

**BORON CONCENTRATION IN HUMIC AND FULVIC ACID EXTRACTS
OF SOIL EPIPEDON IN SAN VITALE PINEWOOD (RAVENNA, ITALY)**

**LE CONTENU EN BORE DES ACIDES FULVIQUES ET HUMIQUES
EXTRAITS DES EPIPEDONS DE SOLS
DE LA PINÈDE DE SAINT VITALE (RAVENNE, ITALIE)**

**DETERMINAZIONE DEL BORO IN ACIDI UMICI E FULVICI
ESTRATTI DAGLI EPIPEDON DI SUOLI
DELLA PINETA DI SAN VITALE (RAVENNA, ITALIA)**

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Abstract

Humified Soil Organic Matter (SOM) plays a crucial role in the assessment of soil quality since it makes up a significant part of the total organic carbon and nitrogen in soils. High concentrations of humic and fulvic acids may be presents in soils and subordinately in sediments. These substances can potentially act as a significant reservoir of adsorbed boron as well as a source of this element to soil solution, rivers, and lakes. The aim of this study was to investigate boron in humic substances (e.g. humic and fulvic acids) of soil epipedon. The San Vitale pinewood was selected as the study site and three samples - classified as Sodic Psammaquents and Typic Psammaquents - of the A1-horizon epipedon were analyzed for humic and fulvic acids and boron contents. The knowledge of the mechanisms of boron interaction with SOM is important for a better understanding of the water/rock interaction in the superficial soil environment, and to envisage the application of the blending of boron into humic acid granules in agricultural practices.

Keywords: *boron; SOM; humic acid; fulvic acid*

Résumé

La matière organique humifiée joue un rôle qui est crucial dans l'évaluation de la qualité du sol, car elle constitue une partie importante de la teneur en carbone organique total et de l'azote dans les sols, récalcitrants à micro-organismes. De hautes concentrations d'acides humiques et fulviques peuvent être présentes dans les sols et les sédiments. Ces substances peuvent devenir importantes comme réservoirs de bore adsorbé, ainsi que d'une sources de cet élément pour l'eau du sol, les rivières et les lacs. L'objectif de cette étude est d'étudier le bore sur les

substances humiques (acides humiques et fulviques) de l'épipédon du sol. La pinède de Saint Vitale a été choisie comme site d'étude et trois échantillons – classés comme Sodic and Typic Psammaquents - de l'horizon épipédon A1 ont été analysés pour leurs teneurs en acides fulviques et humiques et en bore. La connaissance du mécanisme d'interaction du bore avec les substances organiques du sol est importante afin de mieux comprendre l'interaction de l'eau avec la roche dans l'environnement peu profound ainsi que pour envisager l'application de granules de la combinaison du bore avec l'acide humique dans les pratiques agricoles.

Clés-mots: *bore; matière organique humifiée; acide humique; acide fulvique*

Riassunto

La sostanza organica umificata ha un ruolo strategico nella valutazione della qualità del suolo, poiché costituisce una frazione significativa del carbonio organico totale e dell'azoto nel suolo. Gli acidi umici e fulvici possono avere tenore elevato sia nei suoli che nei sedimenti e rappresentare quindi una superficie di adsorbimento per il boro. Le sostanze umiche possono potenzialmente essere quindi una significativa riserva di boro.

Lo studio di questo lavoro è stato di investigare la presenza del boro sulle sostanze umiche estratte dall'epipedon del suolo. La pineta di San Vitale è stata scelta come sito di studio ed i campioni dell'epipedon (orizzonte A1) di tre suoli Sodic and Tipic Psammaquents sono stati analizzati per determinare il contenuto di boro sugli acidi umici e fulvici estratti. La conoscenza del meccanismo di interazione del boro con la sostanza organica del suolo è importante nello studio dell'interazione tra acqua e roccia in ambiente superficiale e nell'applicazione dei composti umico-borati nelle pratiche agricole.

Parole chiave: *boro; sostanza organica umificata; acido humico; acido fulvico*

Introduction

Soil organic matter (SOM) consists of a complex and rather resistant mixture of brown or dark brown amorphous and colloidal organic compounds that result from microbial decomposition and synthesis, and that has chemical and physical properties of great significance to soils and plants. In soils SOM plays an important role, because it holds particles together and stabilizes the soil reducing the risk of erosion, aids crop growth by improving the soil's ability to store and transmit air and water, it stores and supplies many nutrients needed for the growth of plants and soil organisms, and prevents or minimizes compaction. Moreover, organic matter retains carbon from the atmosphere, and reduces the negative environmental effects of pesticides, heavy metals, and many other pollutants.

Together with those compounds that are relatively easily decomposed by microorganisms, SOM also includes carbohydrates, amino acids, lipids and humic substances (HS), that formed by the breaking down of organic structures and synthesis of new ones.

HS are complex substances of high molecular weight, which are resistant to further decomposition. Consequently they tend to accumulate in the soil, where they make up 60-80% of total SOM. Most HS are dark and are hence responsible for the dark color that is commonly associated with soils of high organic matter content. HS possess carboxyl groups (-COOH), which give them their acidic character and make them effective in buffering soil pH. HS are divided into three general groups: fulvic acids, humic acids, and humin. These three groups are nondescript mixtures of many chemical compounds and should not be considered as distinct chemical entities. Humins are particles with large molecular weight, relatively small specific surface area, relatively low number of carboxyl groups, and are inactive.

Humic acids are smaller in size than humins (approximately colloid-sized) and have more carboxyl groups than humins. Fulvic acids are the smallest in size among humic substances, and have a large number of carboxyl groups per unit mass. For this reason they are the most active among humic substances. The percentage of the various humic fractions varies considerably from one soil type to another. The humus of forest soils is characterized by a high content of fulvic acids while the humus of peat and grassland soils is high in humic acids. The humic acid / fulvic acid ratio usually, but not always, decreases with increasing depth.

Boron is an essential micronutrient for plants, but the concentration range between deficient and toxic is narrower than for any other nutrient element. Together with inorganic compounds (oxides, hydroxides, clay minerals) organic matter represents one of the significant boron-adsorbing surface. SOM adsorbs more boron than mineral soils on a weight basis (Gu & Lowe, 1990); this process increases with increasing pH, and reaches a maximum at pH=9 (Gu & Lowe, 1990, Lemarchand et al., 2005).

Boron adsorption reaches equilibrium rapidly (hours) and is enhanced by increasing ionic strength of the solution. Plants respond directly to boron content in soil solution, and only indirectly to boron adsorbed onto soil constituents, such as that blending boron into humic acid has been suggested as an ecological friendly method in agricultural practices (Hughes, <http://garudabd.org/sites/garudabd.org/files/Boron.pdf>). The interaction of boron with soil organic matter, including humic and fulvic acids, is likely to exert a significant control on boron chemical and isotopic budgets in superficial environments.

The aim of this work is to investigate boron in humic substances (e.g. humic and fulvic acids) extracted by soil epipedon. In this study we have determined the boron content in humic and fulvic acids (HA and FA, respectively) isolated by soil epipedon in San Vitale pinewood (Ravenna, Italy), a site of community importance. The San Vitale soil is affected by contamination from saline waters and from atmospheric deposition.

The soil profiles here studied are classified as Sodic Psammaquents (Pin 3 and Pin 9) and Typic Psammaquents (Pin 5). The humic substances are extracted from epipedon (A-1 horizon), that is more rich of organic carbon than the deeper

horizons. In fact the salinity and B concentrations in soils affect the plant growing and the microbial communities.

Methods

The extraction of humic substances is carried out using NaOH 1M Suprapur, with a 1:10 ratio (wt/vol) for 24 hours at 65°C. After centrifugation, the samples are filtered at 0.45µm (Millipore system). The supernatant is acidified at pH<2 and the HA fraction is separated by centrifugation. The FA fraction is separated from PVP using solid-chromatography, according to Ciavatta et al. (1990). The HA and FA fractions are dialyzed at 6000-8000 Da, and 1000 Da, respectively and are then frozen-dried.

To separate boron from the organic matrix the analytical method reported in Tonarini et al. (1997) is followed. This method was set up for the measurement of the 11B/10B ratio, by Positive Thermal Ionization Mass Spectrometry (PTIMS). This procedure is used because it allows to purify boron from the matrix for ICP analyses, and an aliquot of the purified solution can be stored for further B-isotope analyses. HA and FA are dried, weighted (about 0.1 and 0.02 g, respectively), then mixed with boron free-K₂CO₃ (1:5 wt ratio) in Pt-Ir crucibles.

The samples are then fused, and the fusion cake dissolved in ultra-purified water. The insoluble phase is centrifuged, and a weighted amount of the supernatant processed according to the three-steps ion-exchange procedure, described in Tonarini et al., (1997). HNO₃ (2%) is then added to the eluted, and the solution finally analyzed by ICP-OES.

Results

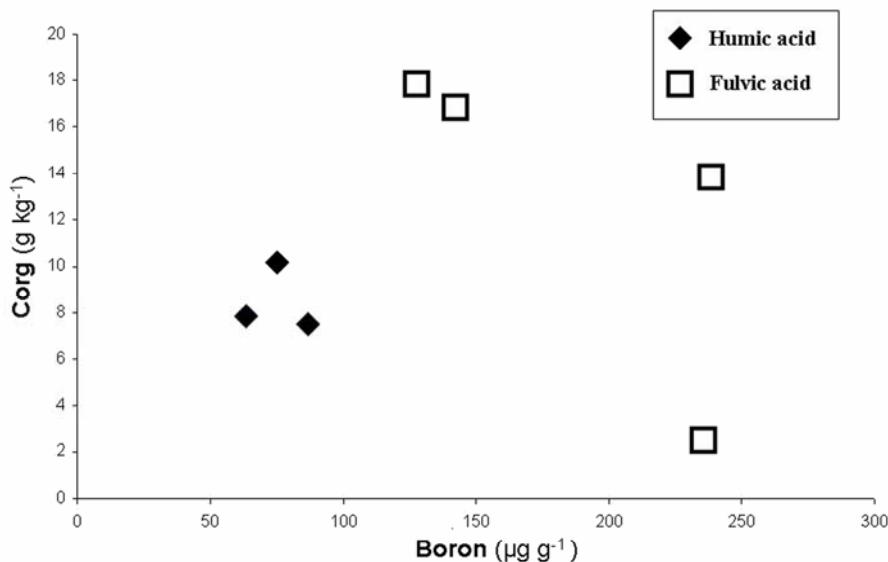
Soils of the San Vitale Pinewood highlight a typical A/C sequence. The organic-mineral horizons (A1 and A2), about 20 cm thick, usually have a darker colour, between 2,5Y3/1 and 2,5Y5/4 (dry colour). As far as total organic carbon (TOC) is concerned, the soils of the San Vitale Pinewood are characterized by significant rates in the A1 epipedon (139 to 31 g of Corg.kg⁻¹), but show a sudden decrease already in A2 horizon, below 5 cm of depth, with rates between 51 and 5 g kg⁻¹.

Boron concentration measured in humic substances ranges between 65 and 240 µg of B g-1organic acid. FA samples are enriched in boron respect to HA. Data gained on the three soil samples do not highlight a clear correlation between boron and organic carbon (Fig.1). Results of this study confirm that boron sorption on HA and FA is significant, and could play an important role in the boron geochemical cycle.

To evaluate the influence of SOM on boron sorption (and release) in soils further investigation is required in soils from different geological context and/or areas with different soils use and management. These preliminary data encourage carrying on sophisticated analyses, including PTIMS (11B/10B ratio), and 11B NMR. In contrast with the extensive literature on the isotopic fractionation of boron during geological processes, boron chemical and isotopic analyses in plants and living

organisms are extremely scarce, and little is known about boron isotope fractionation associated with biological uptake (Weiser et al., 2001).

Figure 1 - Boron and Organic Carbon in humic and fulvic acids separated from soil epipedon of the San Vitale pinewood.



Few studies looked into the structure of surface (organic and inorganic) complexes formed by boron and measured the isotopic fractionation related to these interaction. Recently Lemachand et al (2005) characterized carboxylic and phenolic sites in humic acid, which strongly adsorb B-containing species such as B(OH)₃, while Tossel (2006) investigates the stable complexes that form during boron adsorption on humic acids.

The investigation of the B-Organic complex (and O, H, N) remains a main point to constrain the boron budget in the superficial continental environment, and the correct boron supply in agriculture.

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References

- CIAVATTA C., GOVI M., VITTORI ANTISARI L., SEQUI P. (1990) Characterization of humified compounds by extraction and fractionation on solid polyvinil pirrolidone. *J. Chrom.* 509: 141-146.
- GU B. & LOWE L. (1990) Studies on the adsorption of boron on humic acids. *Can. J. Soil Sci.*, 70: 305-311.
- LEMARCHAND E., SCHOTT J., & GAILLARDET J. (2005) Boron isotopic fractionation related to boron sorption on humic acid and the structure of surface complex formed. *Geochim. Cosmochim. Acta*. 69, (14): 3519-3533.
- TONARINI S., PENNISI M., & LEEMAN W.P. (1997) Precise boron isotopic analyses of complex silicate (rock) samples using alkali carbonate fusion and ion-exchange separation. *Chemical Geology*, 142: 129-137.
- TOSSEL J.A. (2006) Boric acid adsorption on humic acids: ab initio calculation of structures, stabilities, ^{11}B NMR and ^{10}B , ^{11}B isotopic fractionations of surface complexes. *Geochim. Cosmochim. Acta*, 70: 5089-5103.
- WEISER M.E., IYER S.S., KROUSE H.R., CANTAGALLO M.I. (2001) Variations in the boron isotope composition of Coffea Arabica beans. *Applied Geochem.* 16: 317-322.