Chapter 4

DISSOLVED ORGANIC MATTER

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4.1 Introduction

Organic matter, which is present in every drop of natural water, in every particle of suspended material, can be conveniently classified into two categories; dissolved and particulate. The latter embrace material having a diameter greater than 0.45μm whereas, the former includes true dissolved matter together with colloidal materials that passed through 0.45μm membrane filter. The quantity of dissolved organic matter (DOM) greatly exceeds that of suspended or particulate organic matter (Romankevich, 1984). Dissolved organic matter, the supply and loss of which are generally balanced, is converted into a suspended state when utilized by organisms as structural material.

Dissolved organic carbon (DOC) in marine and freshwater ecosystems is one of Earth's largest actively cycled reservoirs of organic matter (Bushaw et al., 1996). The ecological significance of DOC in aquatic ecosystems include the following. DOC affects acid-base chemistry and controls the pH of many wetland waters (McKnight et al.1985). Because natural DOM is acidic and is a powerful agent for complexation of metals, it plays an important role in mineral weathering, metal toxicity and metal export (Mierle and Ingram, 1991), influencing the cycling of metals such as copper, mercury, and aluminum which, in turn can affect the concentration of trace metals found in aquatic organisms. It is a major mode of export for N and P (Qualls et al., 2002; Hedin et al., 1995) in many ecosystems and influences the availability of some forms of phosphorus and nitrogen (Bushaw et al. 1996). It is a potential source of carbon for microbial growth (Travnik, 1992) and a source of energy and nutrients to the microbial food chain. By attenuating UV radiation, it protects aquatic organisms from the harmful effects of this radiation. It also restricts the depth of the euphotic zone, stabilizes the depth of the thermocline, and depresses primary productivity in lakes (Quinby, 2000). The oxidation of organic matter affects the redox potential which, in turn, can have dramatic effects on biological and chemical processes (Breck, 1974). Under special conditions of limited circulation, complete utilization of oxygen can result in permanent or temporary reducing environments such as the Cariaco Trench, Santa Barbara basin and Black Sea. Thus, dissolved organic substances play a vital role in biological (productivity), chemical (metal complexation, flocculation and
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Dissolved Organic Matter (DOM) and geological (sedimentation and early diagenesis) processes (Hayase and Shinozuka, 1995).

DOM modifies the air-sea and sediment-water interfaces. It is responsible for the foaming of seawater (Garrett, 1972) and the damping of capillary waves causing sea slicks (Barger et al; 1974). Ion exchange (Rashid, 1969), calcite precipitation (Chave and Suess, 1970) and the surface charge on particles (Neihof and Loeb, 1974) may be influenced by the organic matter. In spite of this supreme importance of organic carbon, our understanding of the distribution and cycling of organic matter in the ocean is still in a very early stage.

Enrichment of DOC in the water column occurs through degradation/transformation of particulate organic carbon (POC), either in the water column or in bottom sediments by leaching processes, desorption of POC due to modification of environmental conditions (salinity, pH, etc.) and diffusion from interstitial water. DOC elimination processes in estuarine environment include flocculation, adsorption and degradation (Mannino and Harvey 1999). The production of soluble organic nutrients is, of course, biological but dissolution and sorption are mainly by geochemical mechanisms. Various mechanisms of retention of soluble organic nutrients can be classified as geochemical, hydrological, and biological. The most important geochemical mechanisms leading to the retention of dissolved organic nutrients is the equilibrium adsorption to Fe and Al oxyhydroxides, clays and/or whole mineral soil samples (Qualls el al., 2002). Hydrogen bonding is important in controlling soluble organic - solid organic interactions sorption (Qualls et al., 2002). Van der Waals forces are also often implicated in organic - organic sorption behavior (Leenheer, 1991). Adsorption/desorption had the effect of buffering concentrations of DOM in both mineral and organic horizons. Microbial dissolution is undoubtedly important in decomposition of cellulose, hemicellulose, proteins, and lignin in the sense that these macromolecules must be broken down into monomers which can enter the cell.

Sunlight penetrating the water surface can promote transformations of dissolved organic matter (DOM) in the photic zone. Photochemical transformations of DOM may have an important impact. Photochemical oxidation of biologically refractory DOM may form biologically labile products, thereby providing a potentially important removal mechanism for this pool of DOM. DOM is known to
be an important light absorbing component of natural waters, and hence has an important role in aquatic photochemical processes. Photochemical transformations of DOM occur upon direct absorption of UV and visible light by organic chromopores in aquatic environments (Frimmel, 1994).

Much of the emphasis on the cycling and leaching of nutrients in mangroves has been focused on inorganic nutrients. Relatively little is known about DOC cycling in coastal wetlands, although wetlands have high DOC concentrations and could be an important source to estuaries. Although it is understood that the high concentrations of DOM originate from plants, yet to our knowledge, there has been not enough seasonal study focusing on composition of DOC constituents in a wetland or tidal stream. Organic matter in mangrove environments is composed of labile and refractory compounds whose relative importance might have profound implications for organic matter diagenesis and turnover (Rowe and Deming, 1985; Fabiano et al., 1995). Conversely, the refractory fraction of OM is largely composed of complex macromolecules (like humic and fulvic acids and complex polymers), which are degraded slowly, subjected to burial, and thus lost in the short-term for the benthic food webs (Fabiano and Danovaro, 1994). This residual fraction of the organic carbon is that part of which is not accounted for by lipids, proteins and carbohydrates and consists of complex molecules like tannin and lignin, humic substances etc. A significantly improved understanding of the biogeochemical roles of carbon is of critical importance to major societal issues such as regional and global climate, the sustainability of major ecosystems, and environmental quality.

4.2 Labile Organic Constituents

A small and perhaps variable component of the dissolved organic material consists of the compounds typically associated with the general biochemical machinery of living organisms. Included in this group are amino acids, proteins, carbohydrates, lipids etc. Carbohydrates and amino acids are biochemical components comprising substantial portions of living biomass (80% of algal carbon and 65% of terrestrial plant carbon) and are important components of DOM in freshwater, in estuaries, and in the ocean. Many of these compounds are essential in life processes and probably undergo fairly rapid biological transformations and
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Degradation and hence highly are highly labile (Fichez, 1991; Danovaro et al., 1993; Fabiano et al., 1995; Cividanes et al., 2002).

Amino acids

Amino acids in aquatic systems have been categorized in terms of dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA). DCAA represent the largest well-defined molecular forms of dissolved organic matter and the largest identified component of dissolved organic nitrogen in water (Keil and Kirchman, 1993) engaged in key roles in carbon and nitrogen cycling in aquatic environments. Most amino acids in living organisms are present as constituents of proteins (Billen, 1984). Little is known about the chemical structure of DCAA, which may contain many types of bound amino acids. These types include proteins and oligopeptides (Lee and Bada, 1977), amino acids adsorbed to clays or other materials (Hedges and Hare, 1987) or amino acids in humic and fulvic substances (Poutanen and Morris, 1985). Amino acids and proteins associated with melanoidins, also contribute to the DCAA pool (Keil and Kirchman, 1991).

DFAA are important intermediates in the aquatic nitrogen cycle (Lomstln et al., 1998). They are the building blocks of proteins and thereby represent one of the most important organic nitrogen components in most aquatic organisms (Landen and Hall, 2000). Free amino acids are constantly being released into surface waters as excreta from a variety of aquatic organisms and by the hydrolysis and decomposition of biological detritus. The low DFAA concentrations detected imply that these molecules are rapidly removed, either by living organisms, which utilize the amino acids as a food source, or by reaction with some other organic material to produce more complex polymeric material, or by absorption onto particulate matter. Free amino acids can provide much carbon and nitrogen for bacterial growth, which in turn suggests that the DFAA is a large component of the labile DOM flux (Hoch and Kirchman 1995; Rich et al., 1996).

The dissolved combined amino acids might serve, as a food source for aquatic organisms in a manner similar to free amino acids, although the direct utilization of combined amino acids by the biota has not been demonstrated. Since the amino acids present are produced by living organisms, they should originally all be of the L-configuration. However, during the time which these compounds
remain in water they may undergo partial racemization (Bada and Lee, 1977) and the extent of this racemization could be an indicator of the age of dissolved combined amino acids in aquatic systems. The DCAA have shown to support some bacterial growth (Rosenstock and Simon 1993). The DCAA pool probably plays an important role in cycling of nitrogen in a variety of aquatic systems (Coffin, 1989; Burdige and Martens, 1988).

The mean concentration of total dissolved amino acids in seawater (free and combined) is approximately 50 µg/l (range of 20 to 250 µg/l) (Millero and Sohn, 1992). Sigleo and Macko (1985) found that the total DFAA concentrations in the Patuxent estuary, Maryland, USA were around 0.06 to 0.2 µM. The DCAA were most abundant than the DFAA by a factor of 50 (Sigleo and Macko, 1985). Jorgensen, (1982) reported greater seasonal differences in a shallow estuary in Denmark where DFAA concentrations of 0.7 to 2.5 µM occurred in spring and fall, and lower concentrations (0.2 µM) in summer and winter.

Monthly and seasonal data of the present study for both dissolved total amino acids (DTAA) and dissolved combined amino acids (DCAA) showed similar variation, since DCAA was the major component of DTAA with only a minor fraction of free amino acids (Table A.8; A.9; and A.10). The concentration of free amino acids is normally 4 to 10 times less than that of the combined forms (Millero and Sohn, 1992). The DFAA were utilized at about the same rate as they were formed by extracellular release, or by the hydrolysis of DCAA and particulates (Sigleo and Macko, 1985).

Maximum values of DTAA and DCAA were at Station R, in the month of December '00 and were 73.44 mg/l and 72.98 mg/l respectively. In September and
November, the rainfall was very low. The low rainfall during September and December reduces the possibility of flushing and causes the amino acid components to remain in the system itself. This could explain the high values observed. For DFAA, the maximum was at Station 1, in September '00 (3.46mg/l). In September, the condition might quite favorable for the hydrolysis of combined amino acids to free forms either biologically or chemically. But in December, the environmental condition might not be favorable for intense biological or chemical activity for DCAA hydrolysis resulting in its accumulation. Lowest values were at Station 1 in July for DTAA (2.58mg/l); at Station R in October, (00) for DCAA (2.24mg/l) and at Station R in July, (00) for DFAA (0.463mg/l), which might be due to the dilution effect of rainfall.

DTAA and DCAA values at Station R showed significantly higher concentrations, the highest being observed during post monsoon (Fig 4.1 and 4.3). This might be due to fish processing activities at this station. The lowest was observed at Station 3 during premonsoon for both these fractions. Annual mean values observed were 16.6mg/l, 23.29mg/l, 13.97mg/l and 28.25mg/l at Station 1, 2, 3 and R respectively for DCAA. Similarly for DTAA they were 17.52mg/l, 24.20mg/l, 14.71mg/l and 28.89mg/l. The lower values at Station 3 might be due to increased flushing activity at this site.

Amino acids are intermediates in degradation reactions and the fate of amino acids is not always easy to predict. When water temperature increased or input of fresh organic matter increased, followed by increased mineralisation rates, no obvious pool of amino acids was built up. Most probably there was an increased production of DFAA, but higher benthic activity will also increase the assimilation and degradation rates, and amino acids are commonly used by benthic microorganisms as a nitrogen source. However, bacterial assimilation and degradation is not the only sink for amino acids; there are other additional sinks of which adsorption is one of the larger, which may be an important way to preserve amino acids from further degradation (Landen and Hall, 1998). It has been observed that basic amino acids become enriched on particles by adsorption onto clay minerals through ionic interactions (Henrichs and Sugas 1993; Hedges et al. 1994). The polyelectrolytic nature of humics, major components of riverine and estuarine DOM, may also mediate adsorption of basic amino acids such as lysine as well as
arginine. Adsorption of hydrophobic dissolved organic matter to surfaces is higher than that of hydrophilic dissolved organic matter (Cosovic and Vojvodic, 1989). Hydrophobicity of DCAA may also be important for understanding DCAA turnover. With rumen bacteria, degradation of peptides has been positively correlated with the surface hydrophobicity of the peptide (Chen et al., 1987). In aquatic systems, hydrophobicity may partially determine the role of proteins in biogeochemical reactions (Keil and Kirchman, 1991).

A variety of marine and estuarine phytoplankton and bacteria can use dissolved organic nitrogen (DON) as a source of nitrogen (Antia et al., 1991; Keil and Kirchman, 1991). Extracellular amino acid oxidation is an important pathway of \( \text{NH}_4^+ \) regeneration and uptake in oligotrophic and low nutrient systems enriched in the labile DON (Mulholland et al., 1998). Amino acid oxidase activity is widespread across a variety of coastal and oceanic ecosystems. Under low inorganic nutrient conditions amino acid oxidation appears to represent a potentially significant, and usually ignored source of nitrogen for phytoplankton and cyanobacteria. This adds further support to the growing recognition that labile DON is cycled rapidly (Antia et al., 1991; Bronk et al., 1994). DFAA readily adsorb onto complex dissolved organic matter and particularly to polysaccharides thereby making them readily available for bacterioplankton utilization while reducing the availability of the adsorbed compared to non-adsorbed DFAA by several orders of magnitude (Schuster et al., 1998).

For free amino acid (Fig 4.2), Station 1 showed the highest value during monsoon and the lowest at Station R during postmonsoon. Annual mean values were 0.924 mg/l, 0.906 mg/l, 0.747 mg/l, and 0.632 mg/l at Station 1, 2, 3 and R respectively, with an overall mean value of 0.826 mg/l. ANOVA showed that there is no significant difference between Stations (Table B.3). Low background concentrations of monosaccharides and DFAA are typically observed in natural waters because these compounds are utilized too rapidly to accumulate (Skoog et al., 1999). Even though Station R showed high values for DTAA and DCAA, DFAA values were the lowest at the same Station. This may be due to the fact that at Station R free amino acids were rapidly removed as soon as they were formed. The limited variation in concentration of DFAA is most likely due to microbial utilization. In other words, DFAA are maintained at a low constant level because they are utilized by microbes very
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... (Millero and Sohn, 1992). Another possible sink for amino acids is sorption. Adsorption to organic material has been shown to be important for amino acids compared to adsorption to clay minerals (Rosenfeld, 1979a,b). Keil and Kirchman (1993) proposed that the decreases they observed in dissolved free amino acids at the Delaware bay turbidity maximum resulted from the adsorption of free amino acids onto <0.2 um clay minerals, DOM or both. It is apparent that sorption/desorption processes occur at sufficiently short time scales to affect the molecular weight distribution of DOC within turbid regions. Therefore it is likely that there is a relationship between adsorption and input of organic material, which vary seasonally as a function of variations of primary production. When amino acids have become adsorbed to a sediment particle they can survive degradation and be preserved for long time in the sediment (Gorden and Millero, 1985). One explanation for the lower DFAA concentrations may be that adsorption of amino acids was higher in the sediment. A conclusion made by Henrichs et al. (1984), from their study of Peruvian sediments, was that the major features of DFAA concentrations and compositions were probably due to production and consumption by bacteria. The seasonal signal of DFAA was suppressed probably due to removal processes (e.g., adsorption, degradation, assimilation) occurring simultaneously with DFAA production, induced by organic matter input and increase of temperature (Landen and Hall, 1998).

Correlation data (Table C4a-d) showed that at Station 1, DTAA was found to be negatively correlated to DO and polysaccharide. The inverse relationship of polysaccharide with amino acids suggest preferential utilization of one by microbes than the other. The inverse relationship with DO at this station might be due to high zooplankton or faunal activity, respiration of which depletes most of the DO and release amino acids as their excreta. Another possible reason is the introduction of large amount of organic matters either mangrove detritus itself or through the sewage decomposition of which also depletes DO. The positive relationship with proteins suggests that DTAA comprise mostly of proteins. At Station 2, the high correlation value of DTAA with DCAA showed that the former is composed mostly of combined forms. DTAA showed strong positive relationship with TSS at Station 3, might be due to TSS at this station may be rich in total amino acids. The strong positive relationship with proteins at Station R also give evidence that total amino acids at this station also is composed mostly of proteins. During
premonsoon (Table C.5a-c), the total amino acid content was dependent on temperature as observed from their inverse relationship. An increase in temperature during premonsoon enhances biological activity decreasing the total amino acid content. A positive relationship was obtained with combined amino acid. During monsoon, DTAA showed a positive correlation with TSS. During postmonsoon, the correlation data showed a pH dependence of total amino acids.

DCAA at Station 1 showed inverse relationship with DO. Alkalinity, an directly related to proteins and total amino acids (Table C.4a-d). At Station 2 the combined amino acids showed relationships only with total and free amino acid and at Station 3 with TSS and total amino acids. Similarly at Station R, only proteins and total amino acids showed the positive relationship with DCAA. The inverse relation of DCAA with temperature during premonsoon (Table C.5a-c) is in accordance with the fact that the degradation of amino acids by hydrolysis (either biologically or chemically) increases with temperature. The positive relationship with lipids suggests that they may be from same source with same rate of leaching and degradation. The positive relationship of DCAA with TSS may be due to the fact that latter may be rich in combined amino acids. A significantly high positive correlation was observed with pH during postmonsoon. Proteins showed a positive relation with combined amino acids, suggesting that combined amino acids were mainly in the form of proteins.

The direct relationship of DFAA with pH at Station 2 (Table C.4a-d), may be due to the fact that formation of free amino acids by the hydrolysis of combined amino acids is pH dependent. The direct relationship of DFAA with monosaccharides may be due to the same rate of formation and decomposition of these two in the aquatic system. The tannin and lignin also showed a positive relationship with free amino acids at Station 2, which may be due to the fact that leaching of tannin and lignin also take place at the same rate as that of free amino acids. Free amino acids showed positive relationship with total amino acids at Station 2, which might be due to the enrichment of total amino acid pool with free amino acids. At Station 3, DFAA showed relationship with POC, which explains that the POC was rich in amino acids. Seasonal correlation data (Table C.5a-c) showed that during monsoon there existed a direct relationship with salinity and inverse relationship with DO. The inverse relationship with DO suggests increased
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Degradation of free amino acids in aerobic conditions. During postmonsoon, free amino acids showed negative relation with proteins suggesting the hydrolysis of proteins to free amino acids. Direct relationship with POC and monosaccharides may be due to the fact that organic matter may be enriched with free acids and formation and degradation of free amino acids and monosaccharides takes place at the same rate.

Proteins

Proteins account for more than about 50% of the organic matter (Romankevich, 1984) and 85% of the organic nitrogen (Billen, 1984) of aquatic organisms.

The results of the present study are furnished in the (Table A.11). Maximum (28.03mg/l) was observed at Station 1 in May '00 and minimum (0.079mg/l) in July and October '99 at Station 3. Mean values of each station were 11.39mg/l, 11.76mg/l, 7.6mg/l and 11.26mg/l for Station 1, 2, 3 and R respectively.

ANOVA showed significant difference between seasons, but not between stations (Table B.3). At all the four stations monsoon concentrations were the least (Fig. 4.4a). According to Chergui and Pattee (1990), the leaves entering the water in summer and autumn were most readily colonized by microorganisms. Temperature influenced overall processing most clearly by its impact on leaching. This leaching might have increased the protein concentration in non-monsoon seasons. Photochemical processes may also be important in the cycling of some biochemical compounds (Keiber and Mopper, 1987). Station 3 showed lowest concentration at all the three seasons. Except at Station R, all the three stations showed maximum during premonsoon. The high value at Station 2 might be due to its isolated environment with creeks and channels were blocked by roots, pneumatophores and also by barriers built to prevent the loss of prawn seeds. So the tidal activity was minimum at this site with low flushing out of organic matter forcing it to remain in the system itself. There is increasing evidence that, contrary to the traditional view of proteins as very labile molecules, certain protein species of bacterial origin present in the aquatic environment, may be particularly resistant to degradation (Nagata et al., 1998) suggesting that some protein components are resistant to enzymatic attack and accumulate in water.
Although high molecular organic materials were refractory to microbial attack, they tend to adhere onto the surface of detritus and consequently, particles are easily formed from high molecular weight materials. Partial hydrolysis of macromolecules such as proteins and other compounds may take place on the detrital surfaces under the influence of bacterial enzymes and consequently, a portion of the macromolecules may be transformed into low molecular weight compounds easily utilized by aquatic organisms (Ogura, 1977). Macromolecular DOM may not be directly utilized by aquatic organisms, but they may be transformed into smaller compounds on detrital surfaces by bacterial activity and take part in food chains in aquatic systems.

Previous studies have shown that absorption of polymeric DOM such as protein to surfaces can occur very rapidly and that the absorption can have substantial effects on degradation rates of organic material (van Loosdrecht et al., 1990, Fletcher, 1991). Keil and Kirchman (1994) suggested that DOM absorbed to colloids was less easily degraded than freely dissolved DOM. Marine colloids and sub micron particles provide large surface areas which could have substantial implications for biochemical cycling in marine environment. Effect of surface area on degradation of organic matter could be stimulative, neutral (no effect), or even inhibitory (van Loosdrecht et al., 1990, Fletcher, 1991), but the results appear to vary depending on several factors, including concentration of organic matter on the surface and the nature of the interactions between organic matter and surfaces (Van Loosdrecht et al., 1990, Griffith and Fletcher, 1991, Taylor, 1995).

Proteolytic enzymes bound to bacterial cells are suggested to be responsible for high turnover of dissolved proteins (Hollibaugh and Azam, 1983). To utilize adsorbed proteins, bacteria need to remove protein molecules from the surface. Hydrolysis of adsorbed proteins are initiated only when the affinity of bacterial proteases or ‘protein-binding proteins’ that bind to proteaceous substrates exceeds the bond strength between proteins and surfaces (Nagata and Kirchman, 1996). Mechanisms underlying variation in degradability of dissolved proteins and other dissolved organic components in aquatic systems are not well understood. One hypothesis is that labile protein is transformed into less labile protein due to abiotic modifications including adsorption, condensation and photochemical reaction (Keil and Kirchman, 1994; Nagta and Kirchman, 1996). Recent research has suggested
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The association of proteins with other organic components may affect greatly the degradability of proteins in water (Keil and Kirchman, 1994; Nagta and Kirchman, 1996). Because of the slow turnover and close association of proteins with other macromolecules including polysaccharides, proteins probably have more chances to be modified geochemically, which may result in the formation of refractory proteins (Keil and Kirchman, 1994). This process is significantly enhanced by radiation, especially in the ultra violet (UV) range (300 to 400 nm) (Keil and Kirchman, 1994), while originally refractive DOM becomes labile upon UV exposure (Lindell et al., 1995, 1996; Wetzel et al., 1995, Graneli et al., 1996, Kaiser and Herndl, 1997, Reitner et al., 1997). However not only the light alters the availability of the labile DOM. It has been shown that labile DOM also becomes refractory due to sorption processes in sediments (Keil et al., 1994, Hedges and Keil, 1995). Thus structures of macromolecular organic complexes and their interactions with bacterial assemblages could substantially influence storage, turnover and transport of dissolved organic matter especially proteins. Thus small polypeptides and free amino acids could condense and undergo a variety of reactions to produce large molecular weight DCAA (Hedges, 1978; Carlson et al., 1985; Yamamoto and Ishiwatari, 1989).

Correlation data (Table C.4a-d) showed that proteins at Station 1 exhibited positive relation with TSS, hardness, DTAA, DCAA) and POC and at Station 2, only hardness showed a strong relationship with proteins. At Station 3, pH, hardness and salinity determined the fate of proteins and at Station R this role was taken by temperature and salinity. Temperature is an important factor in controlling leaching of DOC. Based on seasonal patterns of concentration, Gneve (1991) proposed that production of dissolved organic matter increased exponentially with temperature. Christ and David (1996) showed that DOC leaching increased exponentially with temperature between 3°C and 28°C. These observed temperature effects could be due to the effect of temperature on microbial dissolution, solubility products, sorption equilibria, or even increases in diffusion out of particle matrices (Christ and David, 1996). Protein at Station R was enriched in DTAA and DCAA as observed from their direct relationships. Seasonal correlation data (Table C.5a-c) showed a negative correlation of proteins with temperature and positive relationship with tannin and lignin in the monsoon
periods. Increase in temperature may increase the hydrolysis of proteins to amino acids and presence of tannin and lignin may exert an inhibitory effect on the degradation of proteins during premonsoon. During postmonsoon, proteins showed a negative correlation with POC and free amino acids and positive relationship to pH. Thus, during postmonsoon organic matter may be poor in proteins, by its hydrolysis to amino acids at low pH conditions. Positive relationship with DCAA during the same period may be due to the enrichment of combined amino acids with proteins. In the present study, dissolved proteins contributed 21.9% to 92.6% to DCAA pool, with a mean value of 55.7%. Mean values for each station were 64.6%, 55.8%, 59.8% and 42.8%. Thus the DCAA pool of Station 1 was the most protein enriched one.

From the diurnal data (Fig. 4.4b), protein content of Station 2 was observed to be higher than that at Station 1 showing that Station 2 was enriched with more protein. High protein content of Station R might be due to the impact of neighboring fish processing activities. Besides, all the three Stations showed the lowest values at 1300hrs. The release of organic nitrogenous compounds has been often reported to occur during active phytoplankton growth (Bronk and Glibert, 1991; Bjørnson, 1998). However, such a low value observed at this time may be due to heterotrophic grazing and growth, and consequently bacterial DON consumption (Lara et al., 1997), which may increase simultaneously with primary production. Climate warming and acidification result in faster degradation of high molecular weight substances. On the other hand, UV-B radiation has been found to break down these substances and make them available to bacterial degradation. At
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hours, Station 2 had higher protein content than Station 1 (except a small increase at 4.00hrs at Station 1). ANOVA showed only a slight difference with time and no difference between stations (Table B.4).

From the correlation table for diurnal variation (Table C.6a-c) it is clear that temperature regulated the protein concentration at Station 1. Here increase in temperature may cause an increase in leaching of protein molecules from detritus. The inverse relationship of protein and chlorophyll suggests that they were of different source, the source of proteins might be other than primary production. Proteins might have produced from benthic animals, fishes, prawns etc. The correlation shown by proteins with tannin and lignin suggests they were of same source and also by the hindrance to protein decomposition in presence of phenolic compounds at Station 1. Direct relationship of proteins with humic acid suggests increased humification of the system with accumulation of these compounds onto the humics. Suppression of protein degradation in presence of humic substances may be another reason. Qualls and Richardson, (2002) observed that the humic substances appeared to inhibit biodegradation of the other fractions of the DOC since hydrophilic organic acids decomposed faster when isolated from the humic substances. At Station 2 also, proteins showed direct relationship with tannin and lignin which suggests either their common source or suppression of degradation. As at Station 1, the protein content of Station R was very much dependent on temperature and hardness. But here inverse relationship was found with temperature, which suggests that the increased temperature lead to increased microbial or chemical degradation of proteins in reference site.

Carbohydrates

Carbohydrates are the largest identified fraction of organic matter in the aquatic systems, accounting for 20-30% in surface waters (Pakulski and Benner, 1994; Benner et al., 1992; Skoog and Benner, 1997) and occur as monosaccharides, oligosaccharides and polysaccharides. They are versatile molecules that serve as energy, storage, and structural components of cells. In aquatic systems, chemical energy is stored in the form of phytoplankton-derived carbohydrates, and this in turn provides energy to non-photosynthesising organisms through the processes of glycolysis and respiration (Witter and Luther, 2002). Glucose and to a lesser extent the other dissolved neutral monosaccharide components probably fuel a large fraction of bacterial respiration (Rich et al., 1996). Previous investigation
suggested that polysaccharides are highly labile and cycled rapidly in the water column (Ittekkot et al., 1981). Carbohydrates, especially free glucose, have been shown to be biologically reactive molecules (Skoog and Benner, 1997). They have been found to vary geographically, seasonally, diurnally, and with depth and are primarily derived from phytoplankton and vascular plants.

- **Dissolved Monosaccharides (DMCHO)**

  In the present study monthly data showed a highest monosaccharide concentration of 22.28mg/l at Station R in May'00 and lowest (0.783mg/l) at Station 3 in the same month (Table A.12). In the seasonal graph peak was observed at Station 2 during monsoon and minimum at Station R during monsoon (Fig.4.5a). A second largest peak was observed at Station R during premonsoon. DMCHO showed an overall mean value of 5.08mg/l. Station wise mean values were 5.02mg/l, 6.36mg/l, 4.72mg/l and 4.65mg/l for Station 1, 2, 3 and R respectively.

  In general, Station 2 showed the highest and Station 3 the least monosaccharide concentration. The difference was attributed to the difference in tidal activity between these two sites. The semi-enclosed area at Station 2 entails that exclusively local processes within this sector could cause the strong increases of nutrient and DOC concentrations during ebb. Creek- and rain-water are the only water sources to this mangrove swamp. The characteristic tidal signature of all parameters can therefore not be attributed to simple mixing processes of different water bodies. After inundation or rainfall, water can, however, be stored in the mangrove sediment and released again during ebb. During storage its composition is highly influenced by biogeochemical processes in the sediment. But exactly reverse is the case of Station 3., where high tidal flushing removes most of the dissolved organic nutrients from this region replacing it with fresh water.

  The concentration profile of dissolved carbohydrates reflects the balance between production of dissolved carbohydrates via solubilization/hydrolysis from POC and consumption of carbohydrates by fermentative bacteria. Once hydrolyzed, most monosaccharides would likely be remineralized rapidly (Sawyer and King, 1993; Rich et al., 1996). The major monosaccharide utilizers were found to be the microheterotrophs, namely bacteria, yeasts, and possibly some algae. Their concentrations were found to be highly variable with respect to tidal cycle (Millero and Sohn, 1992). The rates at which organic carbon is actually remineralized via sulfate reduction are therefore somewhat slower than the rates at
which three specific enzymes could potentially produce monosaccharides from high-molecular weight polysaccharides. These three specific enzymes are pullulanase, laminarinase, and xylanase.

From the correlation data (Table C.4a-d) it is clear that monosaccharides at Station 1 decrease with increase of temperature, DO and alkalinity. Increase of temperature, dissolved oxygen and alkalinity might have resulted in increased microbial utilisation of monosaccharides. Polysaccharides showed a negative correlation with monosaccharides. This may be due to the hydrolysis of polysaccharides to monosaccharides. The rate-limiting step for the consumption of monosaccharides would thus be the hydrolysis of polysaccharides. At Station 2, positive correlation with TSS suggests that suspended matter may be composed mostly of particulate monosaccharides and monosaccharides may be formed by the leaching from the suspended particle. The direct relationship of tannin and lignin and free amino acids with monosaccharides suggest either their same rate of formation from the same source i.e., mangroves or suppression of monosaccharide decomposition. Station R also showed a strong relationship with tannin and lignin, which suggests the export and same rate of leaching of mangrove detritus or suppression by phenolic compounds. Monosaccharides during postmonsoon (Table C.5a-c) showed significant positive correlation with POC suggesting the enrichment of organic matter with monosaccharides. Positive correlation of monosaccharides with free amino acids suggests that their source and fate are influenced by common environmental parameters. Negative relationship with pH may be due to their formation by acid hydrolysis of polysaccharides in the same season.

![Figure 4.5a: Spatial and seasonal variation of dissolved monosaccharides](image1)

![Figure 4.5b: Spatial and diurnal variation of dissolved monosaccharides](image2)
In the diurnal study, at 1100hrs., all the three Stations showed the highest monosaccharide concentration and similar to proteins, at 1300hrs. all showed the lowest values (Fig. 4.5b). Highest (9.76mg/l) and lowest (1.29mg/l) DMCHO were observed at Station R (average being 3.28mg/l). Station 1 and 2 showed almost similar variations. Omitting the values at 2300hrs. and 800hrs., variations at Station R resembled that of the other two. Thus, for the three stations maximum was observed at 1100hrs. and minimum at 1300hrs. This may be due to the increase in primary production followed by intense microbial degradation with increase in temperature and light radiation. Dissolved carbohydrates which are largely released by phytoplankton, with smaller contributions from zooplankton and bacterial excretion, as well as other minor sources, are important bacterial substrates and their levels are controlled largely by biological processes (Millero and Sohn, 1992). ANOVA showed a significant difference with time and no difference with stations (Table B.4).

Diurnal correlation data (Table C.6a-c) showed an inverse relationship for monosaccharides with total lipids suggesting preferential utilisation of the former by microbes at Station 1. At Station R, DMCHO showed a positive relationship with chlorophyll, which might be due to the fact that monosaccharides were the result of primary productivity. Direct relationship of monosaccharides with humic substances at Station R may be due to the suppression of monosaccharide degradation in presence of humic substances.

- **Dissolved Polysaccharides (DPCHO)**

The present study showed monthly variation with highest (88.04mg/l) at Station R for the month of December'00 and lowest (3.28mg/l) in August'99 at Station 2. In December, all Stations showed the highest polysaccharide concentration (Table A.13).

The seasonal graph revealed that premonsoon was associated with lowest polysaccharide concentration at all the four stations (Fig. 4.6a). The peak was shown by Station R during postmonsoon and lowest by Station 1 during
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premonsoon, with a mean value of 24.69mg/l. Mean values of the four Stations were 22.26mg/l, 24.13mg/l, 25.85mg/l and 25.93mg/l.

DPCHO at Station 1 showed positive relationship with DO and negative with monosaccharides (Table C.4a-d). This suggests the formation of monosaccharides by the hydrolysis of polysaccharides. At Station 2 also polysaccharides were very much dependent on DO. The direct relationship with DO suggests the microbial degradation of mangrove detritus in aerobic condition resulting in the leaching of polysaccharides. Positive relationship with humic substances during monsoon (Table C.5a-c) may be due to the humification of system with the accumulation of polysaccharides, or may be due the inhibitory effect exerted by humic substances on the degradation of the macromolecules.

![Image of bar chart and line graph showing spatial and seasonal variation of dissolved polysaccharides.](image1)

![Image of line graph showing spatial and diurnal variation of dissolved polysaccharides.](image2)

Diurnal variation showed the highest polysaccharide concentration of 23.59mg/l and lowest of 1.84mg/l at 1000hrs. at Station 1 and Station R respectively (Fig. 4.6b). Mean value observed was 9.39mg/l. From the diurnal correlation coefficients (Table C.6a-c), it is understood that polysaccharides showed positive correlation with TSS and inverse relationship with POC. This means that TSS may be enriched with particulate polysaccharides, leaching of the same would lead to the increased level of dissolved polysaccharides, DPCHO. The same process might have resulted in the simultaneous depletion of POC. Polysaccharide showed positive correlation coefficients with chlorophyll and humic acid at Station R, similar to that of monosaccharide relationships.
Chapter 4

Lipids

There are significant variations in the flux and composition of lipids in the water column as a function of surface water productivity, the species composition of plankton in the surface waters, diagenesis and remineralization of organic matter as it transits the water column towards the sediments and alteration processes at the water – sediment interface. Sources and composition appear to be important factors in degradation of fatty acids. Selective degradation of particulate lipids determines the composition of dissolved high-molecular mass lipids. Differential lability of individual lipids promotes selective release of particulate lipids to the HDOM pool with subsequent selective utilization within the DOM pool (Mannino and Harvey, 1999). Nagata and Kirchman (1992) have shown that heterotrophic flagellates grazing on bacteria release lipid-rich macromolecular DOM.

Monthly data of the present study showed a peak lipid content (4.74mg/l) at Station 2 in September'00 and the minimum 0.017mg/l was observed at Station 1 in April'99 (Table A.14). Mean values at Station 1, 2, 3 and R were 0.757mg/l, 0.822mg/l, 0.388mg/l and 0.430mg/l respectively. As in the case of proteins and carbohydrates, lipids were also maximum at Station 2 and minimum at Station 3. ANOVA results confirmed the significant variation between stations, but there was no difference between seasons (Table B.3). Although lipids comprised a small fraction of dissolved organic matter, the amounts observed were significant.

Correlation data for the four stations (Table C.4a-d) showed positive relationship of lipids with dissolved oxygen, which suggests their microbial or chemical leaching from organic matter in oxic media. Total lipids during premonsoon (Table C.5a-c) showed inverse relationship with temperature and significant positive relationships with particulate organic carbon, and combined amino acids. These correlations suggest that lipid degradation might take place rapidly at moderately high temperatures at the same rate as that of combined amino acids. Also, organic matter might be enriched with lipids. During monsoon, lipids showed positive relationships with pH and tannin and lignin. This may be due to the lipid accumulation in dissolved phase at high pH and in the presence of inhibitory compounds like tannin and lignin.
In the diurnal study all the three stations showed a low value at 1100hrs., with the lowest of all of 0.091mg/l at Station R (Fig. 4.7b). A high value (1.86mg/l) was observed at 2100hrs. at Station R. Station 1 and Station 2 varied almost similarly in terms of lipid content. ANOVA showed a difference in lipid content with time but no difference between stations (Table B.4). Diurnal correlation data showed (Table C.6a-c) conservative behavior of lipids with salinity at Station 1. Lipids also showed inverse relationship with monosaccharides, which suggests the preferential utilisation of the latter by microbes. At Station 2 the lipid concentration showed inverse relationship with pH, i.e., low pH leading to an increased leaching of lipids from particles and vice versa. The positive relationship shown by lipids to tannin and lignin suggests their same source. In reference site the inverse relation of lipids with DO suggests that the decomposition of lipids take place in the presence of DO and in anoxic condition, accumulation of lipids might occur. The inverse relationship of lipids in reference site with humic substances suggests lipids might take part in humification process.

4.3 Variation of Refractory Organic Constituents

Tannin and Lignin (T&L)

Phenolic compounds, especially lignin, are unique constituent of vascular plants (Sarkanen and Ludwig 1971) that is typically found to be resistant to microbial degradation (Benner et al. 1986). Therefore, lignin can be useful as biomarkers for vascular plant derived organic matter in heterogenous samples such
as sediments, dissolved organic matter (Meyers-Schulte and Hedges 1986, Hamilton and Hedges 1988). The nutritional quality of the dissolved organic matter declines with increase in lignin and cellulose contents. The seasonal changes in the concentrations of natural phenolic material in aquatic ecosystems may be driven by climatic patterns that control hydrologic transport of detrital organic matter from the watershed. If climatic patterns shift significantly because of global-scale changes, the associated changes in concentrations of natural phenolic material could seriously affect the functional relationships of aquatic ecosystems.

In the present study, monthly data showed maximum T&L (2.82mg/l) at Station 1 in October’99 and minimum (0.1117mg/l) at Station R in the month of April’00 (Table A.15). Seasonal variation showed the lowest T&L concentration at Station R during postmonsoon and highest at Station 2 during monsoon (Fig 4.8a). The mean values of T&L were 1.22mg/l, 1.4mg/l, 0.533mg/l, and 0.564mg/l at Station 1, 2, 3 and R respectively. Low concentration at Station R might be due to the import of T&L from adjacent mangrove areas and subsequent removal from the water column. Several workers (Day et al., 1953 ; Woodward et al., 1963) have noticed that lignin – like compounds may be removed in large quantities from the water media by adsorption onto the microbial cell wall and also by processes like coagulation, sorption on particulates, dilution by receiving waters etc. The breakdown of mangrove plant tissues gives rise to a considerable variety of tannin and lignin compounds. These are readily moved about within the environment by processes like tidal effect, runoff etc. It would, therefore, seem reasonable to expect a proportion of the organic material in the estuarine waters to consist of phenolic materials like tannins and lignins. Since the mangrove environment is characterized by a number of creeks and channels through which mangrove empty their water to the neighboring estuaries, it is possible to identify mangrove-derived organic matter in the estuarine site through the identification and quantification of these compounds. Tannin and lignin of estuarine site, Station R was low compared to the first two mangrove sites, i.e., Stations 1 and 2. This may be due to the fact that tannin and lignin are of mangrove origin and a major fraction of these substances get removed from the estuarine water by precipitation or degradation as it is transported away from the mangrove environment. Dilution effect may also be one of the reason for the low estuarine concentration. Another possible reason is the adsorption by clay
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minerals, organic matter etc. The concentration at Station 3 was the lowest even when compared to the estuarine site even though it is a mangrove area. The low tannin and lignin content at this Station might be due to the increased tidal activity at this site, which flushes out the organic matter as quickly as it is formed. The lignin-derived phenols in dissolved organic matter was far below the values found in sedimentary organic matter or litter. This might be due to the removal of these inhibitory compounds from the water column either by precipitation, coagulation or by adsorption onto clay minerals.

In a previous study by Kalesh et al., 2001, along the west coast of India, tannin and lignin (T&L) levels varied between 80μg/l and 147μg/l. They found a positive and negative correlation with dissolved oxygen in surface and bottom waters respectively and a negative and positive correlation with salinity at low water depths and in deep waters respectively. They suggested that substances such as lignins behave more conservatively in higher salinity waters.

Correlation data (Table C.4a-d) showed that tannin and lignin was directly proportional to monosaccharides at Station 1, Station 2 and Station R which suggest the same rate of their leaching from the mangrove plant litter and proved that mangrove detritus are exported to the neighboring estuaries. It may also be due to the decreased rate of decomposition of labile compounds in presence of tannin and lignin. At Station 2, tannin and lignin was also related to TSS and free amino acids. Thus at Station 2 tannin and lignin may be formed at the same rate as that of monosaccharides and free amino acids by leaching from TSS. Here also hindrance to amino acid decomposition by tannin and lignin may be another reason. More the suspended solids more the tannin and lignin formed in the dissolved phase. During premonsoon (Table C.5a-c), tannin and lignin showed positive correlation with dissolved proteins and during monsoon with pH and total lipids. Positive correlations with proteins and lipids may be due to the accumulation of these two by low rate of degradation in presence of phenolic compounds. During postmonsoon, tannin and lignin showed an inverse relationship with temperature. This may due to the removal of tannin and lignin from the water column by increased microbial or chemical degradation at moderately high temperatures.
Diurnal variation of T&L showed a range from 0.12 mg/l (Station R, 1300 hrs.) to 4.69 mg/l (Station 2, 200 hrs.) with a mean value of 1.55 mg/l (Fig. 4.8b). Similar to proteins and DMCHO, at 13.00 hrs., T&L values for the three Stations were also the lowest. Except at 200 hrs., mangrove sites showed higher concentrations of T&L as expected. The higher concentration at 200 hrs. in the reference site may be due to the export of mangrove detritus with the retrieving tide (since at 200 hrs., low tide was observed). The two mangrove sites showed similar variation. Apart from the values at 200 hrs. and 800 hrs., the Station R also showed a variation similar to the mangrove site which also strengthen the hypothesis that mangroves export organic matter to the adjacent estuary. Exactly like protein, except at 1100 hrs., Station 2 showed higher values than the other mangrove site, Station 1. This also confirms the fact that Station 2 produces more organic matter and is more productive than Station 1. ANOVA showed slight difference between stations and with time (Table B.4).

In the diurnal correlation data (Table C.6a-c), tannin and lignin showed a negative relationship with temperature and chlorophyll at Station 1 and direct relationship with pH. Thus tannin and lignin content at Station 1 is pH dependent. Negative relationship between tannin and lignin with chlorophyll suggests they were of different sources i.e., chlorophyll was from live plankton and tannin and lignin from mangrove detritus. Direct relationship of proteins with tannin and lignin suggests their similar source, i.e., mangroves and/or low rate of decomposition of former in presence of the latter. In reference site tannin and
'lignin is negatively related only to POC, which suggests tannin and lignin may be formed by the leaching of POC in this site.

**Humic Substances**

The bulk of the dissolved organic material has not been completely characterized. Large, complex humic-acid-like molecules, possibly of terrestrial origin and of considerable age, probably constitute the largest fraction. This material is probably relatively inert and therefore plays no active role in biological cycles. The source of this material and the mechanisms by which it is generated are, however, essentially unknown. Dissolved humic substances are assumed to be recalcitrant biopolymers representing at least 10% of the total marine dissolved organic carbon (DOC) pool (Ishiwatari, 1992). The occurrence of humic substance in the dissolved form is attributed to the elution of the soil organic matter (Sardessai, 1989). Shanmukappa and Neelakantan (1989) reported a mean humic acid concentration of 10.01 mg/l in mangrove habitats of Karwar, west coast of India.

In the present study, mangrove stations showed higher humic acid content than estuarine site, Station R. Monthly data (Table C.16) showed highest (28.46 mg/l) in May'00 and minimum (1.62 mg/l) in November'00, at Station R. Mean value of each station were 9.59 mg/l, 11.13 mg/l, 4.73 mg/l and 2.74 mg/l respectively, the overall mean value being 6.96 mg/l.

All the stations showed their maximum during premonsoon (Fig. 4.9a) and might be due to increased productivity and litter fall. With low surface runoff and rainfall effect, the degraded organic matter remains within the system itself and tend to accumulate in it. Except at Station R, all the Stations showed the trend premonsoon > monsoon > postmonsoon. The trend Station 2 > Station 1 > Station 3 > Station R was observed during all the three seasons. ANOVA also confirmed that the fluctuation between seasons and stations are significant (Table B.3). As the mangrove sites contain high organic matter, there is an increased chance of production of humic substance, which explain the high concentration of humic substance in these sites. Low values at Station R may be due to low humification and also by the subsequent removal by adsorption. The loss of dissolved humic acids in the dissolved phase could result from adsorption of humic acids onto fine
particles (Ertel et al., 1986). Low values at Station 3 may also due to the same removal process and also by the dilution effect of the active tidal waters.

Humic substances showed no significant correlations at the four stations (Table C.4a-d). But seasonal correlation data (Table C.5a-c) showed that during monsoon humic substances exhibited a positive relationship with polysaccharides, which might be due to the slow degradation of polysaccharides in presence of humic substances. During postmonsoon a negative relationships with DO and a positive relationship with tannin and lignin was observed. This might be due to the accumulation of humic substances along with tannin and lignin in low oxygen condition during this season.

In the diurnal data (Fig. 4.9b) the highest value of 11.94mg/l was observed at 1000hrs. at Station 2 and the lowest value of 1.75mg/l at 13.00hrs at Station R, showing a mean value of 7.39mg/l. At Station 1, the diurnal correlation data (Table C.6a-c) showed an inverse relationship of humic substance with chlorophyll and TSS suggesting that increased primary productivity produce fresh samples of TSS with a little or no humic substance leaching out of it. Humification might occur during the period of low productivity when the TSS contains humified compounds, which leaches to the water column. Humic substance concentration was round to be directly correlated to proteins and polysaccharides at Station 1. Direct relationship of proteins and polysaccharides suggests that accumulation of proteins and polysaccharides results in humification of dissolved organic matter. It may also due to the suppression of protein and polysaccharide hydrolysis and subsequent
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Accumulation in presence of humic substances. The humic substances appeared to exhibit biodegradation of the other fractions of the DOC since hydrophilic organic acids decomposed faster when isolated from the humic substances (Qualls and Richardson, 2002). At Station 2, humic substances showed an inverse relationship with salinity. At Station R, humic substance was positively correlated to chlorophyll and very high with monosaccharides. This might be due to the fact that increased primary production and accumulation of monosaccharides results in humification of dissolved organic matter or it may be due to the fact that increased humic substance in the dissolved phase suppresses the decomposition of monosaccharides, which tend to accumulate in the water column. The inverse relationship of lipids with humic substance might be due to the participation and subsequent removal of lipids in humification process at this reference site.

4.3 Summary

Concentrations of dissolved organic nutrients are influenced by local plant production, decomposition, and sorption equilibrium with particulate matter and sediment. While biological factors, plant production and microbial decomposition are important in producing potentially soluble organic nutrients, physicochemical sorption equilibria, hydrology, and degradation by solar radiation are also likely to control the concentration of this material. Dissolved organic matter concentrations were generally low at Station 3 as a result of dilution with low-DOC water from nearby freshwater sources, flushing by increased tidal activity and also due to low plant density. Flushing of organic matter during rain events also influenced dissolved organic matter concentrations. Variability in rainfall accounted for the variability in DOM concentrations at Stations 1 and 2. The concentrations of Station 3 were comparable with that at the estuarine reference site, Station R except for proteins and amino acids. But at Stations 1 and 2, anthropogenic inputs, isolated condition with low to medium tidal activity and high plant density contributed to the accumulation of organic matter and its constituents. Organic matter load of these sites was higher than that of the neighboring estuary. Concentrations of proteins and amino acids at this reference site were, however, similar to that at the mangrove site, which can be attributed to the fish processing activities at that site.
Chapter 4

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