CHEMICAL CHARACTERIZATION OF MUCILAGE SAMPLES FROM THE GULF OF ELEFSIS, GREECE

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Abstract

Chemical analyses of mucus macroaggregates collected in the gulf of Elefsis, Greece, depicted a low protein content and the presence of degradation products. IR and ^{1}H-NMR spectroscopy demonstrated the presence of carbohydrates, aliphatic components, characteristic groups such as ester and amide groups and organosilicon compounds. The role of carbohydrate exudates by diatoms in the development and flocculation of diatom blooms was also investigated by observing biological specimens with epifluorescence microscopy using ConA lectine conjugated with fluoresceine.

Keywords : Chemical Analysis, Blooms.

Incidents of marine mucilage appearance, attributed to blooms of phytoplanktonic organisms, represented by gelatinous aggregates suspended in water column, are common in several areas of the Mediterranean, such as the Aegean Sea. However, the chemical composition of mucus aggregates remains only partly elucidated. Regarding their role in marine chemistry, the formation of mucilage participates in the cycling of marine organic matter and contributes to the agglomeration of its dissolved forms (DOM) towards colloidal and successively into particulate forms (POM) [1]. Through their various complexing sites, mucilage may act as a natural surfactant resulting in effective floatation, which could be progressively transformed into a flocculant able to accumulate various substances, among which dissolved metals, influencing their biogeochemical cycling [2]. The present study presents the results of chemical and spectroscopic analyses of mucus macroaggregates collected during an extraordinary phytoplankton bloom event in the summer of 2003 in the gulf of Elefsis, aiming at a systematic approach of the structure and evolution of these aggregates.

Well developed macroaggregates were collected by scuba diving both from the surface and the sea bottom of the western coastal part of the Elefsis gulf. The samples were freeze-dried and subsequently extracted several times with Milli-Q water in order to remove salts. Following centrifugation, they were relyophilized and afterwards used for C, N, heavy metal (Cd, Cu, Zn), IR and ^{1}H-NMR analyses.

C and N contents of samples were determined using an EA 1180 CHN Fisons Instruments elemental analyser. Heavy metals were determined employing GFAAS following wet digestion of samples. For spectroscopic analyses the dried samples were extracted twice with diethyl ether to remove lipids and pigments and the residue gently evaporated to dryness. IR spectra were obtained by a Perkin Elmer system 883 using KBr pellets and ^{1}H-NMR spectra by a Unity Plus Varian operating at 299.95 MHz, following solubilization of the sample in a percentage equal to 80-85% in deuterated trifluoroacetic acid (TFA). To observe polysaccharides in specimens, epifluorescence microscopy (Olympus BX51) was used to determine the nature of released polysaccharides by incubating samples for 1 h with 25 μg ml^{-1} Con-A (Sigma) conjugated with fluoresceine isothiocyanate (FITC), a lectin from *Concanavalia ensiformis*, which specifically binds glucose and mannose residues [3].

Macroaggregates are characterised by a rather high OC/N ratio (Table 1), indicating a relatively low protein content of the samples and the presence of degradation products [4]. However, the higher Cu and Zn concentrations detected in the mucilaginous mass from the sea surface compared to those of the bottom one (Table 1), may be attributed to a combination of atmospheric input of metal particles and the properties of the surface mucus, which formed stable foams floating on the sea surface, in the interface between atmosphere and seawater.

The IR spectra showed an almost identical composition for surface and bottom samples, both characterised by the presence of carbohydrates and proteins as major fractions of the mucilaginous mass. In the ^{1}H-NMR spectra, following calculation of H integrals, four major classes of compounds are identified: carbohydrates (36%), aliphatic chains (45%), substances with ester and amide groups (14%) and organosilicon compounds deriving from diatoms (4%). The presence of aromatic structures was very limited (1%).

As indicated spectroscopically, mucilaginous macroaggregates consist mainly of carbohydrates which appear in a lower percentage than aliphatic chains, since they degrade faster. Microscopy observations by using labelled lectine demonstrated clearly that the lysis of phytoplankton cells releases in seawater polysaccharides in the form of glue [3], dominating the formation of Transparent Exopolymer Particles (TEP), which possess a critical role in the coagulation of blooms. The increase in the concentration of TEP and the simultaneous decrease in the complexing capacities of dissolved metals during the mucilage appearance [5] point to a possible mechanism according to which TEP contribute to the aggregation, among others, of dissolved organic substances that may, otherwise, act as ligands of metal ions.

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Tab. 1. C, N and heavy metal content of mucilage samples collected from the surface and the bottom of the Elefsis gulf.

Chemical Parameter	Surface	Bottom
%C	11.5 ± 0.2	11.3 ± 0.1
%OC	2.64 ± 0.10	1.64 ± 0.01
%N	0.330 ± 0.040	0.150 ± 0.017
OC/N	8.00	10.9
N/C	0.029	0.013
Cd (µg/g)	0.11 ± 0.03	0.17 ± 0.02
Cu (µg/g)	12.1 ± 2.6	3.87 ± 0.90
Zn (µg/g)	69.1 ± 1.0	34.0 ± 3.6

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