

XXV<sup>th</sup> Congress and Plenary Assembly of ICSEM, Split  
(22-30, Oct. 1976)

Chemical Oceanography Committee

Dissolved Free Amino Acids in Northern Adriatic Waters

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Dissolved organic matter (DOM) represents a mass which is 300 times greater than organized matter from the sea. It is ecologically right to propose that this niche should be filled, and it was Pütter who in 1909 proposed that marine organisms do use the DOM for their nutrition. This question has been actualized in the past two decades and a considerable evidence has been accumulated recently, supporting this idea (for review see Stephens, 1972). Although the free amino acids represent only about 5% of the DOM considerable attention has been paid to that particular fraction since it represents and especially nutritive value as direct building block for the synthesis of new proteins in addition to the other metabolic purposes. The demonstrated ability of many common genera of phytoplankters to take up and assimilate a variety of amino acids (Wheeler et al., 1974; Schell, 1974; North, 1975) was proposed to be mediated by a mechanism for active transport of amino acids

called  $\gamma$ -glutamyl cycle. The presence of all five enzymes of that cycle has been demonstrated in a natural population of phytoplankton (Kurelec et al., in press), in Nereids from the sedentary fauna (our unpublished work) and in sponge Geodia cydonium (to be referred at this meeting). Since our investigations were made in Northern Adriatic, we were interested in the actual concentration of dissolved free amino acids (DFAA) in order to understand and interpret our results, and secondly, in order to relate that organic nitrogen fraction to the primary production of marine water of that area. Therefore we conducted measurements of DFAA quantitatively as well as qualitatively in 1974, 1975 and 1976 at typical stations in Northern Adriatic.

Samples were collected from 0,5 and 25-40 m depth at station 1 (Rovinj), 6 (Trieste), 10 (Venice) and 20 ("open sea" from Middle Adriatic) about 5 km off shore from June 1974 to August 1976. The samples were passed through 0.45  $\mu$ m Millipore filter, frozen and analysed within a week.

Total DFAA concentration was estimated using fluorescamine reagent by adopting a general method described by Stein et al. (1973). As a sample we used 10 ml of filtered sea water concentrated to 1 ml. Excitation of the fluorescence was at 390 nm and emission was read at 475 nm using a Perkin-Elmer fluorometer equipped with two monochromators. Similarly concentrated artificial sea water with added amino acids in the ratio as found by Bohling in Deutsche Bucht, Station Elbe 1, on the April 16, 1969 (Bohling, 1970) summing up 3.542  $\mu$ M/l, and dilutions of it, served as our standard in those estimations. The average amount of the total primary amines demonstrated in 49 samples was  $1.138 \pm 0.638$   $\mu$ M/l sea water, varying irregularly either with depth, geographical position or season. Extreme values were 2.920  $\mu$ M/l and 0.170  $\mu$ M/l. An exceptionally high concentration (7.500  $\mu$ M/l) was found in a water of Pula-harbour during a red-tide blow in July 9, 1974.

In our recent estimations, the procedure described by North (1975) was used. The standard curve was done this time with glycine added to artificial sea water. Boric acid and buffer of pH 10 was used. Relative fluorescence was read against quinine sulphate (0.250  $\mu\text{g/ml}$ ) set up as 100% fluorescence in a Zeiss spectrofluorometer PMO3. Using this method we found in July 27, 1976, that the concentration of surface DFAA increased moving shoreward: 1.20  $\mu\text{M/l}$  (2.800 m), 1.55 (2.400), 2.44 (2.000 m), 2.22 (1.600 m), 2.60 (1.200 m), 2.24 (800 m) and 3.00 (400 m).

The two-stage preparatory method as modified by Bohling (1970) with the Beckman-Amino acid analyser Unichrom 120B was used for the quantitative analyses. 17 most extreme samples representing 1 l of sea water revealed the constant composition of DFAA which was characterized by a strong predominance of serine and glycine, over alanine, ornithine and urea. Asparatic acid, threonine, proline, glutamic acid, valine, methionine, leucines, phenylalanine, thyrosine, lysine, histidine and arginine were also found, however in much smaller concentrations.

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