation capacity curve obtained for the peat humic substances, using a Pb (II) standard metal solution and the tangential flow ultrafiltration procedure. The CC was obtained by plotting the concentration (mmol L^{-1}) of the free metal species against the total metal concentration. The curve shows a slope alteration towards the end, and the CC was obtained by the intersection of the two linear sections of the graph [21–24]. The complexation
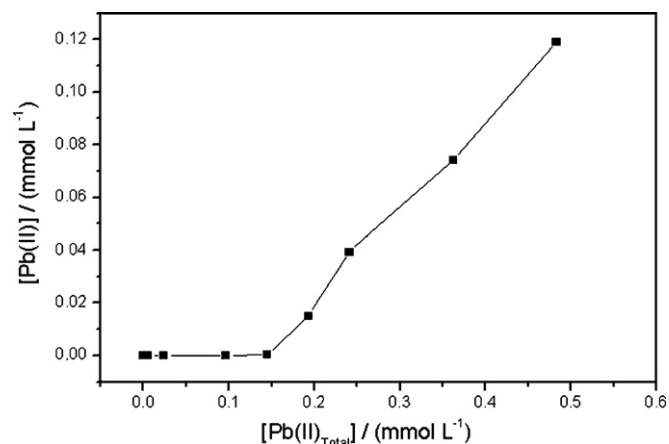

Fig. 2. Complexation capacity curve of peat HS for Pb(II), using the tangential ultrafiltration technique. Conditions: sample concentration 100 mg L^{-1}; pH 5.0; 0.10 mol L^{-1} NaNO$_3$.

capacity results gave the following increasing order of affinity of the HS for the metals: Zn < Co < Ca < Mg < Mn < Ni < Pb < Mo < Al < Cu (Table 2).

3.3. Competition of non-essential metals for humic substances enriched with macro and micronutrients

Figs. 3 and 4 show the behaviors of the macro and micronutrients complexed by the humic substances, in the presence of the non-essential metals Al(III) and Pb(II), respectively. Release of the nutrient species occurred when non-essential metals were complexed by the HS. It appears that release of essential metals occurs after occupation of complexation sites by non-essential metals, when the affinity for HS of the latter is greater than that of the nutrient species.

Zn(II), Co(II), Mn(II) and Ni(II), only required to urease enzyme, were the essential trace metals most readily exchanged by the humic substances, indicating that the HS possessed greater affinity for the non-essential metals (Al and Pb) than for these essen-

Table 2
Complexation capacity of peat humic substances for macro and micronutrients, and for non-essential metals.

Metal	Complexation capacity (mmol metal g^{-1} TOC)
Ca	4.25
Mg	4.33
Zn	2.10
Co	3.07
Ni	4.58
Mn	4.52
Cu(II)	13.75
Mo	13.37
Al(III)	13.59
Pb(II)	8.33

(■) Macronutrients, (□) non-essential metals and (▨) micronutrients.
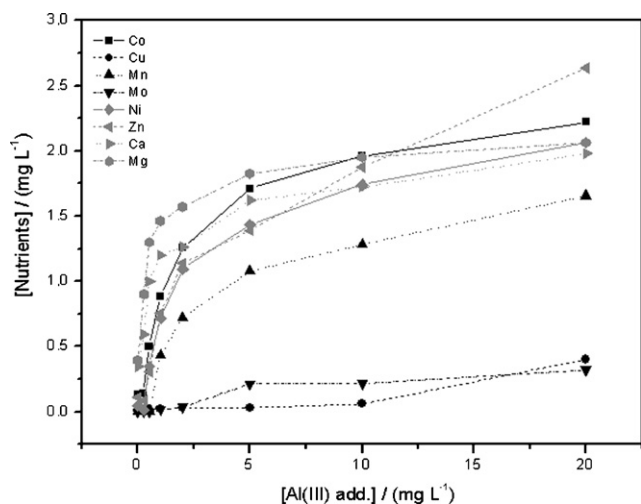
Fig. 3. Competition for Al(III) of HS ligand sites enriched with macro and micronutrients.

tial trace metals. Mo(II) and Cu(II) were only slightly exchanged, indicating their high affinity for humic substances. HS–Zn, HS–Co, HS–Ni and HS–Mn complexes were therefore less stable than HS–Al or HS–Pb, while HS–Mo and HS–Cu were more stable than the non-essential metal complexes. This is in agreement with the findings of Burba et al. [25,26] who highlighted the high stability of Cu complexes, both in the presence of other metals in aquatic and terrestrial environments, as well as in competition for chelating agents other than HS (such as EDTA).

The exchange behavior of the macronutrients was similar to that of the micronutrients. When exchanged with Al(III) and Pb(II), the most labile metal was Mg(II), although Ca(II) was also exchanged, inferring that HS-non-essential metal complexes were more stable than HS–Ca or HS–Mg complex. In some cases, the amount of essential metals released was greater than the amount added, which can be explained by occupation by the non-essential metals not only of the sites occupied by the nutrients during the 24 h contact period, but also of sites containing metals originally complexed with the HS. Metals for which the CC is higher tend to form more stable complexes, so that when non-essential metals compete for sites complexed with nutrients, they exchange, and the nutrients are released. Such behavior was not seen in the competition between
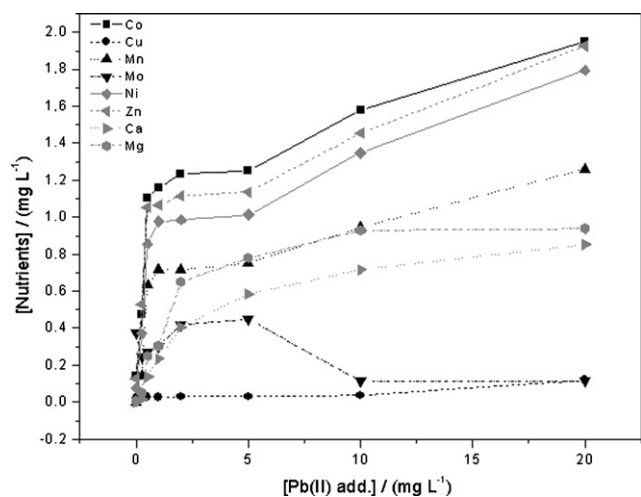
non-essential metals and Cu(II) ions, due to the high stability of the HS–Cu complex.

4. Conclusions

Complexation capacity studies have shown that humic substances possess a strong affinity for the non-essential metals Pb(II) and Al(III), forming stable complexes. Competition for humic substance ligand sites between macro and micronutrients, on the one hand, and non-essential metals, on the other hand, showed high affinity of HS for Al(III) and Pb(II), with release of complexed nutrients. Although HS are already used commercially as organic fertilizers, further studies of methods of HS enrichment, aimed at avoiding losses, are highly desirable from environmental and economic perspectives.

Application of nutrient-enriched peat humic substances to agricultural soils containing high levels of non-essential metals is of interest since this material acts as a fertilizer, releasing nutrients, while at the same time complexing metals that could be prejudicial to the growth and development of crops.

Acknowledgements

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Fig. 4. Competition for Pb(II) of HS ligand sites enriched with macro and micronutrients.

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