I - EXECUTIVE SUMMARY

This synthesis, based on inputs received from all the participants, was consolidated by Gerhard Herndl in the aftermath of the workshop.

TRANSFORMATION AND PERSISTENCE OF DOC

Introduction

The transformations and fate of dissolved organic molecules in marine systems have been the subject of numerous studies, yet clearly there are still important gaps in our understanding of this issue. Generally, more attention has been paid toward understanding biological processes, like microbial degradation and utilization, than abiotic processes, like photochemical degradation. Our view on the general reactivity of dissolved organic matter (DOM) in the ocean has changed dramatically over the past decade. It has been shown that the bulk of the oceanic DOM is below 1000 Da and this low molecular weight DOM is largely refractory to microbial degradation (Amon and Benner, 1996). Conversely, the DOM pool larger than 1000 Da consists of labile and semi-labile molecules preferentially utilized by prokaryotes, largely bacteria, although archaea have been shown to take up at least fractions of the DOM pool as well (Ouverney and Fuhrman, 2000; Herndl et al., 2005).

Biological uptake of DOM by heterotrophic microorganisms, ultimately converts organic molecules to carbon dioxide and water. When molecules are too large (typically greater than 600 Da) to pass across bacterial cell membranes, they have to be first hydrolyzed by bacterial exoenzymes. The hydrolysis products are then transported into cells and metabolized. Photochemical degradation may lead directly to bioavailable DOM or indirectly affect the ability of organic molecules or their “conversion products” to persist in the water column. Here we consider the biological and abiotic processes that may govern the fate and persistence of dissolved organic compounds in the Mediterranean Sea.

Turnover of DOM in the Mediterranean Sea is likely to differ from that in other marine environments, for several reasons:

1) An essential characteristic of the surface waters of the Mediterranean Sea is that phosphorus (P) is the major nutrient limiting primary production (Krom et al., 2004; Thingstad et al., 2005). The limitation of P has important implications for the biotic processes determining the fate of autochthonous dissolved organic carbon (DOC), as bacterial degradation may be P-limited as well. The unusual N/P ratio in the Mediterranean could be due to the process of nitrogen fixation and/or the contribution of atmospheric deposition of nutrients (Herut et al., 1999, 2002; Ridame and Guieu, 2002; Ridame et al., 2003; Özsoy, 2003). Atmospheric input (Saharan dust) of N and P could cause an imbalance of N/P and contribute to a higher N/P ratio. The lack of significant denitrification also contributes to a high N/P ratio and to the highly unusual P limitation of the primary productivity in the eastern Mediterranean (Krom et al., 2004). Moreover, atmospheric input can influence primary production (Bonnet and Guieu, 2004) and are important for the iron cycle in the Mediterranean Sea (Guieu et al., 2002) and globally (Jickells et al., 2005). The deposition of Saharan dust provides iron, which impacts succession and bloom formation and composition.
2) A distinct characteristic of the deep waters in the Mediterranean Sea is their relatively high temperature of 12°C, while deep waters in other oceanic basins generally have much lower temperatures, commonly between 2-4°C. The high temperatures in deep waters of the Mediterranean Sea likely increase the biological degradation of DOC by heterotrophic prokaryotes.

3) Atmospheric deposition, especially of Saharan dust, into the Mediterranean is high, and includes particles from natural and anthropogenic sources. It is an important source of various microelements and nutrients that might influence microbial community structure and activity. Because of a lack in our knowledge and understanding of these complex interactions and their impact on the marine biogeochemistry, biology and climate there is a need for more intense research in this area.

Seasonal accumulation of autochthonous DOC in the Mediterranean Sea
The accumulation of DOC in surface waters is a frequently observed phenomenon and it has been suggested that the lack of P inhibits the efficient biological degradation of DOC (Copin-Montegut and Avril, 1993; Thingstad et al., 1997). In the North-Western Mediterranean Sea (DYFAMED-Site), the major part of autochthonously produced DOC is transported to deep waters. Copin-Montegut and Avril (1993) estimated that the input of surface DOC to deep waters is roughly 2-fold higher than the input of POC and represents therefore the principal export term. Thus, deep waters receive a substantial input of DOC that presumably is highly bioreactive.

The fate of this DOC in deep waters has not been studied so far. The biological degradation of DOC originating from the surface within the deep Mediterranean basin is probably enhanced by the mixing of the P-depleted surface waters with nutrient-rich deep waters. Differences in the prokaryotic community could support or retard the transformation and mineralization of surface DOC in deep waters of the Mediterranean Sea. A recent study from the Eastern Mediterranean Sea indicates that distinctly different prokaryotic communities, as determined by 16S rRNA fingerprints, are present in the surface, the intermediate and the deep water of the Eastern Mediterranean Sea (Moeseneder et al., 2001). In the Sargasso Sea, DOC that accumulated during summer was shown to be degradable by a prokaryotic community originating from deep waters (Carlson et al., 2004). This raises the question whether deep water prokaryotes have different functional capabilities for the degradation of DOC than prokaryotes from near-surface waters.

The injection of surface DOC to deep waters could enhance the degradation of bio-resistant deep water DOC due to co-metabolism. This pathway, however, is poorly understood.

Alloclothonous sources of DOC to the Mediterranean Sea
The Mediterranean Sea receives DOC from the Atlantic and the Black Sea, and of riverine and atmospheric origin (Figure 1). The only export of Mediterranean waters occurs through the Strait of Gibraltar. The DOC concentration of deep waters leaving the Mediterranean basin is lower than that of incoming Atlantic waters (Dafner et al., 2001a), thus the net inflow of DOC from the neighboring oceans is larger than the outflow.

DOC also enters the Mediterranean Sea via river inflow but, as in other regions, some riverine DOC is removed by physical (see below), biological and photochemical processes before it reaches the Mediterranean Sea. Gross estimates indicate that 3.3-13.5 x 10^10 mol C a year of labile (susceptible to rapid bacterial degradation) DOC is exported from European rivers to the entire Mediterranean Sea (Sempéré et al., 2000). The labile fraction defined as the carbon equivalent of measured amino acids and neutral sugars is relatively higher during periods of low sediment load whereas biodegraded organic substances dominate at high discharge rates. Photochemical oxidation reactions may change the bioavailability of riverine dissolved organic compounds (Mopper et al., 1991). Recent studies showed that in summer, high solar radiation in concert with high NO3/NO2 concentrations in coastal northwestern Mediterranean Sea may induce substantial production of OH radicals which can in turn oxidize labile organic molecules (Tedetti et al., unpubl. data). Similarly, aggregation of dissolved organic compounds in the colloidal size range to particles may enhance sedimentation in estuarine environments and accumulate near the bottom, forming distinct nepheloid layers (Sempéré et al., 1994).
Atmospheric input is another important pathway for the transport of natural and anthropogenic material from continents to the ocean (Duce et al., 1991; Jickells, 1995). Organic carbon from the atmosphere (e.g., black carbon) enters the marine environment through the sea surface microlayer, where it resides for varying periods of time. Photochemical and biological processes have a profound impact on the concentration and characterization of this organic carbon at the air-sea interface. This deposition seems to be very important for oligotrophic areas and semi-enclosed seas such as the Mediterranean Sea (Bergametti et al., 1989, 1992; Guerzoni et al., 1999). However, although all these different sources import DOC into the Mediterranean Sea, the Mediterranean Sea represents a net sink of organic carbon, suggesting that loss processes must be high.

Removal of DOC in the Mediterranean Sea

As noted above, P limitation in surface waters may lead to low microbial degradation rates compared to other marine areas.

Organically enriched muds (sapropels) in the eastern Mediterranean basin could represent a sink of DOC. However, their importance for the fate of DOC is unknown thus far.

High intensity solar radiation will render photochemical processes an important removal pathway of DOC, especially for DOC of riverine origin. Even though riverine DOC is already substantially photochemically altered when it reaches marine systems (Miller and Zepp, 1995; Granelli et al., 1996; Blough and Del Vecchio, 2002; Obernosterer and Benner, 2004), the interplay between photochemical and biological processes will also have important implications on the further fate of riverine DOC once it enters the Mediterranean Sea. A number of studies have shown that the photochemical transformation of DOC results in increases in heterotrophic prokaryotic production and respiration (e.g., Miller and Moran, 1997; Obernosterer and Herndl, 2000; Smith and Benner, 2005). It has been concluded from these studies that the interaction of photochemical and biological processes can enhance the rates of DOC degradation.

In the Mediterranean Sea, the input from the atmosphere and rivers provides particulate matter, which acts as surface for sorption of DOC. Most dissolved organic compounds are particle-reactive in seawater. Adsorption of DOC onto non-living particles leads to an overall enhanced concentration of organic matter on the particle as compared to the ambient water. Such particles may be biogenic (opal, calcium carbonate, or detritus) or entirely abiotic (clays, iron oxyhydroxides). As such, non-living particles exhibit a broad size spectrum; they—and their adsorbed organic compounds—will be characterized as “particulate” (i.e., larger than the cut-off used to delineate particulate from dissolved matter) or “dissolved” (smaller than the cut-off). Thus, DOC can be “transferred” to the POC pool simply by sorption to larger particles, or by sorption to smaller particles which form larger particles via aggregation.

Depending on their characteristics, the particles may affect the adsorbed organic substances via oxidation or reductive processes. The particles may be important for the preservation of organic molecules or may play an important catalytic role in photochemical processes. It is well known that some dissolved organic (e.g., humics) and inorganic substances (e.g., nitrate, ferric ions), and suspended minerals (clays, zeolites, TiO₂, ZnO) may accelerate the photochemical degradation of different organic compounds in natural waters (Bajt et al., 1993; Richard and Boule, 1994).
Due to the high content of different metal oxides in natural sediments and suspended matter (Wgrzynek et al., 1997), these processes might be particularly important in shallow coastal Mediterranean waters.

The availability of an organic substance for microbial uptake may also be altered significantly once it is associated with particles. For example, the bioavailability of a molecule may be reduced if its sorption to a particle alters its tertiary structure or changes the nature or concentration of its dominant functional groups. Such changes may make the compound unrecognizable for exoenzymes that would have been able to hydrolyze the compound in its free (i.e., not adsorbed) form. If the sorption of organic molecules to particles reduces their bioavailability (a testable assumption for a range of inorganic and organic particles and a broad spectrum of organic molecules), then the conversion of its “labile” to “refractory” (or at least relatively persistent) status may reflect in part the time needed for an organic molecule to adsorb to particles. Formation of particles due to scavenging and aggregation of DOC by bubbles, however, increases the bacterial utilization of the DOC (Kepkay and Johnson, 1989). At present we can only speculate about most of these processes, but they are quantifiable in controlled experiments.

**Mucilage phenomena**

In some coastal areas, the accumulation of autochthonous DOC results in the formation of macroaggregates. This so-called mucilage formation is of particular importance in the Northern Adriatic Sea where it has been studied over the past 10 years. Even though not all responsible mechanisms have been identified thus far, there is evidence from *in situ* and laboratory studies that the high N/P ratio in surface waters of the northern Adriatic Sea accounts to some extent to the development of this phenomenon (Kaltenböck and Herndl, 1992; Obernosterer and Herndl, 1995; Smodlaka et al., 2004).

Eutrophication of the Northern Adriatic (Vollenweider et al., 1992; Vollenweider and Rinaldi, 1995 and references therein), due to the run-off from the Po and other rivers, causes hyper-production of phytoplankton during spring/summer at rates that far exceed the grazing potential of herbivores or rate of decomposition by bacteria. Consequently, large standing stocks of phytoplankton build up and extracellular polymers, mainly polysaccharides, accumulate in the water column, especially in the euphotic layer above the thermocline. The hallmark of the mucilage phenomenon is the rapid (1-100 h) appearance of enormous amounts of gelatinous organic matter that can be generally defined as macrogels (Figure 2). While a large number of

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**Fig. 2. Biophysical scenario of giant gel formation in the Northern Adriatic.**
hypotheses on the cause and mechanism of massive mucilage events have been presented (see Vollenweider et al., 1992; Vollenweider and Rinaldi, 1995; Funari et al., 1999 and references therein), the mechanism of the transformation of DOC to macroaggregates is still not well understood. The formation of such large mucus aggregates at such high rates (within a few hours) cannot be explained by biological process (Žutić et al., 2004). There is a high probability that mucilage formation is preceded by the accumulation of a precursor pool of organic matter until a critical concentration is reached (Žutić and Svetlicic, 2000). The abiotic formation of gel particles from dissolved precursors has been shown (Passow, 2000). It seems that the imbalance of the N/P ratio is causing an increasing phytoplankton extracellular production which is essential for the development of this phenomenon (Smolldaka et al., 2004). The macrogel formation involves the transformation of macromolecular DOM into colloidal organic matter (COM) and further into macrogel by aggregation process (Kovac et al., 2004). In addition, a biophysical scenario was proposed that features self-organization of extracellular polymers into presursor particles that transform into macrogels by first order phase transition (Žutić and Svetlicic, 2000; Žutić et al., 2004; Svetlicic et al., 2005c) (Figure 2).

The chemical characteristics of northern Adriatic macroaggregates, as indicated by spectroscopic studies, are similar to marine high molecular weight DOM (>1 kDa) and to phytoplankton exudates. The structure mainly reflects the primary composition of phytoplankton material including proteins, carbohydrates and lipids (Hedges et al., 2002; Nguyen et al., 2003). The general chemical structure of macroaggregates includes four major classes of organic structural elements: carbohydrates, ester and amide functional groups, aliphatic, and organo-silicon components (Kovac et al., 2002, 2004). The aromatic C moities are much lower then those observed in riverine (Hedges et al., 1992) and estuarine HMW-DOM (van Heemst et al., 2000), and is similar to oceanic HMW-DOM (Benner et al., 1992). Thus, macroaggregates probably originate from a high concentration of dissolved macromolecules of phytoplankton exudates (Kovac et al., 2004). The macroaggregate gel is stabilized by interactions with particles such as calcite, quartz, clay minerals, phytoplankton cells and their remains (Kovac et al., 2004, 2005).

The phenomenon referred to as «mucilage of the Northern Adriatic» has been observed infrequently over the past three centuries but its intensity and frequency of appearance have increased since the major event of 1989 (see Figure 1 in Žutić, this volume). Similar events were noted in recent years in other coastal areas of Mediterranean: along the Dalmatian coast (Stachowitsch et al., 1990), in Greece (Gotsis-Skretas, 1995), Sicily (Calvo et al., 1995), along the Tyrrhenian coast (Innamorati, 1995; Mecozzi et al., 2001), and the Black Sea (Moncheva, pers. comm.), but at such a massive scale the phenomenon has been unique to the Northern Adriatic.

**Persistence of DOC: Mediterranean vs. Atlantic**

It is possible to estimate the turnover rates of DOC in ocean basins if one assumes that the overall DOC concentration does not change over time (i.e., there is a steady state between production and loss). Then, one can calculate the turnover rates of DOC by combining the average age of the DOC (14C measurements) with the DOC production rate, calculated as the fraction of total annual primary production released as DOC (on an ocean basin scale). If the calculated loss rate of DOC for the Atlantic is not appreciably different from that of the Mediterranean, then one can assume that the unique features of the Mediterranean (warm temperature, short residence time of the water, high N/P ratio) do not affect the long-term fate of the DOC. If the calculated turnover rates do differ appreciably between the two water bodies, then the Mediterranean could become an important “natural laboratory” to explore reasons for these differences. The initial focus should be on the factors that are obviously different between the Mediterranean and the Atlantic. Assuming a steady state, a DOC concentration of 70 µM in the layer 0-200 m and a concentration of 54 µM below, the residence time of DOC in the Mediterranean Sea was estimated (by dividing the DOC reservoir by the overall input) to range between 103 and 149 years (Sempéré et al., 2000).
**RECOMMENDATIONS FOR FUTURE RESEARCH**

1) A number of previous studies indicate that primarily P limits the biological degradation of surface water DOC in the Mediterranean Sea. However, P-limitation occurs even at relatively high concentrations of dissolved organic phosphorus (DOP) (Rassoulzadegan and Thingstad, this volume). This raises the question of the bioreactivity of the DOP pool in the surface waters of the Mediterranean Sea.

2) As a consequence of the accumulation of DOC during summer in the surface waters of the Mediterranean Sea, the DOC is exposed to relatively high intensities of solar radiation over a prolonged period of time. It has been shown recently that exposure of ‘young’, phytoplankton-derived DOC to solar radiation results in a decrease in its biological reactivity (Benner and Biddanda, 1998; Obernosterer et al., 1999). This decrease has been attributed to phototransformation and photomineralization of the bioreactive DOC component (Obernosterer and Benner, 2004). To better understand the fate of surface DOC transported to deeper waters during winter convective overturn, the impact of photochemical processes needs to be addressed more intensively.

3) A broader interest in the mechanistic basis of the mucilage phenomenon is to prognosticate whether such uncoupling between production and degradation in the carbon cycle may be expected in the future elsewhere in the ocean as well, e.g., due to eutrophication coupled with specific trophodynamic and environmental conditions. Further research on the mechanism of macrogel formation and its fate is important for the more general understanding of abiotic processes in the transformation of DOM and their role in the Mediterranean Sea. Additionally, the macroaggregates in the Northern Adriatic offer a rare opportunity to study the assembling of macromolecular DOM into macrogels.

4) Few studies exist on the prokaryotic growth efficiency in the Mediterranean Sea. How P-limitation impacts prokaryotic growth efficiency is important for the understanding of the cycling of DOC in the Mediterranean Sea.

5) Several studies on the atmospheric input of biogenic and anthropogenic compounds into the Mediterranean Sea have already been conducted in its western part (Bergametti et al., 1989, 1992; Remoudaki et al., 1991; Molinaroli et al., 1999; Rodríguez et al., 2002; Migon et al., 2002; Ridame and Guieu, 2002) and also in the Eastern Mediterranean region (Levin and Ganor, 1996; Ganor and Foner, 1996; Mihalopoulos et al., 1997; Herut et al., 1999, 2002; Markaki et al., 2003; Krom et al., 2004). However, additional research is needed to elucidate the relation between the atmospheric inputs and the biological and biogeochemical consequences for the Mediterranean Sea.

6) Atmospheric input could potentially increase P limitation, because N rather than P is delivered through the atmosphere. However, this hypothesis is controversial.

7) Determination of the age of Mediterranean DOC through $^{14}$C analytical techniques and the link between the overall hydrographic regime of the main water masses and the age of the DOM and its transformation.