New and traditional analytical tools for the study of soils and humic acids

Paula C. Dabas

School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956 (1113), Buenos Aires, Argentina, Email pdabas@ffyb.uba.ar.

Abstract
A variety of analytical methods have been used for the study of different soils of Argentina. These methods are based on the general physicochemical properties of the material under study such as its oxidation-reduction capacity, the presence of ionisable functional groups and, its migration in an electric field among other. The determination of the activity of some soil enzymes was analyzed in air dried soils samples. The samples were oxidized by a new heterogeneous phase, catalytic oxidation, using chemically modified electrodes with bimetallic films of metalloporphyrins. The results were analyzed by capillary electrophoresis (CE) and by gas chromatography- mass spectrometry (GC-MS).

Different extraction methods of soil humic components were compared. The usefulness of separation by gel exclusion (SEC) of humic acid was also evaluated. Infrared spectroscopy with Fourier transform (FTIR) spectrometry was done for soils, humic acid and in the fractions obtained by SEC. In addition, redox titrations of the isolated humic acids were also performed.

Key Words
Soils, enzymatic assays, catalytic oxidation, gas chromatography-mass spectrometry analysis, capillary electrophoresis analysis, FTIR.

Introduction

Analysis of soils has acquired increasing importance in recent years, given the economic importance of this material, which affects agriculture and environment. Both, soil and humic acids (HA), represent very complex samples and, their analyses constitute a true challenge for analytical chemists. There are many reports in the literature regarding the analysis of HA and soil and each publication brings something new, mainly due to the characteristics of the sample under study (soil and/or humic substances). In recent years there has been an increasing work using sophisticated instruments which are not available to all laboratories, particularly those in developing countries. It is very important for countries which devote large areas to agronomic activities, to get to know the state of the soil in a short time and at a low cost. Therefore, the aim of the present work was to evaluate the current analytical techniques as well as their potential in the analysis of soils and/or humic substances in Argentina.

Methods

Soil samples from three different geographical areas of Argentina were analysed. The soil samples were air dried and 2 mm sieved.

Enzymatic assays
The urease activity was determined as the amount of NH_4^+ released from 5 g soil after the incubation for 120 min at 37 °C with urea (6 %) in 0.95 M citrate, pH 6.7. The product formed (indophenol blue) by the reaction of the NH_4^+ released and the phenol and hypochlorite in the alkaline medium was measured by spectrophotometry.

The phosphatase activity was evaluated by spectrophotometry as the amount of p-nitrophenol (PNP) released from 5 g soil after incubation at 37 °C for 10 min with the substrate p-nitrophenyl phosphate in MUB buffer pH 6.5 for acidic phosphatase and in MUB buffer pH 11.0 for basic phosphatase. Then 2 M $CaCl_2$ was added and the PNP released was extracted with 0.2 M NaOH.

The cellulose activity was determined in 5 g soil with carboxymethyl-cellulose (0.7 %) as substrate by incubating for 24 h at 50 °C, in 2 M acetate buffer, pH 5.5. Then the glucose released was incubated with (a mixture of 1.25 mM 4-aminophenone, 2.75 mM phenol, ≥ 3000 U/I glucosidase, ≥ 400 U/I peroxidase, pH 7.4) for 10 min at 37 °C. The compound generated was determined by spectrophotometry.

Invertase activity was determined similarly to cellulose activity except that sucrose was used as substrate and the incubation time was for 3 h.

For each enzyme, the activity was quantified using calibration curves corresponding to standards incubated with each soil sample under the same conditions. Measurements were performed by triplicate.

Infrared spectroscopy with Fourier transform (FTIR) spectroscopy
For all the samples analyzed the infrared spectrum was performed on a KBr pellet.

Catalytic oxidation
A gold electrode modified with polymers of metalloprotoporphyrins was used. The synthesis of Me-protoporphyrins was performed following the Adler procedure (Adler 1970) and the Fe^{III}protoporphyrin IX following the Smith procedure (Smith 1975). The electropolymerization of metalloprotoporphyrins were carried out in 0.1 M tetrabutylammonium perchlorate - dichloromethane by potential sweep between 0.00 and + 1.50 V (vs. Ag|AgCl) at 0.05 Vs^{-1}, five cycles; for all the polymers except for Fe^{III}protoporphyrin IX (0.00 to + 1.2 V). An aliquot of commercial HA in 50 mM phosphate buffer pH 5.00 or pH 7.00 centrifuged and filtered through a 0.45 µm membrane filter, was transferred to a glass tube and a gold electrode was immersed in the solution.

A 0.5 g soil sample was transferred to a 50 mL flask and a few milliliters of 0.5 M NaOH added, and after 15 min, the volume was completed with 50 mM phosphate buffer pH 7.00. The suspension was centrifuged and filtered through a 0.45 µm membrane filter. An aliquot of 3.5 mL of the filtered solution was transferred to a glass tube, 0.5 mL of 80 mM H_2O_2 was added and a gold electrode was immersed in the solution. At different time points, the solution was analyzed by gas chromatography (GC) and capillary electrophoresis (CE).

Gas chromatography-mass spectrometry analysis
A HP-5MS (5%)-diphenyl-(95%)-dimethylsiloxane capillary column (30 m x 0.25 µm; Supelco) was employed and the gas carrier was helium (99.995 % pure). The column was initially maintained at 70 °C for 2 minutes, and then the temperature was increased to 230 °C at a rate of 8 °C/minutes, which was held until the end of the analysis. The mass spectrometer operated in the range of *m/z* 50 -550 amu. An aliquot of the aqueous solution was acidified with 6 M HCl and extracted with a sixth volume of chloroform, and the chlorophormic phase was separated by chromatography.

Capillary electrophoresis analysis
Capillary zone electrophoresis mode with UV detection was employed. A fused silica capillary of 60 cm total length x 75 µm i.d. was used. The background electrolyte was a 15 mM borate buffer, pH 10.0. The voltage was 20 kV and the analysis was carried out at room temperature. All the solutions and samples were filtered through a 0.45 µm membrane filter previous to the introduction into the apparatus. The sample was introduced in mode hydrostatic for 30 s. Detection was performed at 214 nm.

Isolation and purification of humic acids.
The soil was washed twice with distilled water to extract the non-humic, water-soluble substances. Then, the extraction was carried out with 0.5 M NaOH by constant shaking under a N_2 gas atmosphere in sealed tubes for no more than 24 hours. Following centrifugation for 25 min at 3000 rpm, the supernatant was acidified with 6 M HCl (pH 0.5- 1) to precipitate the HAs. Then, the HAs were purified by dissolution in 0.1 M NaOH, centrifugation and precipitation of HAs by acidification with 6 M HCl. Finally, the suspension containing the HAs was dialyzed against distilled water until free of chloride and freeze-dried.

Gel permeation chromatography (SEC).
The chromatography was made by passing a solution of HAs in mobile phase (0.02 M borate buffer, pH 9.2) through a glass column (60 cm x 1.5 cm i.d.) containing Sephadex G-75 or Sephacryl S-200 High Resolution. The fractions were collected every 6 and 4 minutes respectively. The absorbance was monitored at 280 nm. In order to obtain enough material, each chromatography was repeated several times. The fraction of every HA eluted with the same volume of mobile phase, was combined and acidified to obtain HA and then was treated in the same way as the AH extracted from soils.

Other determinations.
Redox titrations were performed of the HA isolated from each soil.

Results

The biological activity in cultivated soil shows a significant increase of the enzymatic activity, fundamental catalytic chemical reactions are essential to plant nutrition (Zornoza 2006). Enzymatic determinations were conducted on dry soil (Table 1). This allows working with more homogeneous samples and preserves them without any chemical changes. Zornoza *et al*. determined enzyme activities for urease, phosphatase and glucosidase, in dry soil and in soil rewetted for different times and achieved satisfactory results. These results are encouraging, since if they are combined with the other methods described, they can be used as early markers of soil deterioration (Alexander 1980).

Table 1. Enzymatic determinations

Soil	Urease (µmol/g h)	Sacarase (µmol/g h)	Cellulase (µmol/g) h	acidic phosphatase (µmol/g h)	Basic phosphatase (µmol/g h)
A	1.6	1.6	nd	2.5	0.7
B	2.0	1.7	nd	1.8	0.9
C	1.7	nd	nd	1.7	0.6

*nd= no detectable

The application of monometallic and bimetallic complex of porphyrins to catalyse the oxidation of HA and soil samples has proved very useful (Figure 1), getting the best results with polymers of Coprotoporphyrin films. The mild oxidation of humic substances with catalysts redox showed very promising results, since a relatively simple profile in GC and CE was obtained, providing the ability to categorize the types of soil and possible contamination.

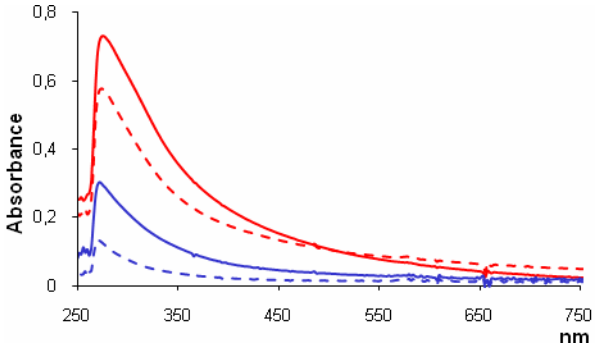

Figure 1. Oxidative degradation of two Argentine soils at different times: A. —— 4.8 h; - - - - 38.4 h; B: —— 7.2 h; - - - - 31.2 h.

Conclusion

Classical analytical chemistry is still very useful for the analysis of complex samples with variable composition such as the material studied in the present work. It can give important information by itself and can be a very useful complement to the results obtained with other techniques. So far, there is not a sole technique that can provide all the information that we need to know about the soils Therefore a lot of work is needed to assemble the different analytical techniques in order to obtain the required information.

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