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Copper complexing ligands and organic matter characterization in the northern Adriatic Sea

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ABSTRACT

The study on dissolved organic ligands capable to complex copper ions (L_T), surface-active substances (SAS) and dissolved organic carbon (DOC) in the Northern Adriatic Sea station (ST 101) under the influence of Po River was conducted in period from 2006–2008. The acidity of surface-active organic material (Ac_r) was followed as well. The results are compared to temperature and salinity distributions. On that way, the contribution of the different pools of ligands capable to complex Cu ions could be determined as well as the influence of aging and transformation of the organic matter. The L_T values in the investigated period were in the range of 40–300 nmol l^{-1}. The range of DOC values for surface and bottom samples were 0.84–1.87 mg l^{-1} and 0.80–1.30 mg l^{-1}, respectively. Total SAS concentrations in the bottom layer were 0.045–0.098 mg l^{-1} in equiv. of Triton-X-100 while those in the surface layer were 0.050–0.143 mg l^{-1} in equiv. of Triton-X-100. The majority of organic ligands responsible for Cu binding in surface water originate from new phytoplankton production promoted by river borne nutrients. Older, transformed organic matter, possessing higher relative acidity, is the main contributor to the pool of organic ligands that bind copper in the bottom samples. It was estimated that ~9% of DOC in surface samples and ~12% of DOC in the bottom samples are present as ligands capable to complex copper ions.

1. Introduction

Organic ligands present in natural waters consist of largely uncharacterized organic material, resulting from the combination of biological and geochemical activities as well as pollution (Buffle, 1988). 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 (Lee and Wakeham, 1988; Hansell and Carlson, 2002). Formation of organic complexes with copper ions influences the availability of copper to aquatic organisms and its association with particles and sediments in the system. All these properties are directly related to the bioavailability and toxicity of copper to biota (Bruland et al., 1991; Buck and Bruland, 2005). The abundance of these inert complexes in natural waters determines the apparent complexing capacity for copper ions (L_T) (van den Berg, 1982; Plavšić et al., 1982). The term apparent signifies that the value determined is valid only under the selected experimental conditions.

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^{-1} (or μM) of carbon. This parameter alone does not provide information on the type of organic matter present. A part of the aquatic organic matter exhibits the property of surfactant activity and is named surface-active substances (SAS), thus accumulating on different phase boundaries (air/water; water/sediments; water/biological membranes). Surfactant activity originates from structural groups that are repulsed from water (hydrophobic groups) and structural groups that have strong affinity for water (hydrophilic groups). Hydrophilic substances may possess surface-active properties that originate from their high molecular weight, as it is the case for polysaccharides (Plavšić and Ćosović, 2000; Gašparović and Ćosović, 2003). Clear evidence of surfactant production by marine phytoplankton cultures based on laboratory and field experiments is also available (Žutić et al., 1981; Zhou et al., 1989; Plavšić et al., 1990; Gašparović et al., 1998). Due to the specific reactivity of 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) (Ćosović et al., 2000).

Regarding the SAS, range typical for the North Adriatic seawater samples expressed in equivalents of calibration substance, is from

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0.04 to 0.18 mg l^{-1} in equiv. of Triton-X-100. Eighty-six percent of North Adriatic seawater samples have SAS concentration $\leq$0.13 mg l^{-1} in equiv. of Triton-X-100, which was concluded based on the monthly measurements in year 2000, on eight sampling stations in the North Adriatic covering the area from western to eastern region of the transect Po River – Rovinj, by the method with *o*-nitrophenol as an electrochemical probe (Gašparović and Ćosović, 2003). The same method of SAS measurements was applied in this investigation. Complexation of copper ions was not systematically investigated in the area of North Adriatic so, the data on L_T could be regarded as first set of data for the area. Data for L_T and the corresponding apparent stability constant (K_{app}) for North Adriatic station are in agreement with the data obtained for the saline layer in the Krka river estuary in the middle Adriatic area (Plavšić et al., 2009), where the results for L_T are in the range from 60 to 120 nM, while log K_{app} values were in the range from 7.4 to 7.9.

Recently, the multivariate statistical methods applied in the paper by Tepić et al. (2009) showed importance of the surface layer for the dynamics of the organic matter in the northern Adriatic. The strong separation of the statistical data groups was observed showing important effect of different depths and processes occurring there. The bottom layer data make a more homogeneous group than the surface layer data, pointing on smaller concentration ranges there than in the surface layer. That is why this investigation was concentrated on the four characteristic parameters used for the organic matter characterization, namely DOC, SAS concentration, acidity (Ac_r) and L_T in surface and bottom layer, on a single station in northern Adriatic Sea, expecting to evaluate the differences regarding the new biological production in the surface layer and the aging of organic material as well as its transformation in the bottom layer on capability to complex Cu. The study was conducted on the surface (0.5 m depth) and at the bottom of the station (~31 m depth) in different seasons in the time period of two years (2006–2008).

2. Materials and methods

2.1. Northern Adriatic sampling station (station ST 101)

The northern Adriatic is a shallow basin, with a maximum depth of less than 50 m, which exhibits a strong eutrophic gradient along transect from the west to the east coast (Degobbis et al., 2005). The western part of the basin is occasionally eutrophic as a result of inputs from Italian rivers, particularly the Po River, which has a mean runoff of 1520 $m^3\,s^{-1}$ and a maximum runoff of 6000 $m^3\,s^{-1}$ (Hopkins et al., 1999). The eastern part of the northern Adriatic is generally oligotrophic, owing to the predominant cyclonic current system, which brings oligotrophic waters from the central Adriatic (Orlić et al., 1992). In addition to the regular climate-driven and seasonally dependent stratification, the water column is additionally influenced by large inputs of freshwaters, often leading to a significant transversal advection of freshwaters towards the Istrian peninsula. As a consequence of the large amounts of river born nutrients (load of total nitrogen is 15.5×10^{10} gyr^{-1} (Pettine et al., 1998) the northern Adriatic is among the biologically most productive areas (120–260 g C m^{-2} year, Zoppini et al., 1995) of the Mediterranean Sea. The phytoplankton community structure is dominated by diatoms, while dinoflagellates are abundant during June–July, the period of nutrients depletion (Aubry et al., 2004). Enhanced eutrophication is coupled with an intensive remineralization of newly produced organic matter in the basin. It was estimated that approximately 10–20% and 27–50% of the daily average primary production were mineralized in sediments in March and August, respectively, which indicated a tight benthic–pelagic coupling (Giordani et al., 2002).

Water samples for analysis were taken approximately monthly in the period from September 2006 to December 2008, in the western part of the northern Adriatic (station ST 101; 44°59′N, 12°19′E; Fig. 1). Samples were collected using research vessel "Vila Velebita", at the depths of 0.5 m and 2 m above bottom (~31 m) with 5-l Niskin bottles.

2.2. Measurements of complexing capacity for copper ions (L_T)

The experiments were performed on the electrochemical system consisting of a 663 VA-Stand (Metrohm, Herisau, Switzerland) connected via an IME663 module to a computer controlled voltammeter (EcoChemie, Utrecht, The Netherlands). The working electrode was a static mercury drop electrode (SMDE, drop surface area 0.54 mm^2). The reference electrode was a double-junction Ag/AgCl (3 M KCl) electrode, and the counter electrode was a glassy carbon rod. The solutions were stirred by a rotating teflon rod during deposition step of measurements. Potential of deposition was −0.6 V *vs.* Ag/AgCl reference electrode, while the deposition time was 60 s. The complexing capacity was determined in untreated seawater samples. Determination was performed by the direct titration method of the sample with increasing amounts of copper ions and their electrochemical response was measured by the method of differential pulse anodic stripping voltammetry (DPASV) (Plavšić et al., 1982; Plavšić, 2003). The peak currents, resulting from the oxidation of voltammetrically labile copper, previously reduced to form amalgam with Hg electrode, are plotted against the added copper concentration yielding a titration graph. The labile copper fraction detected by this technique is hydrated Cu^{2+} or Cu ions bound to inorganic or organic complexes whose dissociation kinetics are so rapid for the applied method (depending on the electrode diffusion layer thickness) that they are detected to be electro-active. Inert copper–ligand complexes do not dissociate and are not reduced at the chosen fixed potential. 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 (Ružić, 1982; van den Berg, 1982). 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 total concentration of binding ligands (i.e. complexing capacity) and *K* is the apparent stability constant. [Cu] and [CuL] are calculated from the titration data, where $[Cu] = I_p/S$ and $[CuL] = [Cu_T] - [Cu]$. In these 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 ions added + copper ions originally present in the sample. The plot of [Cu]/[CuL] *vs.* [Cu] yields a straight line with a slope of $1/L_T$ and intercept $1/KL_T$. The detailed procedure and calculations have been described elsewhere (Plavšić et al., 1982; Ružić, 1982; van den Berg, 1982). The same method of calculation is applied in other papers (Scoullos et al., 2004; Andrade et al., 2006).

2.3. Determination of surface-active substances

Total surface-active substances (SAS_T) were determined by non-destructive electrochemical phase-sensitive alternating current (*a.c.*) voltammetry by in-phase measurements using *o*-nitrophenol as an electrochemical probe. When using the ONP electrochemical probe, the SAS concentration (surfactant activity) in natural seawater samples is expressed as the equivalent concentration of