Scientific paper

The Role of Dissolved Carbohydrates in the Northern Adriatic Macroaggregate Formation

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Abstract

Total dissolved carbohydrates and their monosaccharide and polysaccharide fractions were studied at a fixed sampling point in the northern Adriatic Sea off Pesaro, Italy, in 2002, 2003, and 2004 which were characterized by the presence of macroaggregates, and in 2001 and 2005 when the macroaggregates were absent. During the presence of macroaggregates in 2004, the study was extended to the southern part of the Gulf of Trieste, Slovenia. In parallel, phytoplankton biomass determined from chlorophyll *a* concentration, and nitrogen and phosphorus nutrients were tracked. The concentrations of total dissolved carbohydrates and, above all, dissolved polysaccharides were higher in late spring and early summer before and during the mucilage appearance. Conversely, the concentrations of dissolved monosaccharides remained nearly constant throughout the study period. In addition to higher polysaccharide concentration, other hydrological, chemical and biological factors seem also important for macroaggregate formation in the northern Adriatic Sea. Among them, the DIN/PO_4^{3-} ratios, regularly increasing in spring due to phytoplankton use of nitrate and phosphate, could be indicative of macroaggregates appearance since they were lower in 2001 and 2005 while during the presence of mucilage in 2002, 2003 and 2004 they markedly increased.

Keywords: Dissolved carbohydrates, mucilage, nutrients, phytoplankton, northern Adriatic Sea

1. Introduction

In the late spring and summer, the northern Adriatic Sea is periodically affected by the massive presence of macroaggregates consisting of gelatinous material suspended in the sea surface, in the water column and at the sea bottom. This complex phenomenon has been observed for a long time, since the first record dated back to 1729[1] and continuous reports appeared throughout the 19th and 20th Centuries.[2,3] In the last two decades, gelatinous masses have appeared in the northern Adriatic along the western (Italian) coast as well as eastern (Slovenian and Croatian) coastline.[4,5,6] These mucilaginous masses are found in a variety of stages and forms and can be distributed patchy[7,8] in the water column depending on hydrological conditions including temperature, salinity and currents.[9] The chemical analyses of mucilage organic matter revealed a complex chemical composition mostly due to the presence of heteropolysaccharides as well as other compounds including lipids, proteins and refractory substances.[10–16]

The formation of macroaggregates is not yet completely understood. Several hypotheses have been suggested, including the alteration of the N/P ratio which could cause a change in the rate of phytoplankton growth as well as affect phytoplankton metabolism inducing the production of mucilaginous material.[17–20] Diatoms[21] seem to be the principal producers of macroaggregates although other phytoplankton species, for example dinoflagellates, are also present in aggregates.[15,22,23] It has also been postulated that macroaggregates are produced as a consequence of enhanced production of algal exudates associated to changes of grazing[24] as well as the interactions between phytoplankton and bacteria.[25,26] A hypothesis based on extensive lysis of the diatoms present in mucilage has been also proposed[23,25] since it seems that the organic matter release from cell lysis is higher than from extracellular production of healthy cells. Another hypothesis on mucilage origin in the Adriatic Sea is based on the humin structure of macroaggregates[27].

The mechanism responsible for macrogel formation could be understood in terms of polymer gel theory.[28–30] Formation of marine micro- and macrogels implicates the agglomeration of polymer fibrils and colloids by collision, forming macrogels and larger particles[31] or the spontaneous or induced assembly of polymer chains when the interchain distances become so close that the polymers could interact with each other through chemical bonding or forces, including electrostatic, hydrogen and van der Waals forces, resulting in the formation of crosslinkings.[32,33]

The aim of the present work was to study the distribution of dissolved carbohydrates, in terms of dissolved monosaccharides and polysaccharides in the northern Adriatic in the years of 2001, 2002, 2003, 2004 and 2005, including three years (2002, 2003 and 2004) of mucilage appearance, to decode their role in the macroaggregate formation. The related nutrient and phytoplankton distributions as well as the gross chemical composition of macroaggregates were also investigated.

2. Materials and Methods

2. 1. Samples

Water samples were collected monthly in the northern Adriatic sea 3 km offshore of Pesaro (43°55.50’ N, 12°54.50’ E), Italy, (Fig. 1) in the period 2001–2005 and in the southern part of the Gulf of Trieste 1.5 km offshore of Piran (45°31.46’ N, 13°33.72’ E), Slovenia, weekly to biweekly from May – September 2004. The seawater samples were collected at surface (0.5 m) using polyethylene bottles and frozen at –20 °C.

The macroaggregate samples were collected off Pesaro in surface layer during the mucilage appearance in July 2002, and June 2003 and 2004. In the Gulf of Trieste the macroaggregates were sampled in surface layer in June 2004 during the mucilage event. All mucilage samples occurred in the form of gelatinous surface layer,[7] i.e. macrogel form. The thawed mucilage was separated from the water by centrifugation at 4000 rpm for 20 min at ambient temperature. The sediment was dialyzed using dialyzing tubing bags (12–14 kDa, 24 h) against milli-Q water, dried at 105 °C and stored at –20 C until further processing.

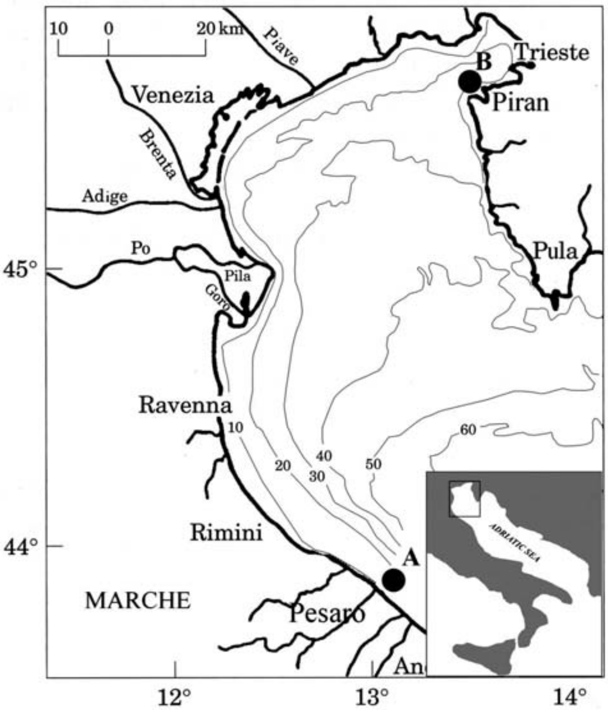

Figure1. Research areas in the northern Adriatic Sea off Pesaro (A, Italy) and in the S part of the Gulf of Trieste (B, Slovenia).

2. 2. Analyses

2. 2. 1. Seawater Samples

Total dissolved carbohydrates (TDCHO) and monosaccharides (MDCHO) in water samples were determined using the 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) method of Johnson and Seiburth.[34] D-glucose was used as a standard. All analyses were performed in triplicate. The precision of measurements was ±5%.

Chlorophyll *a* (Chl *a*) was determined in 90% acetone homogenates of particulate matter collected on Millipore HA membrane filters using the spectrophotometric method of Strickland and Parsons[35] in samples collected off Pesaro and fluorimetrically according to Holm-Hansen et al.[36] in samples from the Gulf of Trieste. The precision of measurements was ±5%. Analyses of phytoplankton species were performed using an inverted microscope according to Utermohl.[37]

NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-} in water samples filtered through Millipore HA membrane filters were determined using the standard colorimetric methods of Parsons et al.[38] The precision of measurements was ±5%. Dissolved inorganic nitrogen (DIN) was calculated as $NO_3^- + NO_2^- + NH_4^+$.

2. 2. 2. Macroaggregates

In the dry residue of macroaggregates, the total carbohydrates, proteins and lipids were analyzed using the MBTH method of Pakulski and Benner,[39] the Comassie Brilliant Blue method of Setchell,[40] after extraction with 0.5 M NaOH, and the gravimetric Folch[41] method, respectively. Standards comprised solutions of D-glucose for carbohydrates and BSA for proteins in mili-Q water. All analyses were performed in triplicate.

2. 3. Dissolved Carbohydrates

In all studied years, i.e. 2001, 2002, 2003, 2004 and 2005, the concentrations of TDCHO and dissolved polysaccharides (PDCHO) showed temporal variations (Fig. 2) which were more pronounced, except for the year 2004, in the years with the presence of mucilage.

They were normally higher in late spring and early summer reaching values of up to 60 µM C-TDCHO. It should be pointed out that during the macroaggregate appearance in 2002 and 2003 the increase of concentrations in late spring and summer was in general higher compared to years of macroaggregate in absence. In 2004, we observed another maximum of carbohydrates in February in ad-

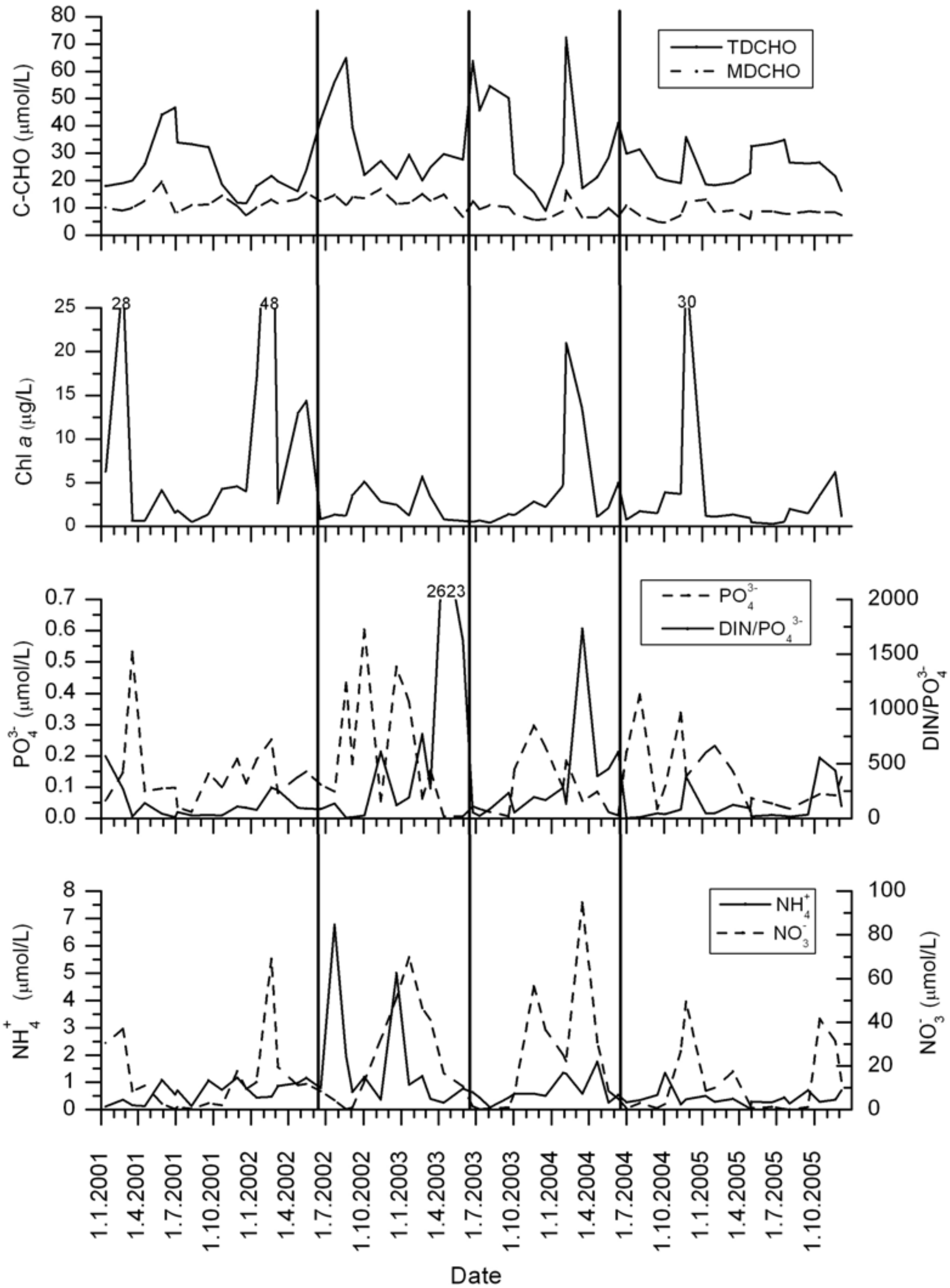

Figure 2. Variations of total dissolved carbohydrates (TDCHO), dissolved monosaccharides (MDCHO), chlorophyll *a*, nitrate, ammonium, phosphate and DIN/PO_4^{3-} ratio in the surface layer (0.5 m) of northern Adriatic off Pesaro. Vertical lines indicate the occurrence of macroaggregates.

dition to the less intense spring/summer increase of carbohydrates in June. The concentrations in other periods were generally lower, averaging 20 µM C-TDCHO, and were just above the background levels reported for the northern Adriatic waters.[42] Conversely, the concentrations of dissolved monosaccharides remained nearly constant throughout the study periods averaging about 10 µM C. The monosaccharides represented only a minor fraction of the total dissolved carbohydrates during late spring and summer in all studied years, averaging about 20–30%, while during other periods their percentage increased to about 30–50%. The concentrations of TDCHO in the Gulf of Trieste measured in spring 2004 (Fig. 3) varied between 21–28 µM C and were lower than those off Pesaro. The monosaccharides represented about 20–30% of TDCHO.

2. 4. Chlorophyll *a* and Phytoplankton

Chl *a* concentrations off Pesaro (Fig. 2) showed pronounced maxima in winter (February) of 2001, 2002, 2003, 2004 as well as in May/June 2002 and June and December 2004. Concentrations of chlorophyll *a* in 2005 were much lower compared to the years when the mucilage appeared. The taxonomic analysis of phytoplankton species off Pesaro revealed that in early spring of 2003, 2004 and 2005 mainly *Skeletonema costatum*, *Cerataulina* and *Pseudo-nitzschia* were present while later in May and June 2003, 2004 and 2005 and July 2003 and 2004 *Gonyaulax fragilis*, *Cylindroteca closterium* and *Gymnodimium* dominated. In July 2005, *Chaetoceros* and *Fibrocapsa japonica* prevailed.

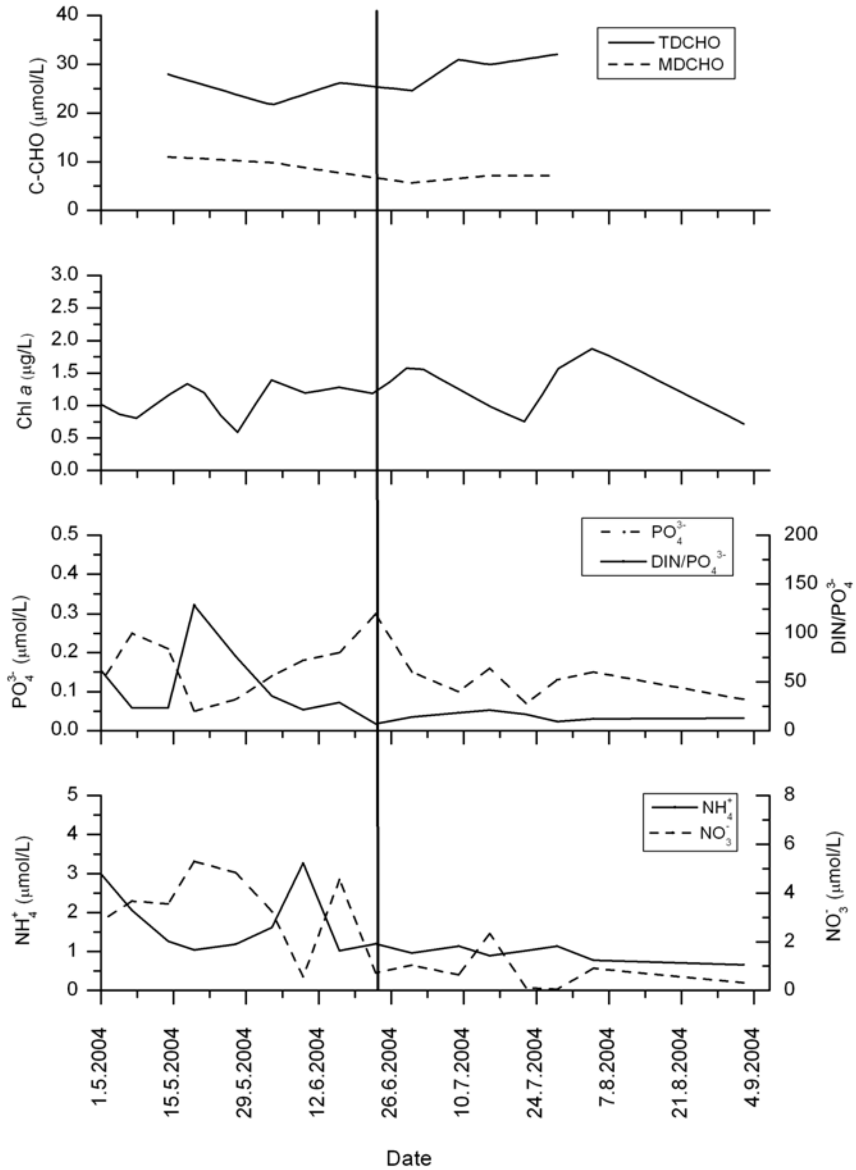

Figure 3. Variations of total dissolved carbohydrates (TDCHO), dissolved monosaccharides (MDCHO), chlorophyll *a*, nitrate, ammonium, dissolved inorganic nitrogen (DIN), phosphate and DIN/PO_4^{3-} ratio in the surface layer (0.5 m) of the Gulf of Trieste. Vertical line indicates the occurrence of macroaggregates (from Kovac et al.).[16]

In the Gulf of Trieste the concentrations of chlorophyll *a* (Fig. 3) showed maximal values in spring (May and June) and summer (end of July and in the beginning of August) of 2004. Taxonomic composition of the phytoplankton blooms showed that nanoflagelates was the dominant group throughout the year except in August when diatoms prevailed. Their abundance increased also during the most pronounced seasonal peak in June coinciding with the mucilage appearance.[43]

2. 5. Nutrients

2. 5. 1. Nitrate and Ammonium

During the entire study off Pesaro (Fig. 2) lower nitrate (<14 μM) concentrations were observed in the period from May to October. The spring nitrate concentrations were higher in the years of mucilage event, i.e. 2002, 2003 and 2004, with some values exceeding 40 μM. The ammonium concentrations (Fig. 2) were low generally not exceeding 2 μM. Nitrate comprised great majority of DIN except during summers when ammonium reached up to 78% of DIN. Similar temporal change of DIN composition in the northern Adriatic, i.e. from high winter nitrate content to summer high ammonium content, was also observed by Cozzi et al.[9]

The nitrate concentrations in the Gulf of Trieste in 2004 (Fig. 3) were higher in spring, and dropped down below 4 μM in late spring and summer. Higher ammonium concentrations, ranging 1.5–3.3 μM, were observed in early spring 2004. In the mid of May, the intense decrease of ammonium concentrations to about 1 μM was noticed (Fig. 3). Nitrate comprised the majority (approx. 70–80%) of DIN in spring 2004 while during summer the ammonium reached up to 90% of DIN.

2. 5. 2. Phosphate

The lowest phosphate concentrations off Pesaro (Fig. 2) were observed in spring. In 2005, low phosphate concentrations were present throughout the whole year. However, the phosphate concentrations were not exceeding 0.63 μM. In the Gulf of Trieste in 2004 (Fig. 3), the phosphate concentrations varied around 0.1 μM in early spring and increased in the mid of May and later in June to about 0.3 μM.

2. 5. 3. DIN/PO_4^{3-} Ratio

The DIN/PO_4^{3-} ratios (molar) off Pesaro (Fig. 2) in 2001 and 2005 varied between 9–555 (average values about 121) while in the years of 2002, 2003 and 2004, characterized by the presence of mucilage in late spring and summer, were higher averaging about 340 (including values over 1000).

The DIN/PO_4^{3-} ratios in the Gulf of Trieste (Fig. 3) dropped down in the first half of May 2004 from about 150 to 20 (molar). In the mid of May it raised up to about 130 (atomic) and in June decreased again to about 10. The summer DIN/PO_4^{3-} ratios were about 14 approaching the Redfield ratio.

2. 6. Macroaggregate Description and Composition

Large macroaggregates appeared in the mid of July 2002 off Pesaro were in the form of creamy surface layer of about 10–15 cm length while in the water column the 7–8 m long filamentous structures prevailed. This extensive phenomenon reached the western central Adriatic Sea. The macroaggregates disappeared definitely in September 2002. In June 2003, the low intensity mucilage phenomenon was observed consisting of surface creamy layer, 1–2 m long stringer in the water column, and accumulation at thermocline (6–7 m deep) and in the bottom water layer. It disappeared at the end of July 2003. In June 2004, extensive mucilage formation was observed consisting of long surface and subsurface horizontal bands, as well as accumulations at the thermocline (5–6 m deep) and in the bottom water layer. It disappeared at the end of July 2004.

The mucilage event in 2004 in the Gulf of Trieste evolved in the mid of May of 2004 in the form of marine snow and small aggregates within the water column. More intense aggregation processes appeared in June 2004 leading to the formation of large quantities of surface aggregates, and subsequently more dense and gelly macroagreggates. They were at the beginning densely populated by single species of dinoflagellates – *Gonyaulax fragilis* but they became later colonised by typical "mucous community" with predominating diatoms, flagellates, dinoflagellates (more empty theca than living cells), cyanobacteria and heterotrophic bacteria, when mucus aggregates assumed their typical appearance (Mozetič, pers. comm.). In the beginning of July only sporadic macroaggregates were present on the sea surface. In the mid of July 2004 the water column was transparent but about 5-m thick highly turbid bottom layer was found due to the accumulated organic matter.

The gross chemical composition of macroaggregate samples collected off Pesaro in the years of 2002, 2003 and 2004 is presented in Table 1. Analyses revealed carbohydrates as the main component varying between 20–25%. Proteins averaged about 12% and lipids about 8% of the macroaggregates. The gross composition of macroaggregates from the Gulf of Trieste in 2004 (Table 1) showed lower contents of carbohydrates, proteins and lipids compared to those sampled off Pesaro probably due to different "maturation stages" and entrapped particle contents.

This was evident also from the comparison of FT-IR spectra of samples collected off Pesaro in 2002 and that from the Gulf of Trieste sampled in 2004 (Fig. 4). The broad band centred between 3700 and 3000 cm^{-1} mostly corresponds to O–H stretching of hydroxyl groups as well as N–H groups. Absorptions in the range 2800–3000 cm^{-1} originating from various aliphatic components were more pronoun-

Table 1: Mean composition (± S.D., n = 3) of mucilage in terms of total carbohydrate, protein and lipid contents (% of dry weight) in samples collected off Pesaro (Italy) in 2002, 2003 and 2004 and in the S part of the Gulf of Trieste (Slovenia) in 2004

Sample	Year	Carbohydrates	Proteins	Lipids
Off Pesaro	2002	21.49 ± 0.70	11.91 ± 0.29	8.28 ± 0.10
	2003	20.23 ± 0.15	11.54 ± 0.06	7.07 ± 0.06
	2004	18.78 ± 0.32	10.22 ± 0.19	7.18 ± 0.08
Gulf of Trieste	2004	13.5 ± 0.58	4.67 ± 0.11	2.24 ± 0.13

ced in spectra of Italian sample. Similarly, a band of a higher intensity at 1440 cm^{-1}, assigned to aliphatic C–H deformation vibrations of methyl and methylene was observed. Signals from carbonates, additionally confirmed by low-intensity bands at 2515, 1795 and 876 cm^{-1}, also contribute the later band. Carbonate component, polysaccharides and silicates (900–1200 cm^{-1}) were relatively lower in samples collected in the Gulf of Trieste in 2004. Higher relative abundance of silicates in Italian sample was mostly due to greater contribution of diatoms. Part of silicates could also originate from the freshwater and atmospheric inputs.

The FT-IR spectra are in accordance with previously reported general composition of the northern Adriatic macroaggregates[14,15,44,45] and also carbohydrate and protein data are similar to formerly published analyses.[46]

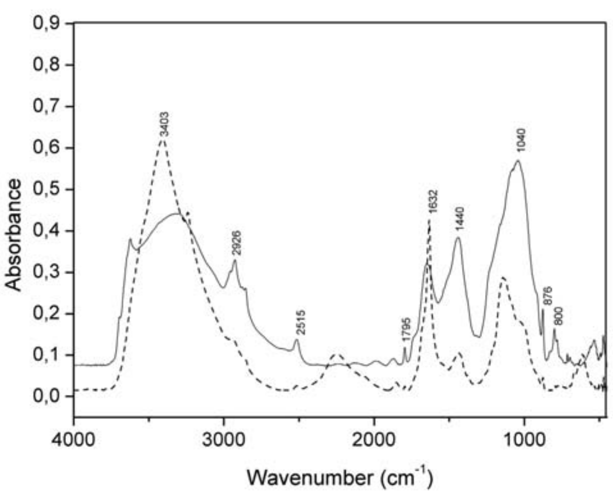

Figure 4. FT-IR spectra of macroaggregates collected off Pesaro, Italy (solid line) and in the Gulf of Trieste, Slovenia (dash line)

3. Discussion

The temporal changes of dissolved carbohydrates, macroaggregate precursor have been studied in two different areas of the northern Adriatic in surface layer, namely eutrophic off Marche and largely oligotrophic in the Gulf of Trieste, where mucilage events recently appeared. During the study we have observed the interannual variation of soluble carbohydrates as well the change of their concentrations, throughout the year. A seasonal pattern of DTCHO concentrations in northern Adriatic, with a marked increase during stratification periods (especially during summer) and the lowest values in the winter, was reported also by Ahel et al.[47] The late spring and summer increase of TDCHO in the northern Adriatic, while the concentration of dissolved monosaccharides remained nearly constant, depends entirely on the increasing presence of dissolved polysaccharides. Similar observations were reported by Nieto-Cid et al.[48] in the coastal waters along NW Iberian peninsula. Børsheim et al.[49] and Myklestad and Børsheim[50] also reported increasing concentrations of TDCHO in late spring and summer in the Norwegian Trondheimsfjord and north Atlantic, respectively, but they found the increasing concentrations of both monosaccharides and polysaccharides during the “productive” season. However, monosaccharide concentrations seem to be less variable than that of soluble polysaccharides which are a result of phytoplankton production.[50] Similar trends of dissolved organic carbon (DOC) increase in late spring and summer were described for the northern Adriatic,[42,51,52] as well as for the Gulf of Trieste[53,54] which is in accordance with the general accumulation of DOC during the “productive” season in mid and high latitude coastal surface waters.[55] Considering the late spring mean DOC surface concentrations off Marche[51] of about 150 µM, it would appear that TDCHO comprise up to 40% of DOC. In the Gulf of Trieste, the mean spring and summer DOC concentration averages 100 µM,[54] hence, TDCHO comprise 20–30% of DOC. Both percentages, higher than the values reported so far in marine research,[50,56,57] confirm the importance of dissolved carbohydrates in the northern Adriatic waters.

The late spring and summer increase of TDCHO and polysaccharides in the northern Adriatic, amounting to more than 40 µM C, leaded to abundant mucilage masses in the years of 2002 and 2004, and in lesser extent in 2003. Our results imply that an increase of soluble polysaccharides in seawater can be the first step towards polymeric chain agglomeration leading to the formation of macroaggregates and the successive appearance of large mucilage masses predominantly composed of carbohydrates (Table 1). The higher concentrations of free macromolecules can favour the formation of more complex and crosslinking chains producing gels[33] and macrogels.[30] The aggregation process can be explained by polymer gel theory[33] through the formation of nanogels and further of microgels[30] and macrogels, i.e. mucous macroaggregates.

During the transformation of DOM to POM the changes in size and reactivity of macromolecular material proceed. Bacterial utilization of high molecular weight compounds is possible only after their hydrolyzation to low molecular compounds of approximately 500–1000 D[58,59] depending on the ability of microbial community to generate specific extracellular enzymes.[60] However, the hypothesis that extracellular enzymatic hydrolysis is a limiting step in macromolecular organic matter degradation in the marine environment is not always confirmed.[61] The uncoupling between phytoplanktonic production and removal processes[62] could be due to macromolecular nature of dissolved organic matter released from phytoplankton.[63] This represents less reactive substrate to bacterial degradation and lead to its concentration and accumulation.[64,65]

Carbohydrates are important component of excreted photosynthetic products[66] so the increase of dissolved carbohydrate concentrations can be related to higher phytoplankton biomass occurring before the macroaggregate appearance.[67] Phytoplankton is known to be a major source of exopolymers [47,50,57,68–70] which are the main precursor of polysacchridic material important for formation of mucilage.[70] Higher concentrations of TDCHO in seawater have been observed during and after the phytoplankton blooms[72] and especially when phytoplankton becomes nutrient limited.[66,73–75] However, the relation between phytoplankton biomass (from Chl *a* concentrations) and concentrations of dissolved carbohydrates is at present rather obscure. Cozzi et al.[9] found higher Chl *a* concentration in the years of mucilage appearance compared to those without mucilage. Conversely, some recent studies indicate no significant increase in phytoplankton biomass in years with mucilage events.[7,76] In our study, the concentrations of Chl *a* (with the same dominant species, i.e. *S. costatum*) in February of 2001 and 2004 off Pesaro were very high, amounting to 28 and 20 μg/L, but only in 2004 they coincided with an increase of TDCHO and successive formation of macroaggregates. The spring Chl *a* peaks were generally lower then the before mentioned values and also the summer concentrations, even in the years of mucilage events, were low. The concentrations of Chl *a* measured in the Gulf of Trieste in 2004, also characterized by mucilage appearance, were 10–20 folds lower than those determined off Pesaro.[77] Considering the phytoplankton response time to environmental conditions of days to weeks, some chemical signals could be missed due to a large temporal scale sampling usually used in mucilage monitoring.

Variability and imbalanced availability of nutrients influence the phytoplankton physiology and have been often connected to macroaggregates events.[9] Various studies reported that the marked variation of DIN/PO_4^{3-} ratios can cause nutritional stress to algae leading to increased polysaccharide execration with a consequent formation of macroaggregates.[10,78] It seems that variable trophic conditions, reflected also by changes DIN/PO_4^{3-} ratios, could be more important that constant nutrient deficiency (such as P-deficiency) and lead to enhanced release of dissolved organic matter and macroaggregate formation.[9] On the other hand, P-limitation may limit the bacterial degradation of exudates[25] allowing their accumulation and aggregation. The northern Adriatic is thought to be primarily phosphorous limited before and after the substantial reduction of phosphorous load by the Po River.[79] Recently, a significant increase of DIN/PO_4^{3-} ratio was observed in the northern Adriatic surface waters.[78,80] The biogeochemical processes occuring in surface layer seem important for macroaggregate formation[79]and organic matter[47–52] transformation and reactivity.

In the northern Adriatic, the months before to mucilage event (March – May) can be characterized as an "incubation" period with favourable condition for organic matter production.[79] The spring increase of DIN/PO_4^{3-} ratio in our study, probably due to phytoplankton use of DIN, mostly[5] as NO_3^-, and PO_4^{3-}, was regularly followed by an increase of TDCHO and PDCHO concentrations (Fig. 2). However, disolved organic phosphorous (DOP) and dissolved organic nitrogen (DON) can be also used by phytoplankton.[81,82] The DIN/PO_4^{3-} ratios showed a moderate increase in spring 2001 and 2005 when the mucilage did not appear, while this ratio increased significantly during the spring of 2002, 2003 and 2004 with the presence of mucilage. Conversely, Degobbis et al.,[79] reporting higher values of DIN/PO_4^{-3} ratio before the mucilage event in the northern Adriatic in 2000 and 2001, believed that the DIN/PO_4^{-3} changes do not trigger the mucilage events. However, they hypothesized that it could have an important role especially during late spring in the pycnoclines and frontal areas between more eutrophic freshened and oligotrophic high salinity waters where P-limitation can alternate with N-limitation.

We found in the temporally short but detailed study in the mostly oligotrophic Gulf of Trieste[83] performed in spring and summer of 2004 that markedly changes of measured parameters were observed in May including first decrease of NO_3^- and later PO_4^{3-} concentrations by phytoplankton. Similar situation was already reported for the mucilage event in 1991[5] and during the occurrence of marine snow shortly after the nutrient exhaustion in mesocosm experiment.[75] The subsequent increase of DIN/PO_4^{3-} ratio was followed by a decrease of Chl *a* concentrations suggesting P limited stress conditions. Approximately one month later the enhanced concentrations of dissolved total carbohydrates and polysaccharides were encountered successively allowing accumulation of dissolved macromolecular organic matter and assembling of macromolecules into macrogels. The interdependence between DIN/PO_4^{3-} ratio and particulate carbohydrate concentrations during the macroaggregate phenomenon was already reported for the Gulf of Trieste in spring of 1991 characterized by mucilage appearance.[5]

High DIN/PO_4^{3-} ratios were also determined in November and December 2003 off Pesaro and similar response, i.e. higher concentrations of carbohydrates reaching 73.2 μM C TDCHO, was observed in February 2004. This value was much higher then that, i.e. 40 μM C, supposed to be necessary for development of mucilage.[47] However, the mucilage did not appear successively but only later the unbalance of DIN/PO_4^{3-} ratio leaded to increase of dissolved carbohydrates in May and June 2004. Also, the carbohydrate concentrations present in the years of mucilage events, i.e. 2002, 2003 and 2004, was not much higher than those observed in the years when the presence of macroaggregates is negligible (2001) or even absent (2005). Lower concentration could be also due to the scavenging processes by larger aggregates resulting in decrease of measured concentrations in the dissolved fraction.[84]

We would expect, however, that increasing the concentration increases the enchances of interactions/contacts between polysaccharides leading to the gel formation. High concentrations seem not to be the only important characteristic related to polysaccharides. Various conformations and shapes that they can adopt in aqueous systems influence their solubility and rheological properties in aqueous media.[85] In solution polysaccharides mostly exist as fluctuating disordered chains and tend to adopt more or less coiled[86] structure. Like other polymers, they can also form helical structures[87] with different number of strands (single or multiple helices) or local ordered conformation and local helices. Chemical and structural polysaccharide characteristics depend also on environmental changes such as ionic strength, temperature, pH and degree of hydration. The additional variability arises from changes in charge density of polysaccharide chain due to enzymatic or/and photochemical modification of groups.[85] The chemical modifications and various polysaccharide mixtures in aqueous media could lead to novel rheological (gel) properties of polysaccharides.[88] Hence, the high concentration of polysaccharides seems to be only one of the prerequisites needed for mucilage formation.[47]

In the Gulf of Trieste (northern Adriatic Sea), the intense aggregation of macroflocs proceed after the rain storm event as a source of particles. The input of riverine and atmospheric particles, probably acting as crosslinking agents and aggregation nucleus, stimulates the formation of huge mucilage masses. Ca^{2+} and Fe^{3+} ions have been already reported to be very efficient cross-linkers between polysaccharides.[33,89] In this way, a three-dimensional polymer network in water is probably stabilized by a formation of a higher degree of ion-bridging between polymers[33] and the entrapment of water in the network resulted in hydrogel structure. The importance of organic matter – mineral associations in mucilage phenomenon (i.e. its formation, evolution and transformation) is confirmed also by the distribution and accumulation of macroaggregates. Intense aggregation processes and further the accumulation of macroaggregates usually coincides with a higher concentration of the dissolved (and colloidal) organic matter and mineral particles at phase boundaries such as the sediment-water and air-sea interfaces, and at pycnocline. When the larger suspended particles or macroaggregates were formed the increasing collision and coagulation rates between them and other suspended particles[84] contributes to clarification of the sea water column which was usually observed during the greater macroaggregate appearance. Their scavenging capacity impacts the cycling of organic matter and the whole plankton community.

Therefore, besides higher polysaccharide concentrations, the presence of particles,[14,15] the specific hydrographic conditions including the formation of gyre, stratification, higher water residence time and reduction of water exchange with the central Adriatic in late spring and summer and lower turbulence,[90] photochemical reactions including photooxidation and photopolymerization,[91] and the specific quality and assemblage of dissolved organic matter[92,93] and exudates are probably required in macroaggregates formation and evolution.

4. Conclusions

Our study indicates that in spring, before the appearance of macroaggregates, high concentrations of dissolved polysaccharides, comprising the great majority of total dissolved carbohydrates, appeared in the waters of the northern Adriatic Sea. Conversely, the dissolved monosaccharide concentrations remained nearly constant during the whole study period. The reason seems to be related to an increase of the DIN/PO_4^{3-} ratio in spring before the macroaggregate appearance, probably due to intense phytoplankton use of nitrate and phosphate, since during the years in which mucilage was not present the DIN/PO_4^{3-} ratio was lower. In addition to higher polysaccharide concentrations and DIN/PO_4^{3-} ratio, hydrological, (photo) chemical and biological factors, such as phytoplankton physiology, seem important for macroaggregate formation. However, the concentration level of dissolved polysaccharides observed before the mucilage appearance and variation of DIN/PO_4^{3-} ratio could be some of relevant indicators to predict the phenomenon of extensive macroaggregate formation which is well known for its negative impact on tourism, fishery and aquaculture in the northern Adriatic Sea. To asses the important temporal changes more frequent sampling scheme is needed for a better understanding this complex phenomenon.

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Povzetek

V severnem Jadranu smo ob obali dežele Marche (Italija) v času sluzenja morja (v letih 2002, 2003 in 2004) in v obdobju brez prisotnosti sluzastih makroagregatov (v letih 2001 in 2005) študirali vlogo raztopljenih ogljikovih hidratov pri nastanku makroagregatov, tj. koncentracije celotnih ogljikovih hidratov ter njihovo monosaharidno in polisaharidno frakcijo. V letu 2004, ko je bil prisoten pojav sluzenja morja, je raziskava potekala tudi v južnem delu Tržaškega zaliva (Slovenija). Vzporedno smo spremljali še fitoplanktonsko biomaso izraženo kot vsebnost klorofila *a* ter koncentracije dušikovih in fosforjevih hranil. Koncentracije celotnih ogljikovih hidratov, predvsem pa polisahridna frakcija, so bile višje v spomladi in zgodaj poleti, tj. pred pojavom sluzenja morja, koncentracije raztopljenih monosaharidov pa se skozi obdobje raziskave niso bistveno spreminjale. Poleg visoke koncentracije raztopljenih polisaharidov so za razvoj makroagregatov v severnem Jadranu pomembni še drugi, tj. hidrološki, kemijski in biološki dejavniki. Razmerje DIN/PO_4^{3-}, ki v spomladanskem obdobju zaradi fitoplanktonske porabe nitrata in fosfata poraste, je lahko indikativno za pojav makroagregatov. Le-to je bilo veliko nižje v letih 2001 in 2005 glede na leta s prisotnostjo makroagregatov (2002, 2003 in 2004).