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Spectroscopic evidence of the marine origin of mucilages in the Northern Adriatic Sea

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Abstract

Vibrational Fourier transform infrared (FTIR) and UV–Visible spectroscopies coupled to thermogravimetry were used to characterise the gelatinous aggregates as well as the humin and humic fractions extracted from the aggregates. Spectroscopic analysis made it possible to identify the main classes of compounds present in the samples. The FTIR spectra of the aggregates showed the presence of carbohydrates, saturated aliphatic compounds, aminic, esteric, amidic (proteins) and ketonic compounds, organic and inorganic phosphates, silica and, in some cases, carbonates. The FTIR spectra of humin and humic acids showed functional groups similar to those found in the spectra of the aggregates.

The high aliphatic character and the absence of aromatic compounds observed in many aggregates sampled far from estuarine areas suggest the marine origin of mucilages. These results were also confirmed by the 270/407 nm (A2/A4) and 465/665 nm (E4/E6) absorbance ratio in humic acids.

Keywords: Humic acids; Humin; Mucilages; Adriatic Sea; IR; Thermogravimetry; UV–Vis

1. Introduction

The accumulation of mucous aggregates has been observed to be particularly significant in the pycnocline determined by the summer warming and by the fresh water stratification (Herndl, 1992; Degobbis et al., 1995).

The major components of mucilage have been identified as polysaccharides and proteins (Posedel and Faganeli, 1991; Giani et al., 1992; Faganeli et al., 1995), but other complex organic fractions such as humic acids and humin also contribute to the mucilage (Faganeli et al., 1995; Mecozzi et al., 2001). The mucilages studied in previous works (Posedel and Faganeli, 1991; Faganeli et al., 1995; Mecozzi et al., 2001) were sampled prevalently in coastal areas highly influenced by riverine inputs and some aggregates were found to contain lignins (Mecozzi et al., 2001), which are usually of terrestrial origin. It was also hypothesised that the zeolites and polycarboxylic acids introduced in the detergents after the elimination of polyphosphates may have played a role in the aggregation processes, enhancing coagulation and flocculation (Pettine et al., 1992). It was thus important to establish if organic matter of terrestrial origin is involved in the aggregation process.

The present study deals with the characterisation of mucilage samples from the Northern Adriatic Sea by Fourier transform infrared (FTIR) and UV–Visible spectroscopies coupled to thermogravimetry (TG), in

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order to obtain information on their origin. IR spectroscopy was used to obtain information on the various components present in the samples and made it possible to estimate roughly their relative amounts on the basis of the corresponding bands; TG analysis made it possible to confirm more precisely the IR spectroscopy.

2. Materials and methods

2.1. Sampling

Macroaggregates were collected in the Northern Adriatic Sea during the summer of 2000, 2001 and 2002 at different depths in different sampling sites as described in Giani et al. (2005). A brief identification of the samples analysed here is reported in Table 1.

The samples were immediately stored at -20 °C until the analyses. The freeze-dried aggregates were desalted by dialysis (Cellusep, 3500 MWCO) and then lyophilised.

Surface sediments were collected by a manual corer at Miramare in the Gulf of Trieste on June 2000 and July 2001. In order to compare humic acids of different origin, marine sediment PACS-2 (NRC), lacustrine sediment (1646-IAEA SL-1), terrestrial humic acid (Sigma Aldrich) and microphytoplankton, mainly dominated by the diatom *Skeletonema costatum*, collected by 30-μm net in the Po river prodelta, were analysed (Table 1).

2.2. Analytical methods

The extraction of humic fractions (humin and humic acids) from aggregates sediments and microphytoplankton was performed in conformity with the methods of the International Humic Substance Society (www.ihss.gatech.edu).

20–500 mg d.w. of samples were shaken with 10 mL of 0.1 mol/L HCl for 1 h. After centrifugation at 4000 rpm, the supernatant was neutralised with 1 mol/L NaOH and then added with an alkaline solution of NaOH (0.1 mol/L) to pH 9. The suspension was shaken for 4 h. The humin precipitate, separated by centrifugation, was washed with deionized MilliQ water and 0.1 mol/L HCl three times. The alkaline extract was acidified at pH ≅ 2 to separate fulvic acids from humic acid. The humic acid precipitate was separated by centrifugation, re-dissolved in 0.1 mol/L NaOH and reprecipitated by 6 mol/L HCl. This procedure was repeated twice. After centrifugation, the precipitate humic acid and humin were dialysed (Cellusep-MFPI laboratory, 3500 D) against MilliQ water and lyophilised.

FTIR and TG analyses were performed on the lyophilised aggregates and on the humic and humin fractions. FTIR absorption spectra were recorded in the 4000–400 cm^{-1} spectral region on a Jasco FTIR-300E spectrophotometer with a spectral resolution of 4 cm^{-1}. The spectra were obtained by the KBr pellet technique (about 0.5% w/w). The vibrational assignments for organic compounds were given according to Anderson (1974), Bellamy (1975) and Parker (1971), those for inorganic compounds according to Hunt et al. (1950), Miller and Wilkins (1952) and Nyquist et al. (1997).

TG measurements were performed by a Mettler TA-STAR, TGA/SDTA 851^{e} thermobalance in air (flow rate 200 mL/min) with a heating rate of 7 °C/min from 25 to 1000 °C. The weight loss in the 25–200 °C range was assigned to water of hydration. This

Table 1
Brief identification of the samples analysed in the present study

Sampling	Location	Date (month/day/year)	Depth of sampling (m)
Mucilaginous aggregates			
Creamy layers–surface aggregate	C09b	June 15, 2000	Surface
Creamy layers–surface aggregate	2009	July 3, 2001	Surface
Creamy layers–surface aggregate	A4V	July 4, 2001	Surface
False bottom	B4V	July 18, 2002	10
Cloud	C04	June 5, 2002	22
Cloud	A07	July 5, 2001	8
Cloud	B4V	July 30, 2002	12
Sedimented cloud	B15	July 6, 2000	45
Sedimented cloud	B04	July 30, 2002	30
Microphytoplankton[a]	River Po prodelta	February–April 2002	
Sediments sampling			
Surface sediments (0–1 cm)	Miramare–Trieste	Jun 6, 1999	Surface
Surface sediments (0–1 cm)	Miramare–Trieste	July 6, 2000	Surface

[a] Mainly dominated by the diatom *S. costatum*.