Speciation studies of copper in the Gulf of Elefsis: the role of the macroalgae *Ulva rigida*

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**Abstract**

The Gulf of Elefsis, situated in the vicinity of Athens, is a high productivity area regarding the biota. One of the most abundant algae existing there is *Ulva rigida*. Controlled laboratory simulation experiments, as well as field investigations indicated that copper is complexed to a high extent with organic substances. A significant part of these organic ligands exhibit surface active properties, i.e. the accumulation on different phase boundaries. The complexing capacity values of samples collected from different microenvironments within the Gulf of Elefsis, determined by the electrochemical method of differential pulse anodic stripping voltammetry (DPASV), vary between 0.04 and 0.43 µM Cu\textsuperscript{2+}. At the same time, the complexing capacities of samples obtained from the laboratory tanks in which *U. rigida* has been placed under controlled conditions, show much higher complexing capacity values (up to 13 µM Cu\textsuperscript{2+}). The surface active substances (SAS) expressed in equivalents of the nonionic surfactant Triton-X-100 were determined by out of phase a.c. voltammetry. Higher amounts of SAS were detected in the tanks containing *U. rigida* (up to 0.70 mg/L eq. T-X-100), in comparison to samples originating from natural microenvironments in the Gulf of Elefsis which had values ranging between 0.10 and 0.17 mg/L eq.T-X-100. These results show that *U. rigida*, contributes considerably to the production of ligands with high metal complexing capacity and high surfactant activity.

**Keywords**: copper complexing capacity, *Ulva rigida*, Gulf of Elefsis, surface active substances

1. Introduction

The abundance of complexing ligands, which are part of dissolved organic carbon (DOC) in seawater, determines the complexing capacity for metal ions (Plavšić et al., 1982). Especially, copper ions form very stable organic complexes and copper is an essential metal ion, so very often the copper complexing capacities are measured in natural waters. The electrochemical technique of choice in the present work is differential pulse anodic stripping voltammetry (DPASV) which is employed for the determination of the complexing capacity. Inert complex as for the applied method of DPASV is the one which does not dissociate under the time scale of the measurement.

Organic ligands present in natural waters consist of a pool of largely uncharacterized macromolecular organic material, resulting from a combination of biological (e.g. phytoplankton exudates and bacterial activity) and geochemical activities (humic and fulvic substances). The organic matter is partly identified and classified into particular groups of compounds such as carbohydrates, amino acids, proteins, fatty acids, lipids and hydrocarbons.

A large part of the dissolved organic matter in natural waters exhibits significant surface active properties. Such properties, i.e. the presence of hydrophobic and hydrophilic groups of organic molecules, may cause their accumulation on different interfaces (e.g. at the boundaries of seawater with the atmosphere, in bubbles, in turbulent intermixing layers, on living and nonliving dispersed particulates and sediments), creating a major part of the surface microlayer and organic coating on mineral particles. Clear evidence of surfactant production by marine phytoplankton cultures based on laboratory and field experiments is also available (Plavšić et al., 1990). Due to the specific reactivity
of surface active substances (SAS) at natural phase boundaries, their distribution and fate in the sea could be different, compared to those of organic matter pool values, i.e. dissolved organic carbon (DOC). SAS could be determined by alternating current out-of-phase voltammetry (Plavšić et al., 1990) and expressed in equivalent concentration of model-calibrating substance, e.g. nonionic surfactant Triton-X-100.

The study area of the present work is the Gulf of Elefsis, a shallow (maximum depth of 33 m) embayment rich in primary productivity, in the vicinity of Athens (Fig. 1). The area has been thoroughly studied in the last 40 years by the University of Athens team (Scoullos and Riley, 1978; Scoullos, 1979, 1983, 1986; Kaberi and Scoullos, 1996; Scoullos and Pavlidou, 1997 etc) and is characterized by a particularly rich biota, with U. rigida being for many years the most abundant alga, anchored on rocky substrates or floating in the water. The highest rate of alga U. rigida biomass decomposition has been observed during summer, when samplings took place.

Fig.1 Sampling sites

2. Materials and Methods
2.1 Water sampling from the field
Seawater samples were collected by scuba diving and placed in plastic bottles extensively prewashed by 10% nitric acid supra pure. The collection took place during the summer of 1999 from five coastal sampling stations located at the northern part of the Saronikos Gulf. Four of them are from within the Gulf of Elefsis while the fifth one is from the area of Piraeus, the largest port of Greece (Fig. 1). All of them were surface samples (0.5 m). At the sampling area of Loutropyrgos, within the Gulf of Elefsis, water samples were collected from two depths: the surface and the bottom near the seabed (maximum depth of 3.0 m), where most of the biomass of U. rigida was accumulated. After their collection, seawater samples were transferred within 1 or 2 h to the laboratory where they were filtered through nitric cellulose Millipore 0.45 µm filters.

2.2 The laboratory simulation experiments
The experiments carried out in the laboratory aimed at the simulation of natural conditions with restricted unknown factors. This was obtained by the use of artificial seawater, enriched with dissolved organic substances originating entirely from the decomposition of U. rigida biomass, under various controlled conditions. For the preparation of artificial seawater, commercially available salt Instant Ocean (Aquarium Systems) and water purified by reverse osmosis (Milli-RO, Millipore) and ion-exchange (Milli-Q, Millipore) were used. In each tank, the salinity was adjusted at 36‰ with the addition of appropriate amounts of salt.
Three distinctive conditions, usually coexisting in nature, were reproduced:
- Condition (1), decomposition of fresh biomass under aeration (aerobic conditions) and plenty of light,
- Condition (2), decomposition of fresh biomass under naturally developed anaerobic conditions,
- Condition (3), decomposition of dried biomass which was also observed to take place in nature.

The experiments were carried out by employing three glass tanks filled with 20 L of artificial water each. For each experiment, 630 g (wet weight) of thoroughly cleaned *U. rigida* were used. The cleaning of *Ulva* included careful removal of epiphytes and thorough rinsing with artificial seawater.

The actual duration of the experiments was 73 days for (1), 67 days for (2), and 58 days for (3).

2.3. Analysis
Electrochemical measurements were carried out by using an Eco Chemie (The Netherlands) voltammetric instrument µ-Autolab II, connected to a Metrohm three electrode cell (VA 663, Metrohm stand, Switzerland). The working electrode was static mercury drop electrode (SMDE).

3. Results
In the seawater of the Gulf of Elefsis copper is to a high extent bound to the organic ligands. The values determined for the apparent complexing capacity for Cu are in the range of 0.04 to 0.43 μM. The concentrations of surface active substances (SAS) in the same waters fluctuate between 0.10 and 0.17 mg/L eq. T-X-100. Samples derived from simulation experiments in tanks, containing the macroalga *U. rigida* show much higher surfactant activities (up to 0.70 mg/L eq. Triton-X-100) and significantly higher CuL values (up to 13 μM) compared to samples collected from the field. The presence of sulfur species (S₂ +S⁰) was detected in the non aerated tanks which contained *Ulva* and these contributed to the complexing capacities through the formation of Cu- sulfides. The aforementioned results show that *Ulva rigida* releases significant quantities of dissolved and colloidal ligands with high complexing affinity for copper influencing its behavior and bioavailability. At the same time part of the organic matter deriving from *Ulva rigida* has a high surfactant activity. Consequently, although the organic ligands originating from *Ulva* in the particular area are not the only or necessarily the main contributors to the determined organic ligands, it is obvious that they represent a very significant component of measured DOC.

4. Conclusions
Surfactant activity of the released organic matter is responsible for the fact that a significant part of the interaction of copper with organic ligands is taking place at different phase boundaries. This influences directly the bioavailability, bioaccumulation, toxicity and transport of copper at these boundaries. The basic trends exhibited in this paper could be extrapolated to other nutrient impacted coastal regions hosting different algal species.
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6. References


