Gel filtration and absorption spectroscopic investigations on humic substances from organic fertilizers

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Summary Three organic fertilizers: a farmyard manure (FYM), humified poplar barks (PBF) and spruce barks (SBF) were investigated by means of chemical analysis and by the study of humic substances extracted by 0.1 M sodium pyrophosphate (pH = 10.0). The organic carbon content and the C/N ratio were higher in SBF, probably as a consequence of the short fermentation period (5 months). Yields of organic substances removed by three consecutive extractions with Na₄P₂O₇ solution were in the order: PBF > FYM > SBF. Nominal molecular weight distribution of total extracts was studied using Sephadex G type gels; TRIS buffer (pH = 9.0) was the eluant, since the use of water produced complex gel-solute interactions. It was shown that SBF had a higher content of small size particles than the other two products which exhibited quite similar elution curves. Slopes of log A(absorbance) vs log λ plots and ratio of absorbances at 465 and 665 nm (E₄₆₅/E₆₆₅) of fractions containing particle of lower size were the steepest and the highest, respectively.

Introduction

The important and beneficial role of organic matter and particularly humic substances in the fertility of cultivated soils is well known. The rising fertilizers and energy costs, the increasing demand for food, the large quantities of organic wastes produced yearly by industry and agriculture have stimulated research on the utilisation of these wastes. Consequently, during the last decade there has been a burst of research on the production of fertilizers from organic wastes in a few countries and, on assessing the agrochemical value of the products, their analytical characterization has also been considered. In this context, organic carbon, mineral micro and macro nutrients and potentially toxic elements were generally determined; however, very little attention has been payed to humic substances. A joint research programme on the conversion of poplar and spruce barks, both separately and mixed with other organic wastes is being carried out by this Institute and the Istituto Piante da Legno e Ambiente (I.P.L.A.). One of its aims is the evaluation of the fertilizing efficiency of products obtained from various starting materials under different fermentation conditions. In this context, attention is given to qualitative and quantitative characteristics of humic substances and their correlation to agrochemical effects. Here we report the first results of gel filtration, visible and UV spectrometry of humic materials extracted from three organic fertilizers.
Materials and methods

Organic fertilizers

Three organic fertilizers were considered: a farmyard manure (FYM) obtained after a composting period of six months; two products obtained from composting spruce bark (SBF: 5 months fermentation) and poplar barks (PBF: 20 months fermentation) respectively. The latter was produced according to a composting process developed by Fassi et al. Samples for analysis were collected from heaps containing about 10 tons of materials: they were cut vertically, at ten random selected sites, and combined to give a gross sample from which a laboratory sample was obtained with the method of coning and quartering. Prior to chemical analysis and extraction of humic substances, laboratory samples were air dried and ground in order to pass a 2-mm sieve.

Chemical analysis

Chemical analysis was performed on air-dried samples. Moisture was determined by heating air-dried samples at 105°C to constant weight. The ash content was weighed after the samples were heated in a muffle-oven at 550°C for 4 hours. The pH of suspensions of organic fertilizer in water (1/5, w/v) was measured with a Metrohm E 388 potentiometer equipped with glass and saturated calomel reference electrodes. Total C and N content of the fertilizers were determined by an automated procedure using the Carlo Erba Elemental Analyzer, Model 1106. The organic carbon content of total extracts, humic acids (HA) and fulvic acids (FA) was determined by refluxing for two hours in a dichromate-sulfuric acid mixture. All metals were determined by atomic absorption spectrophotometry (Perkin-Elmer 5000) of the ashed samples after digestion with concentrated nitric and perchloric acid. Total phosphorus was determined by the Fiske-SubbaRow method.

Extraction of humic substances

Humic materials were extracted from air-dried samples with 0.1 M Na₄P₂O₇ solution (pH = 10.0) by using a fertilizer extractant ratio of 1:100. The mixture was allowed to stand 24 hours and the extraction procedure was repeated three times on the same portion of fertilizer; a total extract was obtained by combining successive extracts. After each extraction, insoluble materials were removed by centrifugation for 15 min at 6000 rpm and the solution obtained was filtered and brought to volume by washing the precipitate with the extracting solution. Extracts were subjected to gel filtration directly or stored in a refrigerator under a nitrogen atmosphere. A portion of the solution obtained by the exhaustive extraction was acidified with HCl to pH = 1.5 to precipitate the HA fraction; after flocculation, the latter was separated from the FA solution by centrifugation, washed with a dilute HCl solution and redissolved in the extracting solution at pH = 10.0.

Sephadex gel filtration

Characterization of the extracted materials by nominal molecular weight distribution was carried out by Sephadex (Pharmacia) gel filtration. The following solutions were gel filtrated: total pyrophosphate extract; HA redissolved in 0.1 M Na₄P₂O₇ solution; FA in solution adjusted to pH = 10.0 with NaOH. No purification treatment such as dialysis or ion-exchange chromatography was employed in order to lower the amount of non-humic substances such as carbohydrates or the ash content. As already pointed out, only a few studies used this approach which has the advantage of avoiding loss of humic substances closely associated with mineral matter and non-humic organics. Since gel-solute interactions on Sephadex G type gels with water as an eluant, especially when extracts were not desalted by dialysis were observed, elution was carried out by using both distilled water and TRIS-HCl buffer (pH = 9.0). The gels (G type, fine grade) were swollen in the eluant according to the 'heating method' and packed into glass columns (Φ = 2.5 cm; l = 40 cm). The void volume (V₀) and the total bed volume (Vₜ) of each column were determined by using a Pharmacia Sample Mixture. A suitable volume of extract was first applied to the lowest G type Sephadex column; three ml fractions were collected and their absorbance measured at 260 and 465 nm. The excluded