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DISTRIBUTION OF AMINO SUGARS IN A LACUSTRINE AND A MARINE ANOXIC ENVIRONMENT

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SUMMARY

Aquatic environments retain a large quantity of carbon and have a significant influence on the global carbon and nitrogen cycles. It is therefore essential to understand the mechanisms responsible for the transformation and accumulation of organic material and to characterize its composition. During the last few decades, environmental conditions like anoxia, nature and origin of contributed material, sedimentation regime, and adsorption to mineral particles were identified as important factors for carbon accumulation. Furthermore, some specific environments are considered to accumulate carbon more efficiently than others, as for instance, anoxic and limnic systems. Although progress has been made in characterizing organic compounds, rather large fractions of the organic matter in aquatic systems still remain unidentified.

This thesis contributes to the understanding of organic matter transformations and accumulation by analysing single organic matter components, in particular amino sugars, in two anoxic systems. The choice of a lacustrine system, Lake Lugano, and a marine system, the Black Sea, allowed the comparison of a freshwater and a salt-water environment. Amino sugars are significant components of organic matter, since they are ubiquitously present in the environment and represent an organic carbon and nitrogen source. This work focuses on the most common amino sugars: glucosamine, galactosamine, mannosamine, and muramic acid. The latter is particularly interesting, because it is an indicator for peptidoglycan, a component of bacterial cell walls.

In both environments studied, the concentrations of muramic acid were higher than in oxic systems. This characteristic was ascribed to higher production of peptidoglycan and slower degradation than in oxic oceans. In the sediments of Lake Lugano, amino sugars accumulated (except muramic acid), while in the Black Sea they were degraded as fast as total organic carbon. Moreover, chlorins, which are considered to be the most reactive compounds in oxic systems, were degraded only partially in the sediments of Lake Lugano, while in the Black Sea they occurred in very low concentrations and showed a high degradation state. The differences between these two anoxic basins indicate that the lack of oxygen can not be the main factor responsible for organic matter accumulation. More likely, higher sedimentation rates, irregular

sedimentation regimes, and mixing within the sediments are crucial factors affecting accumulation of organic compounds in sediments of Lake Lugano.

The ratio of glucosamine to galactosamine in deep waters and in sediments had similar values to ratios reported from highly degraded material of deep oceans and sediments suggesting a common source of this biopolymer in all environments. Since several previous studies indicated an increased contribution to organic material from microbial remnants, it is likely that also this polymer is of prokaryotic origin. Moreover, this biopolymer appeared to be very refractive, because it was present in organic matter of highly degraded environments.

In conclusion, amino sugars were found to be more refractive than fatty acids and amino acids. This suggests that amino sugars are useful to describe a different degradation stage than amino acids, fatty acids, and chlorins and contribute to the understanding of transformation and reactivity of organic material in aquatic environments.

RIASSUNTO

Gli ambienti acquatici ritengono un'enorme quantità di carbonio ed hanno quindi un'influenza rilevante sui cicli globali di carbonio ed azoto. Risulta dunque fondamentale comprendere i meccanismi responsabili delle trasformazioni e dell'accumulazione della materia organica e conoscerne la composizione. Purtroppo, nonostante la presenza di numerosi studi, gran parte della materia organica non è ancora stata identificata. Esistono degli ambienti specifici, in cui la materia organica è accumulata in modo particolarmente efficiente, per esempio, in sistemi anossici e in sistemi lacustri. Le cause dell'accumulazione più efficiente sono ancora incerte, tuttavia esistono diverse ipotesi che suggeriscono un influsso da parte dell'anossia, della natura della materia di sedimentazione, del regime di sedimentazione e dell'adsorbimento a materia inorganica.

Alla luce di queste osservazioni, questo lavoro intende contribuire alla comprensione delle trasformazioni e dell'accumulazione della materia organica in due ambienti anossici tramite l'analisi di componenti della materia organica e, in particolare, di amminozuccheri. La scelta di un sistema lacustre, il Lago di Lugano, e di uno marino, il Mar Nero, permette inoltre di evidenziare le differenze tra un ambiente di acqua dolce ed uno di acqua salata. Gli amminozuccheri sono rilevanti, siccome sono ubiquitari negli ambienti naturali e rappresentano una fonte organica di carbonio e azoto per gli organismi. Questo studio focalizza sugli amminozuccheri più comuni: la glucosamina, la galactosamina, la mannosamina e l'acido muramico. Quest'ultimo è particolarmente interessante, essendo presente solo nel peptidoglicano - un componente della parete cellulare, fungendo così da indicatore per la materia di origine batterica.

In entrambi gli ambienti analizzati, le concentrazioni di amminozuccheri di origine batterica (acido muramico) nella colonna d'acqua e nei sedimenti sono risultate più elevate di quelle riportate in ambienti ossici. Questa caratteristica è stata attribuita soprattutto alla più alta concentrazione di acido muramico nei residui batterici degli ambienti anossici e alla degradazione più lenta rispetto agli oceani. Nei sedimenti del Lago di Lugano, si è constatata un'accumulazione di amminozuccheri (eccetto l'acido muramico), mentre nel mar Nero sono degradati alla stessa velocità della materia organica totale. Inoltre, i prodotti di degradazione della clorofilla, che negli ambienti ossici sono le sostanze più reattive, si ritrovano in concentrazioni

elevate nei sedimenti del Ceresio, mentre i sedimenti del Mar Nero ne sono molto poveri. Queste differenze tra i due ambienti anossici indicano che la mancanza di ossigeno non può essere il fattore principale responsabile per l'accumulazione di materia organica. Piuttosto, i tassi di sedimentazione elevati, il regime di sedimentazione variabile e la presenza di eventi di mescolanza dei sedimenti, sono fattori decisivi per l'accumulazione di materia organica nei sedimenti del Lago di Lugano.

Il rapporto tra la glucosamina e la galactosamina nell'acqua profonda e nei sedimenti è risultato simile a quello trovato nelle colonne d'acqua e nei sedimenti oceanici altamente degradati suggerendo un'origine comune di uno stesso polimero rifrattivo. Siccome molteplici studi precedenti indicano un elevato contributo alla materia organica da parte dei residui microbici, è presumibile che l'origine di questo polimero con composizione costante di glucosamina e galactosamina sia pure batterica.

In conclusione, gli amminozuccheri, risultando più rifrattivi degli acidi grassi e degli amminoacidi, promettono di poter contribuire ulteriormente alla comprensione dei meccanismi di conservazione della materia organica negli ambienti acquatici.

1

INTRODUCTION

There is a growing interest to understand the nature and transformations of organic matter in aquatic systems, since the ocean's waters and sediments represent a huge reservoir of organic carbon and are considered to have an influence on the global carbon and nitrogen cycles (Emerson and Hedges, 1988). In spite of numerous studies, still a large fraction of the marine organic matter remains unidentified (e.g., Benner et al., 1992; Cowie and Hedges, 1994; Aluwihare et al., 1997; McCarthy et al., 1997; Wakeham et al., 1997b; McCarthy et al., 1998; Hedges et al., 2001). In particular, several investigations focus on specific environments considered to accumulate carbon more efficiently, even though their contribution to the total volume of water bodies is small. Two examples are anoxic and limnic environments. Traditionally, anoxic systems are believed to retain carbon more efficiently than oxic systems (Henrichs and Reeburgh, 1987; Hedges and Keil, 1995; Hartnett et al., 1998; Hulthe et al., 1998); however, there is still an ongoing debate about the actual importance of anoxia (Force and McCarty, 1970; Calvert et al., 1991; Lee, 1992; Calvert and Pedersen, 1993). Numerous studies propose that the residence time in the water column, the degradation state of organic matter reaching the sediments, the adsorption on minerals, or a combination of these factors are determining carbon accumulation (Henrichs and Reeburgh, 1987; Hedges and Keil, 1995; Hartnett et al., 1998; Hulthe et al., 1998).

Although lacustrine systems account for only a small percentage of the water body on earth, their role in the global carbon and nitrogen cycles appears to be relevant, because lacustrine systems accumulate carbon more efficiently than oceans (Mulholland and Elwood, 1982; Dean and Gorham, 1998; Müller et al., 2005; Cole et al., 2007). In the present studies, two anoxic systems were chosen: the Black Sea and Lake Lugano. Both basins host permanent anoxic conditions in the deep water. This allows comparing the organic carbon production and

accumulation in a limnic and a marine anoxic environment and evaluating the importance of anoxia.

A possible approach for gathering information on the source, degradation state, and transformation of bulk organic matter is the analysis of the distribution of single components (Meyers and Ishiwatari, 1993; Colombo et al., 1996; Wakeham et al., 1997b). Amino acids, lipids, sugars, and amino sugars are the most important compounds representing about 80% of fresh phytoplankton, up to 11% of fresh dissolved organic carbon (DOC), 3% of refractive DOC (Benner, 2002), and 20% of surface sediment organic carbon ; Fig. 1.1). The distribution of these compounds in the environment is related to sources and transformations that occurred during degradation processes (Dauwe and Middelburg, 1998; Dauwe et al., 1999; Amon et al., 2001). Two groups of these compounds, amino acids and amino sugars, are particularly interesting, because they represent an organic nitrogen source and are consequently not only involved in the carbon cycle but also in the nitrogen cycle (Amon and Benner, 1996; Burdige and Zheng, 1998; Bronk and Ward, 2000).

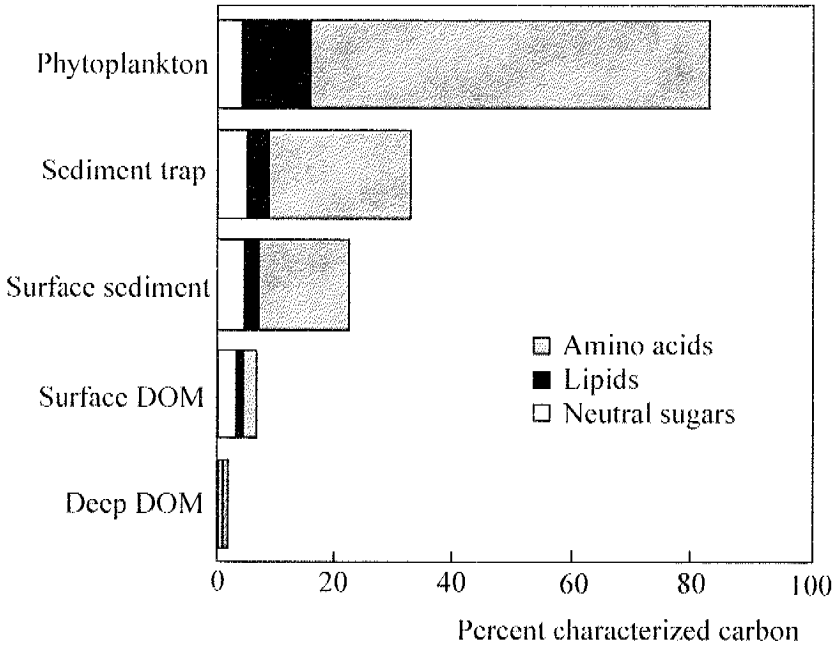


Figure 1.1: Percent characterized carbon as contributions from amino acids, lipids, and neutral sugars in fresh phytoplankton, sediment trap material, surface sediment, surface dissolved organic matter (DOM, < 1000 m), and deep DOM (> 1000 m) in the ocean (Wakeham et al., 1997b; Benner, 2002).

This study focuses on the significance of the, poorly described amino sugars in order to understand of organic matter sources and transformations in Lake Lugano and the Black Sea. Further, amino acids, fatty acids and chlorins, which are widely described in marine environments (e. g., Sanger, 1988; Wakeham and Lee, 1993; Dauwe and Middelburg, 1998; Volkman et al., 1998), are used to substantiate the conclusions drawn from amino sugar analyses. In the following the characteristics of the studied compounds are described and the two analyzed environments are presented.

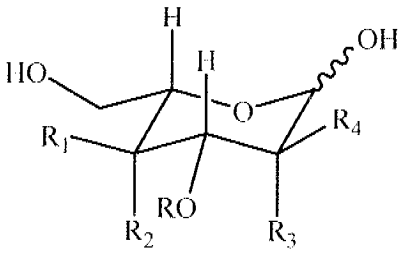
1.1. Amino sugars

The amino sugars glucosamine, galactosamine, mannosamine, and muramic acid (Fig. 1.2) are major components of organic matter as they are found in chitin, peptidoglycan, polysaccharides, glycoproteins, and glycolipids (Sharon, 1965; Mayzaud and Martin, 1975). Chitin is the second most abundant polymer on earth (Cohen, 1987) and the most abundant biopolymer in the ocean (Poulicek et al., 1998). It is a structural polymer of the amino sugar N-acetyl-glucosamine and is produced by a large variety of marine organisms (Muzzarelli, 1977). It is contained mainly in organisms like copepods in concentrations of up to 5% of their total body weight (Mayzaud and Martin, 1975), in algae, and in fungi (Sharon, 1965). Peptidoglycan is an important structural component of prokaryotic cell walls (Brock et al., 1994) and is composed of N-acetylglucosamine, N-acetylmuramic acid, and L- and D-amino acids (Sharon, 1965; Dittmar et al., 2001). The most attention-grabbing amino sugar in peptidoglycan is muramic acid, because so far, it has only been detected in peptidoglycan (Sharon, 1965) and can reach concentrations of 10% of the whole bacterial biomass (Moriarty, 1975).

In soil science, amino sugars play a key role and are well investigated (Zhang and Amelung, 1996; Amelung, 2001; Glaser et al., 2004). In these studies, they were used as biomarkers for microbial residues as well as indicators for land use (Amelung, 2001; Turrion et al., 2002; Amelung, 2003). In contrast, in aquatic environments only a few recent studies focused on the amino sugar distribution and the application of amino sugars as indicators for organic matter production and degradation (Nedoma et al., 1994; Benner and Kaiser, 2003; Davis and Benner, 2005; Niggemann and Schubert, 2006b; Tremblay and Benner, 2006). Recently, Kaiser and Benner (2000) developed a method for measuring amino sugars in seawater samples and

provided the first detailed data on a variety of marine organisms, particulate organic matter, and ultrafiltered dissolved organic matter from various ocean basins and depths (Benner and Kaiser, 2003; Davis and Benner, 2005; Tremblay and Benner, 2006). In sediments, to the best of our knowledge, there are only a few studies describing in detail amino sugar distribution and changes in concentrations with depth (Kemp and Mudrochova, 1973; Liebezeit, 1993; Niggemann and Schubert, 2006b) and a few analyzing amino sugars in the top sediment layer (Dauwe and Middelburg, 1998; Jennerjahn and Ittekkot, 1999; Gupta and Kawahata, 2000).

The interpretation of the origin of amino sugars and their usefulness for the description of organic matter sources and degradation state has been equivocal in different studies (Mimura and Romano, 1985; Müller et al., 1986; Liebezeit, 1993; Jennerjahn and Ittekkot, 1999; Gupta and Kawahata, 2000; Benner and Kaiser, 2003; Niggemann and Schubert, 2006b). In dissolved organic matter from deep water, most amino sugars were attributed to bacterial sources (Benner and Kaiser, 2003), while in sediment trap material a major contribution by chitinaceous material was reported (Müller et al., 1986). In sediments, both a major contribution of bacterial remnants (Liebezeit, 1993; Niggemann and Schubert, 2006b), as well as a significant input of phyto- and zooplanktonic material have been proposed (Dauwe and Middelburg, 1998). The amino sugar distribution along the water column and in the underlying sediments hinted to the degradation of fresh planktonic amino sugars and the increase of bacterial contribution in the bottom water and in the top sediments (Ittekkot et al., 1984a; Ittekkot et al., 1984b; Jennerjahn and Ittekkot, 1999). Moreover, degradation experiments of chitin and peptidoglycan in seawater reported fast degradation of amino sugar-containing organic matter with a loss of 97% of chitin in 70 days and a peptidoglycan turnover of 10-167 days (Nagata et al., 2003; Davis and Benner, 2005). These contrasting opinions on the major amino sugar source confirm the need for further investigations into amino sugars in aquatic environments.



	R	R_1	R_2	R_3	R_4
Glucosamine	H	OH	H	H	NH_2
Galactosamine	H	H	OH	H	NH_2
Mannosamine	H	OH	H	NH_2	H
Muramic acid	Lactyl	OH	H	H	NH_2

Figure 1.2: Structure of the amino sugars glucosamine, galactosamine, mannosamine, and muramic acid.

1.2. Amino acids

Amino acids (Fig. 1.3) are the main nitrogenous compounds in all living organisms. They are ubiquitously present in the environment and their distribution and transformations in the water column and in sediments have been extensively studied (Degens et al., 1967; Lee and Cronin, 1982; Ittekkot et al., 1984a; Ittekkot et al., 1984b; Cowie et al., 1992). Among organic matter components, amino acids are considered to be rather labile (e. g., Lee and Cronin, 1982; Keil et al., 2000) and it has been observed that some amino acids are prone to selective degradation in sediments, while others are conserved (Brown et al., 1972; Burdige and Martens, 1988). Therefore, changes in amino acid distributions are useful to track diagenesis. For instance, the relative abundance of non-protein amino acids (β-alanine and γ-aminobutyric acid) in the amino acid pool increases as the organic material becomes more degraded (Cowie and Hedges, 1994). Similarly, the neutral amino acids glycine and alanine increase in concentration with depth and age (Lee and Cronin, 1982; Burdige and Martens, 1988), while the acidic (aspartic acid and glutamic acid), the sulfur (methionine) and the aromatic (phenylalanine and tyrosine) amino acid concentrations decrease with enhanced degradation (Burdige and Martens, 1988). Moreover, the concentrations of the basic (arginine, histidine) and hydroxilic (serine and threonine) amino acids remain constant. These characteristics have been used to develop a degradation index (DI) (Dauwe and Middelburg, 1998; Dauwe et al., 1999), which attempts to describe the diagenetic state of organic matter. This index has been applied successfully in many marine and some lacustrine environments (Dauwe et al., 1999; Keil et al., 2000; Meckler et al., 2004; Niggemann, 2005; Lomstein et al., 2006). However, it has been observed that in organic matter rich environments the DI always indicates high freshness of organic matter and amino acids appear to rather reflect the source than the diagenetic state of organic material (Keil et al., 2000; Lomstein et al., 2006).

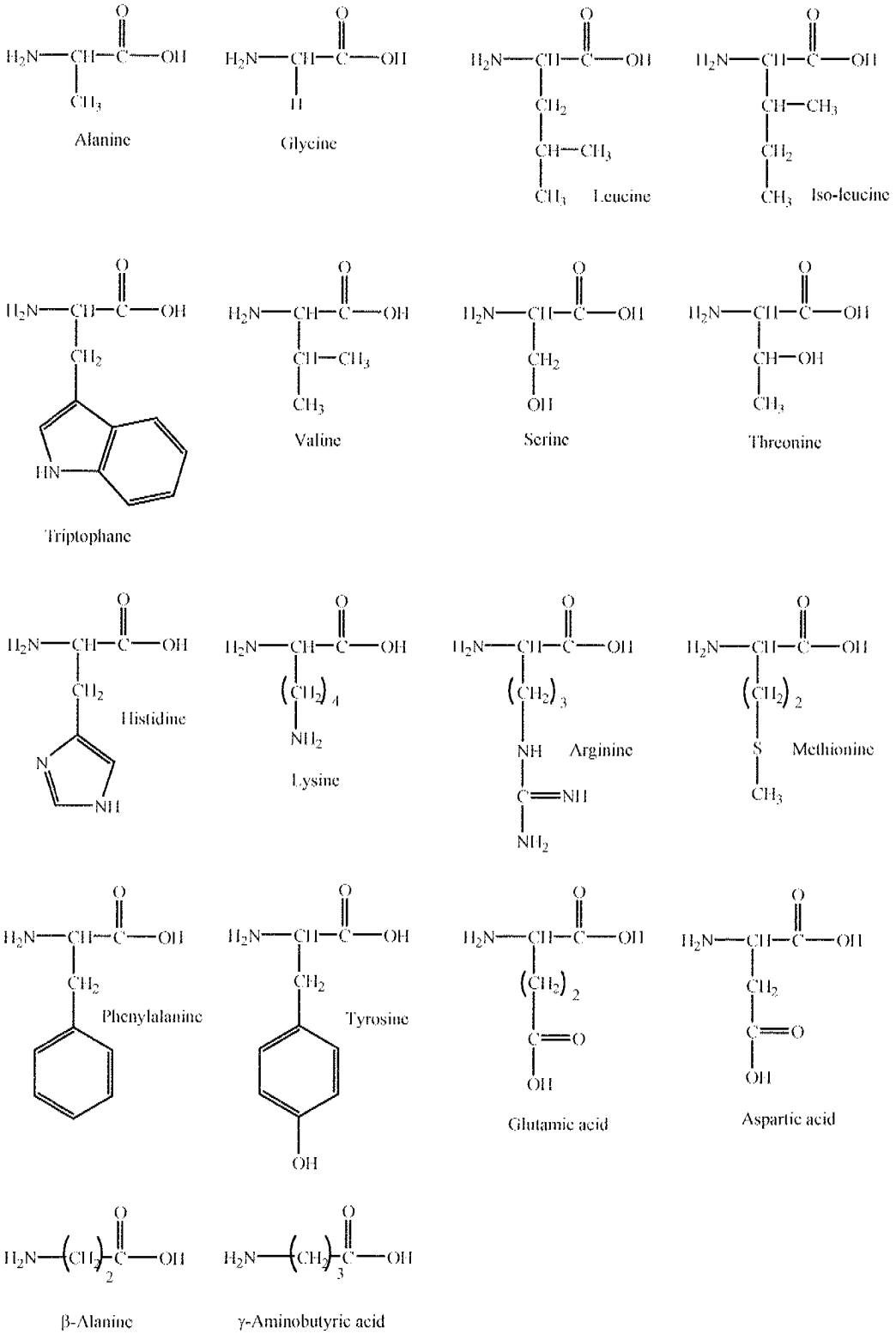


Figure 1.3: Structures of the amino acids analyzed in this work.

1.3. Fatty acids

In aquatic environments, fatty acids occur in much lower concentrations than amino acids. In return, their distribution can reveal the sources and degradation state of organic matter (Beier et al., 1991; Meyers and Ishiwatari, 1993; Wakeham et al., 1997b; Niggemann and Schubert, 2006a). Therefore, the variations in fatty acid distribution and concentration have been widely studied in various different aquatic environments (e.g., Volkman et al., 1980; Meyers and Ishiwatari, 1993; Wakeham et al., 1997b; Niggemann and Schubert, 2006a). Fatty acids (Fig. 1.4) can be divided into different groups: mid-chain saturated, long-chain saturated, monounsaturated, polyunsaturated and branched fatty acids (Niggemann and Schubert, 2006a). Saturated fatty acids are the most abundant and a possible end product of degradation of unsaturated fatty acids. Mid-chain saturated fatty acids are more labile than long-chain fatty acids. Normally mid-chain fatty acid concentrations decrease with sediment depth, while long-chain fatty acids concentrations are constant. Long-chain fatty acids are enriched in land plants in comparison to algal material and can therefore be used as indicators for terrigenous input in lake sediments (Meyers and Ishiwatari, 1993). Unsaturated fatty acids are the most labile fatty acids and are degraded rather fast within the water column and in the top sediments (Meyers and Ishiwatari, 1993). Some organisms produce particularly high amounts of specific unsaturated fatty acids, which are used as biomarkers. For instance, the polyunsaturated fatty acid $C_{20:5}$ is present in high concentrations in diatoms (Dunstan et al., 1994) and the $C_{22:6}$ fatty acid in dinoflagellates (Volkman et al., 1989). Another example is given by the $C_{18:1}$ fatty acid: The $C_{18:1\omega9}$ fatty acid has typically higher concentrations in algae and zooplankton than the $C_{18:1\omega7}$ fatty acid which is enriched in bacteria (Volkman et al., 1980; Volkman et al., 1989). Therefore, the $C_{18:1\omega7}$ fatty acid can be used as a bacterial marker and ratios between the $C_{18:1\omega9}$ and the $C_{18:1\omega7}$ fatty acids can indicate bacterial activity (e. g., Thoumelin et al., 1997; Mudge et al., 1998; Camacho-Ibar et al., 2003). Low ratios of $C_{18:1\omega9}$ to $C_{18:1\omega7}$ have also been found in some diatoms and Cryptomonads, which weakens the reliability of the method (Volkman et al., 1980; Volkman et al., 1989). Branched fatty acids are commonly encountered in bacterial lipids and they have lower concentrations in other organisms. Therefore, branched fatty acids are useful to determine the bacterial contribution to organic matter (Cranwell, 1973; Meyers and Ishiwatari, 1993).

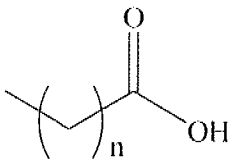
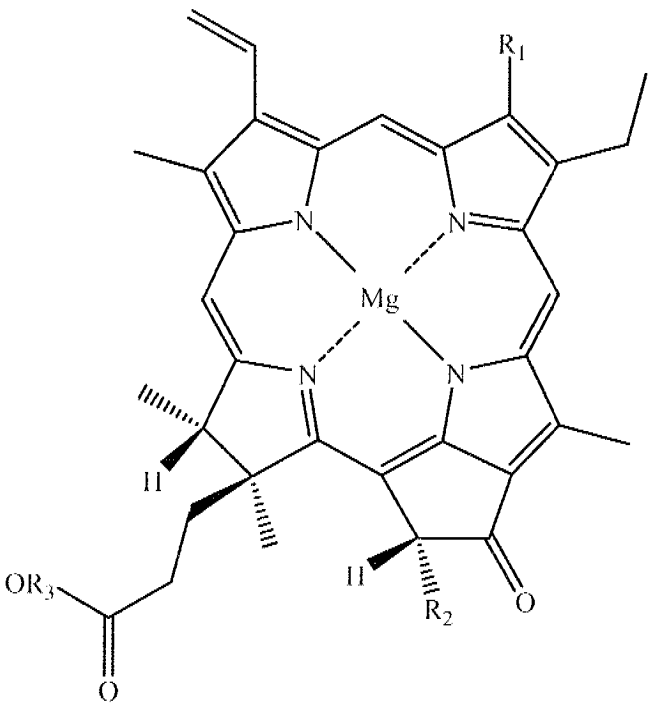


Figure 1.4: Structure of fatty acids. The number of carbon atoms varies between 12 and 40.

1.4. Chlorins

Chlorins (Fig. 1.5) comprise of chlorophyll and its degradation products. They are present in algae, photosynthetic bacteria, and land plants. Their abundance in sediments depends on the input of these organisms and on the preservation of material derived from them (Sanger and Gorham, 1970; Gorham and Sanger, 1972). Chlorins are considered to be easily degraded in the water column (Brown et al., 1972; Colombo et al., 1996). Environmental conditions like water column depth, temperature, oxygen content, light conditions, and sedimentation rate have been shown to influence the degradation processes (Sanger, 1988). In particular, chlorins are degraded faster in warm, light, oxic water than in cold, dark, anoxic water (Sanger, 1988). Recently, an index for organic matter freshness was developed (Schubert et al., 2005). The chlorin index (CI) is based on the ratio of the fluorescence intensity between the acidified extract and the neutral extract. A fresh chlorin extract is rapidly degraded by HCl, while an extract with higher degraded chlorins is not further decomposed. The CI ranges from 0.2 for fresh chlorophyll to 1 for highly degraded chlorins. It has been applied to several marine systems and to sediments of Lake Zug, and has been compared to the amino acid based DI (Meckler et al., 2004; Niggemann, 2005; Schubert et al., 2005). In most cases the CI correlates well with the DI (Schubert et al., 2005), although in Lake Zug sediments no relationship was observed (Meckler et al., 2004). This mismatching has been attributed to the different degradation rates of amino acids and chlorins: Amino acids are considered to be more resistant to degradation than chlorins and, as a consequence, the DI might indicate a more advanced degradation than the CI (Meckler et al., 2004).



	R_1	R_2	R_3	
Chlorophyll *a*	CH_3	$COOCH_3$	Phytyl	
Chlorophyll *b*	CHO	$COOCH_3$	Phytyl	
Pheophytin *a*	CH_3	$COOCH_3$	Phytyl	- Mg + 2H
Pheophytin *b*	CHO	$COOCH_3$	Phytyl	- Mg + 2H
Chlorophyllide *a*	CH_3	$COOCH_3$	H	- Mg + 2H
Chlorophyllide *b*	CHO	$COOCH_3$	H	- Mg + 2H
Pyrochlorin	CH_3	H	H	- Mg + 2H

Figure 1.5: Structure of the most common chlorins.

1.5. Study sites

1.5.1. Lake Lugano

Lake Lugano is a deep, subalpine lake located at the Swiss-Italian border (Fig. 1.6). It is divided into three sub-basins: the northern and the southern basin separated by an artificial dam, and a smaller basin in front of the outlet of the Tresa River. The study site was chosen in the

northern basin. This basin has a volume of 4.69 km^3, a maximum depth of 288 m, and a mean water residence time of 12.3 years. The steep slopes, warm winters, weak winds, and eutrophication cause the meromictic mixing regime that induces permanent anoxic conditions in the lowermost 180 m (Barbieri and Mosello, 1992). Intense investigations on Lake Lugano's chemical and physical characteristics have been conducted (e.g., Barbieri and Polli, 1992; Dominik and Span, 1992; Span et al., 1992; Wüest et al., 1992) and monitoring studies on nutrient concentrations, primary productivity, and planktonic community structure have been performed since the early 1980ties (LSA, 2003). The collected data shows a strong reduction in the nutrient input at the end of the 1980ties that provoked a 50% decrease in the phytoplanctonic biomass and an increase in the zooplanktonic biomass.

Sediments in the northern basin of Lake Lugano have experienced a permanent anoxic condition for more than 50 years (Wüest et al., 1992). Organic carbon concentrations in recent sediments are high (up to 8%) and degradation processes are slow (Span et al., 1992). Occasional turbidites have been observed in the sediment cores and have been attributed to slides resulting from the steep slopes of the basin (Niessen, 1987). A recent study which focused on the transport of particulate matter in the water column showed that particles resuspended from shallower regions contribute to up to 70% of the total resuspeded material (Hofmann and Dominik, 1995).

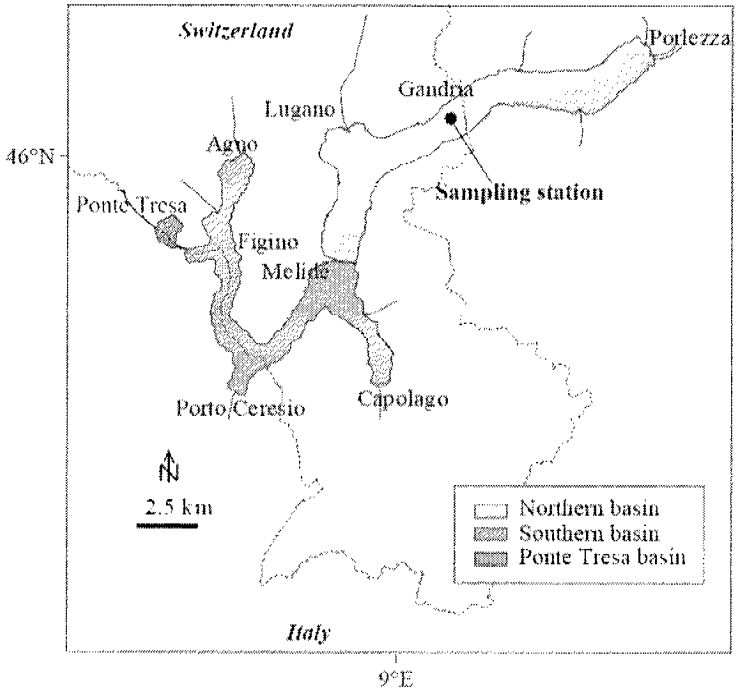


Figure 1.6: Map of Lake Lugano with the sampling station in the northern basin near the village of Gandria.

1.5.2. Black Sea

The Black Sea (Fig. 1.7) is the largest anoxic water basin in the world and has been extensively studied by marine scientists (Various Authors, 1991; Sorokin, 2002). It was a freshwater lake during the Pleistocene, and with the post-glacial rise in global sea level, saline Mediterranean water began to flow through the Bosporus into the Black Sea establishing a pycnocline at 50-100 m depth (Deuser, 1971; Tolmazin, 1985b; Calvert et al., 1987; Murray et al., 1989; Murray, 1991). The seawater inflow maintains a salinity of 22.3‰ in the deep water, whereas major inflows from the Danube, Dnieper, and Dniester establish a more brackish (18‰) layer in the top 50-100 m (Tolmazin, 1985a; Murray et al., 1989; Murray, 1991). The absence of oxygen below 100 to 150 m allows for the occurrence of increased amounts of methane and hydrogen sulfide in the anoxic layer (Sorokin, 2002). These conditions are rarely found in marine environments and make it interesting to study organic matter transformation and accumulation.

The community structure of the prokaryotes present in the anoxic water column, being dominated by anaerobic organisms like, for instance, methane oxidizing archaea and sulphate reducing bacteria, is different from oxic oceans (Madrid et al., 2001; Durisch-Kaiser et al., 2005; Morgan et al., 2006; Neretin et al., 2007).

In the older sediment layers, the earlier oxic conditions of the bottom water can be recognized (Ross et al., 1970), while at present the permanent anoxic conditions keep the upper sediments undisturbed from bioturbation. Within the sediments there are laminae reflecting the sedimentation of diatoms in spring and coccolithophores (*E. huxley*) in summer (Hay et al., 1990). However, a large fraction of the diatoms dissolve in the water column and the surface sediment (Pilskaln, 1991). In deep region sediments, organic matter mainly originates from autochthonous material, while at the coasts and at the North-Western shelf terrigenous influence from the Danube, Dnieper and the Dniester is possible (Stokozov and Buesseler, 1999).

The study sites were located in the North-Western Black Sea in proximity of the Crimean peninsula (Fig. 1.7). The shallow site was in the Dniepr-paleo-delta at a depth of 250 m and the deep site at 1900 m. Based on radioactive tracers studies, both sites are not influenced by riverine input (Stokozov and Buesseler, 1999).

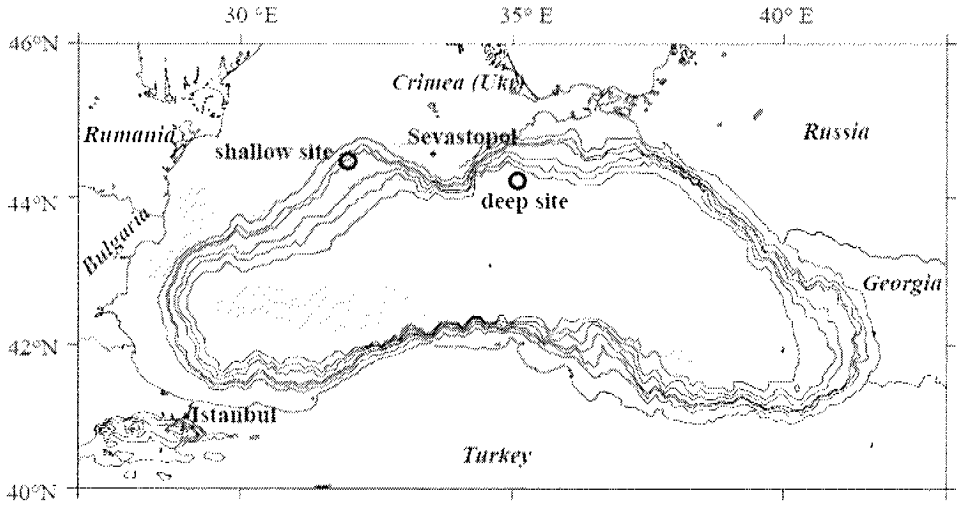


Figure 1.7: Map of the Black Sea with the shallow and deep sampling sites.

1.6. Objectives and outline of the thesis

The goals of this work were to elucidate and compare the characteristics of amino sugars in marine and limnic anoxic systems, to evaluate amino sugars as indicators for organic matter sources and degradation state in water and sediments, and to determine the reactivity of amino sugars in comparison to other organic matter components.

In the following chapter of this work an evaluation of the distribution, reactivity, and degradation state of different organic matter components in sediments of Lake Lugano is presented. The third chapter focuses on amino sugar distribution and transformation in the water column and in sediments of Lake Lugano. In the fourth chapter, amino sugar distribution and characteristics as indicator for organic matter degradation state are discussed over the two study sites of the Black Sea. In the conclusion chapter, the importance of amino sugars, amino acids, fatty acids, and chlorins in the analysis of anoxic environments is commented and ideas for future research are presented.

1.7. Contribution to publications

Chapters 2 to 4 are prepared for submission to Organic Geochemistry and Limnology and Oceanography. During this thesis the collaboration in additional projects led to the publication of the following articles and book chapters:

McGinnis, D. F., Wüest, A., Schubert, C. J., Klauser, L., Lorke, A., Kipfer, R., 2005. Upward flux of methane in the Black Sea: Does it reach the atmosphere? In: *Environmental Hydraulics and Sustainable Water Management* (Eds: Lee and Lam), Taylor and Francis Group, London.

Durisch-Kaiser, E., Klauser, L., Wehrli, B., Schubert, C., 2005. Evidence of intense archaeal and bacterial methanotrophic activity in the Black Sea water column. Applied and Environmental Microbiology, 71: 8099-8106.

Schubert, C. J., Durisch-Kaiser, E., Klauser, L., Vazquez, F., Wehrli, B., Holzner, C. P., Kipfer, R., Schmale, O., Greinert, J., Kuypers, M. M. M., 2006. Recent studies on sources and sinks of methane in the Black Sea. In: *Past and present water column anoxia.* (Ed. Neretin, L. N.), pp. 419-441, Springer.

Schubert, C. J., Durisch-Kaiser, E., Holzner, C. P., Klauser, L., Wehrli, B., Schmale, O., Greinert, J., McGinnis, D. F., De Batist, M., Kipfer, R., 2006. Methanotrophic microbial communities associated with bubble plumes above gas seeps in the Black Sea. Geochemistry Geophysics Geosystems 7, Q04002, doi: 10.1029/2005GC001049.

2

INDICATORS OF ORGANIC MATTER SOURCES AND DEGRADATION STATE IN ANOXIC LAKE SEDIMENTS

Lucia Klauser and Carsten J. Schubert

2.1. Abstract

Since there is evidence that lakes accumulate carbon more efficiently than oceans, the understanding of mechanisms responsible for organic matter (OM) retention in lacustrine sediments is essential. In this work, we discuss the distribution and reactivity of fatty acids, chlorins, and amino acids in anoxic sediments of the subalpine eutrophic Lake Lugano (Switzerland). Further, we apply the Dauwe degradation index (DI) and the chlorin index (CI) to describe the diagenetic state of OM. ^{210}Pb dating revealed periodical turbidites and mixing events in the sediment core. Increased concentrations of long-chain fatty acids in some layers indicated the presence of terrigenous OM. High amounts of algae-specific unsaturated fatty acids and low concentrations of bacterial markers were detected. The DI indicated the presence of very fresh OM and indicated only a moderate degradation with depth. On the other hand, the CI suggested an alternation of fresh and degraded OM. This variability of chlorin degradation state was attributed to the influence from allochthonous material and sediment mixing-events, which increased and decreased the CI, respectively. Degradation of pigments appeared to be highest at the sediment-water interface and the residence time in the top sediment layer appeared to be crucial for degradation of chlorin. Hence, other factors than anoxia, like OM input and sediment mixing, clearly contributed to the conservation of organic compounds in Lake Lugano sediments. The lack of concentration decrease of chlorins in this anoxic system is in strong contrast to down-

core trends in oxic systems, where chlorins are the most labile compounds, while in Lake Lugano sediments, unsaturated fatty acids showed the strongest reactivity followed by saturated fatty acids, amino acids, and finally chlorins.

2.2. Introduction

The study of organic matter (OM) transformations in sediments is central for the comprehension of mechanisms responsible for carbon accumulation. Questions concerning the generation of fossil fuels, the connection of the carbon cycle to climate change, or, on a smaller scale, the problem of eutrophication due to anthropogenic nutrient input have provoked extensive research on OM cycling over the last decades. Most of the investigations on OM degradation in sediments, so far, have been conducted in marine environments. Recently, however, it has been shown that lakes have a higher storage capacity of carbon than oceans and therefore their role in the global carbon cycle is of high interest (Mulholland and Elwood, 1982; Dean and Gorham, 1998; Müller et al., 2005; Cole et al., 2007). There are some basic differences between lacustrine and marine sediments: the influence of terrestrial OM may be much higher due to direct river inflow. Terrestrial OM contains more refractory organic compounds compared to, for instance, autochthonous produced algal material (Capone and Kiene, 1988). Higher productivity, especially in eutrophic lakes, and often much shallower water depths lead to enhanced burial of OM in lacustrine sediments (Meyers and Ishiwatari, 1993). Finally, the OM oxidation occurs mainly via sulfate reduction in the marine realm (Jorgensen, 1982) and via methanogenesis in freshwater systems (Capone and Kiene, 1988). Environmental conditions such as OM sources, oxygen exposure time, anoxia, and sedimentation rates, have been identified as important parameters influencing OM accumulation processes (Henrichs and Reeburgh, 1987; Hedges and Keil, 1995). Therefore the study of OM rich lacustrine sediments with high sedimentation rates and permanent anoxic conditions can significantly contribute to the understanding of the mechanisms responsible for carbon accumulation.

Compounds frequently used to describe OM sources, degradation state, and reactivity in sediments are amino acids (AA), fatty acids (FA), and chlorins (e. g., Wakeham and Beier, 1991; Meyers and Ishiwatari, 1993; Volkman et al., 1998; Dauwe et al., 1999; Meckler et al., 2004; Niggemann and Schubert, 2006a). AAs are ubiquitously present in the environment and have

similar distributions in all organisms. Furthermore, it has been observed that some AAs (acidic, sulfur, and aromatic) are prone to selective degradation, while others (non-protein and some basic) are accumulated in sediments (Brown et al., 1972; Burdige and Martens, 1988). These effects have been used to develop the Dauwe degradation index (DI, Dauwe and Middelburg, 1998; Dauwe et al., 1999), which allows determining the diagenetic state of OM in sediments. The DI has successfully been applied in many environments (Dauwe et al., 1999; Keil et al., 2000; Meckler et al., 2004; Niggemann and Schubert, 2006a) with the exception of some organic carbon rich sediments where AAs appeared to reflect OM sources rather than degradation state (Keil et al., 2000; Lomstein et al., 2006). In contrast to AAs, FAs have rather low concentrations in sediments. In return, single FAs and their down-core distribution give important information on OM sources and transformations (Volkman et al., 1980; Beier et al., 1991; Wakeham et al., 1997b). For example, long-chain FAs (LC-FA) are indicators for terrigenous-derived OM, branched FAs (BFA) are enriched in bacteria, and the polyunsaturated FA (PUFA) $C_{20:5}$ originates from diatoms (Meyers and Ishiwatari, 1993; Dunstan et al., 1994). Chlorins comprise chlorophyll and its degradation products and are present in algae and photosynthetic bacteria, as well as in higher plants. They are considered to be the most labile among the above described compounds (Brown et al., 1972; Colombo et al., 1996), particularly, under oxic conditions, where they are degraded more rapidly than under anoxic conditions (Sanger, 1988). The recently developed chlorin index (CI) is based on the reactivity of chlorins (Schubert et al., 2005) and is a tool for the estimation of OM degradation state. It has been applied in marine systems and in the sediments of Lake Zug, but only few comparisons of the DI and the CI are present in the literature (Meckler et al., 2004; Schubert et al., 2005; Niggemann and Schubert, 2006a). In many cases, CI correlates well with DI, but in sediments of Lake Zug no correlation was observed. This was attributed to the different degradation rates of AAs and chlorins where the DI is considered to describe a later stage of degradation than the CI (Meckler et al., 2004).

In this study, we will focus on the distribution of AAs, FAs, and chlorins in the completely anoxic sediments of Lake Lugano. The high productivity and high sedimentation rates result in the burial of increased amounts of sedimentary OM. A comparison of the reactivities of various OM components and the evaluation of the DI and the CI as indicators for the degradation state is performed.

2.3. Materials and methods

2.3.1. Study site

Lake Lugano (Fig. 2.1) is a deep subalpine lake located at the border of Switzerland and Italy (271 m above sea level). It is divided into three sub-basins: the northern and the southern basins separated by an artificial dam, and a smaller basin situated in front of the outlet of the River Tresa. The sampling site was located in the deepest area in the northern basin, which has a volume of 4.69 km^3 and a maximum depth of 288 m (Barbieri and Polli, 1992). Due to its morphology with steep slopes and its geographic position protected against strong winds the lake is meromictic and hosts permanent anoxic conditions below 100 m depth (Vollenweider, 1964; Barbieri and Polli, 1992). Eutrophication, which started in the second part of the last century, led to significantly increased primary productivity and changes in the planktonic community structure. The highest primary productivity (> 400 g C $m^{-2}yr^{-1}$) was reached in the 1980's with a planktonic community dominated by cyanophyceae and diatoms (LSA, 2003). After the introduction of severe policies limiting the phosphate input into the lake and efficient sewage treatment, in the 1990's the primary productivity decreased to 300 g C $m^{-2}yr^{-1}$ and nowadays diatoms and cyanophyceae as well as cyclopoida and cladocera predominate and make up about 75% of the total phytoplanktonic and total zooplanktonic biomass, respectively (Barbieri and Simona, 2001).

The sediment total organic carbon (TOC) concentrations reflect the evolution of the lake showing highest concentrations in the top layer and can reach up to 15% (Span et al., 1992; Hofmann, 1996). Most of the sedimentary organic carbon (> 90%) was shown to originate from autochthonous material, like algal residues, copepod shells and microbial detritus (Niessen, 1987). Turbidity currents which deposit sand and silt on the sediment and which are provoked by the steep slopes of the basin are well recognized by light layers in the sediment cores (Niessen, 1987).

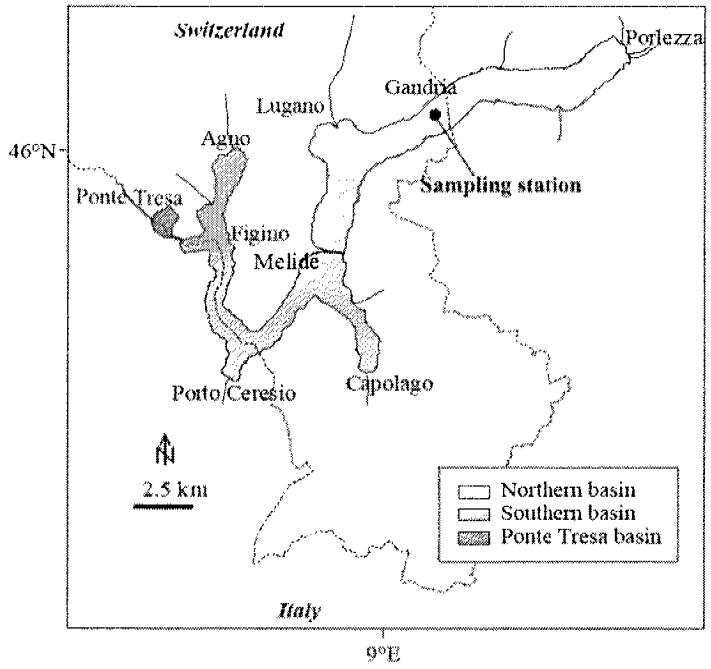


Figure 2.1: Map of Lake Lugano with the sampling station in the northern basin near the village of Gandria.

2.3.2. Sample collection

Sediment samples were collected in March 2004 in the northern basin of Lake Lugano at 286 m depth near the village of Gandria. A sediment core was collected with a gravity corer, sliced on the field in 1 cm-intervals down to 6 cm, at 2 cm-intervals from 6 to 22 cm, and at 3 cm-intervals from 22 to 25 cm, and frozen at -20°C.

2.3.3. Analyses

For supported ^{210}Pb and ^{137}Cs activities, 1-2 g homogenized dry sediments were analyzed on a germanium gamma spectrometer (Canberra) using the line 46.5 keV for ^{210}Pb and 661.7 keV for ^{137}Cs. The precision of the method was 10-15%. Unsupported ^{210}Pb activities were calculated by subtracting the lowest measured activity from the supported activities.

Total carbon (TC) and total nitrogen (TN) concentrations of homogenized sediment samples were measured with a CNS elemental analyzer (Hekatech). The precision of TOC and TN concentrations was 0.2 and 0.3%, respectively. Total inorganic carbon (TIC) concentrations of sediment samples were measured with a CO_2 coulometer (Coulometric Inc., 5011). The precision of TIC measurements was 0.2%. TOC in sediments was calculated as the difference of TC and TIC.

The concentration of total hydrolysable AAs was determined according to the method of Dauwe and Middelburg (1998). Briefly, 50-100 mg freeze-dried and milled sediments were hydrolyzed with 5 mL 6 mol L^{-1} HCl for 24 h at 110°C. The hydrolysates were separated from the sediment by centrifugation and neutralized with 6 mol L^{-1} NaOH. After 1 h the neutralisate was buffered with PO_4^{3-} buffer and amino acids derivatized with ortho-phthaldialdehyde (OPA) in presence of mercaptoethanol (HPLC grade) for 5 min. The fluorescence was then measured on a spectrofluorometer (Cary, Varian) at an excitation wavelength of 340 nm and an emission of 455 nm. A calibration curve was determined with an amino acids standard solution (AA-S-18, Sigma) to quantify the samples. The precision of the AA concentrations was 5%.

The concentrations of 17 single AAs were determined according to the method of Lindroth and Mopper (1979), modified by Cowie and Hedges (1992). 50 to 100 mg freeze-dried and milled sediments were hydrolyzed with 5 mL 6 mol L^{-1} HCl for 24 h at 105°C. The hydrolysates were separated from the sediment by centrifugation and neutralized with 6 mol L^{-1} NaOH. The amino acids were derivatized just before injection with OPA in presence of mercaptoethanol. Separation and detection were achieved on a high-performance liquid chromatograph (Jasco) equipped with a reverse phase column (C18, 150 x 3.9 mm i.d., 4 µm particle size, Waters) and a fluorescence detector at an excitation wavelength of 350 nm and an emission of 460 nm. The eluents (methanol and 40 mmol L^{-1} acetate buffer with 1% tetrahydrofuran) were delivered at a flow rate of 1.5 mL min^{-1} with a gradient with increasing methanol content. The precision of the method lies between 1-8%. Total AA C- and N-normalized yields (AA (%C) and AA (%N)) were calculated from concentrations of single compounds. The DI was determined applying the coefficients determined by Dauwe and Middelburg (1999).

Chlorin concentrations were determined following the method of Schubert et al. (2005). In brief, 5 to 10 mg freeze-dried and milled sediments were extracted three times with 5 mL

acetone in a sonication bath. Acetone was decanted after centrifugation for 10 min at 4°C. Fluorescence was measured on a spectrophotometer (Cary, Varian) at an excitation wavelength of 428 nm and an emission of 671 nm. Phaeophytin for quantification was prepared from fresh chlorophyll *a* that was acidified with 25% HCl. The precision of chlorin concentration determination was 5%. To determine the chlorin index the extracts were acidified with two drops of 25% HCl and measured at the same wavelength. CI was calculated as the ratio between the acidified and the non-acidified sample.

FAs were analyzed using a modified method from Wakeham and Beier (1991). Sediments were extracted three times by sonication with methanol and dichloromethane mixtures with decreasing polarity to obtain the total lipid extracts. Aliquots of the total extracts were saponified after addition of nonadecanoic acid (19:0) as an internal standard. The samples were derivatized with BF_3-MeOH (14%, Sigma) for 2 h at 100°C. After addition of 1 mL nanopure water (previously extracted with dichloromethane), the methyl esters were extracted three times with hexane. Separation was carried out on a gas chromatographic system with a flame ionisation detector (HRGC 5160, Carlo Erba Instruments) equipped with a split-splitless injector and a VF-5ms column (60 m length, 0.25 mm i.d., 0.25 m film thickness, Varian). The carrier gas was H_2 (2.4 mL min^{-1}) and the oven temperature program was initially set at 90°C (1 min), then heated to 140°C at 10°C min^{-1} and to 320°C at 4°C min^{-1}. The final temperature was maintained for 20 min. The injector temperature was 280°C and the detector temperature 320°C. Identification was carried out by comparison of retention times with the standard methylated FA mixtures FAME and BAME (Supelco), and quantification was performed with the internal standard. The precision of the method was 4-8%. Total FA C-normalized yields (FA (%C)) were calculated from C contents of single compounds.

2.4. Results

2.4.1. Age control and bulk organic parameters

2.4.1.1. Sedimentation regime

The sedimentation rate calculated by applying an exponential fit to the unsupported ^{210}Pb activity and excluding turbidite layers that were identified below 18 cm and between 4-5 cm

amounted to 0.5 cm yr^{-1} (Fig. 2.2A, r^2=0.84). A slightly higher sedimentation rate of 0.7 cm yr^{-1} was determined using the ^{137}Cs distribution with the peak caused by the Chernobyl reactor accident clearly recognizable at 12-14 cm (Fig. 2.2B). Both values are in agreement with sedimentation rates calculated in previous studies (Dominik and Span, 1992; Hofmann, 1996). Moreover, the estimation of the sedimentation rate obtained by overlapping the TOC profiles with a core from 1993 (Hofmann, 1996) gave 0.6 cm yr^{-1}, which is in agreement with the values obtained in our measurements.

^{210}Pb activities positively deviating from the exponential fit indicate the presence of younger material mixed within a deeper layer of the sediments (Appleby and Oldfield, 1978). At 4-5 and 6-10 cm the ^{210}Pb activity was higher than the exponential fit suggesting burial of younger material. Layers with lower ^{210}Pb activities than expected by the exponential decay are turbidites. In our core, low ^{210}Pb activities at 4-5 cm and at the bottom (18-25 cm) identified these layers as slumps transported from shallower regions. The steep slopes of Lake Lugano facilitate sediment slides and the formation of turbidites (Niessen, 1987; Sturm, 1990).

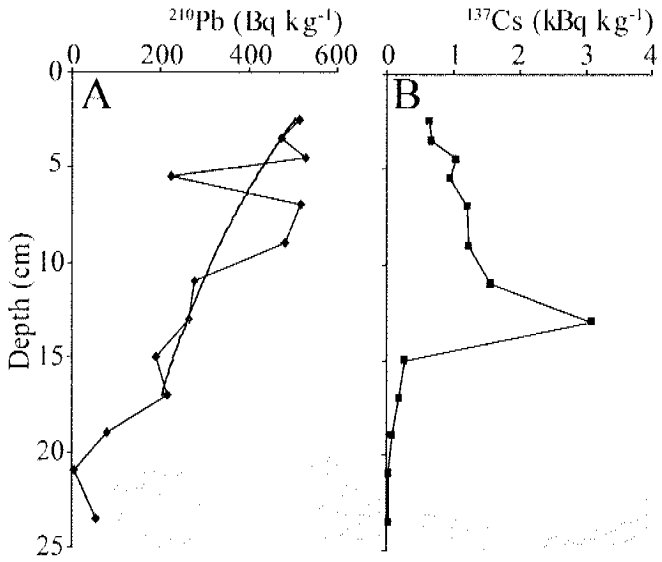


Figure 2.2: A: Unsupported ^{210}Pb activity with exponential fit. B: ^{137}Cs activity. Shading indicates the turbidite layers.

2.4.1.2. Carbon and nitrogen contents

In general, in the top 18 cm, TOC and TN concentrations (Fig. 2.3A) varied from 3.7 to 6.9% and 0.4 to 0.7%, respectively, while in the bottom turbidite layer below 18 cm, TOC and TN concentrations were lower than in the uppermost sediments (1.3-3.0% and 0.2-0.3%, respectively). In the whole sediment core, the molar C/N ratio varied between 9.4 and 12.3, suggesting only minor terrestrial input.

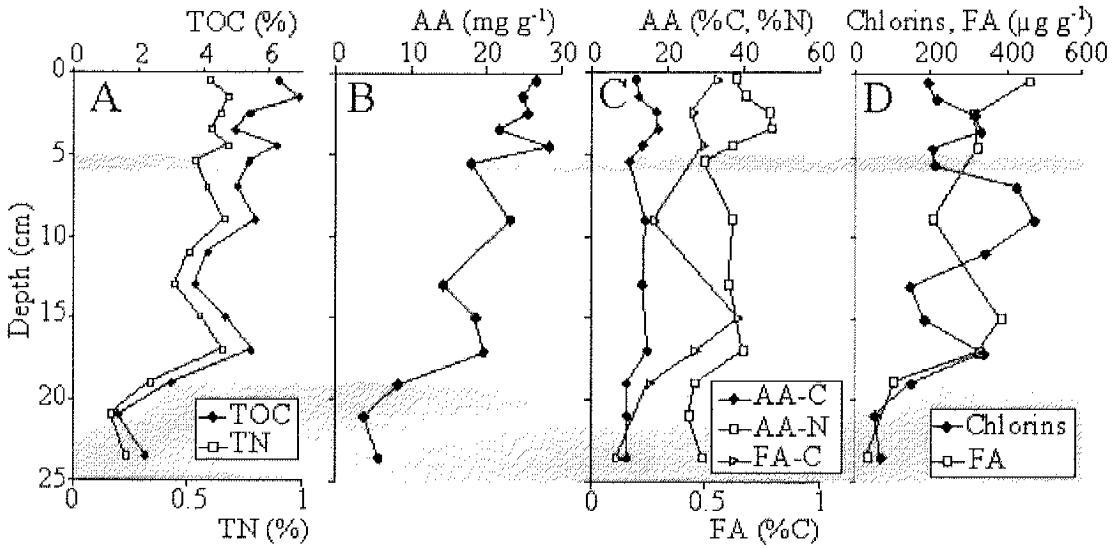


Figure 2.3: A: TOC and TN concentrations in % to dry weight. B: Total hydrolysable amino acid (ΛΛ) concentrations. C: Amino acid C- and N- normalized yields (AA (%C), AA (%N)) and total fatty acids C-normalized yields (FA (%C)). D: Total fatty acid (FA) and chlorin concentrations. Shading indicates the turbidite layers.

2.4.2. Amino acids

AA concentrations varied between 3.8 and 28.2 mg $(g\ dw)^{-1}$ (Fig 2.3B) correlating well with TOC and TN (both r^2=0.91, n=13). AA (%C), and ΛΛ (%N) did not vary strongly with depth (Fig. 2.3C).

The most abundant ΛΛs were aspartic acid (Asp), glycine (Gly), alanine (Ala) and glutamic acid (Glu) all accounting for more than 10 mole% of ΛΛ (Fig. 2.4). In particular, the acidic ΛΛs Asp and Glu had slightly higher concentrations than in other environments (Meckler

et al., 2004; Pantoja et al., 2004; Niggemann and Schubert, 2006a). Most AAs did not show a strong down-core trend. The contribution of the acidic, hydroxy-, and the three neutral AAs valine (Val), triptophane (Trp), and leucine (Leu) slightly decreased in the top 4 cm sediment, while the basic and the non-protein AAs slightly increased. Between 6 and 18 cm AA concentrations were rather constant and in the bottom turbidite phenylalanine (Phe), Lys and methionine (Met) concentrations decreased markedly, while Gly concentrations increased. These slight changes in AAs distributions indicate that there are only few transformations within the sediment and slow degradation. Indeed, DI values were high and decreased only slightly varying between 0.2 and 0.7 in the top 18 cm and between 0 and 0.1 in the bottom of the core (Fig. 2.6A). At 4-5 cm and 6-10 cm increased DI values confirm the intrusion of fresher material as already suggested by the ^{210}Pb activities, while lower values at 5-6 cm and 18-25 cm reflect the presence of turbidite layers with more degraded material.

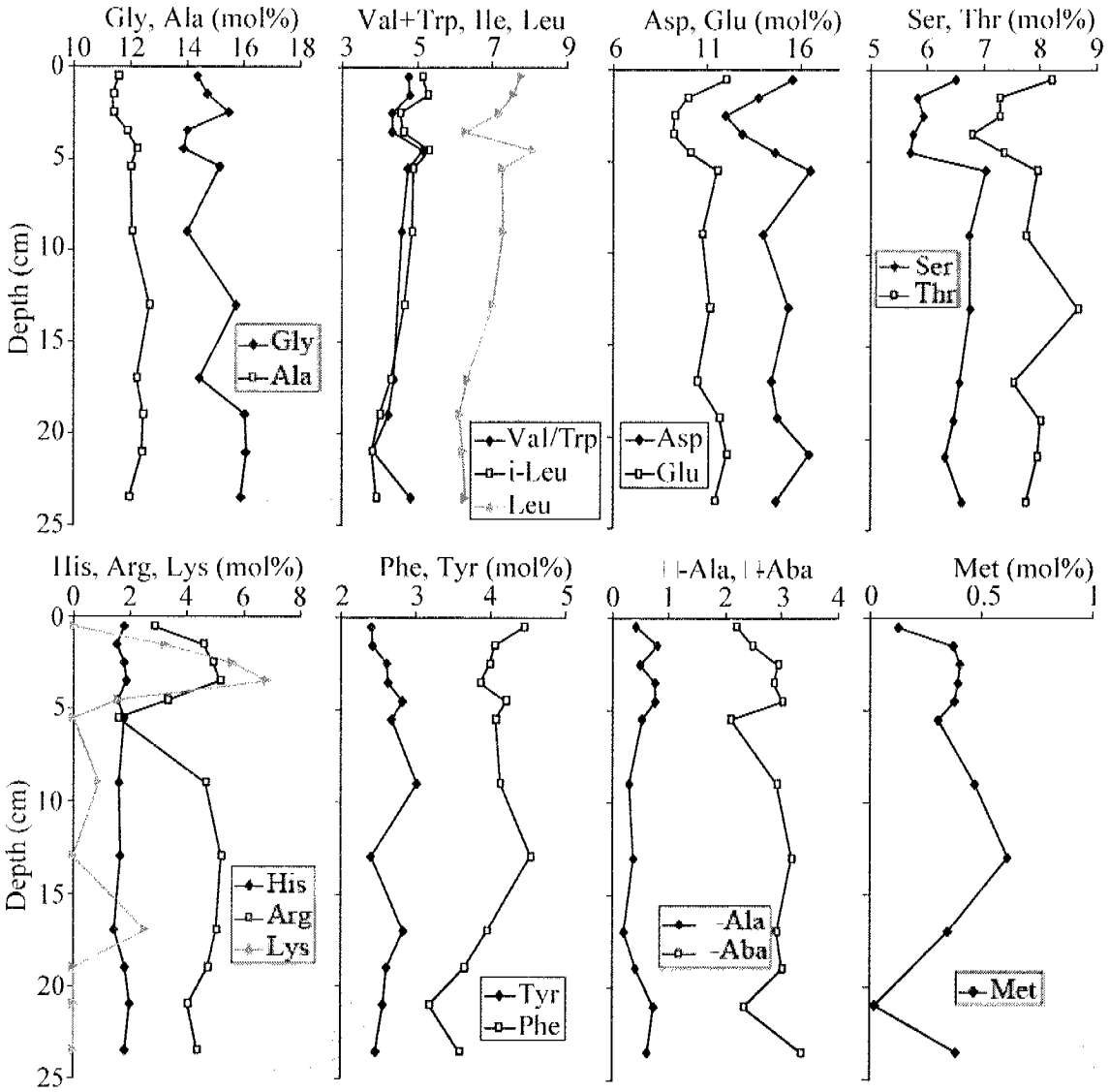


Figure 2.4: Concentrations of single AAs in mole %. Neutral ΛAs: glycine (Gly), alanine (Ala), valine (Val), triptophane (Trp), iso-leucine (Ile), and leucine (Leu). Acidic ΛAs: aspartic acid (Asp) and glutamic acid (Glu). Hydroxylic AAs: serine (Ser) and threonine (Thr). Basic ΛAs: histidine (His), arginine (Arg), and lysine (Lys). Aromatic AAs: phenyalanine (Phe) and tyrosine (Tyr). Non-protein ΛAs: β-alanine (β-Ala) and γ-aminobutyric acid (γ-Aba). Sulfuric ΛA: methionine (Met). Shading indicates the turbidite layers.

2.4.3. Fatty acids

In the top 10 cm total fatty acid (FAs) concentrations decreased with depth, between 14 and 18 cm concentrations were similar to the top sediment, and in the turbidite layer they decreased markedly (Fig. 2.3D). The FA (%C) profile had an analogous trend (Fig. 2.3C).

FAs can be divided into five main groups: mid-chain (MC-FAs), LC-FAs, monounsaturated (MUFAs), PUFAs, and BFAs. MC-FAs comprise chain-lengths from C_{12} to C_{20} and LC-FAs from C_{21} toC_{28}. MUFAs are the sum of $C_{16:1}$, $C_{18:1}$, and $C_{20:1}$, and PUFAs comprise $C_{18:3-2}$, $C_{20:5-2}$, $C_{22:6}$, $C_{22:2}$. For BFA, the sum of the *iso*- and *anteiso*-$C_{15:0}$, the *iso*-$C_{16:0}$, and the *iso*-$C_{17:0}$ was calculated. MC-FAs were the most abundant FAs accounting for 32-53% of FA (Fig. 2.5A). LC-FAs accounted for 25-52% of FA with a peak at 14-16 cm. MUFAs and PUFAs accounted for 3-11% and 3-8% of FA, respectively with the highest contribution at the top of the sediment core. BFAs accounted for 3-9% of FA with highest contributions between 2 and 10 cm. Among PUFAs, we focused on the $C_{20:5}$ and $C_{20:4}$ FAs, as an indicators for OM of diatomic origin (Volkman et al., 1980; Volkman et al., 1989; Dunstan et al., 1994), which is the dominant phytoplankton group in Lake Lugano. Since saturated FAs are less reactive than unsaturated FAs, their profiles are rather constant with depth. Therefore the ratio between the unsaturated $C_{20:x}$ FA and the $C_{20:0}$ FA allows tracking increased inputs of unsaturated compounds. The relative concentrations of the $C_{20:5}$ FA were generally high in the sediment (Fig. 2.5B). In addition, $C_{20:4}$ and $C_{20:2}$ peaked at 16-18 cm with the $C_{20:4}$ having higher concentrations than the $C_{20:0}$ FA.

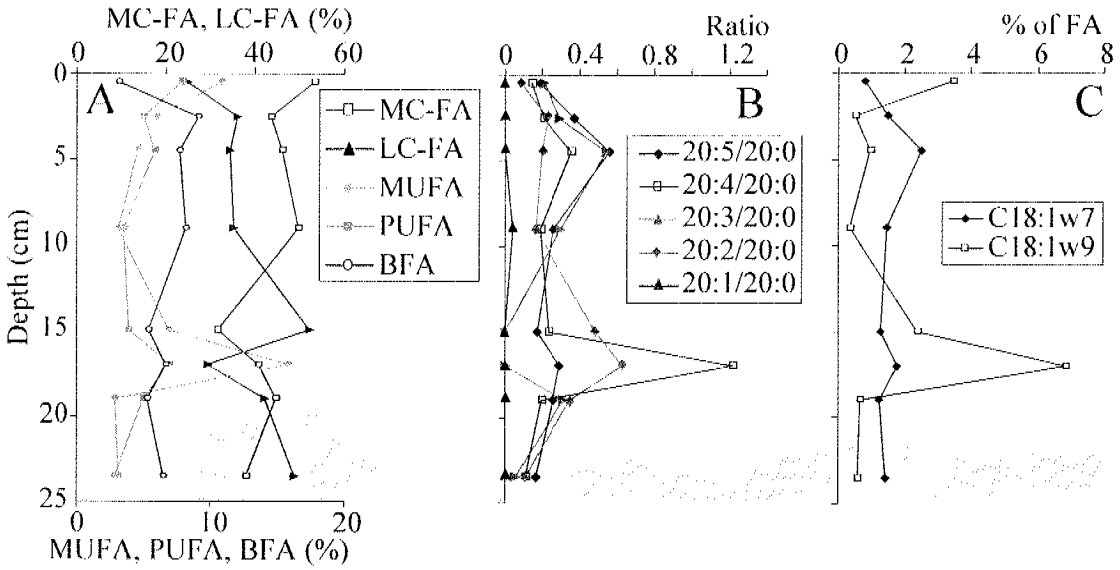


Figure 2.5: A: Contributions to total fatty acids of mid-chain saturated fatty acids (MC-FA), long-chain saturated fatty acids (LC-FA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and branched fatty acids (BFA). B: Ratios of the C_{20} unsaturated FA to the saturated homologue. C: Contributions to total fatty acids (FA) of the fatty acids $C_{18:1w9}$ and $C_{18:1w7}$. Shading indicates the turbidite layers.

2.4.4. Chlorins

Chlorin concentrations varied between 46 and 472 µg $(g\ dw)^{-1}$ with lowest values in the bottom turbidite (Fig. 2.3D). In general, chlorin concentrations were much higher than in Lake Zug where they reached at most 180 µg $(g\ dw)^{-1}$ (Meckler et al., 2004). Moreover, in Lake Zug a clear decreasing trend was observed, while in Lake Lugano there was no clear depth trend.

CIs varied from 0.6 to 0.9 (Fig. 2.6B). The lowest values were from 6 to 12 cm indicating the presence fresh material. The highest values were in the top sediment, at 14-16 cm, and at 20-22 cm and represent degraded material.

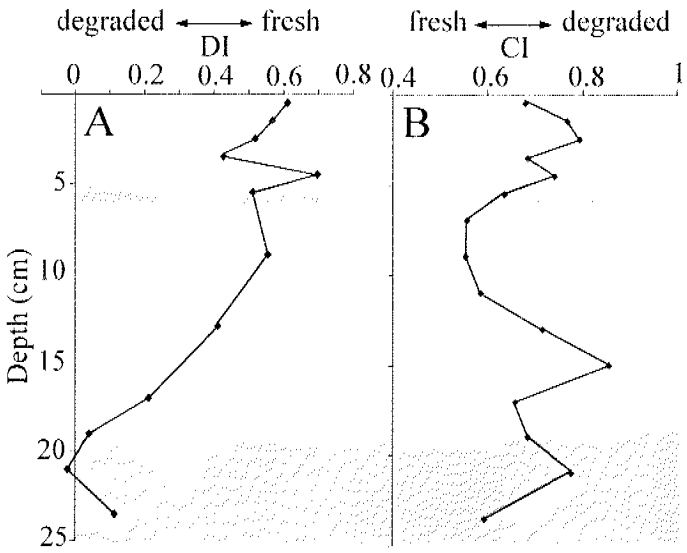


Figure 2.6: A: Degradation indices (DI). B: Chlorin indices (CI). Shading indicates the turbidite layers.

2.5. Discussion

2.5.1. Autochthonous and allochthonous sources of OM

FAs like LC-FAs and the $C_{20:5}$ and $C_{20:4}$ FAs, are in high abundance in specific organisms and can be used as indicators of OM sources in sediments.

Land plants are enriched in LC-FAs and are traditionally used as indicators for terrigenous input in lacustrine systems (Meyers and Ishiwatari, 1993). However, LC-FAs are not exclusively produced by land plants, but also in small quantities by microbes (Gong and Hollander, 1997). Different LC-FA to TOC ratios were reported for riverine and marine samples. In riverine material with an increased terrestrial input, the LC-FAs to TOC ratios was higher (1.5-2.0‰) than in marine sediments (< 1.0‰), where the most important LC-FA source are microbes (Naraoka and Ishiwatari, 2000). Therefore an average ratio of 2.3‰ in Lake Lugano suggests a major terrestrial source of LC-FAs. In Lake Lugano, LC-FAs contributions to FA (Fig. 2.5A) are highest at 14-16 cm and in the turbidite layer, suggesting an increased input of terrestrial debris in these layers. Although, a previous work based on microscope analyses of sediments concluded that the overall contribution by terrestrial OM in sediments of Lake Lugano is lower than 10%

(Niessen, 1987), the regional climatic conditions with heavy rains alternated with dry periods facilitate the periodical input of terrigenous material (Niessen, 1987; Hofmann, 1996). Moreover, during these occasions, the material deposited in deltaic regions, which have a strong terrestrial influence, can be mobilized and transported to deeper regions. The analysis of hydrological data indicated higher average annual flow rates in 1983 and 1984 than in the previous and later years (data from the Federal Office for the Environment). These might have transported more terrestrial material into the water column and the sediments. However, further flood-events occurred in later years which are not reflected in the LC-FAs composition of the sediment core. Therefore we suggest that most likely a combination of a flood-event and transport of deltaic material to deeper waters led to an increased amount of terrestrial material in the sediment.

An enrichment of $C_{20:5}$ and $C_{20:4}$ in sediments is an indicator of the presence of diatom remnants (Volkman et al., 1989; Dunstan et al., 1994; Volkman et al., 1998). In Lake Lugano, relative concentrations of the PUFAs $C_{20:5}$, and $C_{20:4}$ were particularly high (Fig 2.5B) at 4-5 cm and 16-18 cm depth. In fact, in the years from 1983 to 1986 (which correspond to 16-18 cm), biomass production was two times higher than after 1989 and diatoms, the second most abundant phytoplanktonic community, contributed to about 30% of total phytoplanktonic biomass before and about 20% after 1989 (LSA, 2003). Previous microscope analyses described intact diatom remnants in recent sediment samples (Niessen, 1987; Hofmann, 1996). Indeed, diatom frustules are known to build fast sedimenting aggregates which settle within a few weeks, (Hofmann, 1996; M. Simona, pers. comm.). In addition to the $C_{20:4}$ FA, at 16-18 cm also the MUFAs showed higher concentrations (Fig. 2.5A) dominated by the $C_{18:1\omega9}$ FA (Fig. 2.5C) and the $C_{16:1}$ FA. These MUFAs are more enriched in algae than in bacteria (Volkman et al., 1980; Camacho-Ibar et al., 2003), confirming an increased algal input to this layer.

In spite of high $C_{20:5}$ FA concentrations at 4-5 cm, in the period from 1996 to 1998, no particular abundance of diatoms was reported (LSA, 2003), suggesting that an increased production of diatoms is not the only reason for the presence of the $C_{20:5}$ FA in sediments. At 4-5 cm depth, the ^{210}Pb profile indicated input of younger material that was quickly buried. The fast burial probably hindered the degradation of algal material at the water-sediment interface and allowed accumulation of higher amounts of fresh unsaturated FAs than in other layers. This suggests that the degradation capacity in the top sediments is higher than in deeper sediments.

Similarly, in the anoxic sediments of the Black Sea, labile compounds were degraded in the top 20 mm (Sun and Wakeham, 1994).

In summary, the FA biomarkers indicate the presence of terrestrial and algal material in different layers of the sediments. The variability of their distribution reflects the irregularity of the sedimentation regime, which results in having an important influence on the abundance and conservation of OM in deeper sediments. Moreover, most OM transformation occurs in the top sediment; therefore the residence time in this layer has an influence on accumulation of labile compounds.

2.5.2. Indicators of bacterial OM

The relative amount of bacterial OM can be described with the concentrations of BFAs and the $C_{18:1\omega7}$ FA (Volkman et al., 1980; Volkman et al., 1989; Kaneda, 1991). These compounds are of prokaryotic origin and their abundance in sediments hint to bacterial activity and a possible increased degradation of OM.

BFAs are enriched in bacteria and their concentration usually increases with sediment depth and increased bacterial activity (Cranwell, 1973; Kaneda, 1991). In Lake Lugano the concentrations of BFAs are in the lower range of concentrations reported from other sediments (Matsuda and Koyama, 1977; Volkman et al., 1980; Goossens et al., 1989; Meyers and Ishiwatari, 1993; Wakeham et al., 1997a; Niggemann and Schubert, 2006a; Fig. 2.5A). From the surface to 2 cm, the relative amount of BFAs increases suggesting production of bacterial FAs in this layer in concert with OM degradation.

The bacterial FA $C_{18:1\omega7}$ (Fig 2.5C) follows a similar trend as the BFAs and is only present in low concentrations. Consequently, in Lake Lugano sediments, bacterially derived material is a minor component of OM. This is consistent with the high contributions of algal material described in the previous section.

2.5.3. Indicators of OM degradation state

We applied different indicators for OM degradation state: the abundance of the non-protein AAs, β-alanine and γ-aminobutyric acid (β-Ala and γ-Aba; Cowie and Hedges, 1992), the DI, and the CI.

β-Ala and γ-Aba are produced during AA degradation processes and are rather refractive; therefore their concentrations typically increase as diagenesis progresses (Cowie and Hedges, 1994; Keil et al., 2000). In Lake Lugano, the concentrations of these AAs are very low (Fig. 2.4), suggesting an early stage of OM decay, during which this parameter is comparatively insensitive (Cowie and Hedges, 1994; Keil et al., 2000). γ-Aba, which is the degradation product of Glu, has higher concentrations than β-Ala, the degradation product of Asp (Cowie and Hedges, 1994; Dauwe and Middelburg, 1998; Meckler et al., 2004). Since Glu concentrations are lower than Asp concentrations, either Glu has been degraded more efficiently than Asp, or the observed pattern reflects AA distribution of the sources. To our knowledge, no evidence exists for preferential degradation of Glu in anoxic environments. Moreover, other pathways of γ-Aba production might contribute to the high concentrations as has been previously speculated (Lee et al., 2000). Therefore we suggest that the observed Glu and Asp concentrations reflect the AA distribution of the sources.

The DI (Dauwe and Middelburg, 1998; Dauwe et al., 1999) is based on amino acid compositions, while the CI describes pigment reactivity (Schubert et al., 2005). Based on the DI, OM in the top 18 cm is very fresh with decreasing freshness towards deeper sediment layers. The bottom turbidite layer clearly contains material that is more degraded (Fig. 2.6A) in agreement with the above mentioned terrestrial input due to slumps. In general, in Lake Lugano DIs are higher than in Lake Zug and most marine sediments, but still lower than in sediment trap material and fresh plankton (Dauwe et al., 1999; Amon et al., 2001; Meckler et al., 2004; Niggemann and Schubert, 2006a). This suggests that rather fresh and well preserved OM is deposited.

In contrast to the DIs, the CIs are highly variable within the sediment (Fig. 2.6B) hinting to very fresh and highly degraded OM in different sediment layers. The CI varies between 0.2 for chlorophyll and 1 for highly degraded material and correlates with the DI in several marine systems (Schubert et al., 2005). However, in Lake Zug it has already been observed that DI and CI did not correlate, even though they showed a similar trend. This discrepancy was attributed to

the different sources and reactivities of AAs and chlorins (Meckler et al., 2004). However, in Lake Lugano the high disagreement between CI and DI trends suggests that the difference in reactivity can not be the only reason for the lack of correlation.

The CIs were particularly low at 6-10 cm where ^{210}Pb profiles indicated input of younger material suggesting that material that was quickly buried is fresher. As a result, the CI appears to be influenced by the residence time at the water-sediment interface, which is the layer where most degradation processes occur (Sun and Wakeham, 1994). This suggests that chlorins are better degraded in the top sediments than in deeper sediments. Moreover, the CIs were low at 16-18 cm in conjunction to the presence of increased amounts of algal FAs markers indicating an influence by algal input. When more algal material is buried, chlorins are less degraded resulting in lower CIs. This suggests that chlorins derived from algae are barely degraded in the water column and CIs remain very low when they reach the sediments. This might be related to protection of chlorins by algal cell walls.

High CIs were found at 14-16 cm in conjunction to high LC-FA concentrations, which indicate increased terrigenous input. In fact, there is a slight correlation between CI and LC-FA concentrations (Fig. 2.7A, $r^2 = 0.64$). Actually, chlorins originating from land plants have a longer way to the sediments, passing through soils and oxygenated rivers before reaching the lake. This results in a higher exposure time to oxic conditions where chlorin degradation has been shown to be more effective (Sanger and Gorham, 1970; Sanger, 1988). Therefore material transported from rivers contains highly degraded pigments and increases the CI of sedimentary organic matter.

These observations point out that the CI can be strongly influenced by the sedimentation regime and input of terrestrial OM and, therefore, it describes not only the autochthonous, but also the allochthonous sources. In contrast, the DI is less source dependent (Dauwe and Middelburg, 1998; Keil et al., 2000) and can not give information about sources and deposition conditions.

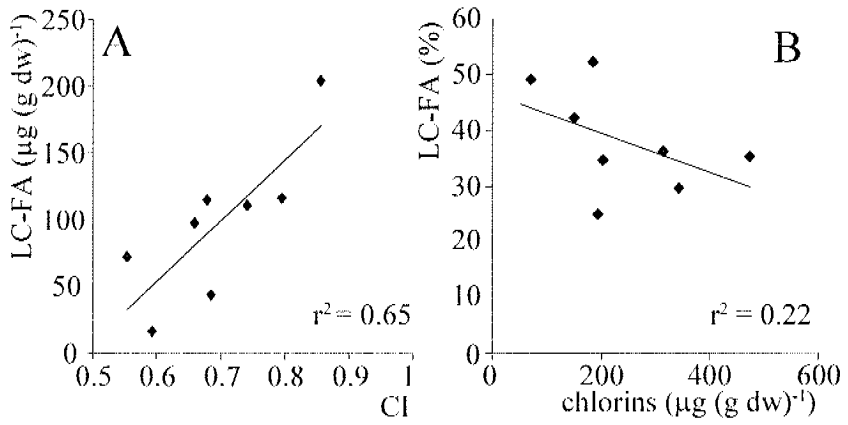


Figure 2.7: A: Long-chain fatty acid concentrations (LC-FA) versus chlorin indices (CI). B: LC-FA contribution to total fatty acids versus chlorins concentrations.

2.5.4. Different reactivity of OM components

We estimated the reactivity of OM components comparing the downcore trends of TOC, TN, AAs, FAs, and chlorins. Since the sedimentation regime and the OM input appeared to be highly variable, a calculation of the degradation rate constant was not reasonable. TOC and TN concentrations showed remarkably low decreases with sediment depth. Similar trends have been observed in completely anoxic sediments (Meyers and Ishiwatari, 1993; Teodoru et al., 2007), but in a seasonally anoxic environment like Lake Zug the down-core decrease in TOC and TN was greater (Meckler et al., 2004). Accordingly, OM in sediments of Lake Lugano appears to be degraded very slowly. However, overlapping the TOC profile with a core from 1993 the concentration in the same layers decreased by up to 3% (Hofmann, 1996) pointing to active OM degradation in the last 20 years.

Indeed, depth profiles of single OM components showed significant decreases indicating degradation of labile compounds. In particular, FA (%C) decreased with depth (Fig. 2.3C) suggesting preferential degradation of FAs in respect to TOC. The strongest decrease in concentration was observed in the MUFAs and the PUFAs (Fig 2.5A) indicating that these are the most labile compounds in Lake Lugano sediments. Among FAs, the less reactive compounds clearly were the LC-FAs, while MC-FAs slightly decreased with depth. In several previous works similar reactivities of FAs have been reported (e. g., Meyers and Ishiwatari, 1993;

Wakeham et al., 1997b). In contrast, AAs, which are considered to be rather labile (e.g., Lee and Cronin, 1982; Keil et al., 2000), are only slightly degraded within the sediments of Lake Lugano (Fig. 2.3B), indicating that they are more resistant to degradation than most FAs. This is consistent with previous observations (Wakeham et al., 1997b).

The most unexpected reactivity is the one of chlorins which did not show a significant decrease with depth (Fig. 2.3D). Therefore they appear to be more resistant to degradation than the other analyzed components, while in earlier studies, chlorins were clearly the most reactive compounds (Brown et al., 1972; Colombo et al., 1996; Meckler et al., 2004). In comparative studies of anoxic and oxic degradation, anoxia affected chlorin degradation very strongly. In fact, under anoxic conditions, chlorins were not completely degraded (Sun et al., 1993; Ding and Sun, 2005). Chlorin preservation is promoted by suboxic to anoxic conditions in the water column especially during periods of high productivity, by high sedimentation rates which quickly bury the detritus, and by low light conditions (Sanger, 1988; Shankle et al., 2002), which are all characteristics of the water column of Lake Lugano. Moreover, chlorin concentrations and degradation state can be influenced by sedimentation of terrestrial material. However, there is only a slight negative correlation between LC-FA (% of FA) and chlorin concentrations (Fig. 2.7B, r^2=0.22), suggesting that, in contrast to chlorin degradation state, chlorin concentrations are only slightly influenced by terrestrial material.

In the previous section, we observed that the input of fast buried younger material to the sediments has an influence on the CI. In the same layers, in particular at 6-10 cm, chlorin concentrations are higher than in the overlaying sediments (Fig. 2.3D). This indicates that the fast input of younger material facilitates accumulation of these organic compounds. Moreover, the increased input of algal material at 16-18 cm is also reflected in higher chlorin concentrations. These observations suggest that faster burial of chlorin-rich material enhances its preservation. Hence, variable sedimentary conditions appear to have an important role in preservation of particular organic compounds in anoxic lake sediments.

In conclusion, in sediments of Lake Lugano, MUFAs and PUFAs are the most labile compounds followed by AAs, MC-FAs, and LC-FAs, while chlorin concentrations and degradation state are highly dependent on different sources and residence time at the water-sediment interface. Consequently, preservation of organic compounds in lacustrine sediments appears to be strongly dependent on variations in the sedimentation regime.

3

AMINO SUGAR DISTRIBUTION IN FRESHWATER SYSTEMS: INSIGHTS FROM LAKE LUGANO

Lucia Klauser and Carsten J. Schubert

3.1. Abstract

Although amino sugars are a significant source of carbon and nitrogen in aquatic systems, only a few studies focused on their transformation and reactivity. Here, we discuss amino sugar distribution in the water column and sediments of a freshwater lake. Lake Lugano represents an appropriate environment to investigate carbon accumulation in lacustrine systems, since it is highly productive and permanently anoxic. The amino sugars, glucosamine, galactosamine, mannosamine and muramic acid were analyzed in sediments, and in the dissolved and particulate fractions of the water column. In sediments, amino sugar concentrations were in the range of other environments and increased with depth, except for muramic acid, which showed the opposite trend. The accumulation of amino sugars in respect to organic carbon in the core was attributed to the input of relatively refractory organic matter, which is most likely of terrigenous origin. In particular, mannosamine concentrations in the sediments correlated with long-chain fatty acids originating from terrestrial material. Further, we revealed that amino sugars are more refractive than amino acids and fatty acids. In the water column, high concentrations of muramic acid indicated a major contribution by bacterial remnants, in particular in the dissolved fraction. Therefore, extraordinarily high mannosamine concentrations in the water column might have an important bacterial origin. Analyses of the ratio between glucosamine and galactosamine

suggested that the reactivity of amino sugars in the dissolved fraction is lower than in the particulate fraction.

3.2. Introduction

Amino sugars (ASs) represent an important source of organic carbon and nitrogen in aquatic systems. In marine environments, up to 0.6% of the dissolved organic carbon in water (Benner, 2002) and up to 2.4% of the total organic carbon (TOC) in sediments is composed of ASs (Niggemann and Schubert, 2006b). Their contribution to organic nitrogen is even higher reaching up to 1.7% of dissolved organic nitrogen in seawater (Benner, 2002), 3.8% of total nitrogen (TN) in marine sediments (Niggemann and Schubert, 2006b), and 25% of TN in freshwater sediments (Kemp and Mudrochova, 1973). ASs largely occur in the form of biopolymers in all organisms and are a significant component of dead organic matter (OM). The most common ASs are glucosamine (GlcN), galactosamine (GalN), mannosamine (ManN) and muramic acid (Mur). GlcN is the deacetylated monomer of chitin, which is the second most abundant biopolymer on earth following cellulose (Cohen, 1987). Together with Mur, GlcN is also a component of peptidoglycan, which is a component of bacterial cell walls (Brock et al., 1994). Since Mur is only present in peptidoglycan, it is a specific indicator for this biopolymer. The second most abundant AS is GalN, the monomer of chondroitin, a polymer present in cartilaginous material (Sharon, 1965). The less common ManN has been found in polysaccharides and glycolipids of bacteria and algae (Sharon, 1965; Yoneyama et al., 1982; Komandrova et al., 2001; Nicolaus et al., 2002; Benner and Kaiser, 2003).

ASs have been studied in detail in soils where they were used as indicators for microbial biomass production and OM degradation state, (Martens and Frankenberger, 1991; Zhang and Amelung, 1996; Coelho et al., 1997; Amelung, 2001; Turrion et al., 2002; Glaser et al., 2004). In aquatic environments, AS studies have focused on total AS concentrations, or only on the most abundant ASs, GlcN and GalN (Degens and Mopper, 1975; Ittekkot et al., 1984a; Ittekkot et al., 1984b; Müller et al., 1986; Liebezeit, 1993; Nedoma et al., 1994; Dauwe and Middelburg, 1998; Jennerjahn and Ittekkot, 1999; Gupta and Kawahata, 2000; Davis and Benner, 2005), while only a few works describe the distribution of Mur and/or ManN (Moriarty, 1977; Mimura and Romano, 1985; Benner and Kaiser, 2003; Niggemann and Schubert, 2006b). These authors

provide different insights to the sources and fate of ASs. In some studies, Mur concentrations correlated with bacterial abundances and were used to derive numbers of living bacteria, suggesting rapid decomposition of peptidoglycan (Moriarty, 1977; Mimura and Romano, 1985). In contrast, in other studies of marine dissolved OM (DOM), a substantial detrital contribution to ASs was observed (Liebezeit, 1993; Benner and Kaiser, 2003; Niggemann and Schubert, 2006b). Further investigations indicated a preferential preservation of chitinaceous material in sediment trap material and in sediments, and attributed this to a higher resistance of AS polymers to degradation (Degens and Mopper, 1975; Müller et al., 1986; Dauwe and Middelburg, 1998).

The studies describing AS concentrations in freshwater all focused on GlcN and GalN (Kemp and Mudrochova, 1972; Kemp and Mudrochova, 1973; Dungworth et al., 1977; Chudoba et al., 1986; Nedoma et al., 1994). In Lake Ontario sediments, ASs accumulated with depth and accounted for up to 25% of sedimentary nitrogen at 7.5 m (Kemp and Mudrochova, 1972; Kemp and Mudrochova, 1973). This high content was attributed to the large input especially from zooplankton in the form of chitin and its higher resistance to degradation in comparison to amino acid-polymers. On the other hand, Dungworth et al. (1977), who analyzed the same core as Kemp and Mudrochova (1972), determined 100 times lower GalN and GlcN concentration and observed a larger decrease with depth than for amino acids, suggesting that ASs are more prone to degradation. These very contrasting results were attributed to the lack of standardization of extraction procedures and the lack of detailed studies on AS-containing polymers degradation processes (Dungworth et al., 1977). Dungworth et al. (1977) hydrolyzed the sediment samples with much stronger acid than Kemp and Mudrochova (1972) so that an important fraction of ASs was probably not accounted for in that study.

All these publications indicate that there is an urgent need for investigations on AS distribution, degradation, accumulation, and sources in aquatic systems. In this work, we have chosen to examine the water column and the sediments of an environment, which has slow degradation processes. Therefore, we opted for Lake Lugano, an anoxic lacustrine environment with high primary production and carbon accumulation in the sediments.

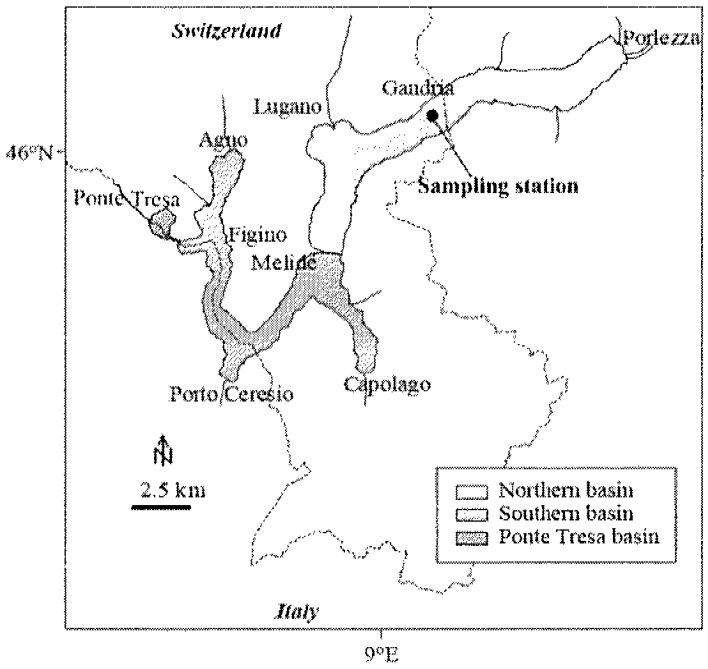


Figure 3.1: Map of Lake Lugano with the sampling station in the Northern basin offshore from the village of Gandria.

Lake Lugano is a deep subalpine lake located at the border of Switzerland and Italy (Fig. 3.1, 46°00' N, 3°30' E, 271 m above sea level). The sampling site was chosen in the deepest area in the northern basin (286 m). The morphology with steep slopes and the geographic location, which protects against strong winds, ensure an oligomictic to meromictic regime. Eutrophication, which started in the second half of the last century, has reduced the mixing layer from 200 m to 100 m water depth (Vollenweider, 1964). The predominant organisms in the water column are diatoms and cyanobacteria, making up about 75% of the total phytoplanktonic biomass, and cyclopoida and cladocera, accounting for 75% of zooplanktonic biomass (Barbieri and Simona, 2001; LSA, 2003). The sediments reflect the evolution of the lake and are characterized by high TOC concentrations of up to 6.9% (this work) in the uppermost layers. Turbidity currents, that deposit sand and silt on the sediment, are provoked by sediment slides on the steep slopes of the basin. These are easily recognizable by homogeneous layers along the sediment core. In the sediments analyzed in this study there are two turbidite layers at 5-6 cm and 18-25 cm. In the upper part of the sediments, at 4-5 cm and 6-10 cm, sedimentary material was

mixed, burying younger OM in more profound layers. These characteristics, in addition to high sedimentation rates (0.6 cm yr^{-1}) and anoxia are the main causes for a low organic matter degradation state in the uppermost sediment. In contrast, in the bottom turbidite layer, Dauwe degradation indices reflect more enhanced degradation of OM (Klauser and Schubert, Chapter 2).

In this study, we aim to evaluate AS degradation in respect to other OM components and their contribution to carbon accumulation in lake sediments. In addition, we examine possible sources of ASs in the water column and in sediments of highly productive lakes.

3.3. Material and methods

3.3.1. Sample collection

Water and sediment samples were collected in March 2004 (spring samples) in the northern basin of Lake Lugano at a 286 m deep station near the village of Gandria (Fig. 3.1). Additional water samples were taken in September 2005 (fall samples). The location off Gandria was chosen, because monitoring data has been available for the last 30 years and permanent anoxic conditions have been described (LSA, 2003).

Profiles of conductivity, temperature, and depth were measured with a CTD profiler (Ocean seven 501, Hydronaut). For AS analysis, 50 mL unfiltered water samples were collected with Niskin bottles, poisoned with 2 drops of saturated $HgCl_2$ solution and stored at 4°C. For bacterial counts, 25 mL samples were poisoned with 0.5 mL formaldehyde solution (36.5%) saturated with borax buffer, transported to the laboratory and filtered on the same day. For TOC analysis, 15 mL samples were collected in acid-rinsed polypropylene-tubes, acidified with 37% HCl to pH~3, and stored at 4°C until measurement.

Water (20-80 L) was filtered with an in-situ pump (McLane) using pre-combusted glass fiber filters (GFFs) with a nominal pore size of 0.7 µm (Schleicher & Schuell). The filters were frozen immediately after sampling. We chose a nominal size of 0.7 µm since 0.2 µm filters clog up very quickly, and the amount of POM needed for our analysis would not be sufficient.

For AS and TOC analysis, a sediment core was retrieved with a gravity corer, sliced on the boat in 1 cm-intervals from the surface to 6 cm and 2 cm-intervals from 6 to 25 cm, transferred into glass vials, and frozen at -20°C.

3.3.2. Analytical methods

For standard solutions, D-(+)-glucosamine hydrochloride, D-(+)-mannosamine hydrochloride, and muramic acid were obtained from Sigma Chemicals, and D-(+)-galactosamine hydrochloride from Fluka. 3-Acetamido-3-deoxy-D-glucose was obtained from TRC. For storage, preparation and separation of samples, hydrochloric acid (37%), ammonium hydroxide (27%), sodium acetate and sodium chloride, formaldehyde (36.5%), 4',6-diamidino-2-phenyl-indol-dihydrochloride (DAPI) and $HgCl_2$ were obtained from Fluka. Carbonate-poor sodium hydroxide solution (50% w/w) was obtained from J. T. Backer. Chitin in the form of crab shell flakes was purchased from Sigma. AG 50W X8 (100-200 mesh and 200-400 mesh) resins and AG11 A8 (50-100 mesh) resin were purchased from Bio-Rad. Deionized water was obtained from a Nanopure ultrafiltration system (Skan AG). The internal standard, 3-amino-3-deoxyglucose hydrochloride (Glc3N), was prepared from 3-acetamido-3-deoxy-D-glucose (Kaiser and Benner, 2000).

AS were analyzed according to the method developed by Kaiser and Benner (2000) slightly adjusted for the purpose of this study. Unfiltered water samples were hydrolyzed with 3 mol L^{-1} HCl at 100°C for 5 hours. Sediment samples and organism-samples (zooplankton, *Stephanodiscus minutulus*, *Fragilaria crotonensis*) were freeze dried, milled, and hydrolyzed at 100°C with 3 mol L^{-1} HCl for 5 hours and with 6 mol L^{-1} HCl for 2, 5 and 10 hours. For particulate AS (PAS) analysis, 1/8 slices of filters were hydrolyzed at 100°C with 3 mol L^{-1} HCl for 5 hours and with 6 mol L^{-1} HCl for 2, 5 and 10 hours. After hydrolysis the sediment and filter samples were centrifuged for 10 minutes, the liquid was decanted with a pipette and 1 mL water was mixed with the remaining sediment. After 10 min sonication and 10 min centrifugation the decanted water was added to the first part of the sample. Filters were additionally squeezed in a syringe. In sediments, hydrolysis with 6 mol L^{-1} HCl for 5 hours yielded highest recoveries for GlcN, GalN, and ManN and 3 mol L^{-1} HCl for 5 hours gave best results for Mur. Therefore, sediment samples were hydrolyzed separately for GlcN, GalN, and ManN and for Mur. For PAS, highest recoveries for all ASs were obtained with 6 mol L^{-1} HCl after 10 hours. After hydrolysis and centrifugation, all samples were neutralized and desalted with cation exchange resins. Separation and quantification was performed using a metal-free chromatography system (Jasco) equipped with a PAD ED50 detector (Dionex) with a gold working electrode, and a pH Ag/AgCl

reference electrode on a Dionex CarboPac PA1 column (250 mm x 4 mm i.d.) with a CarboPac PA1 guard column (50 mm x 4 mm i.d.). The following detector waveform for pulsed integrated amperometry was used: E_{DET}=0.10 V (t_{DEL}= 200 ms, t_{INT}=200 ms), E_{RED}=-2 V (t_{RED}=10 ms), E_{OX}= 0.6 V (t_{OX}=0 ms), and E_{END}=-0.1 V (t_{END}=60 ms). GalN, GlcN, and ManN were separated with 12 mmol L^{-1} NaOH under isocratic conditions; Mur was separated with 99.6 mmol L^{-1} NaOH and 100 mmol L^{-1} sodium acetate with isocratic conditions with a flow rate of 1 mL min^{-1}. ASs were quantified in relation to the internal standard with a calibration curve. The precision of the method was 3-8% for GlcN, GalN, and ManN and 17% for Mur. The detection limit of water samples after cleanup was 10 nmol L^{-1} for GlcN, GalN and ManN, and 30 nmol L^{-1} for Mur.

TOC concentrations in water samples were determined by high temperature catalytic oxidation with a Shimadzu 5050A analyzer (Benner and Strom, 1993). Total carbon (TC) and TN concentrations in sediment samples were measured with a CNS elemental analyzer (Hekatech). Total inorganic carbon (TIC) concentrations of sediment samples were measured with a CO_2 coulometer (Coulometric Inc., 5011). TOC was calculated as the difference between TC and TIC.

Total AS C- and N-normalized yields (AS (%C) and AS (%N)) were calculated from non-acetylated ASs, which is the form measured after hydrolysis. However, many natural forms of ASs are acetylated and have consequently two more carbon atoms. Therefore our calculations of AS (%C) are potentially underestimated by up to 25%.

For bacterial abundances, water sample aliquots of 1 and 5 mL were gently vacuum-filtered through polycarbonate filters with 0.2 µm pore size and 25 mm diameter (Millipore). The filters were placed in clean petri dishes, sealed, and stored frozen at -20°C. Filters were stained with 20 µL of a 5 mg L^{-1} DAPI-solution for 15 minutes in the dark and then washed gently with sterile water (Porter and Feig, 1980). After drying, the filters were placed on a slide and mounted in Citifluor solution for counting on an epifluorescence microscope (Axiscope HBO50, 1000x magnification, Zeiss). At least 300 cells per filter were counted.

Sediment bacteria were stained following the method of Hahn et al. (1992). Sediments were fixed with a formaldehyde buffer for 3-16 hours at 4°C, and then washed with buffer and ethanol. The cell suspensions were applied onto Teflon-coated slides prepared with gelatine and dried. Afterwards the slides were covered with 10 µL of a 0.0001% DAPI buffer-solution, incubated for 10 minutes in the dark, and rinsed with water. After drying, the preparations were

mounted in Citifluor solution for counting on an epifluorescence microscope (Axiscope HBO50, 1000x magnification, Zeiss). At least 300 cells per filter were counted.

3.4. Results

3.4.1. Sediment characteristics

The characteristics of the sediment core analyzed in this study have been discussed in greater detail elsewhere (Klauser and Schubert, Chapter 2). In brief, we summarize the characteristics that are important for this study. Two turbidite layers were identified: one at 5-6 cm and the other at 18-25 cm. From 4-5 and 6-10 cm, the presence of younger material is reflected in slightly higher concentrations of TOC and TN (Fig 3.2A). The Dauwe degradation index (DI) indicated a high freshness of OM along the sediment core, except in the bottom turbidite layer (Fig 3.2B). Bacterial abundances were determined down to 18 cm and varied only slightly with values between 2.3×10^8 and 4.5×10^8 cells mL^{-1}. The highest cell density was in the top 4 cm (data not shown).

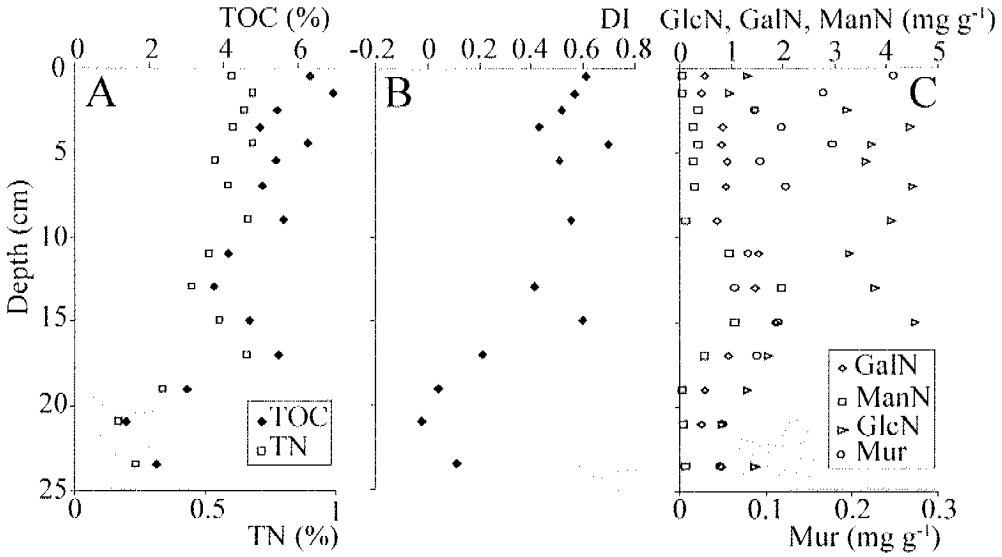


Figure 3.2: A: Total organic carbon (TOC) concentrations, total nitrogen (TN) concentrations in sediments. B: Dauwe degradation index (DI) in sediments. C: Single amino sugar concentrations in sediments. Shading indicates the bottom turbidite layer.

AS concentrations (AS) were lowest in the bottom turbidite layer, while in the top 18 cm, AS concentrations were higher and generally increased with sediment depth (Fig. 3.2C) with the exception of Mur, which decreased with sediment depth. AS (%C) varied between 0.9 and 7.8% and AS (%N) varied between 1.8 and 12.8% (Fig. 3.3A). AS (%C) are similar to values obtained from Lake Ontario (4-7%, Kemp and Mudrochova, 1973) and AS (%N) are slightly lower than in Lake Ontario (5-25%, Kemp and Mudrochova, 1972). Both yields are higher than in marine sediments where AS (%C) vary between 0.4% and 3.2% and AS (%N) between 1.2 and 4.2% (Liebezeit, 1993; Jennerjahn and Ittekkot, 1999; Gupta and Kawahata, 2000; Niggemann and Schubert, 2006b). In most studies, total AS concentrations were calculated from GlcN and GalN contents, while here we have also included ManN and Mur that contribute to at most 2% of AS. Therefore, the data are comparable.

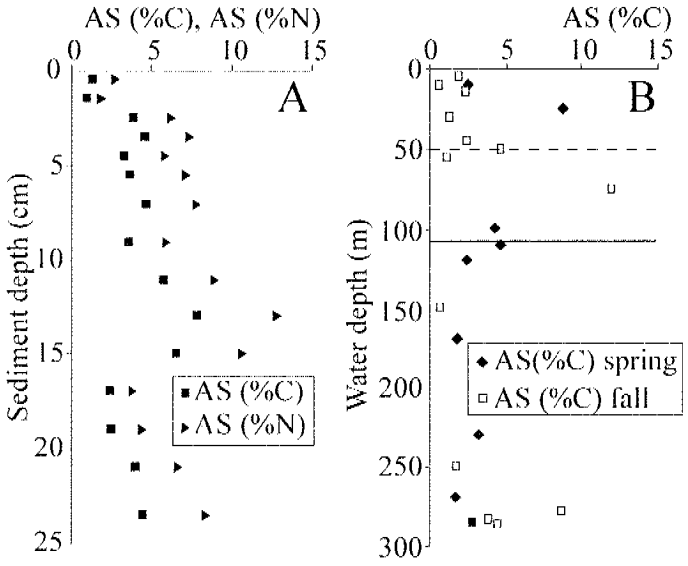


Figure 3.3: A: Total AS C-normalized yields (AS (%C)) in the water column. The continuous line indicates the oxic-anoxic interface in spring and the dashed line in fall. B: AS (%C) and total AS N-normalized yields (AS (%N)) in sediments. Shading indicates the bottom turbidite layer.

3.4.2. Water column characteristics

Physical characteristics of the spring and fall water columns were slightly different. In both seasons the thermal stratification was denoted by a strong temperature gradient around 10-15 m depth, but the suboxic zone reached 110 m in spring and 50 m in fall. In spring, in the anoxic zone, the light transmission had several drops, indicating an accumulation of particles in distinct layers due to a density discontinuity that is caused by a lack of mixing. At the same time, conductivity increased gradually. These are typical conditions for a permanent stratification of the anoxic water body of Lake Lugano (LSA, 2003). In contrast, in fall 2005, the light transmission was rather constant in the anoxic water column and dropped only below 240 m, indicating more mixing than in spring 2004. At the same time, conductivity increased only slightly below 250 m. These data confirm the partial mixing of the anoxic water masses that exceptionally occurred during winter 2004/2005 (M. Simona and C. Holzner, pers. comm.).

Bacterial abundances varied between 9×10^5 and 1.45×10^7 cells mL^{-1} (Fig. 3.4A), and are characteristic for eutrophic lakes (Wetzel, 1983). TOC concentrations varied between 117 and 220 µmol L^{-1} (Fig. 3.4B) with highest values in the oxic layer and a slight increase close to the sediment. The latter suggests resuspension of sedimentary material. The highest TOC concentration in the water column of spring at 60 m depth is probably due to a contamination of the sample and is, therefore, not considered in the following discussion.

AS concentrations in unfiltered water were generally up to 1000 times higher than in the particulate fraction. Consequently, in Lake Lugano most ASs were present in the fraction with a particle size < 0.7 µm and data from the unfiltered water mainly represent the distribution of DOM. Therefore, in the following, we will refer to the unfiltered water fraction as the dissolved fraction and to the 0.7 µm-fraction as the particulate fraction. Dissolved AS concentrations (DAS) were very variable and showed no seasonal trends, although the water column characteristics differed markedly (Fig. 3.5). This suggests that DASs are not influenced by seasonal changes of the physical characteristics in the water column.

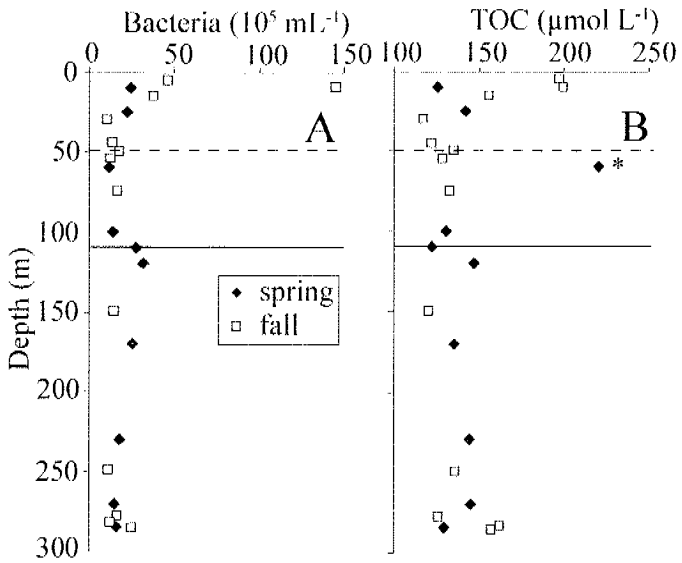


Figure 3.4: Total organic carbon (TOC) concentration (left) and bacterial abundance (right) in the water column of March 2004 (spring) and September 2005 (fall). The continuous line indicates the oxic-anoxic interface in spring and the dashed line in fall. The sample signed with the * was probably contaminated.

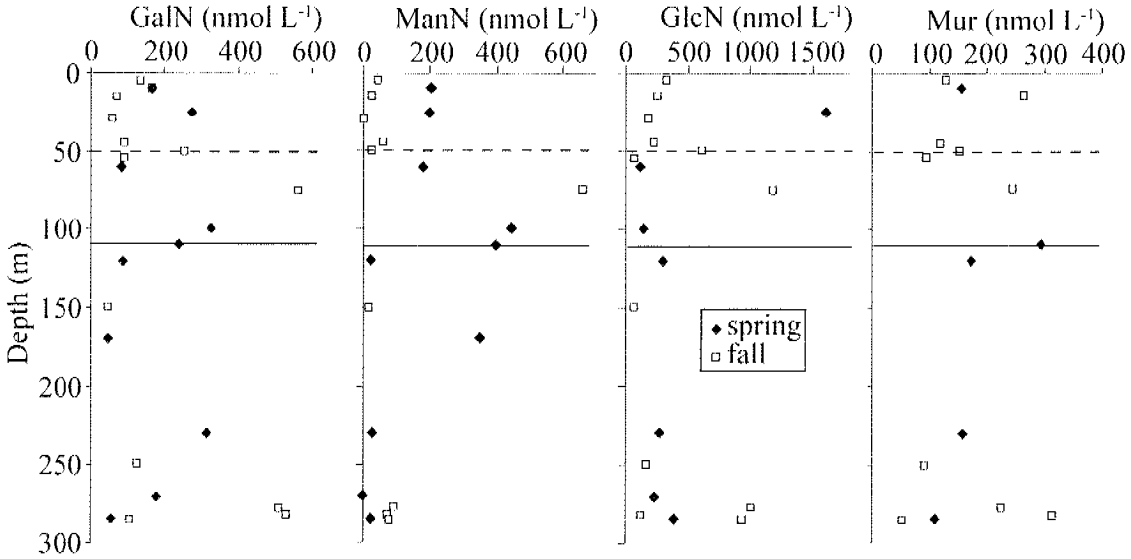


Figure 3.5: Single amino sugar concentrations in the dissolved fraction of the water column in spring and fall. The continuous line indicates the oxic-anoxic interface in spring and the dashed line in fall.

Overall, the total dissolved AS C-normalized yields (DAS (%C)) were between 0.5 and 11.9% (Fig. 3.3B), which is slightly higher than relative concentrations found in other freshwater environments (Nedoma et al., 1994). Below, we compare the concentrations of some DASs with DOM and ultrafiltered-DOM (UDOM, 1-100 nm particle size) from the ocean, because these are the only existing data on single AS concentrations to date. We are aware, however, that different filter pore sizes were used to recover DOM in those studies. The most abundant DAS was GlcN with an average C-normalized yield of 1.8±1.9 and the second most abundant was GalN with 0.8±0.7 %C, representing a slightly higher contribution to OC than in the ocean's DOM (Benner and Kaiser, 2003; Davis and Benner, 2005). ManN concentrations reached 659 nmol L^{-1} and stand for the first ever determined values in water. In marine UDOM, ManN concentrations were below the detection limit (10 nmol L^{-1}, Benner and Kaiser, 2003). Mur C-normalized yields were up to two orders of magnitude higher than in marine UDOM, indicating the presence of elevated amounts of bacterial OM. Particulate AS (PAS) concentrations (Fig. 3.6) were similar to concentrations found in other freshwater systems (Nedoma et al., 1994).

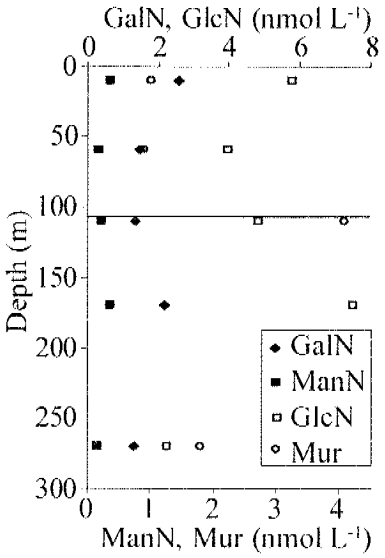


Figure 3.6: Single amino sugar concentrations in particulate OM (POM, > 0.7 µm) of the water column in spring. The continuous line indicates the oxic-anoxic interface.

3.5. Discussion

3.5.1. ASs accumulation in the sediment

Even though ASs are considered to be quickly degraded in aquatic systems (Benner and Kaiser, 2003; Niggemann and Schubert, 2006b), the concentrations of ASs (in particular of GlcN, GalN, and ManN) increased with sediments depth, suggesting an accumulation (Fig. 3.2C). This enrichment could have two reasons: either ASs are produced in the sediments, or they are more refractive than other OM components. If ASs were produced in the sediments by microorganisms, the indicators for bacterial OM like Mur should increase with sediments depth. However, Mur concentrations clearly decrease with sediment depth. Moreover, concentrations of fatty acid indicators for bacterial OM did not increase with sediment depth, neither (Klauser and Schubert, Chapter 2). This suggests that the increase in concentrations of GlcN, GalN, and ManN in sediments is not due to in-situ production, but to preferential preservation.

Indeed, comparing AS concentrations to the TOC, TN, amino acids, and fatty acids we observed that ASs are more refractive than the other OM components. In fact, calculating the ratios of ASs to these other components, we observed a clearly increasing down-core trend (Fig. 3.7). As a result, ASs are preferentially preserved over TOC, TN, amino acids and fatty acids. This suggests that the bioavailability of C and N from ASs is lower. As a result, ASs might be particularly interesting for tracking refractive OM in aquatic systems.

In the bottom turbidite layer, the ratios of AS to TOC, TN, amino acids and fatty acids clearly decrease in respect to the uppermost layer, suggesting that ASs are more degraded than above. The OM in turbidites is generally more degraded than the OM in the rest of the sediments, because it originates from littoral regions. These are often oxygenated and the resuspension into the water column on the way to the deep sediments enhances degradation processes. This suggests that ASs degradation is effective in turbidites. It is also possible that other sources of ASs, containing less ASs or where ASS are degraded more easily, are more abundant in the turbidite layer, resulting in better degradation of ASs.

Enrichment of ASs with sediment depth has also been reported in Lake Ontario (Kemp and Mudrochova, 1972). There, ASs (GlcN and GalN) were enriched with sediment depth attaining up to 25% of total N at 7.5 m depth (Kemp and Mudrochova, 1972). In contrast, in

marine environments, constant or decreasing contributions of AS (%C) were observed with depth (Liebezeit, 1993; Niggemann and Schubert, 2006b). Apparently, in sediments of Lake Lugano and of Lake Ontario, ASs are degraded slower than bulk C and N, while in the studied marine sediments they are degraded faster (or equally) than bulk C and N (Liebezeit, 1993; Niggemann and Schubert, 2006b). Several factors could be responsible for this difference: residence time in the water column, oxygen availability in sediments, and different OM input, or a combination of these factors.

Residence time in the water column. Lake Lugano and Lake Ontario have about the same water depth (220 m and 300 m, respectively). Marine sediments studied off the coast of Peru varied in depth from 102 m to 1278 m (Niggemann and Schubert, 2006b). This shows that there is no cut-off depth where the AS profiles would change from a decreasing to an increasing trend. This suggests that water depth is not the reason for AS accumulation in the sediments of the two lakes. Moreover, in both lakes ASs are accumulated, although sedimentation rates are different: 0.6 cm yr^{-1} in Lake Lugano (Klauser and Schubert, Chapter 2) and 0.1 cm yr^{-1} in Lake Ontario (Kemp and Harper, 1976). In addition, although sedimentation rates off Peru are similar to Lake Ontario sediments, the AS (%C) trends are opposite (Reimers and Suess, 1983). Altogether this suggests that the residence time in the water column is not the main reason for the differences between the lacustrine and the marine systems.

Oxygen availability. The oxygen availabilities are very different in the sediments of the studied environments. Lake Lugano sediments are permanently anoxic: the water above the sediments has not experienced any mixing in the last 50-60 years (Wüest et al., 1992; LSA, 2003). Lake Ontario is dimictic and oxygen reaches the sediment twice a year. The sediments off Peru are situated in an oxygen depleted region (Niggemann and Schubert, 2006b). Additionally, laboratory experiments on chitin degradation reported no significant difference between oxic and anoxic environments (Poulicek and Jeuniaux, 1991). As a result, oxygen does not have a major influence on AS accumulation in sediments.

OM input. A further reason for the diverse pattern between the two lakes and the marine sediments could be the difference in OM input. Autochthonous input depends on species distribution. As shown in Figure 3.8, AS ratios of different species are rather variable and might be different in every environment. It is, therefore, possible that a particular species contains a biopolymer that is more difficult to degrade. This would accumulate only in lake sediments.

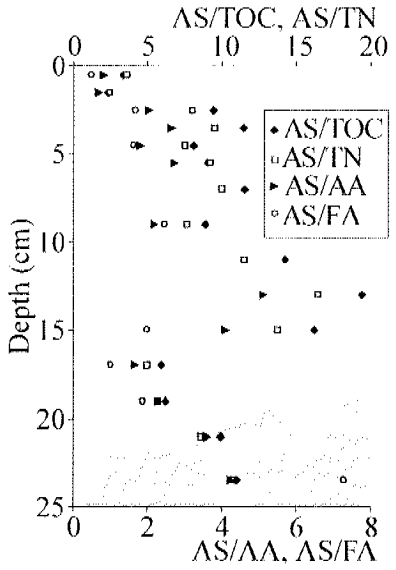


Figure 3.7: Ratio of total AS concentrations in sediment versus total organic carbon (TOC) in ngAS/10gOC, total nitrogen (TN) in ngAS/100gN, total amino acids (AA) in mgAS/100gAA, and total fatty acids (FA) in gAS/10gFA. Shading indicates the bottom turbidite layer.

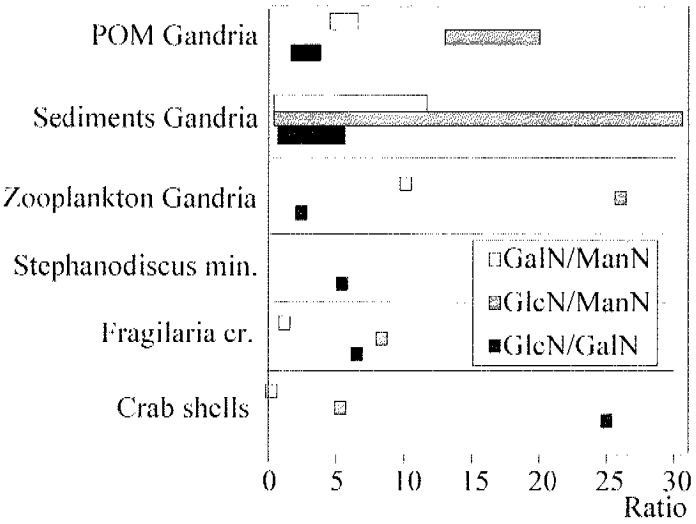


Figure 3.8: AS ratios ranges of unfiltered water, particulate organic matter (POM), and sediments, and ratios of zooplankton of Lake Lugano, *Fragilaria crotonensis* and *Stephanodiscus minutulus* (the most abundant diatom species in Spring 2004), and crab shell flakes.

Further, we examined the abundance of terrigenous input, which can be especially important in lacustrine systems. In Lake Lugano, fatty acid analyses showed that especially in the

deeper core (between 9 and 16 cm) the long-chain fatty acids, which are markers for higher land plants (Meyers and Ishiwatari, 1993), are enriched (Klauser and Schubert, Chapter 2, Fig. 3.5A). In this layer, the accumulation of ASs is particularly high and might, therefore, be also attributed to a higher input of terrigenous material. In fact, AS (%C) and AS (%N) significantly correlate with long-chain fatty acids contributions to total fatty acids (r^2=0.74, p=0.006 and 0.79, p=0.003, respectively; Fig. 3.9A+B). Possibly, allochthonous AS-containing polymers are less degradable than the autochthonous material. This is the case for allochthonous lipids and fatty acids (Meyers and Ishiwatari, 1993). However, to our knowledge, no information on biodegradability of allochthonous AS-polymers is available. The only indication we have is that the degradation proneness of chitin is affected by the macromolecules associated with it, which differ depending on the source (Poulicek et al., 1998). Supposing that AS-polymers of terrigenous origin are associated to different macromolecules, it is possible that ASs from allochthonous sources are less degradable than those from autochthonous sources and, therefore, contribute to the accumulation of ASs. In addition, the core analyzed from Lake Ontario, originates from an area with increased terrigenous contribution in deeper sediment layers (Silliman et al., 1996). This would confirm a terrigenous origin of ASs that accumulate in lake sediments.

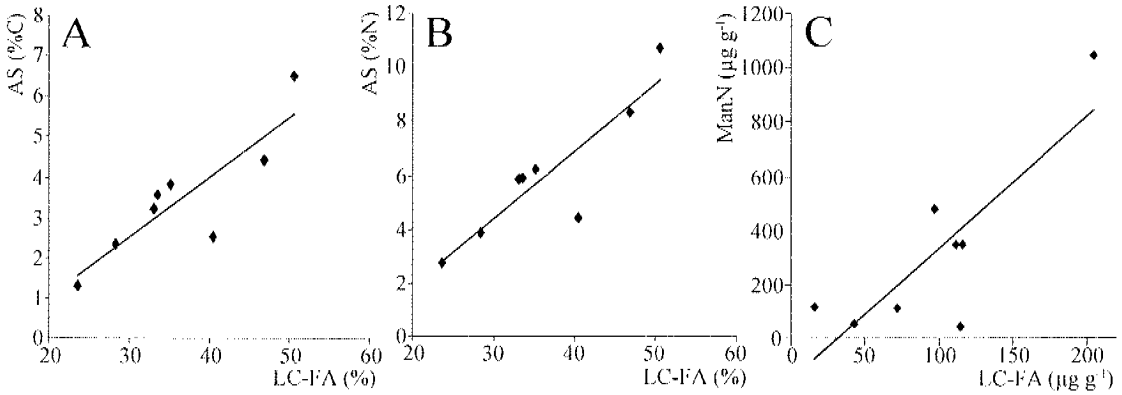


Figure 3.9: A: Linear regression between long-chain fatty acids (LC-FA) and AS (%C), r^2= 0.74, p= 0.006. B: Linear regression between long-chain fatty acids and AS (%N), r^2= 0.79, p= 0.003. C: Linear regression between long-chain fatty acids and ManN concentrations, r^2= 0.69, p= 0.01.

3.5.2. Unexpected abundance of ManN

In this study, we present relatively high concentrations of ManN in the water column. This is in contrast to studies on marine water columns, where the amount of ManN is low (Müller et al., 1986; Benner and Kaiser, 2003). Since ManN was not found in all samples and in further samples of the Black Sea there are only traces of ManN (Klauser and Schubert, Chapter 4), we can exclude a systematic methodological error.

In POM, ManN concentrations were about five times lower than GalN concentrations (Fig. 3.5), while in unfiltered water they were mostly as high as GalN in spring and up to 7 times lower than GalN in fall (Fig. 3.4). Consequently, ManN was mainly present in spring in DOM. There are two possible explanations for the high ManN abundances: either there are sources with a high ManN content, or there is a selective preservation of ManN. Several studies describe ManN concentrations in aquatic and terrestrial environments and organisms (Yoneyama et al., 1982; Martens and Frankenberger, 1991; Zhang and Amelung, 1996; Coelho et al., 1997; Amelung, 2001; Komandrova et al., 2001; Nicolaus et al., 2002; Turrion et al., 2002; Benner and Kaiser, 2003; Glaser et al., 2004; Niggemann and Schubert, 2006b). However, ManN was found in higher concentrations than GalN only in some marine phototrophic algae and bacteria, in some species of bacillus, and in the top layer of a forest soil (Yoneyama et al., 1982; Komandrova et al., 2001; Turrion et al., 2002; Benner and Kaiser, 2003). In order to find possible sources of ManN in Lake Lugano in spring, we analyzed two diatom species (*Fragilaria crotonensis* and *Stephanodiscus minutulus*) and a springtime zooplankton sample (80% *Cyclops sp.*). Further, we compared of the zooplankton sample to chitinaceous material by analyzing crab shell flakes from king crabs.

The lowest GalN/ManN ratios were determined in crab shell flakes and in *Fragilaria crotonensis* (Fig. 3.8), whereas zooplankton showed a much higher ratio. In contrast to the high ManN content of *Fragilaria crotonensis*, *Stephanodiscus minutulus* contained no ManN, indicating that the abundance of ManN in organisms of the same species is highly variable. The same holds true for the difference between the ratios in crab shell flakes and zooplankton from Lake Lugano. Therefore, no obvious source was determined and ManN could still originate from a combination of algae and zooplankton, or from bacteria.

The other possible reason for high ManN concentrations is selective preservation. However, the low ManN concentrations in fall are in contrast to an accumulation. Moreover, if ManN was more refractive than GalN, it would accumulate in more degraded sediments, while in Lake Lugano sediments the GalN/ManN ratios increase in the bottom layer (18-25 cm) where OM is highly degraded (Fig. 3.2C).

In general ManN was present in sediments in much lower concentrations than GalN, except for the layer from 12 to 14 cm, where long-chain fatty acids are enriched (Klauser and Schubert, Chapter 2). Indeed, ManN concentrations correlated slightly with high long-chain fatty acid concentrations (r^2=0.69, p=0.01, Fig 3.9C). Since long-chain fatty acids are markers for land plants (Meyers and Ishiwatari, 1993), ManN might be related to a terrigenous source in the sediments.

In summary, ManN does not appear to be more refractive than GalN nor GlcN and might originate from terrigenous material and/or from autochthonous organisms.

3.5.3. Bacterial source of ASs

In Lake Lugano, high Mur concentrations in DOM, POM, and sediments indicate the presence of high amounts of bacterial OM. Similarly, in marine environments, bacterial remnants are considered to make up a great fraction of OM (McCarthy et al., 1998; Ogawa et al., 2001; Benner and Kaiser, 2003; Niggemann and Schubert, 2006b). Based on bacterial abundances, we estimated the fraction of Mur originating from living bacteria. It has been determined that average carbon content of prokaryotic cells in lakes varies between 20 and 30 fg C $cell^{-1}$ (Fagerbakke et al., 1996) and average Mur yield in bacteria is 12.6 nmol $(mg\ C)^{-1}$ (Benner and Kaiser, 2003). Applying the highest carbon content of 30 fg C $cell^{-1}$ for the cells in the water column and in sediments, we estimated a maximum percentage of Mur from living cells of 0.1% to 2% in the water column and of 6% to 15% in sediments. These estimates are probably too high, because archaea (5-12% of total cells) were included in bacterial counts, but do not contain Mur.

These results confirm that Mur originates mainly from bacterial remnants. Previous studies reported living bacterial contributions to Mur of 10-15% in POM (0.1-60 µm) in deep water samples (Benner and Kaiser, 2003) and of 3-17% in sediments (Niggemann and Schubert, 2006b). The sediment values are comparable to our estimations, while in the water column the

contributions of living bacteria to Mur are 10 times lower than in the marine studies. This indicates that dead bacterial remnants are more abundant in Lake Lugano than in oxic marine water columns. In addition, it might indicate that a lot of the other ASs originate from bacterial remnants. The reasons for this high abundance can be a slower degradation of peptidoglycan in Lake Lugano. Previous studies revealed that Mur is quickly degraded in oxic seawater (Nagata et al., 2003; Tremblay and Benner, 2006), however, to our knowledge, no studies on anoxic environments exist at the present time.

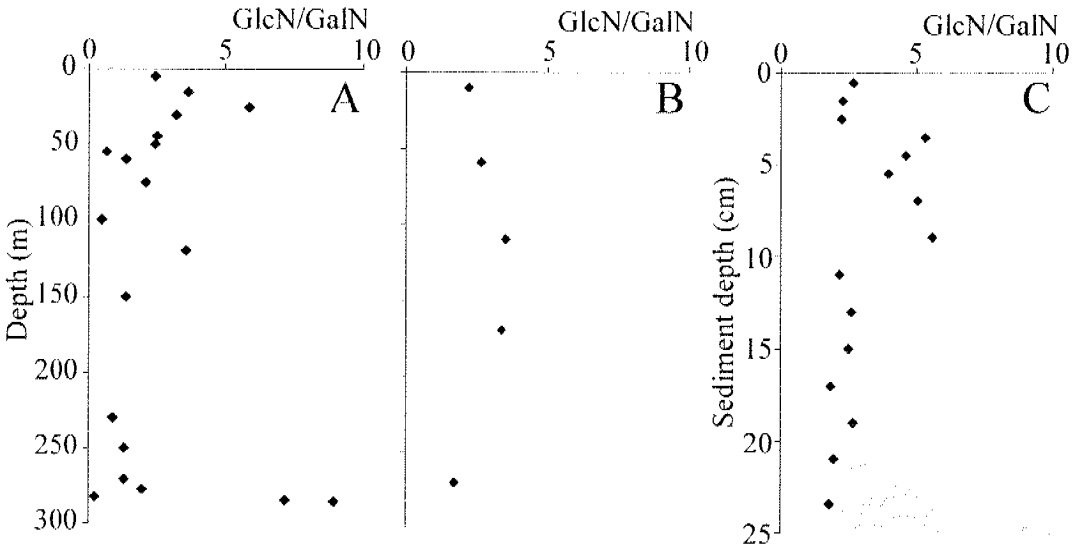


Figure 3.10: GlcN/GalN ratios in the dissolved water fraction (A), in the particulate matter fraction (B), and in sediments (C). Shading indicates the bottom turbidite layer.

3.5.4. Reactivity of ASs in DOM, POM, and sediments

In Lake Lugano, DAS and PAS concentrations do not decrease with water depth suggesting slow reactivity or a high production of ASs in the water column. To estimate the changes in AS distribution and reactivity, we looked at the ratio between GlcN and GalN in each fraction. In DOM the ratio varied strongly from 0.5 to 8.9 (Fig. 3.10A), in POM it ranged from 1.9 to 4.0 (Fig. 3.10B), while in the sediments it varied between 2.2 and 5.6 (Fig. 3.10C). As a result, POM and sediments are similar and have lower variations in AS composition than DOM.

This suggests that ASs in POM and sediments have similar sources and a similar reactivity, while ASs in DOM have different sources and reactivity. In contrast, in marine systems UDOM, DOM, POM, and in sediments all have rather constant and similar GlcN/GalN ratios (Liebezeit, 1993; Benner and Kaiser, 2003; Davis and Benner, 2005; Niggemann and Schubert, 2006b). Therefore, DOM of Lake Lugano appears to have a different composition and reactivity than DOM from the open oceans. This could be due to faster production along the whole water column, or to slower degradation. In the previous section, we revealed a higher contribution from bacterial remnants to OM than in marine OM, especially in the water column. This hints to a slower degradation of bacterial remnants in DOM that provokes also the higher variability in GlcN/GalN ratios. In contrast, in POM and sediments, the lower variability of GlcN/GalN ratios suggests a better reactivity of freshly produced OM in these fractions.

4

AMINO SUGARS IN THE WATER COLUMN AND IN SEDIMENTS OF THE BLACK SEA

Lucia Klauser and Carsten J. Schubert

4.1. Abstract

Amino sugars are ubiquitously present in the environment and represent an organic source of carbon and nitrogen. In this study, we analyzed the amino sugar distribution at two contrasting sites in the water column and in the underlying sediments of the Black Sea. We highlighted the similarities and differences between anoxic and oxic environments and analyzed the differences between the two sites. Further, we evaluated amino sugars reactivity in the water column and in sediments. The amino sugar carbon normalized yields in the Black Sea were similar to oxic marine environments; however, the distribution in the water column was more variable. Exceptionally high concentrations of muramic acid (Mur) in the dissolved fraction indicated significant production of bacterial organic matter and slow degradation. Despite that, Mur resulted to be the most reactive amino sugar in the dissolved water fraction and in the sediments. Similarly to oxic systems, the ratio between glucosamine and galactosamine (GlcN and GalN) in the Black Sea stabilized to a value around 1.4 in the deep water column and in the sediments, suggesting the presence of a biopolymer with constant composition of GlcN and GalN. At the deep site, amino sugars were transformed along the water column reaching a constant GlcN-GalN composition, while in the water column of the shallow site amino sugar composition in the dissolved fraction was more variable. The accumulation of amino sugars in the slope sediments

was ascribed to the shorter water column and higher sedimentation rates than at the deep site and the shorter water column.

4.2. Introduction

Amino sugars (ASs) are important components of organic matter (OM) in aquatic systems. These compounds merit a detailed analysis of occurrence and fate under different redox conditions because they form fairly stable biopolymers such as chitin and peptidoglycan and contribute to up to 1.7% of total dissolved organic nitrogen in the water column (Benner, 2002) and 3.8% of total nitrogen (TN) in marine sediments (Niggemann and Schubert, 2006b). Glucosamine (GlcN), galactosamine (GalN), mannosamine (ManN) and muramic acid (Mur) are the most common ASs in aquatic systems. GlcN is the main contributor to total AS concentrations, as it is the monomer of chitin, the second most abundant biopolymer on earth following cellulose (Cohen, 1987). Zooplankton and phytoplankton are the major producers of chitin in aquatic systems, but GlcN is also the main component of bacterial OM (Brock et al., 1994). The second most abundant AS is GalN, present in zoo- and phytoplankton, as well as in bacteria (Sharon, 1965). ManN is less recurrent and has been detected in polysaccharides and glycolipids of bacteria, algae, and zooplankton (Sharon, 1965; Yoneyama et al., 1982; Komandrova et al., 2001; Nicolaus et al., 2002; Benner and Kaiser, 2003; Klauser and Schubert, Chapter 3). Mur is only found in peptidoglycan from bacterial biomass and is therefore a specific indicator for this biopolymer (Brock et al., 1994).

In general, the AS distribution in DOM of ocean waters is similar in all studied environments. In particular, ratios of GlcN to GalN were fairly constant especially in deep water (Benner and Kaiser, 2003; Davis and Benner, 2005; Kawasaki and Benner, 2006; Tremblay and Benner, 2006). The origin of this GlcN-GalN polymer was attributed to a bacterial source, since previous studies reported a main bacterial origin of DOM in the ocean (Boon et al., 1998; McCarthy et al., 1998; Ogawa et al., 2001; Benner and Kaiser, 2003). Moreover, this biopolymer is considered to be very refractive. Mur was shown to originate mainly from freshly decaying peptidoglycan (Benner and Kaiser, 2003); degradation experiments showed that peptidoglycan was degraded within 10-167 days (Nagata et al., 2003).

So far, there are only few studies comparing AS distribution in water and the underlying sediments (Jennerjahn and Ittekkot, 1999; Klauser and Schubert, Chapter 3). Jennerjahn and Ittekkot (1999) compared AS concentrations of suspended material, sedimenting particles, and surface sediments and showed that bacterial contribution to OM increased in sediments compared to the water column suggesting that OM in sediments was more degraded than in the water column. It was shown that ASs degradation processes occurred, in particular, at the water-sediment interface (Jennerjahn and Ittekkot, 1999). In contrast, other studies suggested high contributions from chitinaceous material, i. e. originating form phyto- and zooplankton, in settling particles and sediments (Degens and Mopper, 1975; Müller et al., 1986; Dauwe and Middelburg, 1998). In general, GlcN to GalN ratios were similar in sediments and in deep water columns suggesting the presence of the same refractive biopolymer (Degens and Mopper, 1975; Müller et al., 1986; Liebezeit, 1993; Dauwe and Middelburg, 1998; Benner and Kaiser, 2003; Davis and Benner, 2005; Niggemann and Schubert, 2006b; Tremblay and Benner, 2006; Klauser and Schubert, Chapter 3).

Within the sediments, recent studies illustrated different degradation patterns and sources of ASs. In some environments, ASs were degraded as fast as bulk OC or even faster (Liebezeit, 1993; Niggemann and Schubert, 2006b), while in others they were enriched in comparison to amino acids (Degens and Mopper, 1975; Müller et al., 1986; Dauwe and Middelburg, 1998). Further, a major bacterial origin was suggested in Arctic sediments and in sediments off Peru (Liebezeit, 1993; Niggemann and Schubert, 2006b), while in several basins a major contribution from phyto- and zooplanktonic chitin was described (Degens and Mopper, 1975; Müller et al., 1986; Dauwe and Middelburg, 1998). Chitin was shown to be resistant to degradation, especially when combined with other polymers (Poulicek and Jeuniaux, 1991). However, degradation experiments in water revealed an almost complete degradation of chitin particles (96%) within 70 days of incubation (Davis and Benner, 2005) and similar chitin degradation rates in oxic and anoxic sediments (Boyer, 1994; Poulicek et al., 1998).

The uncertain nature and reactivity of ASs in aquatic environments motivated us to analyze AS distribution in an anoxic system, often described as environment with increased burial efficiency of organic C (Henrichs and Reeburgh, 1987; Emerson and Hedges, 1988; Harvey et al., 1995; Hedges and Keil, 1995). The Black Sea offered, additionally, the opportunity of sites with different characteristics. We focused on a shallow site with high TOC concentrations

in the sediments and a deep site with lower TOC concentrations. These stations are located in the North-Western region, near the Crimea peninsula (Fig. 4.1). Both sites are not affected by terrigenous input and have similar primary productions (Stokozov and Buesseler, 1999; Sorokin, 2002). The shallow station at the slope has about a three times higher sedimentation rate than the deep station in the abyssal plain (e. g., Teodoru et al., 2007).

These two environments promised to be adequate to answer the following questions: Are AS distributions in an anoxic ocean basin different from those observed in typical oxic ocean waters? What is the reactivity of ASs in the water column and in the sediments? Are ASs accumulated in anoxic sediment?

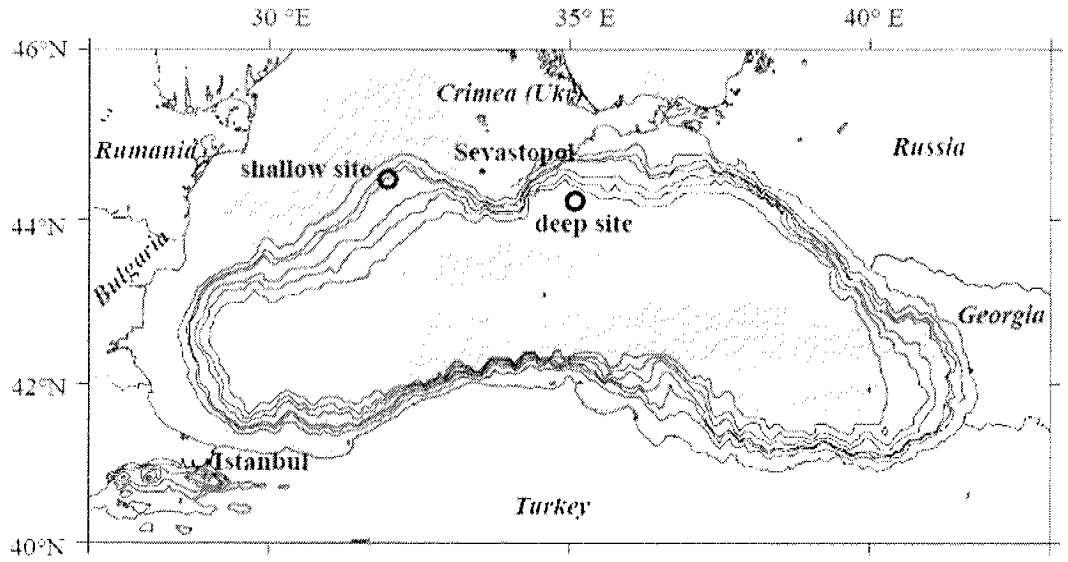


Figure 4.1: Map of the Black Sea showing the shallow and the deep study sites.

4.3. Materials and methods

4.3.1. Sample collection

Water, particulate, and sediment samples were collected during the cruise on the R/V Prof. Vodyanitsky in June 2004 in the North-Western Black Sea at a 2000 m deep station east and a shallow station west of Crimea at 250 m water depth (Fig. 4.1). Both stations were not influenced by riverine input (Stokozov and Buesseler, 1999).

For AS analysis, unfiltered water samples were collected with Niskin bottles, 50 mL aliquots were poisoned with 2 drops of saturated $HgCl_2$ solution and stored at 4°C. For bacterial counts, 25 mL samples were poisoned with 0.5 mL formaldehyde solution (36.5%), filtrated the same day and stored frozen. For TOC analysis, 15 mL samples were collected in acid-rinsed PP-tubes, acidified with 30% HCl to pH~3, and stored at 4°C.

Particulate water samples were collected by pumping 50-1100 L water with an in-situ pump (McLane) through double layered pre-combusted glass fiber filters (GFFs) with a nominal pore size of 0.7 µm (Wathman). The filters were frozen immediately after sampling. The two layers of filters should allow sampling of increased amounts of bacterial OM. This fraction will be referred to as the particulate organic matter (POM) fraction.

We are aware that POM comprises also smaller particles which formed aggregates and that some particles < 0.7 µm (like some free living bacteria) pass through the filter. Moreover, in the top water column filters could be clogged faster with algal and zooplanktonic material, while in deep water bacterial matter might contribute more to total mass. These samples reflect the composition of slowly settling suspended material and are not comparable to sediment trap material, which is typically dominated by fecal pellets and larger particles.

Sediment cores were retrieved with a gravity corer. The top 10 cm were sliced in 1 cm-intervals and the section from 10 to 35 cm was sampled in 2 cm-intervals, transferred into glass vials, and frozen immediately after sampling at -20°C.

4.3.2. Analytical methods

Standard solutions of D-(+)-glucosamine hydrochloride, D-(+)-mannosamine hydrochloride, muramic acid (Sigma), D-(+)-galactosamine hydrochloride (Fluka), and 3-Acetamido-3-deoxy-D-glucose (TRC) were prepared for each analytical run. Analytical grade reagents (hydrochloric acid (37%), ammonium hydroxide (27%), sodium acetate, sodium chloride, formaldehyde (36.5%), 4',6-Diamidino-2-phenyl-indol-dihydrochloride (DAPI) and $HgCl_2$, (Fluka) and (50% w/w) carbonate-poor sodium hydroxide solution (J. T. Backer)) were used for sample preparation and storage. Deionized water was retrieved from a Nanopure ultrafiltration system (Skan AG). The internal standard, 3-amino-3-deoxyglucose hydrochloride (Glc3N), was prepared from 3-Acetamido-3-deoxy-D-glucose (Kaiser and Benner, 2000).

ASs were measured using the method developed by Kaiser and Benner (2000), slightly adjusted for sediments and POM (Klauser and Schubert, Chapter 3). Water samples were hydrolyzed with 3 mol L^{-1} HCl at 100°C for 5 hours. Sediment samples were freeze dried, milled, and hydrolyzed for 5 hours at 100°C with 6 mol L^{-1} HCl for GlcN, GalN, and ManN and with 3 mol L^{-1} HCl for Mur. POM samples were hydrolyzed at 100°C with 6 mol L^{-1} HCl for 10 hours. After hydrolysis, samples were neutralized and desalted with cation exchange resins (AG 50W X8, 100-200 mesh and 200-400 mesh and AG11 A8, 50-100 mesh, from Bio-Rad). Separation and quantification was performed with a metal-free chromatography system (Jasco) equipped with a PAD ED50 detector (Dionex) with a gold working electrode and a pH Ag/AgCl reference electrode on a Dionex CarboPac PA1 column (250 mm x 4 mm i.d.) with a CarboPac PA1 guard column (50 mm x 4 mm i.d.). GalN, GlcN and ManN, were separated with 12 mmol L^{-1} NaOH under isocratic conditions and Mur with 99.6 mmol L^{-1} NaOH and 100 mmol L^{-1} sodium acetate under isocratic conditions with a flow rate of 1 mL min^{-1}. ASs were quantified with an internal standard and a calibration curve from standard solutions. The precision of the method was 3-8% for GlcN, GalN, and ManN and 17% for Mur. The detection limit of water samples after cleanup was 10 nmol L^{-1} for GlcN, GalN and ManN and 30 nmol L^{-1} for Mur.

TOC concentrations of water samples were determined by high temperature catalytic oxidation with a Shimadzu 5050A analyzer (Benner and Strom, 1993) with a precision of 0.5%. Total carbon (TC) and total nitrogen (TN) concentrations of sediment samples were measured with a CNS elemental analyzer (Hekatech). The precision of the method was 0.2% for TC and

0.3% for TN. Total inorganic carbon (TIC) concentrations of sediment samples were quantified with a CO_2 coulometer (Coulometric Inc., 5011) with a precision of 0.2%. TOC was calculated as the difference between TC and TIC.

AS C- and N-normalized yields (AS (%C) and AS (%N)) were calculated assuming that all ASs were in the non-acetylated form, which is the form measured after hydrolysis. However, in nature ASs often occur in acetylated forms with two additional carbon atoms. Therefore our calculations of AS (%C) are potentially underestimated by up to 25%.

To determine bacterial abundances, water sample aliquots of 10 mL were gently vacuum-filtered through polycarbonate filters with 0.2 µm pore size and 25 mm diameter (Millipore). The filters were placed in clean petridishes, sealed, and stored frozen at -20°C. Filters were stained with 20 µL of a 5 mg L^{-1} DAPI-solution for 15 min in the dark and then washed gently with sterile water (Porter and Feig, 1980). After drying, the filters were placed on a slide and mounted in Citifluor solution for counting on an epifluorescence microscope (Axiscope HBO50, 1000x magnification, Zeiss). At least 200 cells per slide were counted.

Chlorin concentrations were determined following the method of Schubert et al. (2005). Briefly, 5-10 mg freeze-dried and milled sediments were extracted three times with 5 mL acetone through sonication. Acetone was decanted after centrifugation for 10 min at 4°C. Fluorescence was measured on a spectrophotometer (Cary, Varian) at an excitation wavelength of 428 nm and emission of 671 nm. Acidified chlorophyll *a* standards were used for quantification of chlorins. The precision of the method was 5%. To determine the chlorin index (CI) the extracts were additionally acidified with two drops of 25% HCl. The CI was calculated as the ratio of the fluorescence intensity of the acidified and the neutral extract.

Total amino acid concentrations (AA) were determined according to the method of Dauwe and Middelburg (1998), where it is described in detail. Briefly, 50-100 mg freeze-dried and milled sediments were hydrolyzed with 5 mL of 6 mol L^{-1} HCl for 24 h at 110°C. The hydrolysate was separated from the sediment by centrifugation and neutralized with 6 mol L^{-1} NaOH. After 1 h the neutralisate was buffered with PO_4^{3-} buffer and AAs derivatized with ortho-phthaldialdehyde (OPA) in presence of mercaptoethanol (HPLC grade) for 5 min. The fluorescence was then measured on a spectrofluorometer (Cary, Varian) at an excitation wavelength of 340 nm and an emission wavelength of 455 nm. A calibration curve was

determined with an amino acids standard solution (AA-S-18, Sigma) to quantify the samples. The precision of the method was 5%.

4.4. Results

4.4.1. Water column

At the shallow and the deep site, oxygen was present only in the uppermost water column, whereas the bottom water was completely anoxic. The suboxic zone was between 90 and 120 m and 70 and 140 m at the shallow and the deep station, respectively. Density increased in the anoxic zone, suggesting a stable stratification of the deep water column. The light transmission decreased steadily at the deep site below 800 m water depth, suggesting an increase in particle concentrations.

In general, the particulate fraction had about 30 times lower organic carbon concentrations and up to 100 times lower AS concentrations than the unfiltered water fraction. This suggests that ASs from particles do not influence considerably the AS concentration measured in unfiltered water samples. Therefore in the following we will refer to the unfiltered water samples as the dissolved fraction.

Dissolved organic carbon (DOC) concentrations were on average slightly higher at the shallow station than at the deep station (Table 4.1) and were 3-4 times higher than in oxic marine environments (e. g. Benner and Kaiser, 2003; Davis and Benner, 2005). Bacterial abundances varied from 2.8 to 5.1×10^5 cells mL^{-1} with highest concentrations at the surface and at the oxic-anoxic interface (data not shown). Dissolved amino sugar (DAS) concentrations were slightly lower in the water column of the shallow site than in the water column of the deep site. They showed highest concentrations at the surface and at 1500 m (Table 4.1). In particular, GlcN concentrations were up to two times higher at the abyssal site than at the slope site where they were similar to GalN concentrations. In contrast, at the deep station GalN concentrations were two to four times lower than GlcN concentrations. ManN was detected at the deepest sample with a concentration of 214 nmol L^{-1} and only detected in low concentration (up to 20 nmol L^{-1}) in a few shallower samples. Surprisingly, Mur concentrations were very high reaching similar values as the other ASs. DAS C-normalized yields (DAS (%C)) varied between 0.4 and 2.3% (Fig. 4.2A

and B) similar to concentrations found in other environments (Benner and Kaiser, 2003; Davis and Benner, 2005). The GlcN/GalN ratio average was much lower at the slope site compared to the profundal site (1.2±0.7 and 2.2±1.1, respectively). In samples below 1000 m, the GlcN/GalN ratio levelled off to 1.4 (Fig. 4.6), a value found also in other aquatic systems (Benner and Kaiser, 2003; Davis and Benner, 2005; Niggemann and Schubert, 2006b).

Particulate organic carbon (POC) concentrations were similar at the two stations (Table 4.2) and in the range of other environments (Benner and Kaiser, 2003; Davis and Benner, 2005). Particulate amino sugar (PAS) concentrations were on average five times higher in the shallow site than in the deep site (Table 4.2). The values of the slope station were in the upper range of other studies, while the values of the abyssal station were in the lower range (Benner and Kaiser, 2003; Davis and Benner, 2005). Particulate GlcN was the most abundant AS in both sites and GalN was more abundant at the shallow than at the deep site. The GlcN/GalN ratios were higher at the shallow than at the deep site. ManN was detected in very low concentrations accounting for at most 12 mole% of PASs in the deepest sample of the shallow station. Mur concentrations were similar in the two sites, but relative to PAS they were very high in the abyssal water column reaching similar concentrations to the other ASs. PAS C-normalized yields (PAS (%C)) varied between 0.1 and 1.4% (Fig. 4.2A and B). At the shallow station, PAS (%C) were similar to DAS (%C); while at the deep site they were 10-20 times lower than DAS (%C).

Table 4.1: DOC and dissolved amino sugar concentrations in the water column of the shallow and the deep stations of the Black Sea.

Depth (m)	DOC (µmol L^{-1})	GlcN (nmol L^{-1})	GalN (nmol L^{-1})	ManN (nmol L^{-1})	Mur (nmol L^{-1})
shallow					
10	286	112	86.5	bd	114
50	315	85.3	46.9	10.3	139
70	244	185	102	20.0	117
85	239	102	44.1	bd	57.2
95	240	55.1	41.4	bd	139
105	230	67.4	35.1	bd	77.2
120	250	31.0	106	bd	73.4
135	197	54.1	60.3	bd	55.8
150	204	109	112	bd	107
170	210	37.8	21.1	bd	72.4
200	234	50.7	232	bd	nd
250	252	47.7	62.6	bd	48.1
deep					
50	246	139	56.7	8.7	299
75	225	248	85.2	20.6	81.1
90	209	83.1	32.2	3.4	54.2
110	198	69.7	28.6	4.3	77.5
125	313	22.6	78.5	12.8	116
150	220	40.5	63.9	bd	36.9
200	212	103	25.1	bd	112
300	158	48.2	nd	nd	71.0
700	205	167	85.6	bd	155
1100	196	124	48.5	7.9	52.6
1500	221	237	192	37.1	58.4
1900	206	287	223	214	65.8

nd = not determined
bd = below detection limit

Table 4.2: POC and particulate amino sugar concentrations in the water column of the shallow and the deep stations of the Black Sea.

Depth (m)	POC (μmolg L^{-1})	GlcN (nmol L^{-1})	GalN (nmol L^{-1})	Mur (nmol L^{-1})	ManN (nmol L^{-1})
shallow					
5	8.8	6.96	1.50	0.61	0.62
65	2.7	1.71	0.39	0.12	0.16
85	3.7	1.05	0.41	0.14	0.14
90	2.1	2.08	0.51	0.18	0.22
100	1.6	2.00	0.50	0.17	0.35
120	1.5	2.32	0.66	0.11	0.35
170	2.0	0.94	0.23	0.25	0.20
deep					
50	1.4	0.10	0.06	0.16	0.01
75	1.3	0.48	0.17	0.14	0.02
90	1.6	0.16	0.07	0.05	0.01
110	4.4	0.46	0.13	0.32	0.03
150	2.2	0.12	0.07	0.26	0.01
300	1.7	0.16	0.09	0.18	0.01
1100	1.7	0.63	0.38	0.33	0.04
1900	1.4	0.42	0.24	0.28	0.03

4.4.2. Sediments

The characteristics of the cores varied widely between the two stations. At the deep site, sediments were dark and fluffy in the top 5 cm and light and compact below 5 cm, while at the shallow site the whole core was rather dark. TOC, TN and AA concentrations were higher at the slope station compared to the abyssal one (Table 4.3). In both cores TIC concentrations were lower than 5% (data not shown). At the deep site TOC concentrations had an eight-fold decrease from the top 5 cm to the rest of the sediment, while no degradation was apparent from the down-core profile of the slope core. In addition, a lower C/N ratio (6.7±0.6) in the deep sediments than in the shallow sediments (10.5±0.4) might indicate a higher degradation state of OM. The CIs were very high (0.9-1) in all samples from both sites indicating enhanced OM degradation.

AS concentrations measured in the slope core (Table 4.3) were similar to marine sediments and were much higher than in the deep core, where also AS (%C) and AS (%N) were lower in comparison to other marine environments (Liebezeit, 1993; Dauwe and Middelburg, 1998; Niggemann and Schubert, 2006b). GlcN was the most abundant AS in both sites (Table 4.3). GalN reached concentration levels of GlcN in the shallow site, like in the dissolved fraction of the water column. The GlcN/GalN ratios were very stable and varied between 0.7 and

1.9 (Fig. 4.6). ManN was detected in very low concentrations in both sites. Mur had similar concentrations in the two sites and was the only AS with a clear decreasing trend with sediment depth. Relatively to total ASs, Mur concentrations were much higher at the deep site than at the shallow site.

Table 4.3: TOC, TN, total amino acids (AA), and amino sugar concentrations in sediments of the shallow and the deep stations of the Black Sea. bd = below detection limit, nd = not determined.

Depth (cm)	TOC (%)	TN (%)	AA (mg g^{-1})	GlcN (µg g^{-1})	GalN (µg g^{-1})	ManN (µg g^{-1})	Mur (µg g^{-1})
shallow							
0.5	2.2	0.2	7.2	509	432	21.2	4.7
2.5	2.1	0.2	6.9	778	489	81.1	3.7
3.5	1.9	0.2	4.8	261	310	7.3	1.4
4.5	nd	nd	6.1	964	536	137	1.7
5.5	2.0	0.2	6.0	816	424	117	3.8
6.5	1.8	0.2	6.2	688	358	86.9	2.7
7.5	2.0	0.2	4.7	221	247	26.5	1.8
8.5	1.7	0.2	4.7	232	178	16.8	1.8
9.5	1.8	0.2	3.4	316	275	bd	1.5
11	1.6	0.2	4.3	263	283	6.0	1.4
13	2.3	0.3	5.6	516	406	bd	0.6
15	2.0	0.2	nd	425	308	bd	1.0
17	2.2	0.3	6.0	335	331	13.7	1.4
19	2.1	0.2	nd	408	364	16.6	bd
21	2.3	0.3	5.8	515	417	70.3	bd
23	2.3	0.3	nd	517	424	7.5	bd
26	2.3	0.2	5.5	451	364	5.9	bd
30	2.5	0.3	6.5	588	415	10.1	bd
deep							
2.5	4.8	0.5	14.1	68.4	40.2	3.0	55.8
5.5	0.6	0.1	1.2	16.9	12.2	0.4	13.9
6.5	0.5	0.1	1.3	14.8	12.0	0.4	3.4
7.5	0.6	0.1	1.0	12.9	11.8	0.5	2.6
8.5	0.4	0.1	0.8	14.1	12.0	0.1	5.7
9.5	0.2	0.0	nd	7.8	7.3	bd	1.8
11	0.4	0.1	0.8	13.9	10.8	bd	1.2
13	0.4	0.1	nd	10.0	9.5	bd	4.7
15	0.5	0.1	0.9	8.6	11.7	bd	4.0
17	0.3	0.1	nd	10.9	10.1	bd	1.7
19	0.6	0.1	1.0	7.5	8.2	bd	3.2
21	0.6	0.1	nd	21.6	16.2	0.9	5.6
25	0.5	0.1	0.7	18.8	13.7	0.7	1.9
27	0.5	0.1	nd	19.2	13.4	0.5	2.9
29	0.6	0.1	nd	33.2	23.0	2.2	3.6
33	0.7	0.1	1.2	27.6	19.0	1.7	4.8

4.5. Discussion

4.5.1. AS characteristics in the water column of the Black Sea

In the Black Sea water column, DOC and DAS concentrations (Table 4.1) were generally higher than in oxic marine environments, while the DAS (%C) (Fig. 4.2A and B) were similar (Benner and Kaiser, 2003; Davis and Benner, 2005). High DOC concentrations are characteristic for anoxic environments (Otsuki and Hanya, 1972; Nguyen and Harvey, 1997). Otsuki and Hanya (1972) described a higher production of DOM (< 0.45 µm) during anaerobic degradation of algae compared to aerobic degradation, and Nguyen and Harvey (1997) detected higher concentrations of dissolved protein material under anoxic degradation compared to oxic decay.

In the Black Sea, DAS concentrations do not show a depth-trend and have very variable values along the whole water column, while PAS concentrations vary only slightly with depth (Table 4.1 and 4.2). This is in contrast to oxic environments, where both fractions clearly decrease with depth (Benner and Kaiser, 2003). This divergence can have two reasons: increased production of DAS and/or slower degradation. The total DAS amount is higher than in oxic waters, but the relative concentrations (DAS (%C)) are similar. The only AS showing a higher C-normalized yield than in oxic systems is Mur (Fig. 4.3 and 4.4). Similarly, in the anoxic water column of Lake Lugano Mur concentrations were unexpectedly high (Klauser and Schubert, Chapter 3). Mur is a component of bacterial cell walls and is an indicator for bacterial detritus. In oxic environments, Mur concentrations were low suggesting quick degradation of peptidoglycan (Benner and Kaiser, 2003). Indeed, degradation experiments in oxygenated water confirmed high turnover (10-167 days) for peptidoglycan (Nagata et al., 2003). Since the abundance of living bacteria in the Black Sea water column is similar to oxic waters (Schink, 1999), we ascribed the high concentrations of Mur to the presence of undegraded peptidoglycan. Apparently, in the anoxic water column of the Black Sea Mur degradation is slower and/or production is higher. So far, peptidoglycan degradation experiments have been performed only in oxic environments (Jorgensen et al., 2003; Nagata et al., 2003; Shibata et al., 2006; Veuger et al., 2006) allowing no conclusive verification of the hypothesis of higher Mur preservation in anoxic environments.

The second hypothesis suggests an increased source of Mur in anoxic systems. It is known that in anoxic sediments the abundance of gram positive bacteria is higher than in oxic

sediments (Benner, pers. comm.; Bowman et al., 2000) and in oxic water most bacteria are gram negative (Moriarty and Hayward, 1982; Benner and Kaiser, 2003). Recent studies suggest that gram positive bacteria communities are abundant in the anoxic water column of the Black Sea (Madrid et al., 2001; Durisch-Kaiser et al., 2005; Morgan et al., 2006). These bacteria have a cell wall containing up to five times more Mur per mg C than gram negative bacteria (Moriarty, 1975; Benner and Kaiser, 2003). As a result, a higher abundance of gram positive bacteria could also explain the high Mur concentrations in the water column of the Black Sea.

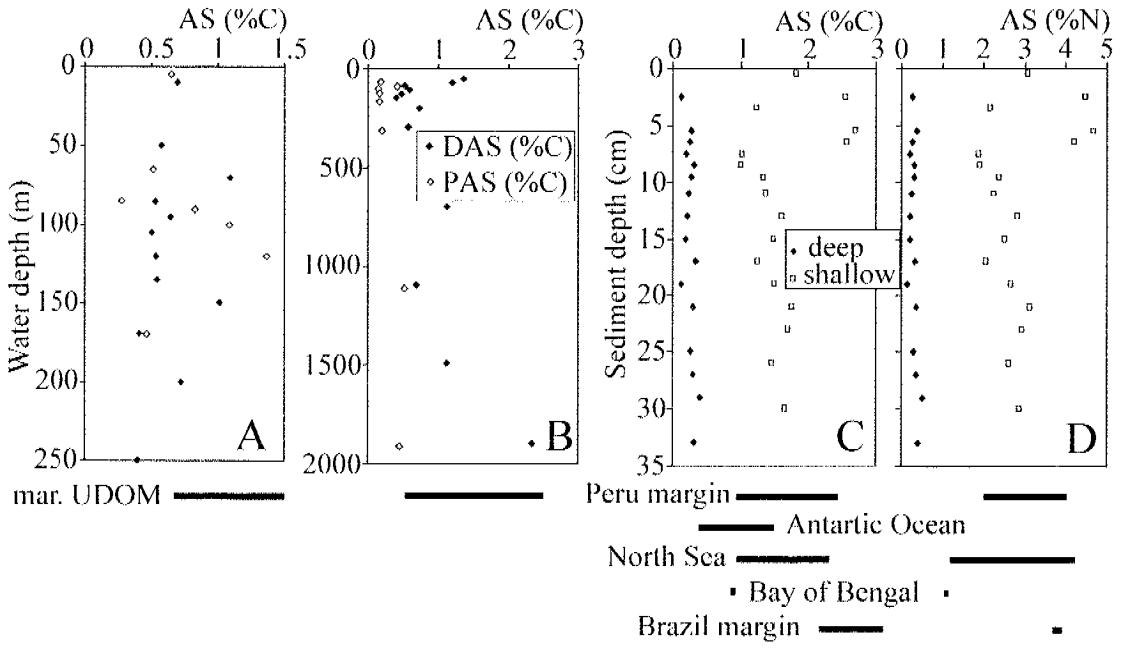


Figure 4.2: Water column. A: Amino sugar C-normalized yields in DOM (DAS (%C)) and POM (PAS (%C)) of the shallow site in comparison to literature data (Benner and Kaiser, 2003). B: Amino sugar C-normalized yields in DOM (DAS (%C)) and POM (PAS (%C)) of the deep site in comparison to literature data (Benner and Kaiser, 2003). C: Amino sugar C-normalized yields (AS (%C)) in the shallow and deep sites in comparison to literature data. D: Amino sugar N-normalized yields (AS (%N)) in the shallow and deep sites in comparison to literature data (Peru margin: Niggemann and Schubert (2006b); Antarctic Ocean: Liebezeit (1993); North Sea: Dauwe and Middelburg (1998); Bay of Bengal: Gupta et al. (1997); Brazil margin: Jennerjahn and Ittekkot (1999)).

4.5.2. Reactivity of ASs

Carbon-normalized yields of ASs allow estimating their reactivity in respect to total organic material. In the water column, DAS (%C) are rather constant with depth, except in the deep station, where their yields increase markedly below 1000 m (Fig. 4.2A and B). Similarly, PAS (%C) of the deep station - with lower values than DAS (%C) - increased in deep samples (Fig. 4.2B). At the shallow station, PAS (%C) have similar values as DAS (%C) and are highest at the oxic-anoxic interface (Fig. 4.2A). Overall, this suggests that there is a slight production of DAS and PAS in respect to OC in the deep water. This is in contrast to observation in oxic environments, where AS yields generally decreased with water depth (Benner and Kaiser, 2003; Davis and Benner, 2005), indicating that ASs were more reactive than bulk OC.

Single AS yields had slightly different trends. GlcN (%C) showed a similar trend as bulk ASs, GalN (%C) behaved similar to the GlcN, but were slightly less variable, and ManN was present only in very low concentrations (Fig. 4.3 and 4.4). Mur (%C) in the dissolved fraction showed no clear trend but had lowest values in the deeper water at both sites, while Mur (%C) in the particulate fraction was constant at the shallow station and increased with depth at the deeper station. The divergence between the profiles of C-normalized GlcN, GalN, and Mur suggests that they have different reactivities assuming that they were only formed in the uppermost productive water column. While GlcN and GalN have the same reactivity as bulk OC, Mur is more reactive than the bulk DOC pool, but less reactive than the bulk POC pool. There are two possible explanations for this difference. Either Mur in POM is mainly related to living bacteria, and is, therefore, not degraded with depth. Or peptidoglycan in the particulate fraction is combined in aggregates and is more difficult to degrade than smaller polymers found in the dissolved fraction.

Based on bacterial abundances, we estimated the fraction of Mur in particulate material originating from living bacteria. It has been determined that average carbon content of prokaryotic cells in marine bacteria is about 12.4 fg C $cell^{-1}$ (Fukuda et al. 1998) and average Mur yield in bacteria is 12.6 nmol $(mg\ C)^{-1}$ (Benner and Kaiser, 2003). Applying these values and assuming that all bacteria have been retained on the 0.7 µm filters and are in the POM fraction, 15-62% of particulate Mur originate from living bacteria (Fig. 4.5). However, this is rather unlikely, because the diameter of free living cells in Black Sea probes ranged from 0.34-0.44 µm, supposing spherical shapes (Morgan et al., 2006). Moreover, the amount of Mur originating from

bacteria in POM decreases with depth, suggesting an increasing contribution from bacterial detritus with depth. Therefore, we conclude that peptidoglycan is a major component of DOM as well as of POM, but that smaller Mur-polymers (in DOM) are consumed more easily than larger polymers or aggregates (in POM).

In the sediments, AS (%C) were very low and constant with depth at the abyssal station, while they increased slightly in the slope sediments (Fig. 4.2C). AS (%N) had the same trend (Fig. 4.2D). This suggests that in the core of the shallow station ASs are accumulated in respect to total OM, while in the abyssal core ASs have the same reactivity as TOC and TN.

Similar to the water column, single sedimentary ASs showed different depth trends. GlcN and GalN (%C) had the same trend as bulk ASs in both sites. ManN was present only in low concentrations. Mur (%C) showed, in contrast to GlcN and GalN (%C), a slight decrease in both sites (Fig. 4.3 and 4.4) and, therefore, appears to be more prone to degradation. These results are consistent with a previous study, where Mur was suggested to be degraded easier than other ASs during degradation of plant debris in water (Tremblay and Benner, 2006).

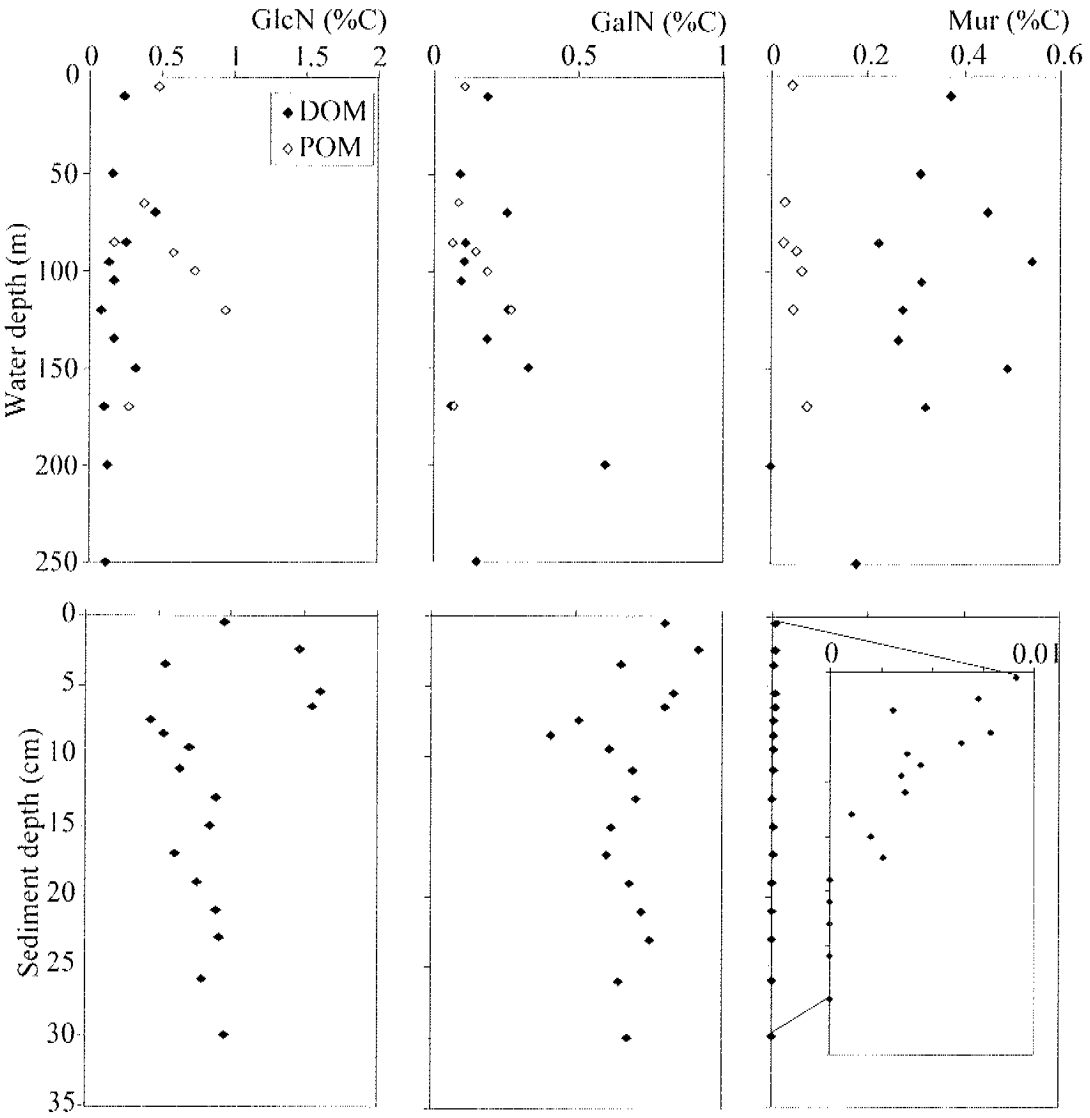


Figure 4.3: C-normalized yields of single ΛSs in the water column (top) and in sediments (bottom) of the shallow site.

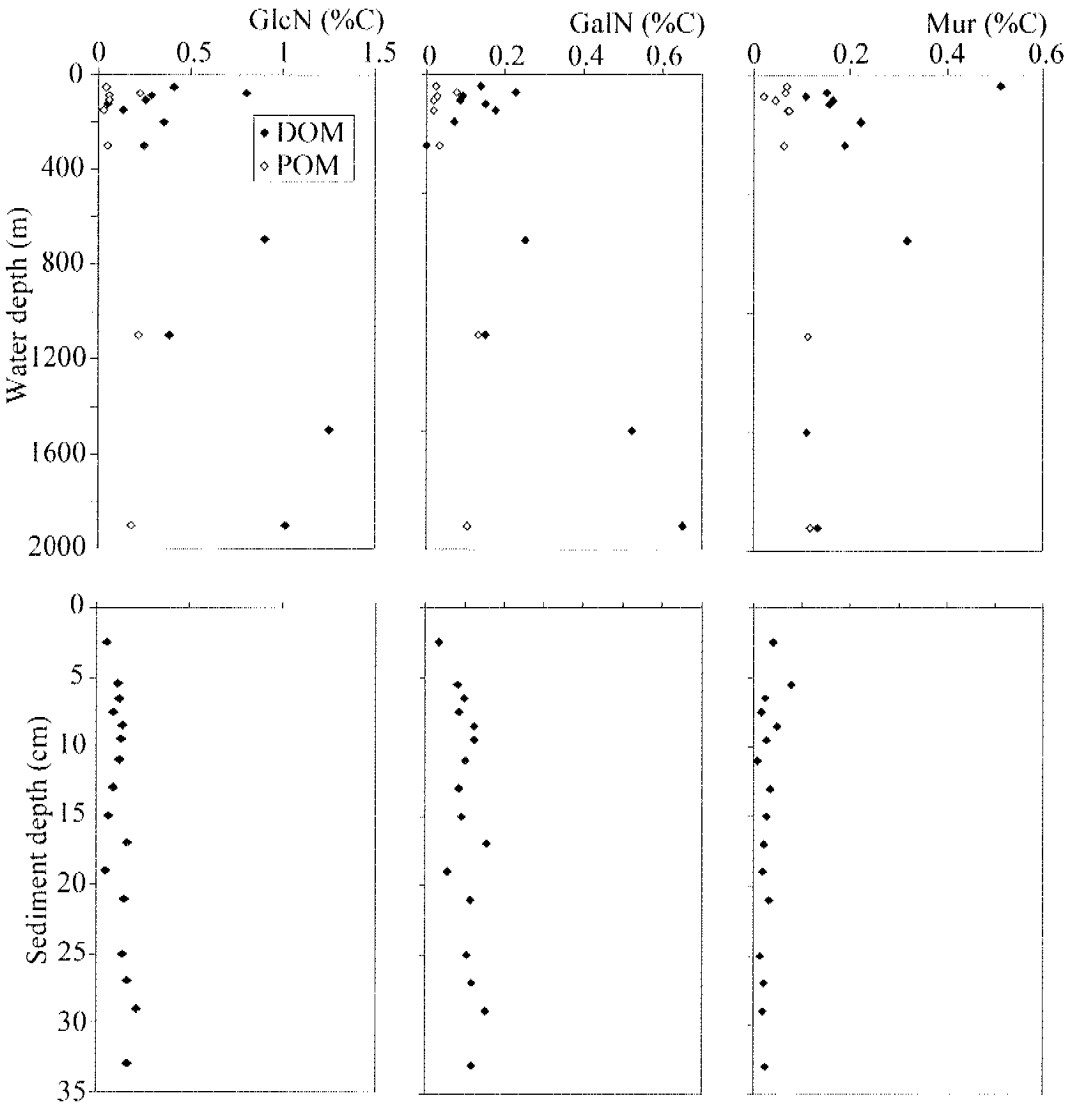


Figure 4.4: C-normalized yields of single ASs in the water column (top) and in sediments (bottom) of the deep site.

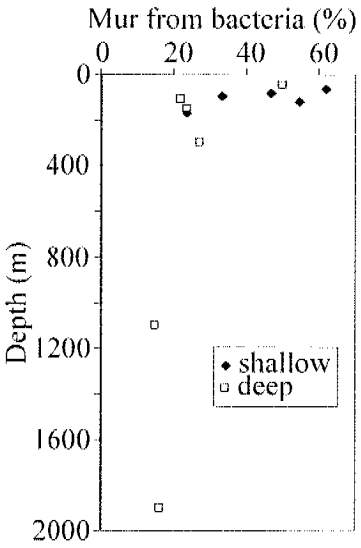


Figure 4.5: Mur derived from living bacteria in POM samples assuming that all bacteria were completely retained on the 0.7 µm filter.

4.5.3. Differences between water column and sediments

Similarly to the investigated oxic ocean water columns, the GlcN/GalN ratios in the Black Sea stabilized in the deep water below 1000 m to a value of about 1.4 (Benner and Kaiser, 2003; Davis and Benner, 2005). This relation was also found in the sediments (Fig 4.6). The same GlcN/GalN ratio has been reported from deep water and sediments of different systems suggesting the presence of a refractory biopolymer with a similar source in all environments (Gupta et al., 1997; Jennerjahn and Ittekkot, 1999; Benner and Kaiser, 2003; Davis and Benner, 2005; Unger et al., 2005; Niggemann and Schubert, 2006b; Tremblay and Benner, 2006; Klauser and Schubert, Chapter 3). This suggests that, GlcN and GalN produced in shallow water depths are substituted by a biopolymer with a constant composition in the deep water. Increasing GlcN and GalN concentrations in the deep water indicate production of these compounds. GalN concentrations increase even stronger relative to GlcN between 700 and 1900 m leading to the observed lower and constant ratio compared to the shallower waters. This suggests that a relatively refractive compound is produced and accumulated in the deep water of the Black Sea. This is in contrast to oxic environments where, although a GlcN and GalN ratio of 1.4 is observed, this biopolymer is not accumulated.

The GlcN to GalN ratios in the sediments of the deep site are similar to the ratios in the bottom water samples, but AS (%C) are about 10 times lower (Fig. 4.2). This suggests strong AS degradation at the water-sediment interface. While constant AS (%C) within the sediment core indicate no preferential degradation of amino sugars in the sediments but do not exclude transformation.

At the shallow station we observed a different pattern of AS composition and transformations. The GlcN to GalN ratios in the bottom water do not reach a constant value of 1.4 (Fig. 4.6) suggesting that ASs are not transformed in the water column to the polymer with constant GlcN and GalN composition. Since in the sediments the GlcN to GalN ratio is constant (about 1.3), transformation processes must occur at the water-sediment interface. Despite these AS transformations at the water-sediment interface, the AS (%C) in the sediments are higher than in the water column (Fig. 4.3). This indicates that sedimenting particles are enriched in ASs in respect to dissolved particles suggesting that the short water column of this site allows settling of increased amounts of ASs. Indeed, the shallow site has about a three times higher sedimentation rate than the deep site (Teodoru et al., 2007). As a result, high sedimentation rates and a shorter water column contribute to AS accumulation in the sediments of the shallow region, while the long water column and the low sedimentation rates of the deep region allow degradation of a major fraction of ASs already in the water column.

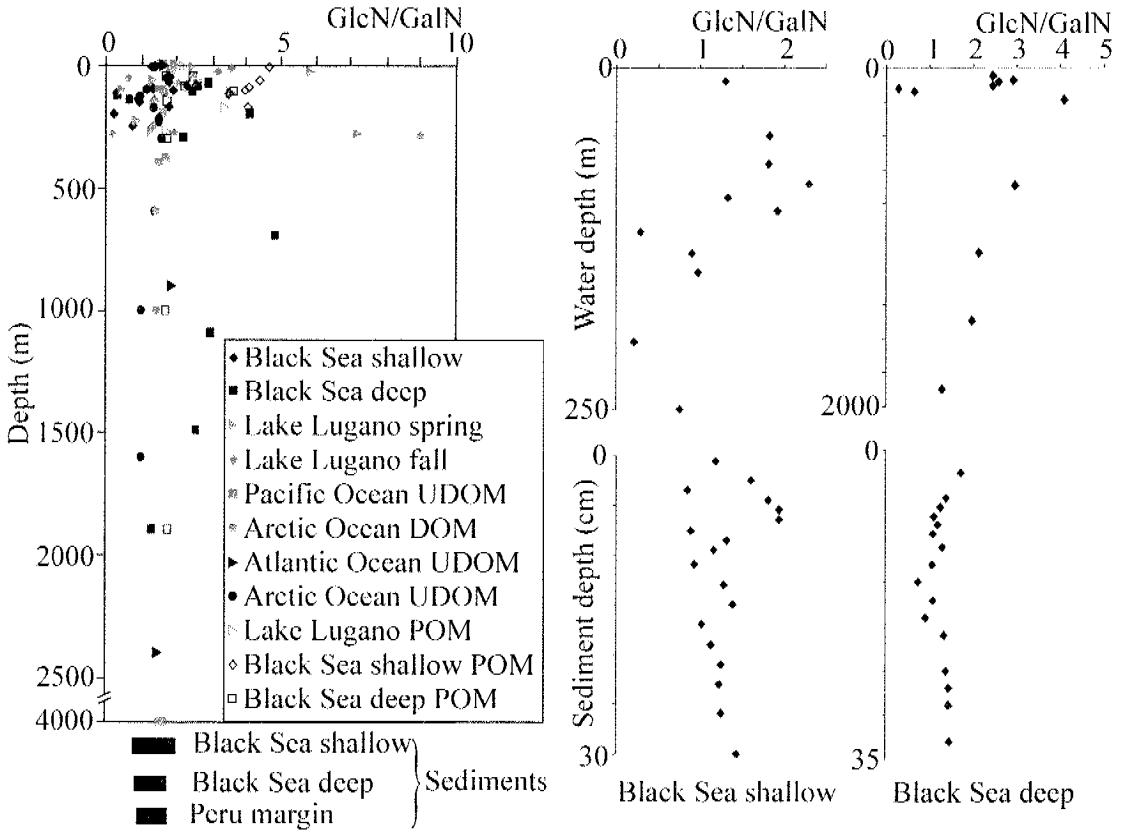


Figure 4.6: GlcN/GalN ratios in the water column and in the sediments of the Black Sea compared to ratios of different environments (Lake Lugano: Klauser and Schubert, Chapter 3, Pacific, Atlantic, and Arctic Ocean UDOM: Benner and Kaiser, 2003; Arctic Ocean DOM: Davis and Benner (2005); Peru margin: Niggemann and Schubert (2006)).

5

CONCLUSIONS AND FUTURE PERSPECTIVES

5.1. Conclusions

This work contributed to the understanding of organic matter transformations in anoxic aquatic environments describing and analyzing the distribution of amino sugars in water and sediments of Lake Lugano and the Black Sea. These compounds could complement traditional indicators for organic matter quality such as amino acids, fatty acids and chlorins. In the following, the most important findings are summarized and discussed with respect to their broad significance in biogeochemistry. In addition, possible directions for further investigations are presented.

5.1.1. Amino sugars characteristics in marine and limnic anoxic systems

Overall, the amino sugar distribution of the two anoxic systems, Lake Lugano and the Black Sea, showed similarities as well as differences to oxic environments. In analogy to oxic environments, the ratio of glucosamine (GlcN) to galactosamine (GalN) had a constant value in the deep water column (> 1000 m) and in sediments. This stable composition of GlcN and GalN in all different environments suggests the presence of a biopolymer with a common source and refractive behavior.

In contrast to oxic systems, the muramic acid (Mur) concentrations were much higher in Lake Lugano and the Black Sea. This suggests an increased production of peptidoglycan and/or a slower degradation in these anoxic environments (Benner and Kaiser, 2003). Although a study of only two anoxic systems should not be generalized, Lake Lugano and the Black Sea represent two very different environments. The first is highly productive and has high sedimentation rates

in the 100 m-short oxic water column resulting in fast burial of high amounts of organic matter. The second has much lower sedimentation rates and variable carbon accumulation rates in different regions (Teodoru et al., 2007).

The main differences in amino sugar distribution between these two anoxic systems were the amino sugar distribution in the sediments and the presence of mannosamine (ManN) in the water column of Lake Lugano. In Lake Lugano sediments, the total amino sugar carbon normalized yields correlated with the relative concentrations of long-chain fatty acids, which are indicators of terrigenous material. This suggests that amino sugars of terrestrial origin are more resistant to degradation than autochthonous amino sugars. In contrast, in the sediments of the Black Sea amino sugars were not accumulated (or accumulated only slightly) with depth and no influence from terrestrial material was observed (Stokozov and Buesseler, 1999).

In sediments of Lake Lugano, ManN concentrations slightly correlated with long-chain fatty acid concentrations suggesting that ManN might originate from a terrestrial source. Therefore also in the water column, ManN most probably originates from terrigenous material. This would explain the absence of ManN in the other studied environments, because those are not influenced by terrestrial input (Benner and Kaiser, 2003). However, an autochthonous source for ManN in Lake Lugano could not be completely excluded.

Besides the presence of refractive terrestrial material, in Lake Lugano the variability of the sedimentation regime appeared to enhance accumulation of organic compounds, as well. In the sediments, very reactive compounds like chlorins were degraded only slightly during burial of fresh algal debris. In contrast, in the sediments of the Black Sea, the sedimentation regime was more regular facilitating degradation of organic compounds (e. g., Calvert et al., 1991; Teodoru et al., 2007). In particular, at the deep site, low concentrations of heavily degraded organic matter indicated that low sedimentation rates through a deep basin facilitate degradation processes.

In summary, amino sugar concentrations are generally high in productive anoxic basins with a short water column, while they are well consumed in deep basins. Ami no sugars-biopolymers of terrestrial origin and variable sedimentation regime might play an important role in the degradation state in the sediments.

5.1.2. Amino sugar sources

High Mur concentrations in Lake Lugano and in the Black Sea suggested the importance of the contribution by bacterial organic matter. This is in agreement with previous studies in the Black Sea (Wakeham and Beier, 1991) which encountered high concentrations of bacterial fatty acids. Moreover, several authors provided evidence that dissolved organic matter is of major prokaryotic origin (Boon et al., 1998; McCarthy et al., 1998; Ogawa et al., 2001; Benner and Kaiser, 2003)

The presence of a biopolymer with similar GlcN and GalN composition in the deep water column and in sediments, where organic matter is more degraded, hints also to a possible prokaryotic source (Benner and Kaiser, 2003; Davis and Benner, 2005; Niggemann and Schubert, 2006). On the other hand, in shallow waters fresh GlcN and GalN are mainly of algal origin. In fact, in highly productive regions a relationship between primary production and GlcN and GalN concentrations in particulate organic matter was observed (Davis and Benner, 2005). Therefore the GlcN-GalN biopolymer might also be produced by phytoplankton in a very refractive form, which is not modified by bacterial reworking. In systems with an allochthonous influence like Lake Lugano, the additional presence of refractive terrestrial organic matter renders the ascription to a specific source even more difficult.

The sources of ManN are still uncertain, because only few studies reported high concentrations (Yoneyama et al., 1982; Komandrova et al., 2001; Turrion et al., 2002; Benner and Kaiser, 2003). In our analysis of Lake Lugano, we ascribed ManN mainly to a terrigenous source.

5.1.3. Reactivity of organic matter components

5.1.3.1. Reactivity of fatty acids, amino acids, and chlorins

The different organic matter components analyzed in Lake Lugano sediments showed distinct reactivities based on their variation in concentration with depth. Unsaturated fatty acids were the most reactive components, followed by mid-chain fatty acids, amino acids, and long-chain fatty acids. This succession of reactivity is in agreement to previous investigations (Wakeham et al., 1997b). In contrast, chlorin is better preserved in the sediments of Lake Lugano

than other components, while in oxic environments chlorins are clearly the most reactive organic matter component (Brown et al., 1972; Colombo et al., 1996; Meckler et al., 2004). Therefore chlorin degradation appears to be strongly affected by anoxia (Sun et al., 1993; Ding and Sun, 2005). In Lake Lugano other factors, like the variability of the sedimentation regime and sediment mixing were probably responsible for chlorin preservation. Because in the Black Sea chlorins were degraded to a similar degree as in oxic environments, anoxia alone can not be the main explanation for reduced chlorin degradation in Lake Lugano.

5.1.3.2. Reactivity of amino sugars

In Lake Lugano sediments, amino sugars appeared to be more refractive than amino acids and fatty acids. Consequently, one would expect an enrichment of amino sugars in comparison to amino acids in more degraded material. Based on the chlorin index and on C/N ratios, the sediments of the three sites, analyzed in this work, show increasing organic matter degradation state in the following order: Lake Lugano, shallow Black Sea site and deep Black Sea site. In contrast to what was expected, the amino sugar to amino acid ratio decreases in more degraded sediments (Table 5.1) suggesting that higher amounts of amino acids are preserved. Possibly, the variable amino acid and amino sugar composition of source material and further effects, like protection by matrix and adsorption to particles contribute to preferential preservation of polymers with variable composition of amino acids and amino sugars (Hedges and Hare, 1987).

Table 5.1: Minimal and maximal values of amino sugar to amino acid ratios in sediments.

Site	amino sugars/amino acids
Lake Lugano	0.06-0.5
Black Sea shallow	0.1-0.2
Black Sea deep	0.01-0.04

5.1.4. Carbon accumulation in anoxic systems

Even though, traditionally, anoxic systems are considered to accumulate organic matter more efficiently (Henrichs and Reeburgh, 1987; Hedges and Keil, 1995; Hartnett et al., 1998; Hulthe et al., 1998), several authors challenged this theory (e. g., Foree and McCarty, 1970; Calvert et al., 1991; Lee, 1992; Calvert and Pedersen, 1993). Here we showed that other factors were governing carbon preservation in the anoxic Lake Lugano, such as sedimentation regime, the nature of input material, and internal slumps. In the Black Sea study, we presented evidence that the sedimentary organic material was highly degraded, even though it was deposited under anoxia. This indicates that anoxia in combination to other factors enhances carbon preservation.

5.2. Future perspectives

This work represents the first amino sugar characterization in specific anoxic environments. It is obvious that, for a generalization and an evaluation of the role of amino sugars in carbon accumulation processes, a larger amount of data in several sites of different environments is required. The identification of specific amino sugar sources, for instance the source of ManN in Lake Lugano, and the characterization of the nature of amino sugar polymers will increase the understanding of amino sugar degradation and accumulation. Degradation experiments with different components (e. g., microbes, phyto- and zooplankton, chitin, peptidoglycan, and terrestrial debris) and environmental conditions (e.g., temperature, light, oxygen availability, and physical mixing) would allow evaluating the role of single environmental factors. For example, oxygen availability, adsorption to mineral particles, sedimentation rates, mixing of freshly deposited sediment, and degradation state of settling particles. Degradation experiments would also help to elucidate the nature of the refractive biopolymer of constant GlcN and GalN composition found in deep waters and sediments of all the studied environments. In addition to the analysis of amino sugar concentration changes with time during the experiment, acid hydrolysis-tests with variable acid strengths would give a rough estimation of the presence of different Mur-, GlcN-, and GalN-polymers. Recent degradation experiments focused on the degradation of plant debris in water (Tremblay and Benner, 2006), on

chitin (Poulicek et al., 1998; Davis and Benner, 2005), and on peptidoglycan degradation in marine environments (Jorgensen et al., 2003; Nagata et al., 2003; Veuger et al., 2006).

An interesting finding of this thesis was the high amount of amino sugars in the dissolved fraction of Lake Lugano and the Black Sea. However, the polymeric nature of the components and, in particular, the chain-length distribution and the reactivity of the different chains of amino sugar-containing polymers were not determined. These analyses would allow estimating the bioavailability of polymers with different chain-length. Several methods for the determination of polymer sizes exist: light scattering, filtration, cumulative sedimentation analysis, and laser scanning microscopy (Muzzarelli et al., 1987; Beri et al., 1993; Bloem et al., 1995; Wu et al., 1995; Knaul et al., 1998; van Hee et al., 2004). The most common method for the determination of polymer size is laser light scattering (Wu et al., 1995) that has been successfully applied in pure chitosan solutions (Muzzarelli et al., 1987; Beri et al., 1993; Knaul et al., 1998). Filtration and size exclusion chromatography are fast methods to separate polymers of different chain lengths. The determination of the nature of the polymer demands a further step of hydrolysis and analysis of monomers. This method has been recently described for the analysis of peptidoglycan (Shibata et al., 2006). Further, cumulative sedimentation combined with Mur detection has been described recently for the quantification of solid cell material in different size fractions (van Hee et al., 2004). In addition, the method developed for the determination of cell numbers, sizes, widths, and lengths in soil samples using laser scanning microscopy might be modified for different polymers (Bloem et al., 1995).

The difference in amino sugar content between the water column and the sediments of the two sites in the Black Sea, suggested that there might be a strong variability in amino sugar degradation at the water-sediment interface. Focused studies on degradation processes of settling particles at the sediment-water interface could, therefore, elucidate the mechanisms of amino sugar degradation and preservation in water, sediments, and at their interface.

The analysis of the natural carbon and nitrogen isotopic ratios in different organisms and in aquatic environment could constrain the sources of amino sugars. For ^{13}C a GC-IR/MS method has been developed for soil samples (Glaser and Gross, 2005), while the low natural abundance of ^{15}N poses problems for the development of a suitable method.

In conclusion, this work showed that amino sugars have different characteristics from traditional indicators for organic matter degradation state, such as amino acids and fatty acids. In

particular, muramic acid is a biomarker for rather fresh organic material of bacterial origin, while glucosamine and galactosamine can be indicators for organic matter degradation state. Therefore, amino sugars will be useful tools for the description of organic matter sources and degradation state.

6

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