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# COPPER COMPLEXATION AND SURFACTANT ACTIVITY OF ORGANIC MATTER IN COASTAL SEAWATER AND SURFACE MICROLAYER SAMPLES FROM NORTH NORWEGIAN FJORDS AND NW MEDITERRANEAN REGION

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## SUMMARY

Organic ligands capable of complexing copper ions (CCu) were measured in selected sea surface microlayer (ML) and underlying water (UW) samples from the NW Mediterranean coast and north Norwegian fjords. A part of these organic ligands exhibit the property of surface activity, i.e. they accumulate on different phase boundaries (air/water; water/sediments; water/biological membranes), and are determined as surface-active substances (SAS). The presence of Cu complexes with surface-active properties influences the biogeochemical cycle of copper ions at these phase boundaries.

The CCu values for all samples determined by differential pulse anodic stripping voltammetry (DPASV) are in the range 90-1790 nM (being, in general, higher for microlayer samples). The SAS values for the samples range between 0.062-0.216 mg/L eq.T-X-100, while dissolved organic carbon (DOC) values are in the range from 0.9 mg/L C to 3.7 mg/L C. CCu data for North Norwegian fjords (ML and UW samples) are well-correlated with DOC data. CCu data are correlated with SAS data for North Norwegian fjords (ML and UW), but in NW Mediterranean region only in UW samples.

In the marine environments investigated, 1-20 % of DOC is present as ligands capable to complex copper ions. Correlation of SAS vs. DOC data for all the samples has shown that the majority of field data are in properties similar to model fulvic acid and microbial polysaccharide xanthan.

**KEYWORDS:**
dissolved organic ligands, surface-active substances, copper, sea surface microlayer, complexing capacity, voltammetry.

## INTRODUCTION

Organic ligands present in natural waters consist of largely uncharacterized macromolecular organic material, resulting from the combination of biological (phytoplankton and bacterial activity), and geochemical activities (humic and fulvic material) and pollution. The other smaller portion of organic matter is identified and classified into particular groups of compounds, such as carbohydrates, amino acids, proteins, fatty acids, lipids and hydrocarbons. Valuable information concerning the nature and properties of organic substances in natural systems can be obtained by studying the interaction of metal ions with organic matter [1, 2]. The interest is frequently focused on the formation of organic "inert" complexes with metal ions [3], which could influence the availability of metals to aquatic organisms and their association with particles and sediments in the system. The abundance of complexing ligands in natural waters determines the complexing capacity for a metal ion [4, 5]. Very often the metal ion of choice for complexing capacity determination is copper, as it forms strong complexes with organic matter in natural waters (>98 % of the total dissolved copper is complexed [6], and it is an essential element being toxic in elevated concentration range.

The concentration of the dissolved organic matter in natural waters is measured as a general parameter i.e. dissolved organic carbon (DOC), expressed in mg/L (or µM) of carbon. This parameter alone does not provide information on the type of organic matter present. A part of the organic matter exhibits the property of surface activity, thus accumulating on different phase boundaries (air/water; water/sediments; water/biological membranes). Surface active substances (SAS) are organic substances of specific structure, possessing structural groups that are repulsed from water (hydrophobic groups) and structural groups that have strong affinity for water (hydrophilic groups). Surfactant activity of some hydrophilic substances originates from their high molecular weight, as is the case with polysaccharides [7, 8]. Sea surface micro-layer (ML) is a unique bio-physico-chemical environment. Sea surface is covered by natural films that influence air-sea fluxes of particles and heat with particularly strong importance on gas fluxes. The adsorbed surfactant films on the air-sea interface reduce air-sea gas exchange by impeding molecular diffusion across the interface and by influencing the hydrodynamic characteristics of water motion at the interface [9]. The natural sea surface films are a complex mixture of molecules covering a wide range of solubility, surface activity and molecular masses with an apparent structural organization exhibiting a spatial and temporal variability [10, 11]. Many dissolved and particulate organic substances, such as lipids, carbohydrates and proteins, as well as trace elements, bacteria, phyto- and zoo-plankton are concentrated at the phase boundary [9]. Phytoplankton exudates and their degradation products are the major sources of marine surfactants [8, 12]. Chemical composition of ML is dependant on the OM fractionation that originates from in-situ production, from the subphase (i.e. bulk of seawater) and from the atmospheric deposition [13]. The concentration of Cu bound to SAS is correlated well with estimated rates of primary production. Concentration of SAS ligands bound to Cu was increased by a factor of fifty during the spring bloom relative to winter levels [14]. The presence of significant amount of surface-active copper organic complexes represent a reservoir of copper species in sea surface microlayer where they accumulate, and a significant portion of the copper will be cycled through the microlayer before removal to deeper water [15].

We have measured the complexation of copper ions in the selected microlayer and underlying seawater samples from the NW Mediterranean coast and north Norwegian fjords. The aim of the work was to compare the data on organic matter characteristics in these two contrasting environments, which have different seawater temperatures and sun-light regimes as well as anthropogenic influences that are important for the organic matter composition and behavior at seawater/atmosphere phase boundary. The concentration of the complexing ligands and their abundance regarding their part in the dissolved organic carbon (DOC) fraction, which exhibits surface active properties (SAS), is discussed. On that way, the contribution of the different pools of ligands capable to complex Cu ions could be determined and discussed regarding the fate of both ligands and copper ions in these selected environments of the coastal seas.

## MATERIALS AND METHODS

### Study sites

In subartic Norwegian fjords, the samples were collected in July 2001, August 2002 and July 2003 (Tromsø region (Fig. 1), i.e. Ullsfjord and Balsfjord [16]. Sampling was performed, with a Garrett-type screen [17]. The screen of stainless steel was made of 0.25 mm diameter wire with 1.0 square mm apertures in the mesh. The screen, rinsed with distilled chloroform and deionized water, was immersed vertically and removed horizontally to the water surface. The thickness of the sampled microlayer was approximately 100–150 µm (which was determined experimentally). Underlying water was collected by immersing 1-L glass bottles at a depth of ~ 0.1 m. Samples were taken either from the coast or from the inflatable boat.

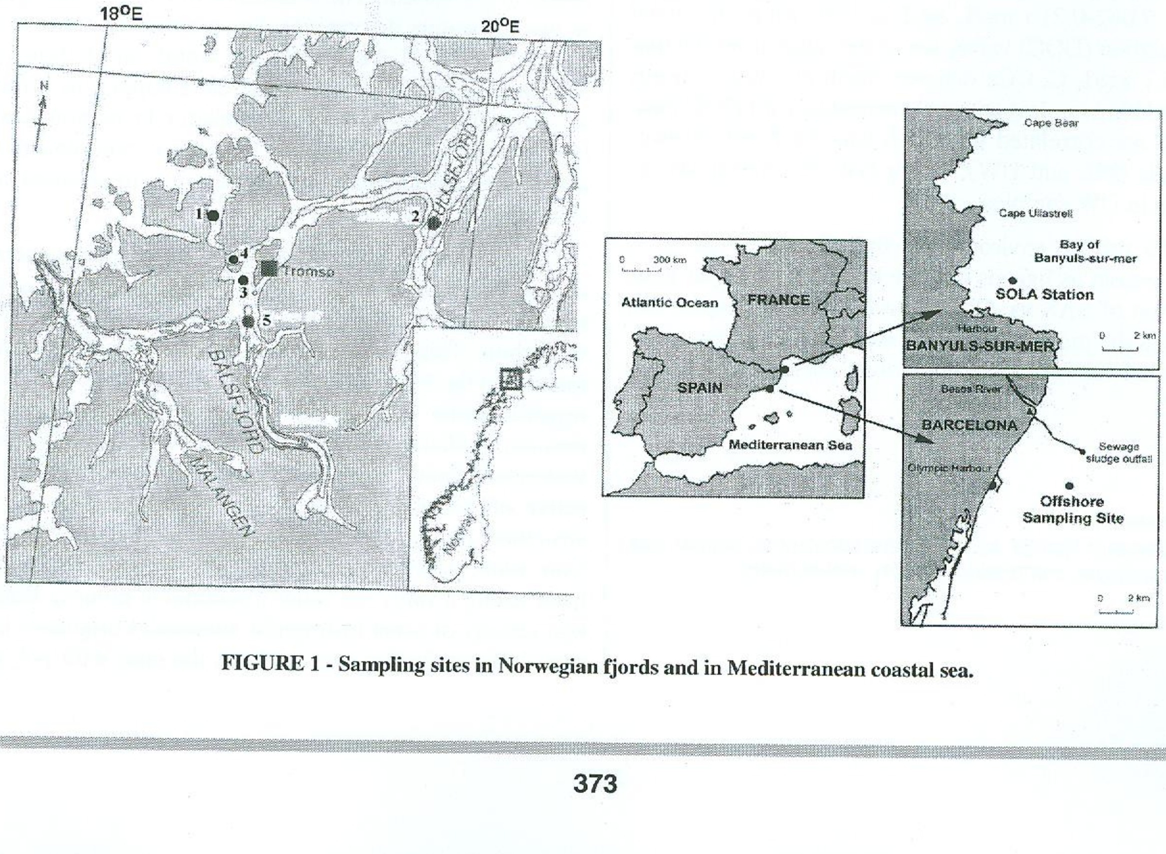

**FIGURE 1 - Sampling sites in Norwegian fjords and in Mediterranean coastal sea.**

Sea surface microlayer and underlying water samples from France and Spain were collected at two coastal stations in the NW Mediterranean sea: the Bay of Banyuls-sur-Mer (France) and off the Olympic harbour in Barcelona (Spain) [20] (Fig.1), in June and July 2002. The Bay of Banyuls-sur-Mer is an oligotrophic area [18-20]. The Barcelona site is moderately eutrophic and heavily impacted by the urban sewage sludge outfall [20, 21]. Microlayer samples were collected with metal screen (thickness of sampled microlayer is 200-400 μm; [20]).

Microlayer and underlying seawater samples were immediately deep-frozen (at -20 °C) after sampling and kept like this until analysis. Before analyzing the samples, they were allowed to stand at room temperature overnight (18-20 °C) for complete thawing.

### DOC analysis

The DOC concentrations were determined by using a sensitive high-temperature catalytic oxidation (HTCO) technique, 1988 proposed by Sugimura and Suzuki [22]. A Model TOC-500 System (Shimadzu) with high sensitive Pt catalyst and non-dispersive infrared (NDIR) detector for $CO_2$ measurements was used. The DOC concentrations were determined in duplicate by filtering parallel samples (2 x 500 ml) through Whatman GF/F filters precombusted at 450 °C for 5 h. The blanks for DOC were less than 3 %, respectively, of the lowest detected concentration. For DOC measurement, the samples were filtered (Whatman GF/F, pore size 0.7 μm), spiked with $HgCl_2$ solution for preservation, and stored in cold dark place until analysis.

### Determination of SAS

### o-nitrophenol as an electrochemical probe

Surface active substances were determined by phase-sensitive alternating current (a.c.) voltammetry by in-phase measurements using o-nitrophenol as an electrochemical probe. The method is based on the fact that electrochemical characteristics of o-nitrophenol (peak potential, height and shape, and pre-peak height) change in significantly different ways depending on the properties of the adsorbed organic substances (hydrophobicity, acidity) [23, 24]. ONP has a voltammetric reduction peak at –0.555 V under the pH conditions of seawater. In the presence of organic matter adsorbed onto the mercury electrode, the ONP peak potential is shifted toward more negative potentials, depending on the concentration and the hydrophobicity of adsorbed organic substances. The surfactant activity (SA) of the complex mixture of organic matter in a natural seawater sample is expressed as the equivalent concentration of the non-ionic surfactant Triton-X-100 (T-X-100), which gives the same effect to the o-nitrophenol peak potential shift ($\Delta E$) as the natural organic substances in the sample. T-X-100 is a high purity, water-soluble, liquid, non-ionic surfactant that has come to be recognized as the performance standard among similar products. It is an octylphenolethoxylate consisting of 9-10 mol of ethylene oxide, and supplied as a 100% active product [25, 26]. The calibration curve for the dependence of the o-nitrophenol peak potential shift on the T-X-100 concentration was presented in a previous paper (Gašparović and Ćosović [12], 2001). The lower limit of detection expressed as T-X-100 concentration is 0.02 mg $L^{-1}$.

### Cu complexing capacity determination

The complexing capacity was determined in non-filtered (untreated) samples comprising both particulate and dissolved ligands. Determination was performed by the direct titration method of the sample with increasing amounts of copper ions and their electrochemical determination by the method of DPASV [5]. The increasing amount of copper ions was added to 25 ml of ML or UW samples at natural pH, and then separately to acidified solutions.

The peak currents, resulting from the oxidation of voltammetric-labile copper, are plotted against the added copper concentration yielding a titration graph. The labile copper fractions detected by this technique are hydrated $Cu^{2+}$ or Cu ions bound to inorganic or organic complexes. Their dissociation kinetics are so rapid for the applied method (depending on the electrode diffusion layer thickness) that they are detected as electroactive. Strong metal-ligand complexes do not dissociate and are not reduced at the chosen fixed potential, and could be regarded as electro-inactive. This provides for labile/inert discrimination data. To obtain complexing capacity values and conditional stability constant, titration data are linearly transformed assuming 1:1 metal-to-ligand complexes [27]. The equation used for calculation is: $[Cu]/[CuL]=[Cu]/L_T+1/KL_T$, where Cu is the copper ion detected by anodic stripping voltammetry, CuL is the copper ion bound in a complex, $L_T$ is the concentration of binding ligands (i.e. complexing capacity), and K is the conditional stability constant. [Cu] and [CuL] are calculated from the titration data, where $[Cu] = I_p/S$ and $[CuL] = [Cu_T]-[Cu]$. In that equations, $I_p$ is the height of DPASV detected copper peak, S is the sensitivity of the DPASV method to labile copper, which corresponds to the slope of the linear portion of the titration graph after all complexing sites are saturated with copper ions, $[Cu_T]$ is the concentration of copper ion added + copper ion originally present in the sample. The plot of [Cu]/[CuL] versus[Cu] yields a straight line with a slope of $1/L_T$ and intercept $1/KL_T$. The detailed procedure (Plavšić [28], 2003; Scoullos et al. [29], 2004, Plavšić et al. [30], 2006) and calculations have been described elsewhere (Ružić [27], 1982; van den Berg [4], 1982; van den Berg and Donat [31], 1992). The same method of calculation was applied in other papers, e.g. Bazzi et al. [6], 2002 and Andrade et al. [32], 2006.

## RESULTS AND DISCUSSION

The measurements of the complexing capacities (i.e. the amount of ligands capable of binding copper ions), total dissolved organic carbon (DOC) and surface-active organic substances could give to us the information about which amount of the total OM is surface-active in its nature, and which part of these ligands is responsible for binding copper ions.

Additional information on the organic ligand characteristics in the samples may be obtained by examining the complexing capacity data normalized in terms of the DOC concentrations (CCu/DOC) measured in the same seawater samples [29, 33]. The value CCu/SAS, i.e. the complexing capacity value data normalized to surfactant activity of the same seawater sample, gives the information on complexing capacity of the part of the organic matter exhibiting at the same time surface-active properties [29]. When normalizations of CCu data to DOC and SAS data are done in the case of DOC, the unit is µmol/mg C, while in the case of SAS, the unit is µmol/mg eq. of model compound T-X-100. Triton –X-100 is regarded, due to its well-characterized properties, as standard surfactant compound when expressing surfactant activity [25, 26].

In North Norwegian fjords underlying seawater and sea surface microlayer samples both CCu and DOC vary considerably, and there is a correlation ($r^2 = 0.5172$) between these two parameters (Fig. 2). For Mediterranean samples (both underlying and microlayer ones), the correlation of CCu vs. DOC is difficult to establish, as for our limited set of data, the correlation line is almost parallel to x- axis.

Regarding the correlation between CCu and SAS, there exists a correlation for underlying seawater samples (NW Mediterranean samples) (Fig.3, line 1; $r^2 = 0.5787$). The same is true for North Norwegian fjords, for the underlying seawater and the sea surface microlayer samples with more significant correlation coefficient (Fig. 3, line 2; $r^2 = 0.6237$). The correlation coefficients for the data presented in Figs. 2 and 3 are not very high, but significant enough to draw certain conclusions. Microlayer samples from North Norwegian fjords follow the same trend in CCu/SAS dependence (line 2, in Fig. 3) like underlying seawater samples indicating that the underlying water in the North Norwegian fjords is probably the main source of organic ligands for corresponding microlayer samples. One of the microlayer samples from Norway is out of range with the other microlayer samples (Fig. 2) with its high value of CCu (1.79 µM). There is no correlation between SAS and CCu for sea-surface microlayer samples from western Mediterranean area indicating probably some input of SAS and/or complexing ligands from different sources e.g. atmosphere. Atmospheric input could be the dominant source of organic matter in the Mediterranean [13], especially in the area of more industrialized NW Mediterranean, from where our samples originated.

In Table 1, the CCu , SAS , DOC, CCu/DOC and CCu/SAS data are presented for underlying seawater and sea surface microlayer samples from the North Norwegian fjords (samples 1-9) and NW Mediterranean (samples 10-16). The mean normalized CCu/DOC value (Table 1) for all underlying seawater samples, regardless of their origin (North Norwegian fjords or Mediterranean), is 0.14, while for microlayer samples the mean value is 0.21, indicating that there are generally more available sites for binding copper ions regarding the total dissolved organic carbon content in sea-surface microlayer, for the measured set of data. Mean value for CCu/SAS for all underlying seawater samples is 2.3 (Table 1), while for all microlayer samples the mean value is 3.6, indicating the presence of more SAS in sea-surface microlayer samples, which at the same time exhibit complexing properties towards copper ions. Samples from Mediterranean area showed higher complexing capacity values and lower SAS values, while the contrary is true for North Norwegian samples (Fig. 3).

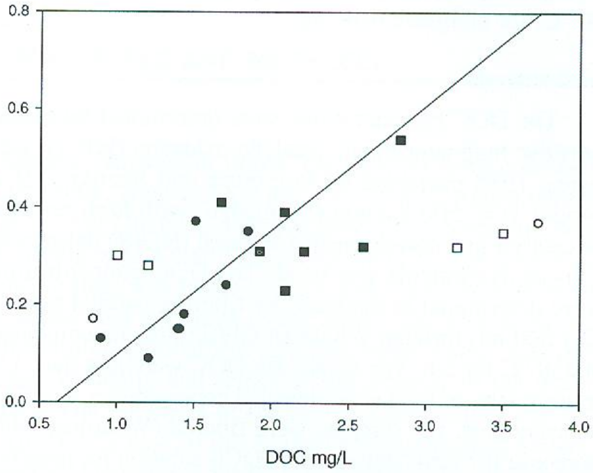

**FIGURE 2 - CCu data *vs.* DOC data for the seawater samples (● - Norwegian samples, ○ - Mediterranean samples) and microlayer samples (■ - Norwegian samples; □ – Mediterranean samples). 1. linear regression line (correlation coefficient $r^2 = 0.5172$) for the underlying seawater and microlayer samples (North Norwegian fjords).**

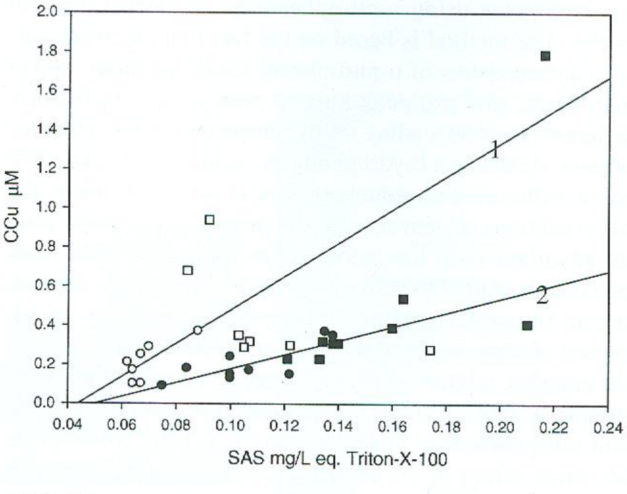

**FIGURE 3 - CCu data *vs.* SAS data for the seawater samples (● - Norwegian samples; ○ - Mediterranean samples) and microlayer samples (■ - Norwegian samples; □ – Mediterranean samples). 1. linear regression line (correlation coefficient $r^2 = 0.5787$) for underlying seawater (Mediterranean samples). 2. linear regression line (corr.coef. $r^2 = 0.6237$) for North Norwegian fjords (underlying seawater and microlayer samples).**

**TABLE 1**

**Copper-complexing capacity (CCu), surface active substances (SAS), dissolved organic carbon (DOC), CCu/DOC and CCu/SAS values for underlying seawater and sea surface microlayer samples for North Norwegian fjords(samples 1-9) and NW Mediterranean (samples 10 -16).**

| | CCu seawater µmol/L | SAS seawater mg/L (eq.T-X-100 | DOC seawater mg/L C | CCu/DOC seawater µmol/mg | CCu/SAS seawater µmol/mg (eq.T-X-100) | CCu microlayer µmol/L | SAS microlayer mg/L (eq.T-X-100) | DOC microlayer mg/L C | CCu/DOC microlayer µmol/mg | CCu/SAS microlayer µmol/mg (eq.T-X-100) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 0.37 | 0.1350 | 1.51 | 0.24 | 2.74 | 0.54 | 0.1640 | 2.83 | 0.19 | 3.29 |
| 2. | 0.35 | 0.1380 | 1.85 | 0.19 | 2.53 | 1.79 | 0.2160 | 2.77 | 0.64 | 8.10 |
| 3. | 0.09 | 0.0750 | 1.21 | 0.07 | 1.20 | 0.39 | 0.1600 | 2.08 | 0.19 | 2.40 |
| 4. | 0.17 | 0.1070 | - | - | 1.59 | 0.23 | 0.1330 | - | - | 1.73 |
| 5. | 0.15 | 0.1000 | 1.41 | 0.11 | 1.50 | 0.31 | 0.1380 | 1.92 | 0.16 | 2.25 |
| 6. | 0.18 | 0.0840 | 1.44 | 0.13 | 2.14 | 0.31 | 0.1400 | 2.21 | 0.14 | 2.21 |
| 7. | 0.24 | 0.1000 | 1.71 | 0.14 | 2.40 | 0.23 | 0.1210 | 2.09 | 0.11 | 1.90 |
| 8. | 0.15 | 0.1220 | 1.40 | 0.11 | 1.23 | 0.32 | 0.1340 | 2.59 | 0.12 | 2.46 |
| 9. | 0.13 | 0.1000 | 0.90 | 0.14 | 1.30 | 0.41 | 0.2100 | 1.67 | 0.25 | 1.95 |
| 10. | 0.29 | 0.0700 | - | - | 4.14 | 0.30 | 0.1220 | 1.0 | 0.29 | 2.45 |
| 11. | 0.17 | 0.0640 | 0.85 | 0.20 | 2.65 | 0.94 | 0.092 | - | - | 10.21 |
| 12. | 0.37 | 0.0880 | 3.73 | 0.10 | 4.20 | 0.35 | 0.1030 | 3.5 | 0.1 | 3.39 |
| 13. | 0.21 | 0.0620 | - | - | 3.13 | 0.28 | 0.1740 | 1.2 | 0.23 | 1.61 |
| 14. | 0.10 | 0.0670 | - | - | 1.49 | 0.32 | 0.1070 | 3.2 | 0.1 | 2.99 |
| 15. | 0.25 | 0.0670 | - | - | 3.73 | 0.68 | 0.0840 | - | - | 8.09 |
| 16. | 0.10 | 0.0640 | - | - | 1.56 | 0.29 | 0.1050 | - | - | 2.76 |

In the sea surface microlayer, solar radiation influences the geochemical cycling of both organic moieties and trace metals. Redox-active trace metals, like Cu, are especially susceptible to photodegradative processes [34]. The photodegradative processes could be especially important in coastal regions, e.g. estuaries, due to the presence of numerous photo-reactive functional groups on humic material (phenolic and carboxylic groups) [35]. In spite of the increased photodegradation of organic ligands in sea-surface microlayers, our results showed ligands enrichment as observed through CCu/DOC and CCu/SAS data.

Copper ions tend to react more with oxygen atoms in carboxylic and/or phenolic types of groups [36]. In general, humic substances constitute the major part of the organic ligands present in natural waters [1, 30], covering wide range of molecular mass distribution, and they are very often the most important copper-binding ligands, although recently more attention is paid to the organic ligands with lower molecular mass, as it was determined that this ligands are responsible for the «strong» complexation of copper ions in natural waters i.e. with $K_{CuL} > 10^{11}$ [37]. According to the new view, humic substances are collections of diverse relatively low-molecular mass components forming dynamic associations stabilized by hydrophobic interactions and hydrogen bonds [38]. Vachet and Callaway [39], who examined two types of Cu-complexing ligands in seawater, i.e. weaker and stronger ones have stated that the MW of Cu ligands is below 1600 Da for weaker and 270 Da for stronger ones. If we assume DOC values of ~2 mg/L (165 µM), complexing capacity values between 0.1 and 2 µM and molecular mass of ligands ~200 Da, we come to amounts of 1-20% DOC being present as ligands capable for binding copper ions (CCu ligands).

CCu of model fulvic acid [30] and model microbial polysaccharide xanthan (polysaccharide polymer of glucose, manose, potassium glucuronate, acetate and pyruvate with an approximate mol. wt. of 2 x $10^6$) were determined in 0.55 M NaCl solution. CCu of 10 mg/L of fulvic acid (with 39% of C, due to the elemental analysis) was 87 nM, while 10 mg/L of added xanthan in 0.55 M NaCl solution did not complex any copper ions. The sensitivity of the electrochemical determination for copper ions in the presence of xanthan was a little bit lower, due to different diffu-sion coefficient of copper ion in the presence of adsorbed xanthan. This is in accordance with other authors who determined that polysaccharides do not form inert complexes with metal ions [40]. This shows that different ligands present in natural samples do not contribute to the same extent to the complexing capacity for copper ions.

Valuable information on the organic matter characteristics may be gained by studies of the adsorption properties of the organic matter present, expressed as SAS normalized in terms of DOC concentration (SAS/DOC) [41]. Higher SAS/DOC ratio indicates prevailing contribution of hydrophobic substances to the OM pool, like lipids and nonionic surfactant Triton-X-100 used in this study, while lower SAS/DOC ratio indicates the presence and contribution of the OM of more hydrophilic type, e.g. fulvic acids, amino acids and polysaccharides. In Fig. 4, the DOC - SAS relationship for the measured samples are presented. The data for all the samples are situated between the lines for model substances of fulvic acid and microbial polysaccharide xanthan, which means that the samples reveal the presence of more hydrophilic type of organic matter usually of biological origin.

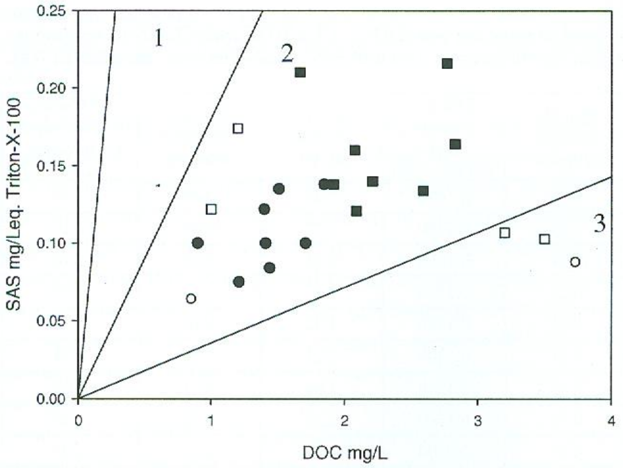

**FIGURE 4 - SAS values *vs.* DOC concentrations eq. to Triton-X-100 obtained for the seawater (● - Norwegian samples; ○ - Mediterranean samples) and microlayer samples (■ - Norwegian samples; □ – Mediterranean samples). Lines correspond to different model substances; no. 1 - nonionic surfactant Triton-X-100; no. 2 - fulvic acid; no. 3 - microbial polysaccharide xanthan.**

## CONCLUSIONS

The CCu for all samples determined (ML and UW) are in the range 90-1790 nM (being, in general, higher for ML samples).

The SAS values for the samples are in the range 0.062-0.216 mg/L eq.T-X-100, while DOC values are in the range from 0.9 C to 3.7 mg/L C. In the marine environments investigated, 1-20 % of DOC is present as ligands capable to complex copper ions.

The samples from Mediterranean area showed higher CCu and lower SAS values, while the contrary is valid for North Norwegian fjords.

The CCu data determined in all North Norwegian seawater samples (ML and UW) show a correlation with DOC data. CCu data vs. SAS data of North Norwegian fjords for UW and ML samples follow the same line, indicating that UW is the main source of ligands for ML in that area.

CCu data for NW Mediterranean seawater are correlated with SAS data only for UW samples. CCu data for the corresponding Mediterranean ML samples do not show correlations with SAS data, indicating some additional/different sources of ligands, probably atmospheric ones.

Correlation of SAS vs. DOC data for all the samples and comparison with selected model substances have shown that the majority of field data are in properties similar to model fulvic acid and microbial polysaccharide xanthan, revealing the presence of more hydrophilic type of organics in UW and ML, usually of biological origin. SAS are responsible for the fact that a significant part of the interaction of copper with organics is taking place at different phase boundaries. This influences directly the bioavailability, bioaccumulation, toxicity and transport of copper ions at these boundaries.

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